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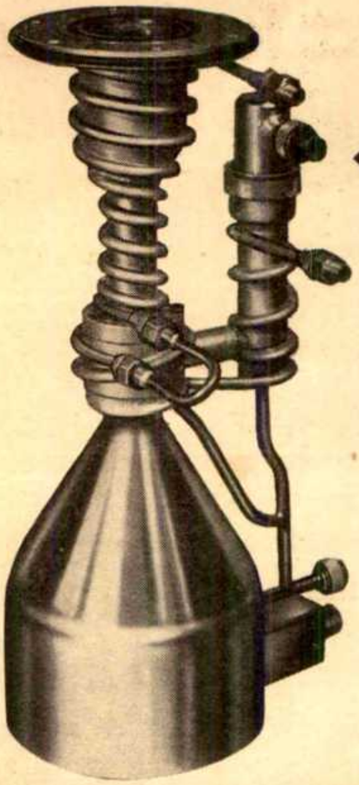

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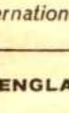

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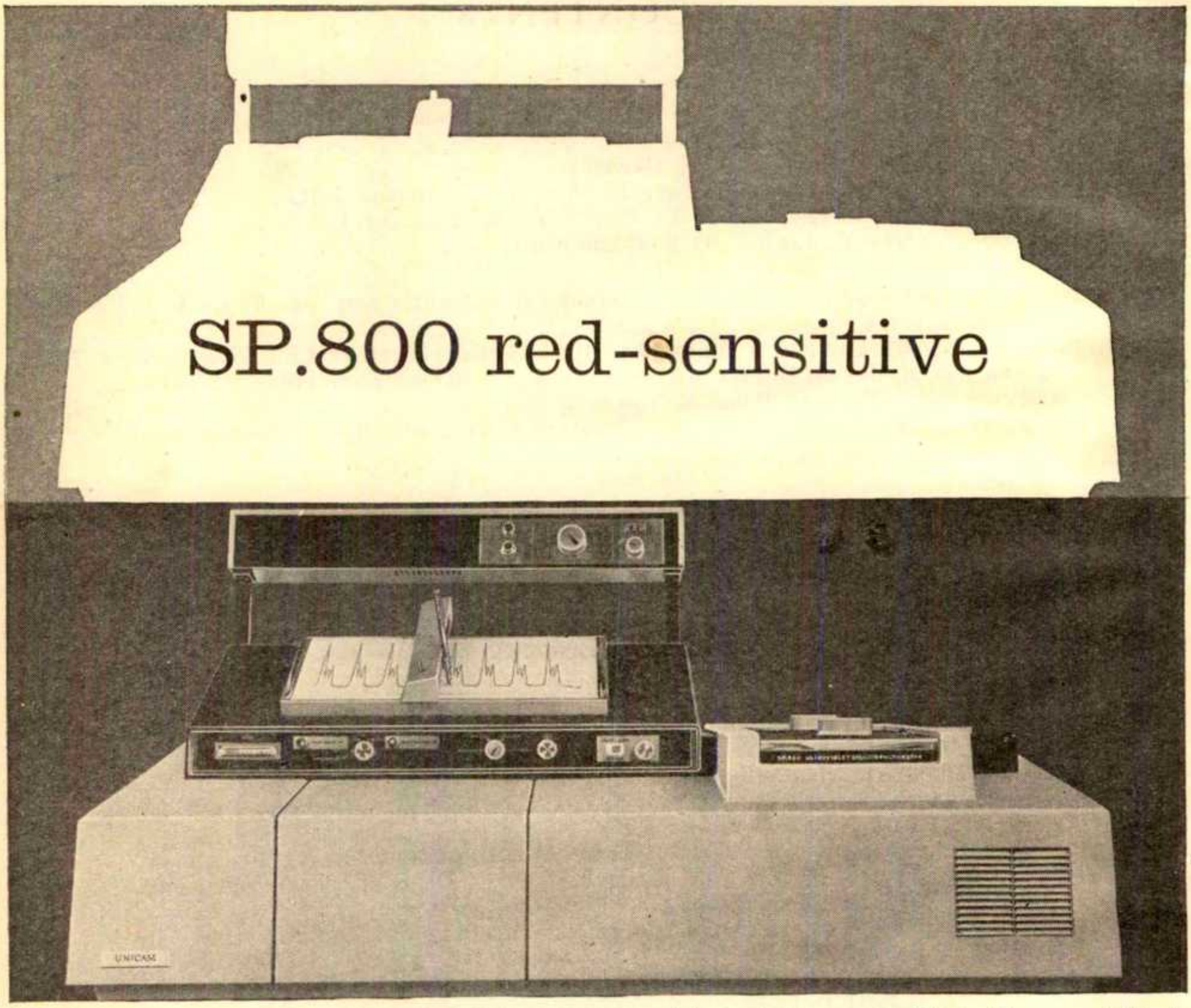

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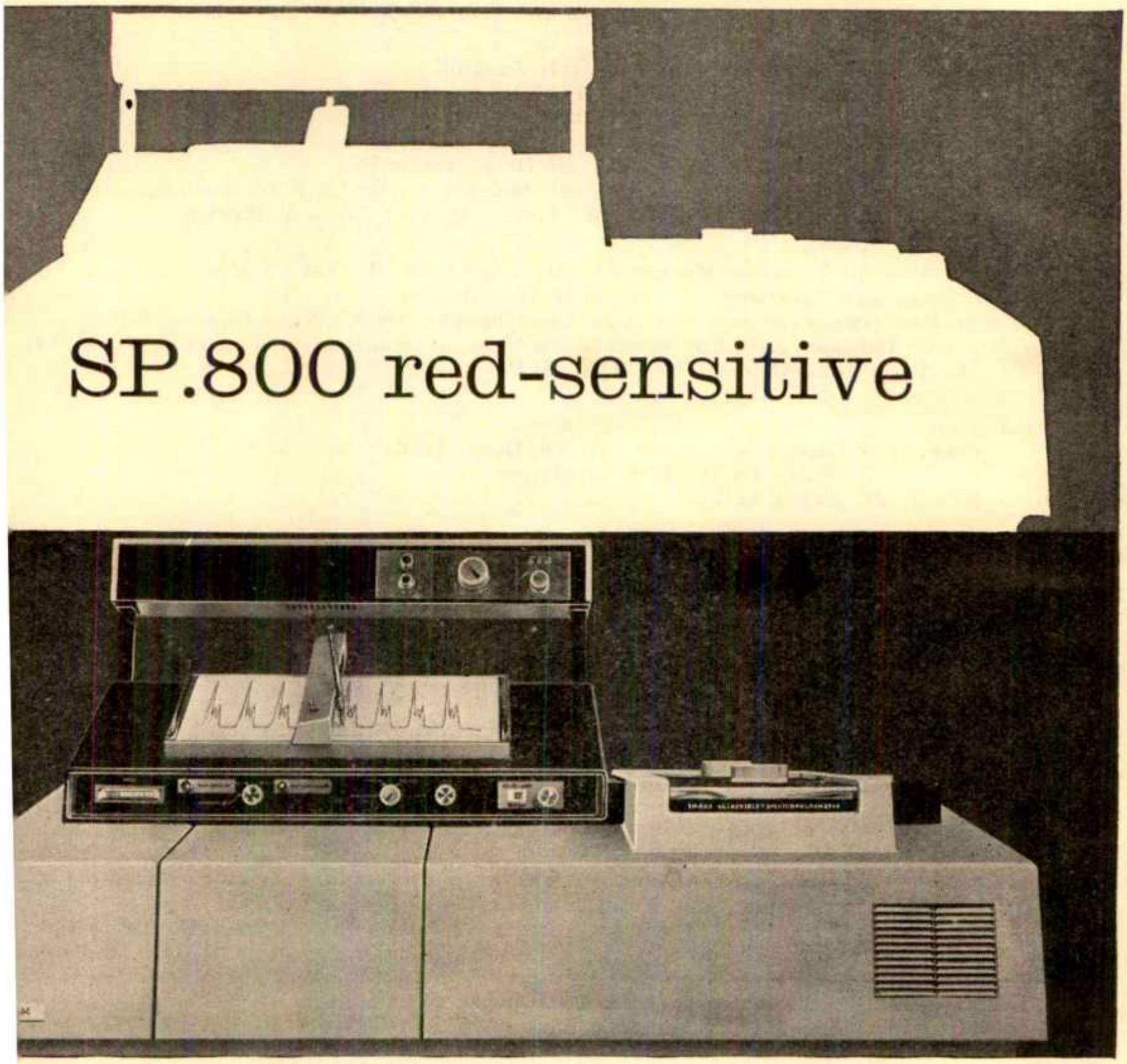

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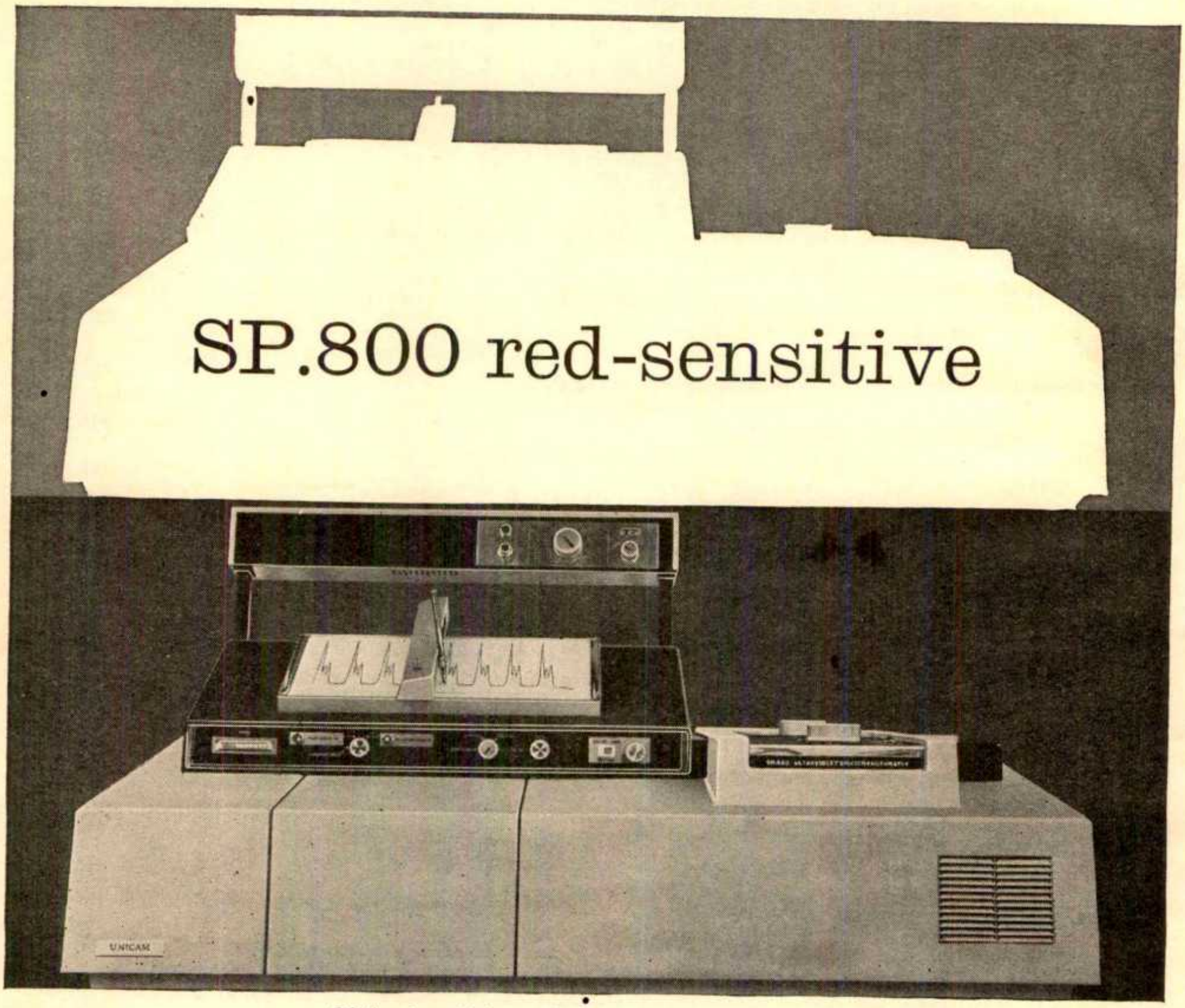

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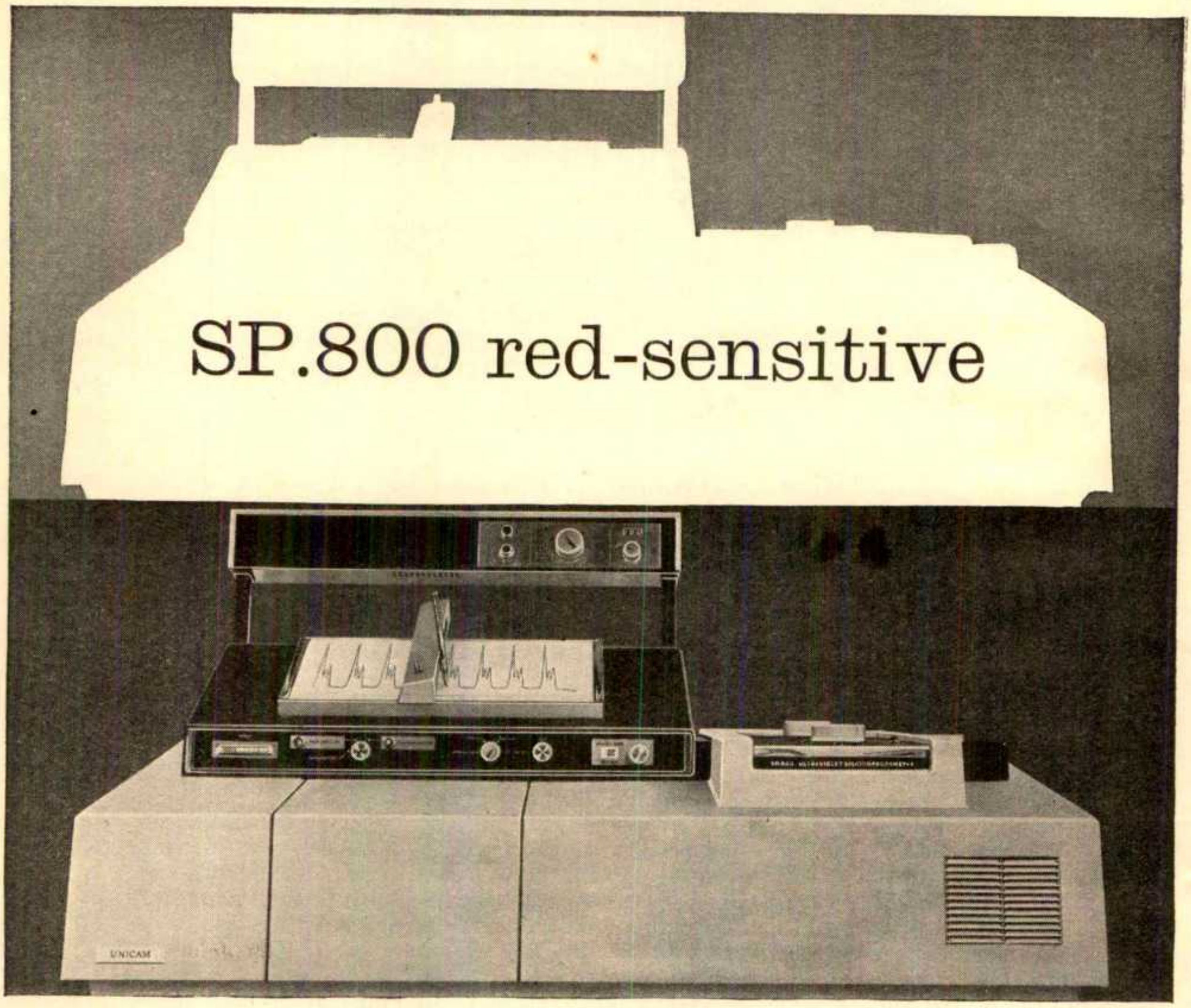

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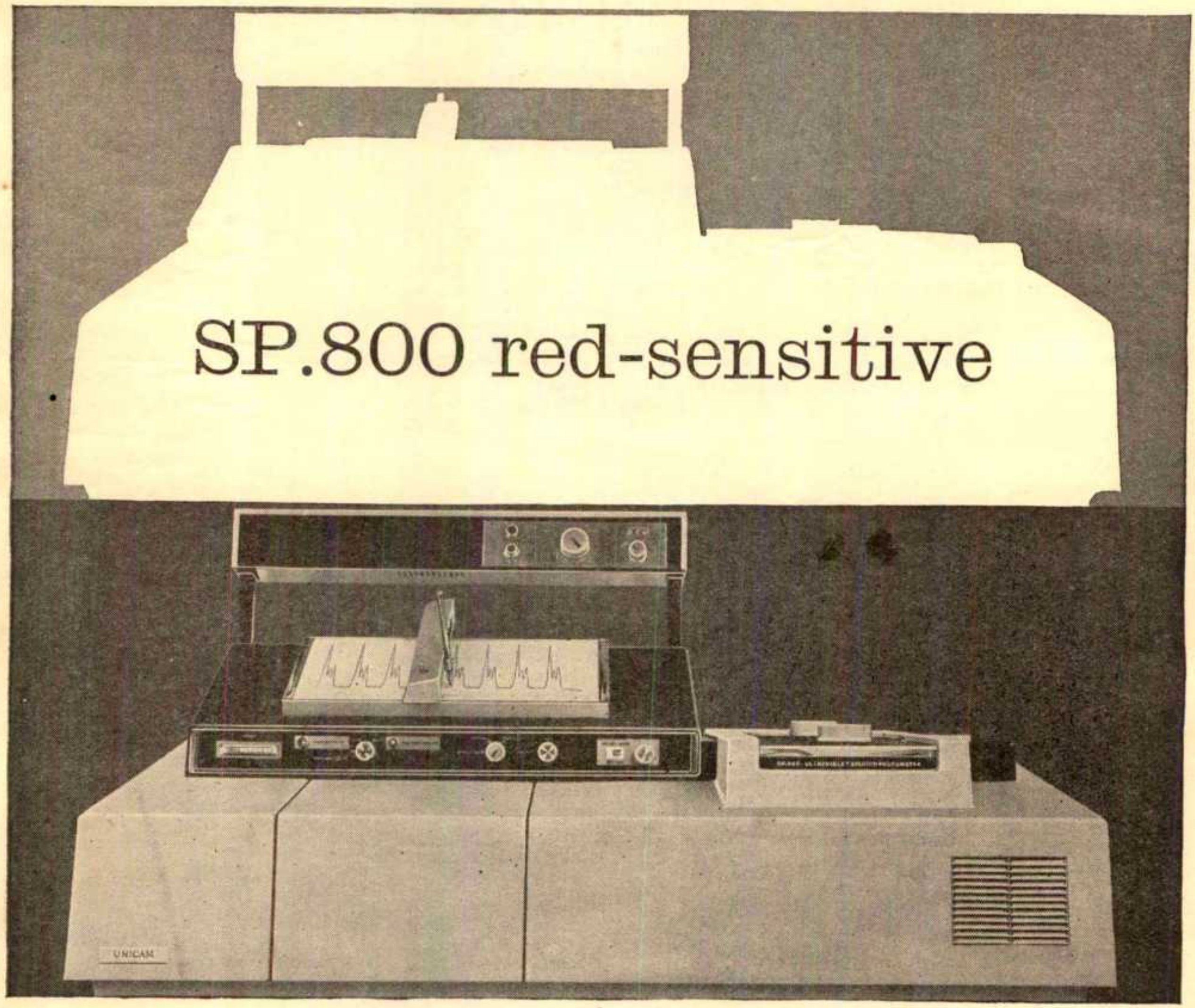

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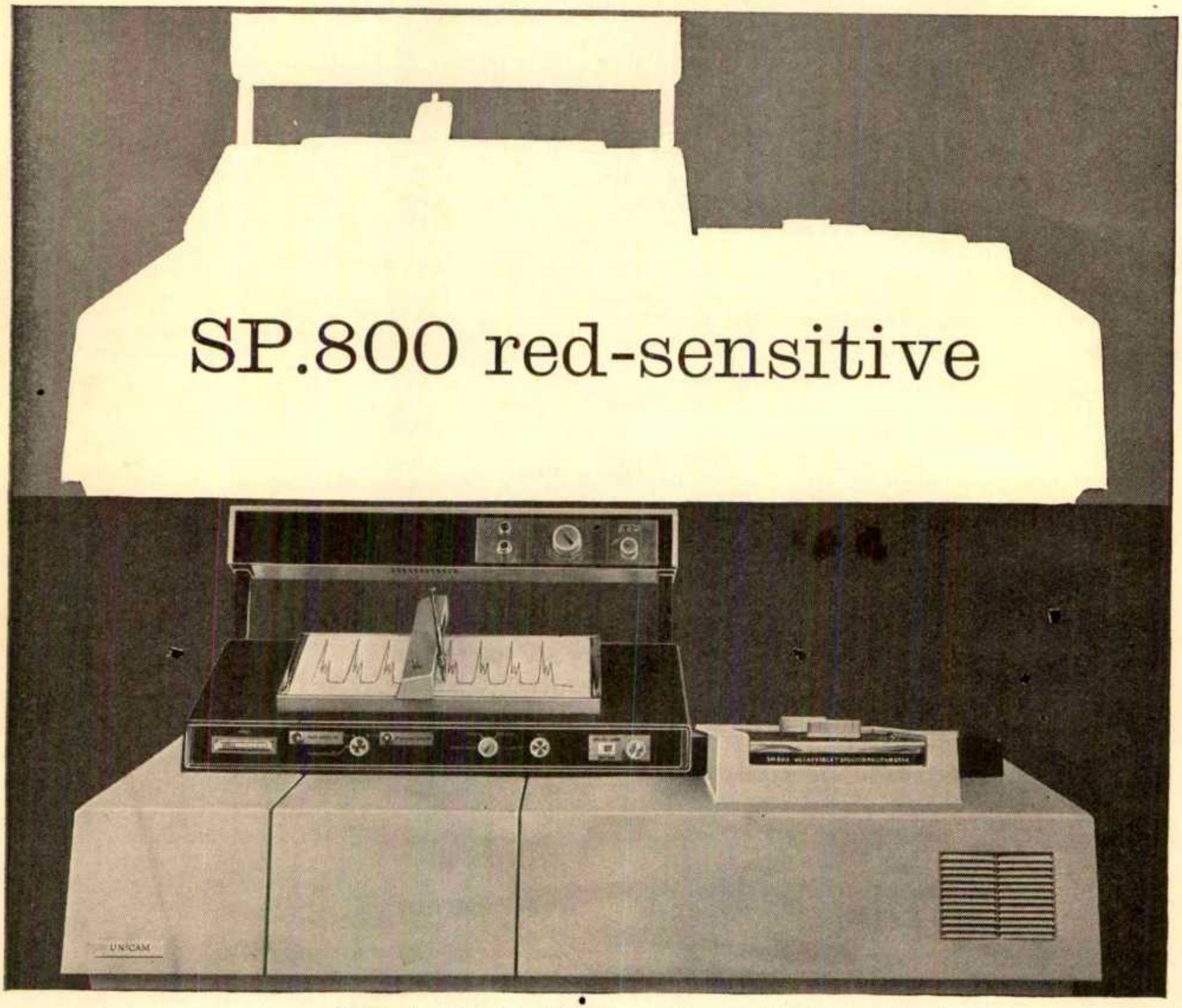

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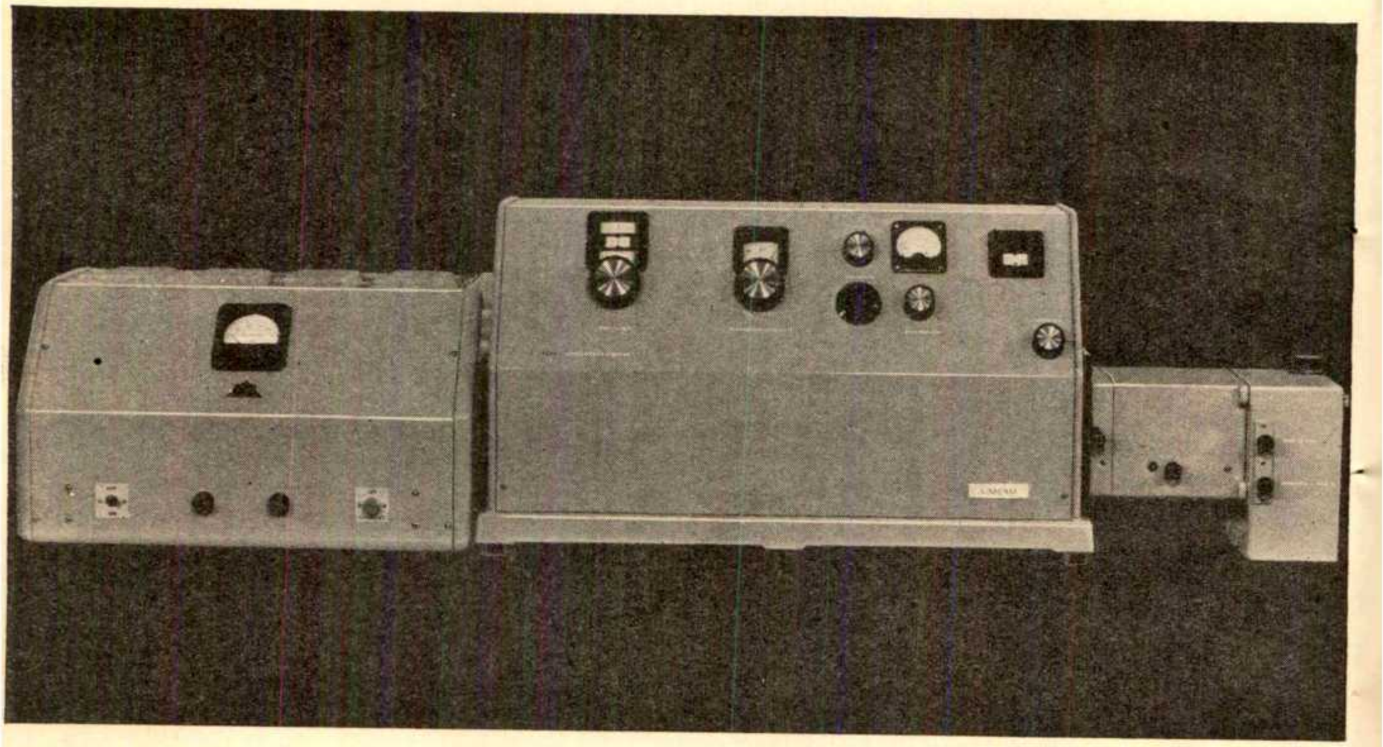

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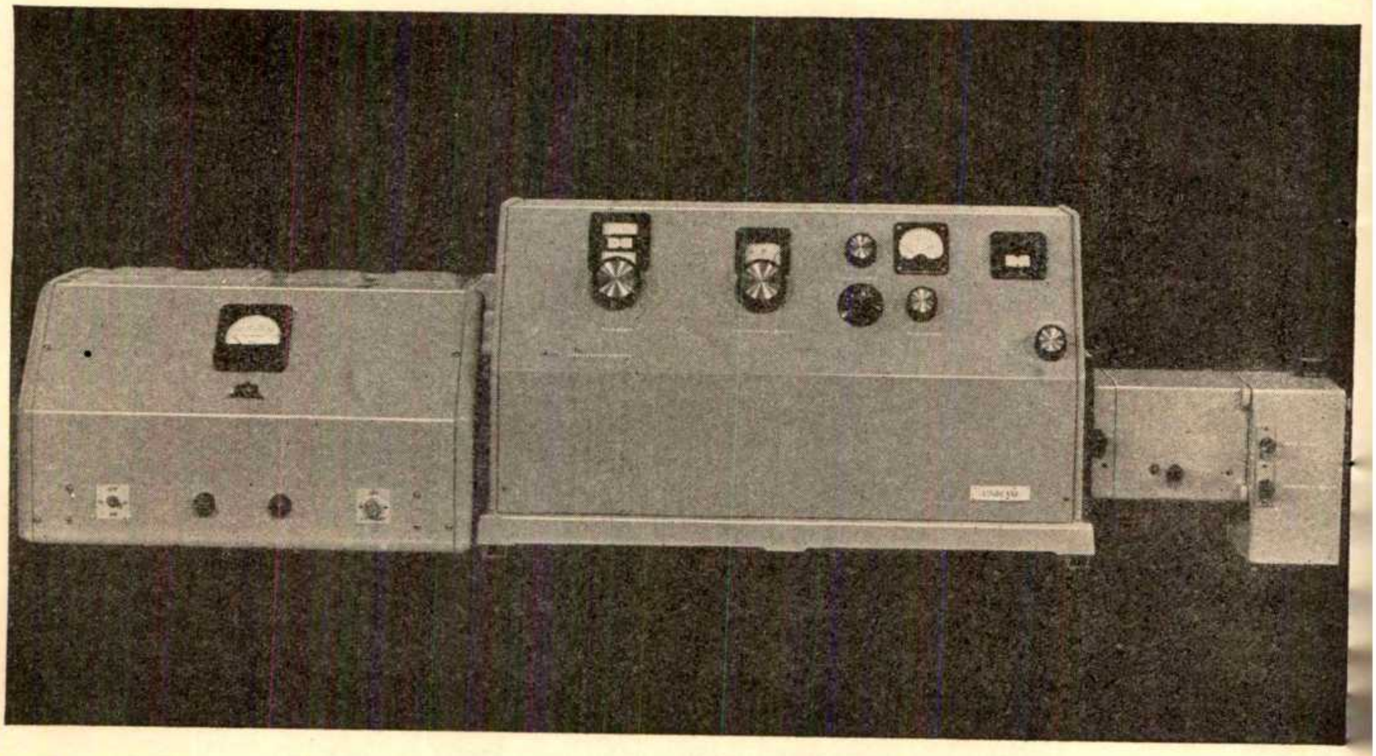

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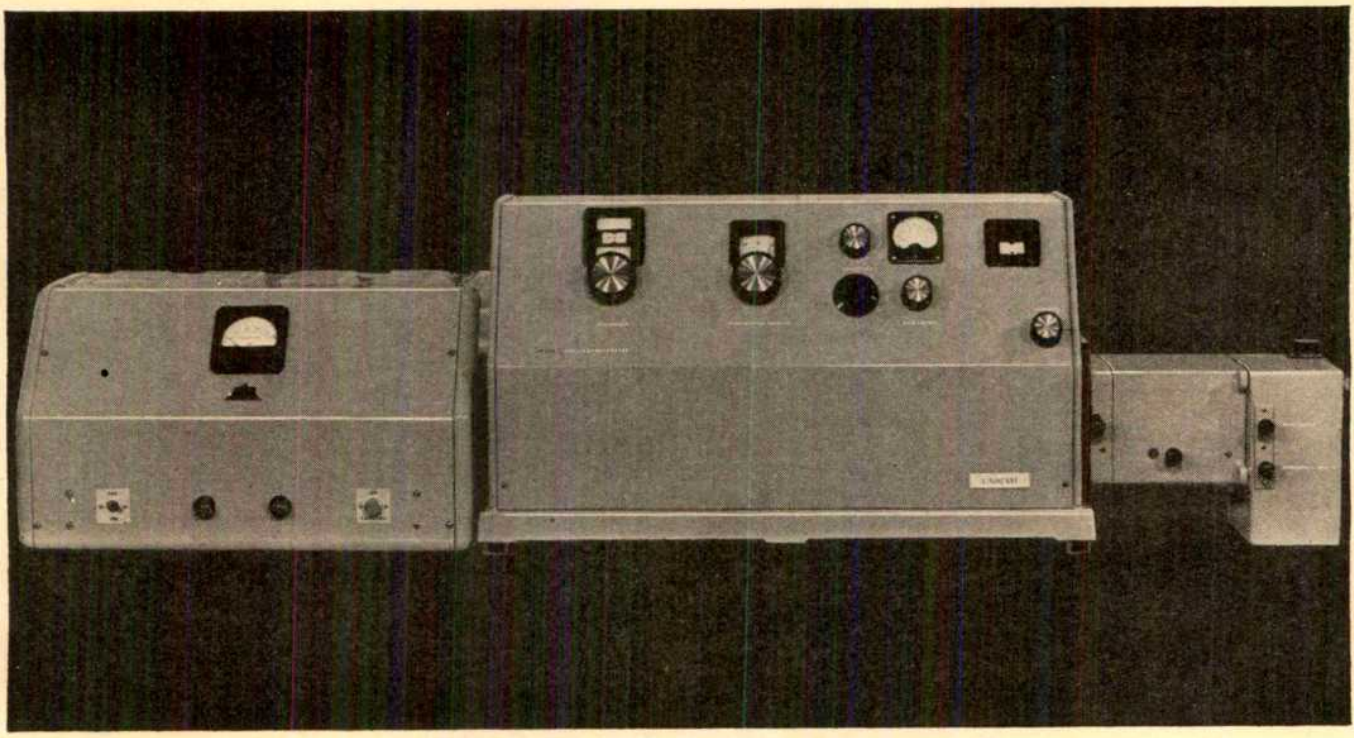

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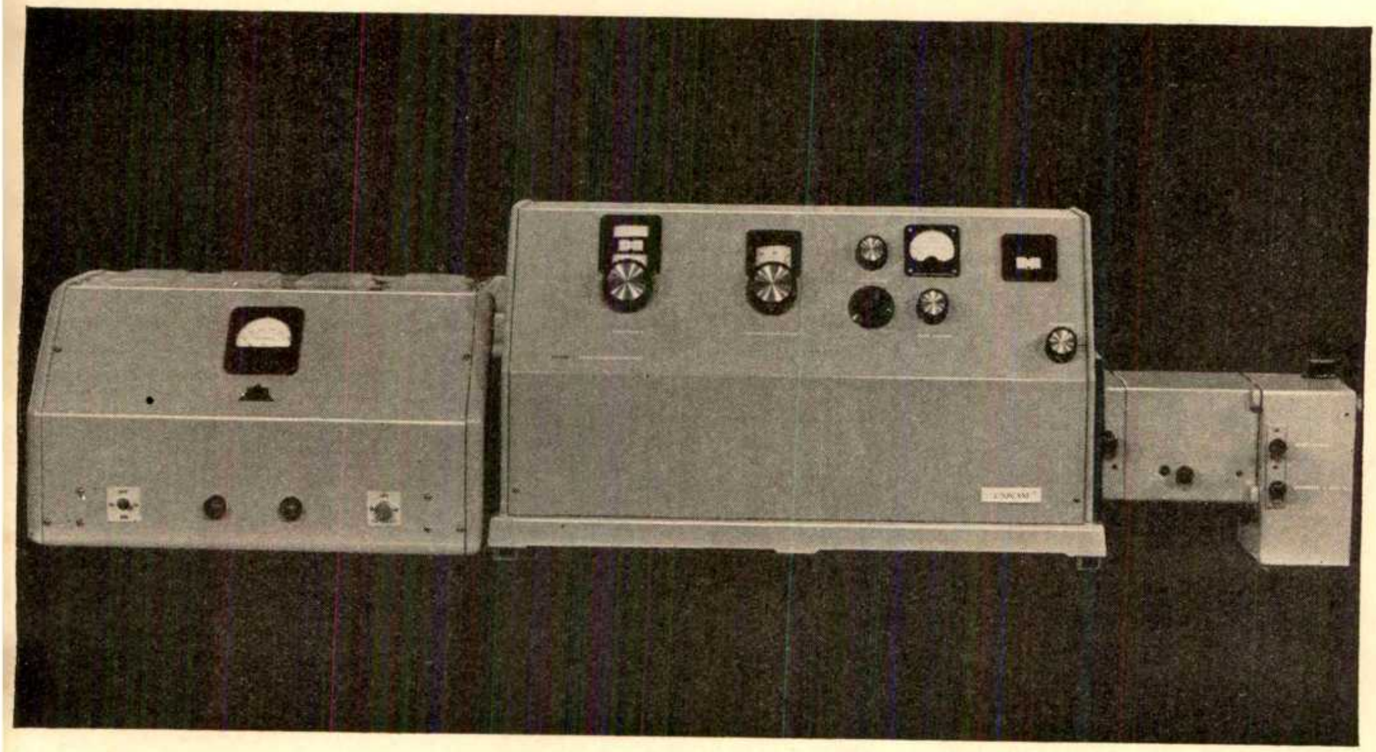

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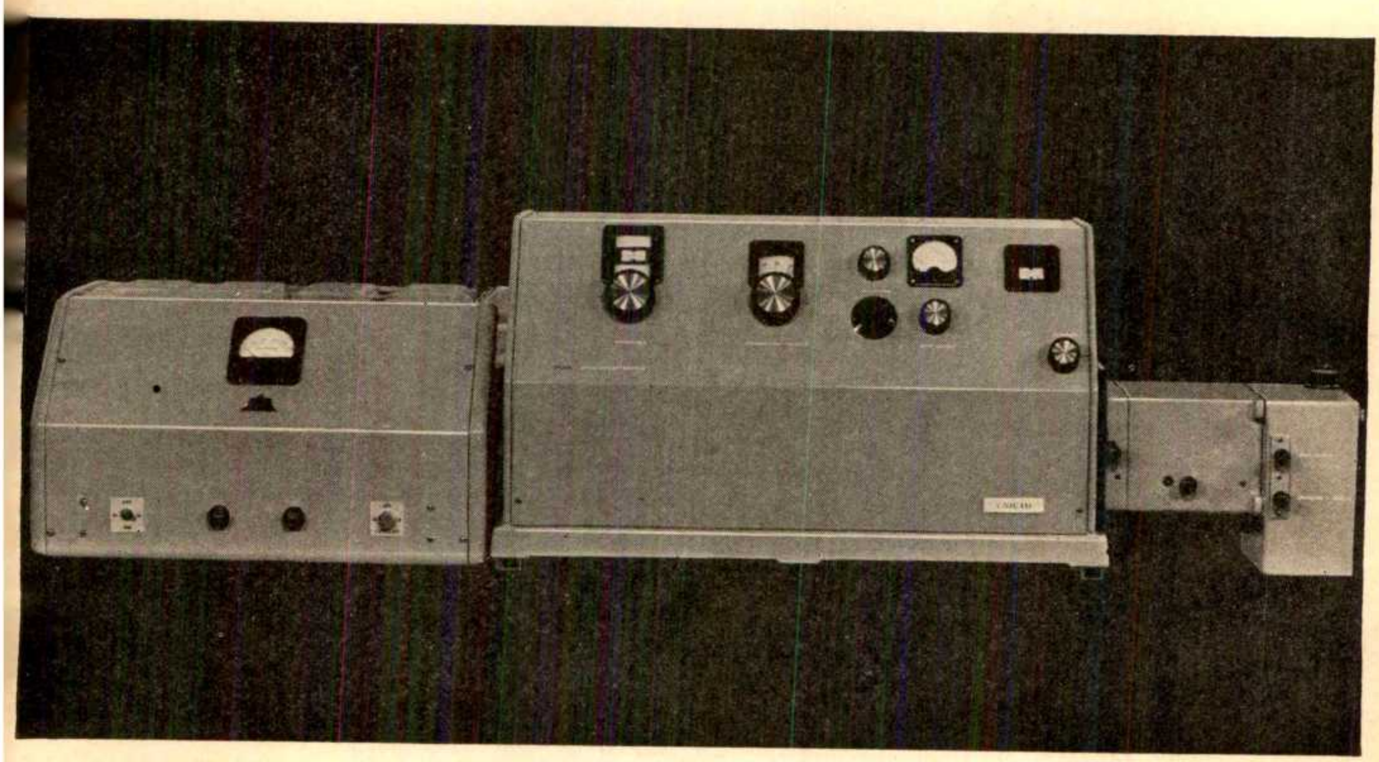

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PROBLEMS OF LEISURE

THE debate in the House of Lords on the problems of leisure on May 13 was recognized to be in direct sequence to the earlier debate on automation on April 8. Although Baroness Burton of Coventry, who opened it, withdrew her motion, the debate essentially developed further some arguments which had been advanced in the earlier debate, and it drew from the Government a reply generally regarded as satisfactory. In spite, however, of the extent to which the two debates were directly related, the second debate covered an even wider field, and its discussion of youth service and the needs of youth was highly relevant to some subsequent incidents of the Whitsuntide week-end. If leisure was seen as an inescapable sequel to the growth of automation, it was recognized that the use of leisure calls for just as much forethought and preparation as the introduction of automation.

Like the Bishop of Coventry in the earlier debate, Baroness Burton emphasized that the two problems were inextricably bound up together, but she added that automation must come at a time of full employment if it was to be acceptable, and that the problem of leisure demanded a completely changed outlook, untrammelled by conventional thinking. Automation and decreasing hours of work made it imperative that the social consequences of increased leisure should receive attention, that people should be helped to use their leisure and provided with the facilities and opportunities for enjoyment. This would mean facing the implications of the inevitable extension of shift working, as well as providing the resources needed, for example, by the Arts Council and the Central Council for Physical Recreation.

The Bishop of Chester reiterated the importance of adequate financial help to the latter Council, the National Playing Fields Association and to other bodies under the Physical Training and Recreation Act, and he did not think that sufficient credit had been given to the Government for what had already been done, particularly since the publication of the report of the Wolfenden Committee on sport. This he was inclined to attribute to an air of improvisation and a lack of continuity in policy; and he thought that there should be an appropriate section in the Department of Education and Science, as well as a Sports Development Council. The challenge to the whole personality which leisure now made could not be met except on the basis of an adequate education in the fullest sense, and this must include the physical as well as cultural and creative activities. The Bishop referred particularly to the need to provide adequately for the needs of the gap between school age and adult-hood noted by the Wolfenden Committee, and also to develop a sense of service such as found expression in voluntary service overseas.

Lord Willis made some reference to the magnitude of the growing demand for recreational facilities, especially for young people. While his suggestion that many of the natural leaders of the youth of to-day had been creamed off in the educational process was later challenged, his sympathetic presentation of the situation of that sector of society included some constructive clues for positive action. We must be concerned with conditions as well as facilities. Legislation for leisure is impossible—unthinkable as well as impracticable—but a scientific revolution calls for a cultural counterpart, and the workers who are trained in new skills for an age of automation must be helped also to find cultural and spiritual development. Lord Willis emphasized that we should start in the schools by arousing interests and stimulating imagination: the service of youth needed overhaul—adequate finance, a big effort to recruit and to train voluntary leaders, and the extension and adequate use of facilities.

Like other speakers, Lord Willis referred appreciatively to the value of such schemes as the Duke of Edinburgh's Award, and he spoke also of the possibilities attending imaginative support of the Arts Council and of the National Theatre, but his final note was the desperate urgency of effective action. Baroness Elliott endorsed much that Lord Willis had said, but thought that the Albemarle Report had led to some improvement in the situation regarding youth leaders; the target of 1,300 new leaders was expected to be reached by 1966, and 142 local education authorities already employed more than 4,000 part-time leaders. She too stressed the urgency of the situation, though she welcomed the increased grants to voluntary organizations by the Department of Education and Science, who had brought in some 38 voluntary organizations since the Albemarle Committee reported. More needed to be done for young adults—she visualized a rather sophisticated and more adult club, with time to talk and to think.

The Bishop of Norwich reminded the House of Lord Bowden's plea for a comprehensive programme of re-education for the nearly 1·5 million of unskilled workers who might well become unemployable if the changes required to maintain the projected growth in our economy were effected. Especially, he stressed the grave possibility of creating and perpetuating large pools of virtually unemployable people; areas of self-perpetuating sub-culture, with all the processes of degeneration built into them. It is this prospect that any educational system which can be regarded as adequate for an industrial society to-day must seek to meet, and the Bishop maintained that this was a vital task of education—to promote interests, a sense of purpose, an enquiring mind and good health—though the home also had its responsibility here. With this, more capital and personal equipment, though not necessarily all, should be provided by the Government for many forms of leisure. In making this suggestion he related the whole debate to the situation revealed in the Newsom Report, *Half Our Future* (*Nature*, 201, 1; 1964). particularly in its chapter "Education in the Slums".

In the conditions described in that chapter, as the Report points out, no adequate education can be given, and the Bishop of Norwich pleaded that it was particularly the children in such areas, and the teenagers in the new housing estates, who should receive first consideration so that they had some chance of fitting properly into a society that professed to value such attributes as orderliness, cleanliness, friendliness, decency and charity. These boys and girls, above all others, needed, as the Newsom Report urged, access to the countryside, the experience of living together in beautiful and civilized surroundings and a chance to respond to the challenge of adventure. They need priority in relation to school journeys, overseas visits and adventure courses. A concern for leisure is a concern for the wholeness and fulfilment of human beings.

It is from that point of view that we can best appreciate the wealth of opportunities for creative thinking, to which Lord Luke referred, offered by the whole problem. He also indicated certain aspects related to the problems of housing generally and also opportunities in which further research might indicate possibilities of greater efficiency and economy with equipment. Baroness Swanborough dealt mainly with the contribution which voluntary service could offer, not simply for growth, but service by youth, particularly to facilitate the participation of youth in voluntary service of all kinds in Britain as well as overseas. The opportunity must be presented with imagination and with some understanding of the conditions under which youth was able to respond, and not simply of the convenience of the voluntary organization itself.

The debate thus covered a wide field, including appropriate reference to the elements of discipline and judgment, and while there were many reservations by other speakers as to the extent of certain developments, it was curious that no one came nearer than the Bishop of Norwich in touching on the argument of Prof. Dennis Gabor's lecture on "Technology, Life and Leisure" to the Royal Institution (*Nature*, 200, 513; 1963), which, like his book *Inventing the Future* (*Nature*, 200, 923; 1963), apart from a reference by Lord Milverton, was also ignored in the earlier debate. The Earl of Bessborough, replying on the debate for the Government, noted that some ten Government departments were interested in the subject and anxious to brief him. Referring to the provision already made, he said that in 1963 the local education authorities provided opportunities for leisure and recreational activities attended by nearly 1·9 million evening students; 1 million had attended evening institutes, and almost 250,000 had enrolled for day-time study in further education establishments. Total expenditure by the Department of Education and Science on adult education in the present financial year was expected to reach about £1 million, apart from the contribution made by the local education authorities and the responsible bodies themselves. On the gap between school and adult life, he said that two-thirds of those in the three age-groups 15–17 were receiving instruction of some kind or another. The possibilities of radio and television in education generally were being examined and he anticipated a definite decision before very long about a wholly educational and experimental channel.

Lord Bessborough also touched on some developments in the public library service and on the support given to the arts through the national museums and galleries, the Arts Council and in other ways—expenditure on which had risen to £11·75 million in 1963–64 from £6·75 million in 1958–59 and was expected to reach £13·5 million in 1964–65. In 1963–64 local education authorities spent £11·25 million on social and physical training under the relevant sections of the 1944 Act, compared with only £6·25 million in 1959–60, nearly half going on the Youth Service. He undertook to ask the Minister to look again at the proposal for a Sports Development Council, and he said that the Minister of Housing and Local Government would like to help the planning authorities to do more to develop recreational facilities in the national parks and to extend the scope of Exchequer grants for that purpose.

It is clear from the debate that the traditional view that education ceases on leaving school or university is already vanishing and that, as Prof. Colin Cherry observed in his lecture "The Scientific Revolution—and Communication" (*Nature*, 200, 308; 1963), education is accepted as a process continuing throughout life. The implications are far from being worked out and account must be taken of them at all stages in our educational system: preparation for leisure, adaptability, cannot be fostered too early, and if curricula are to be very broad and development slower, as Prof. Cherry suggests, examination requirements and entry demands for institutions of higher education must conform with the situation. Education will not make leisure creative, still less meet the needs of the feebler-minded unless it is shaped for the task, and this may call for research, as well as perhaps the use of new tools and techniques.

What could be said of the debate is that it did not focus attention sufficiently sharply on the problem presented by the lowest intelligence group which is threatened most acutely with unemployment as automation extends. This group must, as Prof. Gabor argued, be used for its own good and for the good of society, even if it means some restriction of automation by legislation. That is inescapable, however much we may succeed in fitting the group to enjoy leisure and to make effective use of it.

Probably here more than anywhere else lies the need for creative thought and experiment, and *that* should now be proceeding. Quite apart from the time required to enact any fresh legislation, its initiation by Government is likely to follow rather than precede the recognition by the public of its necessity. Such jobs as those of the dustman, the night watchman, the milkman, the postman and others are unlikely to be protected against automation without some wide public understanding of the situation, and it requires little imagination to visualize the outcry from vested interests which would attend any attempt to give effect to the suggestion of Prof. Gabor that cafeterias and supermarkets should be prohibited. Indeed, even such legal safeguards and prohibitions would not ensure that the posts would be filled by the type of labour in mind without attention to other conditions and a large measure of supporting opinion. What is certain is that automation involves much fresh and constructive thought about its social consequences, and that this should be proceeding as a matter of urgency simultaneously with the technological developments in automation. Only so are either Government or public likely to be ready for action in time to avoid social distress. That could well be also an imperative reason for immediate action to prevent commercial television developing any further obstacles to educational and local broadcasting.

There is a further point made by Prof. Cherry which found an echo in the debate in the House of Lords and which merits elaboration. Reference was made in the debate to the importance of voluntary service, and voluntary service overseas was recognized as providing a valuable outlet for the altruism and idealism of youth. Apart, however, from such satisfactions, the coming of automation is not to be considered entirely as a national affair. There can be no stability if the underdeveloped countries are disregarded. Prof. Cherry referred to the great change in the public conscience wrought by broadcasting and other news services, and to the way in which the great mass of people had now become aware of world conditions, of the increasing disparities and of the mounting political pressures. We cannot fail to consider the needs and position of the underdeveloped countries. he opportunity they offer for disinterested voluntary rvice in many ways and many fields may be no less aportant in its bearing on world stability and co-operaon than on the welfare and fulfilment of the volunteers om Britain who respond to the challenge.

BRAIN MECHANISMS AND MODELS

rogress in Brain Research

ol. 1: Brain Mechanisms. International Colloquium ponsored by the International Brain Research Organizaion (IBRO) on Specific and Unspecific Mechanisms of ensory Motor Integration; Pisa, 1961. Edited by G. Ioruzzi, A. Fessard and H. H. Jasper. Pp. xii+493. 140*s*. ol. 2: Nerve, Brain and Memory Models. Edited by N. Wiener and J. P. Schadé. Pp. viii+280. 80*s*. ol. 3: The Rhinencephalon and Related Structures. Edited by W. Bargmann and J. P. Schadé. Pp. vii+253. 70*s*. (Amsterdam, London and New York: Elsevier Publishing Company, 1963.)

VOLUME 1 of the series *Progress in Brain Research* is the product of a colloquium, sponsored by the International Brain Research Organization (IBRO), on specific and unspecific mechanisms of sensory motor integration. As the colloquium was held in honour of Prof. F. Bremer, it was appropriately concerned with the electrophysiology of the nervous system. There are some two dozen papers. The first is by Sir John Eccles and deals with post-synaptic and pre-synaptic inhibitory actions in the spinal cord. Ragnar Granit writes on recurrent inhibition as a mechanism of control. Among papers of more general interest is one by K.-E. Hagbarth and B. L. Finer on the plasticity of human withdrawal reflexes to noxious skin stimuli in lower limbs. It has been shown electro-myographically that there are two components in the withdrawal reflex—an initial spinal one and another later, probably cerebral. The second can be modified profoundly by hypnotic suggestion, being reduced in amplitude if the subject is told that no pain will be felt, and increased if the contrary is suggested. It was shown to be absent in a patient whose loss of sensibility in the limb was due to hysteria. An investigation by P. Dell shows how the reticular formation which influences alertness and attention is involved in a number of feed-back circuits which tend to maintain it in a state of homeostasis. Alternative sensory pathways and their thalamic integrations are discussed by D. Albe-Fessard and A. Fessard. A. Arduini discusses the tonic discharge of the retina and its central effects, among which is the signalling of the intensity of the steady illumination. The convergence of several sensory modalities on the same cortical neurones is a fact of obvious physiological and probable psychological importance, discussed by R. Jung, H. H. Kornhuber and J. S. Da Fonseca. Among the remaining papers are several on sleep, and the physiological contributions to it made at different levels of the nervous system.

This is mainly a book for specialists; but it provides a broad overall survey of present-day trends in the electrophysiology of the central nervous system, and for the non-specialist there are a few items of considerable general interest.

The development of cybernetics and information theory has provided a temptation to speculate on how the brain might work, which many ingenious minds have found irresistible. Some of the consequences are to be found in Volume 2, which contains the proceedings of a symposium on "Cybernetics of the Nervous System" held at the Royal Dutch Academy of Sciences in 1962. Neurologists, psychiatrists, biologists, engineers, mathematicians and physicists all contributed. There is a paper on the engineering approach to the problem of biological integration by J. D. Cowan from McCulloch's Laboratory at the Massachusetts Institute of Technology. The sequential behaviour of a set of idealized neurones is the subject of P. L. Latour's paper. He investigated nerve nets with mathematical methods similar to those in the theory of linear sequential networks. F. H. George deals with the logical net type representing a sort of idealized or conceptual nervous system. The cybernetic group of the Moscow State University is represented by A. V. Napalkov. P. Müller discusses the characteristics and patterns of learning matrices, and G. W. Zopf the cybernetic significance of the innervation of the accessory motor apparatus of the sensory systems, and the efferent fibres ending on or near the receptor cells. E. Huant approaches the problem of sensory information from the psychiatric point of view, and there are papers concerned with the construction of models explaining some of the parameters of human memory and thinking. G. Pask discusses models of learning and cognition in evolutionary systems and brains, and P. Nayrac puts forward his ideas on the problems of non-linearity in clinical and experimental neurology. Finally, there is a challenging paper by O. D. Wells entitled "Irrelevance of a 'Nervous System'". These are mostly entertaining speculations, and will not become more so until we know more about the finer anatomy and physiology of the neurone.

Volume 3 is a mainly anatomical discussion of the rhinencephalon. F. Valverde writes on fibre degeneration resulting from coagulations in the amygdaloid complex and anterior amygdaloid area, and traces these into three main projecting systems. O. D. Creutzfeldt, F. R. Bell and W. R. Adey investigate the activity of neurones in the amygdala following afferent stimulation, finding that the main input in the anterior area and the basal and cortical nuclei is olfactory, whereas real multi-sensory convergence is the exception.

A. W. Zbronzyna investigates electrical stimulation of the amygdala and shows this to cause autonomic and behavioural effects, which are obtained as components of the defence reaction, and infers the existence of an amygdaloid centre for the defence reaction connected to the integrative centre of this reaction in the hypothalamus. The finer anatomy of the hippocampus is described by T. McLardy and by J. P. Schade and K. Meeter. Other authors discuss the connexions of the olfactory bulb and other anatomical features of the rhinencephalon. The symposium ends with an unresolved debate as to the meaning of this term, A. Brodal wishing to confine it to those structures in the central nervous system which receive fibres from the olfactory bulb. Though by derivation the term rhinencephalon refers directly to the sense of smell, it has come to mean something more for physiologists. In many mammals taste and smell are the primary afferent pathways concerned in feeding and sexual activity, and the parts of the brain in which they terminate are closely linked with pathways which elicit autonomic and emotional responses. Hence to the anatomist the rhinencephalon means one thing, and to the physiologist, another. We may therefore endorse Brodal's concluding suggestion that all who use the term 'rhinencephalon' should put a footnote or make some reference indicating what they mean by it.

BRAIN

RUMFORD REDIVIVUS

Knight of the White Eagle

A Biography of Sir Benjamin Thompson, Count Rumford (1753–1814). By Dr. W. J. Sparrow. Pp. 302+10 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1964.) 50*s*. net.

THIS year marks the one hundred and fiftieth anniversary of the death of Sir Benjamin Thompson Count Rumford, and also sees the publication of the first full-length biography of this remarkable personality which has been written in Britain. This lamentable neglect of "a great man of science, a leader in technology, and a philanthropist of high renown", who inspired the foundation of one of the premier centres of British scientific life, the Royal Institution, is attributed by Dr. Sparrow to the emphasis placed on politics and conflict in the history taught in our schools, and to our national apathy towards the history of science.

In his book, which is an avowed attempt to secure the rehabilitation of Rumford, Dr. Sparrow has set himself three objectives: (1) to tell the story of Rumford's life against the setting of his time; (2) to unravel and exhibit his motives; and (3) to present a balanced account of his scientific work. In pursuit of his aims, Dr. Sparrow, who confesses to an interest in Rumford since his school-days, has during the past twenty years visited all the varied scenes of Rumford's life in both the United States and Europe, and his researches have brought to light a manuscript copy of the journal kept by Rumford on his visit to Munich and Paris in 1801, and an interesting memorandum detailing the younger Boulton's hostile reactions to the Royal Institution. As a result of this dedication to his subject, Dr. Sparrow has been able to compile an authoritative and well-documented account of Rumford's wide-ranging career and manifold activities.

The candid and judicious survey of Rumford's scientific work forms one of the most successful sections of the book. Dr. Sparrow shows clearly that, although Rumford was an 'amateur', in that his scientific work was secondary to his public career, and his strongest motive was not so much scientific curiosity as a passion for order, he was pre-eminent as an apostle of applied science, and outstanding as an experimentalist. Dr. Sparrow admits that Rumford lacked the imagination and mathematical skill of the great theorists, and the compelling curiosity of the greatest experimentalists, but insists that he was far ahead of his time in his advocacy of "the application of science to the common purposes of life", and that his work, while not in the first rank, ensures him a place not far behind the great leaders of science.

Dr. Sparrow traces in careful detail the fascinating story of Benjamin Thompson's rise from a relatively humble origin in America to the eminence of Count of the Holy Roman Empire and Knight of the Order of the White Eagle. His youthful ambiguous behaviour as a Loyalist informer in the American War—his quality as a spy may be gauged from an intelligence report printed in full as an appendix—his escape to England, and his subsequent career as a Junior Minister in Lord North's administration, and later as the right-hand man of the Elector of Bavaria, whose support enabled him to apply his scientific and technological ideas to social ends, are faithfully chronicled with copious references to authorities. But the sober and factual style of Dr. Sparrow's narrative, which is enlivened only by far too infrequent flashes of humour, scarcely evokes that peculiar atmosphere of drama and intrigue which Rumford generated around all his activities. The chapters on the Royal Institution suffer by the omission of an adequate account of the contemporary political and social background, and Rumford's personal reasons for associating himself with the project and later withdrawing from it receive insufficient notice.

It is in his attempt to unravel and exhibit Rumford's motives that Dr. Sparrow is least successful. Admittedly, many details of Rumford's life, linked as it was with such activities as espionage and Freemasonry, are very difficult to ascertain, and Dr. Sparrow resolutely eschews conjecture. He certainly has no illusions about his 'hero', and at times displays a positive distaste for some aspects of his character; at one point he exclaims: "What an unpleasant episode this is" (p. 37), and he hurriedly passes over the details of Rumford's distinctly devious sex-life. But this lack of 'sympathy' with his subject has prevented him from combining into a coherent and convincing whole the many facets of Rumford's complex character. Several crucial decisions in Rumford's career, notably his withdrawal from the Royal Institution in 1802, are reduced to bare statements of fact, such as "He had decided to leave London", instead of emerging as the inevitable result of the interplay of conflicting motives; more than once the reader is left with an impression which is aptly described by a quotation from one of Rumford's own letters: "I cannot help thinking that we are not acquainted with the whole of the secret. There must be something we cannot get at but what that something is I have no conception". But Dr. Sparrow's broad generalization that the two essential clues to Rumford's character are his egotism and his passion for order cannot be questioned.

As a whole, Dr. Sparrow's sound and well-documented study with its comprehensive bibliography and index ably fills an important gap in the library of the history of science, and amply confirms the author's contention that Rumford has for too long been denied his rightful recognition as a great man of science and a leader in technology.

A. H. EWEN

A REASONED VIEW OF SEX

Sex in Society

By Dr. Alex Comfort. Pp. 172. (London: Gerald Duckworth and Co., Ltd., 1963.) 21*s*. net.

SEX in Society, a thoroughly revised version of Dr. Comfort's *Sexual Behaviour in Society*, was first published some fourteen years ago. It will probably be received more favourably than its predecessor because of a much more favourable climate of opinion towards an honest and open examination of the force of sex in our individual and social lives. This does not mean that the reading public has caught up with the author or that his book will be generally acclaimed. Comfort's approach is avowedly anarchistic and, having confounded any religious, humanistic, frustrated and perverted moralists who try to influence public opinion with great conviction and authority on the basis of little substantiated fact, Comfort asks if we really know, for example, whether sexual intercourse can be too frequent, whether masturbation may not be the only suitable alternative to intercourse itself, whether those who give sex instruction and advocate clean-living realize that they probably do more harm than good, and whether strict monogamy is always practicable or even desirable.

In matters of sex the author implies that few Christians live in the twentieth century and most Roman Catholics are still in the sixteenth century, that much English law relating to sex is founded mainly on the prejudices of law-makers, and that many court sentences reflect little but the guilt-complexes of the presiding judges.

Startling as are some of Comfort's views, which are supported by most of the precious few facts available, not many readers would cavil at the reasoned and gentle way in which they are presented. Like all true anarchists Comfort does not expect them to be readily accepted nor to lead to immediate change. What really matters is that a generation has arrived which at long last is prepared to look intelligently and rationally at sex with all its implications for the health of individuals in society. What is also important is that, as genuine sociological investigations get under way, a start may be made to do away with all the feelings of guilt which pervade the sex lives of most civilized peoples.

Although Comfort's book is not free from value-judgments, this clear, sincere and kindly approach to the development of sexual health in individuals and society will stand as one of the outstanding contributions to more dispassionate thinking for many a day to come. It is unfortunate that he can but look through the eyes of a male—this does not explain why Chesser's monumental work on the sexual life and behaviour of British women has apparently been completely ignored. Comfort's book deserves to be followed by one written by an equally enlightened female. Until this occurs, *Sex in Society* could be beneficial reading for all medical, social and legal students, teachers, marriage guidance counsellors, magistrates, judges, and parents. T. H. HAWKINS

WEAK SOURCES OF IONIZING RADIATION

High Sensitivity Counting Techniques

By D. E. Watt and D. Ramsden. (International Series of Monographs on Electronics and Instrumentation, Vol. 20.) Pp. xv+348. (London and New York: Pergamon Press, 1964.) 80*s*. net.

AN increase in the sensitivity of a technique of experimental physics is invariably accompanied by an extension of its range of application. One obvious example is the improvement in the sensitivity of nuclear emulsions which led to the discovery of the π-meson; other examples are to be found throughout the text of *High Sensitivity Counting Techniques*, an account of a special field of nuclear counting. The detection of nuclear radiations is, of course, a rapidly evolving subject, and this book does not attempt to give complete coverage; it is concerned with counters of single particles and with the methods of reduction of the unwanted backgrounds which beset all such instruments. Such a topic might well appear narrow and uninteresting to all but the extreme specialist, but, as the authors point out, the detection of weak activities long known to be important in chronology and in certain problems of nuclear stability has now assumed far wider significance as a result of radioactive tracer work, of the demands of the health physicist and of atmospheric contamination from nuclear explosions. It is the existence of this wider context which has encouraged the authors to place their experience at the disposal of the reader; their interest in the subject was stimulated by S. C. Curran, whose work at Glasgow and at the Atomic Weapons Research Establishment, Aldermaston, has resulted in many of the techniques described in the book.

There is, throughout this volume, an emphasis that high sensitivity in a counter is not necessarily the same as low background, but since the latter reacts favourably on the former, it turns out that most of the book is devoted to the background problem. The origins of counter background are analysed at the outset and the significance of the cosmic ray processes of neutron production and electromagnetic cascade are described. This leads on to the empirical science of shielding and much attention is rightly directed to the anti-coincidence ring of counters with which it is almost essential to surround a low-level counter. This is a recurrent theme throughout the book. There follows a section on the construction of the counter, in which selection of materials for both counter and shield is of prime importance; there is the implication that materials prepared before the age of fission may have to be reserved for the counter-maker of the future. The radiation shield may, in many cases, be elaborate and expensive and there is mention of the construction of special laboratories in which many different low-background counters may be used or tested. Such laboratories share with the anechoic chamber a somewhat eerie quality; in one the heartbeats of a visitor are heard, in the other his content of potassium-40 and cæsium-137 is displayed.

The central chapters of the book deal with the detection of low levels of α- and β-activity. The former problem is simplified by the high ionizing power and short range of the particle; there are surveys of phosphors, gas-filled counters and semi-conductor counters from a practical point of view. The chapter on carbon-14 dating deals with the most widely known of all the applications of low-level counting, and perhaps the most tangible, since the range of times involved is commensurate with recorded history. The nucleide carbon-14 is also used in many tracer investigations, but it is apparently giving way to tritium (^{3}H), which is detected most elegantly and efficiently in a gaseous proportional counter. This latter technique and similar techniques for low-energy β-emitters such as sulphur-35 (and also X-ray and γ-ray sources) are explored in a separate chapter. The limitation arising from the low vapour pressures of many otherwise suitable compounds is mentioned and a high-temperature counter to overcome this is described. The nuclear physicist will recognize the importance of this topic in the investigation of K to L conversion electron ratios in radiative transitions, and of electron-capture to positron ratios in β-decay.

A later chapter is devoted to the more generally applicable techniques of radioactivity; this is largely a description of different counters and of the variants of the anti-coincidence ring; it contains a useful survey of source preparation and of the corrections for scattering and absorption in activity determinations. For the detection of higher energy γ-radiation, the sodium iodide crystal is still pre-eminent, although it is being challenged by the bulk conduction counter (not mentioned in this book). The high-sensitivity aspects of this subject are illustrated by accounts of determination of the lifetime of vanadium-50 and lutetium-176; again screening is of outstanding importance. Finally, a chapter is devoted to whole-body monitoring, a subject the technology of which is rapidly developing. Since 'patient psychology' is involved, high-sensitivity counters are necessary to obtain quick results.

Messrs. Watt and Ramsden have patiently covered a wide field of enterprise. As a result, this book is heavy with factual detail; but while this makes it unsuitable for the general reader, it commends it to the user, be he physicist, biologist, or Civil Defence officer. As a volume in a monograph series, the work is suitably placed; it should be available as a hand-book in every laboratory in which precision techniques of nuclear counting are used. W. E. BURCHAM

THE NORTH AMERICAN LANDSCAPE

Face of North America

The Natural History of a Continent. By Peter Farb. Pp. xv+316. (London: Constable and Co., Ltd., 1963.) 45*s*.

THE author of this admirable book is somewhat of a Gilbert White—but his parish is a continent, and he has supplemented his own far-flung observations of its natural history by information gleaned from "those dedicated custodians of the landscape—the employees of the National Park Service, Forest Service, and National Wildlife Refuge System, and their Canadian counterparts". The result is a popular monograph in the grand manner, written with a fine command of language and a pleasing simplicity of style, which happily combines within its covers the literary requirements of a best-seller and the factual accuracy of a serious work of science. His theme is the inter-dependence of all realms of natural history—the rocks, water, soils and the plant and animal kingdoms; and he develops this topic for all the great topographical divisions of North America, from the barrens of the Canadian arctic to the swamps of the Mississippi delta, from the submergent coast-line of New England to the wave-sculptured headlands of the Pacific shores. A recurrent motif in this scenic symphony is that of evolutionary change resulting from gradual desiccation since the Great Ice Age.

In the first section of his book, Mr. Farb briefly outlines in layman's language the geological processes which have sculptured the surface of the land. Thence he proceeds to explain the forces that have shaped the coasts of the continent, with intimate accounts of some of the more interesting fauna which inhabit these shores. Similar chapters relating physiography and ecology follow on rivers and waterfalls, on lakes and bogs, on hot springs, geysers and caves, on the mountain systems and on the Great Divide. He is at his best in describing the forests—the primeval forests of the Great Smoky Mountains, the redwoods and the giant sequoias of the Pacific Coast. Chapters on "The Drylands" deal with the grassland zones, the Colorado Plateau, and the various sections of the Great American Desert, each main part of the Desert having its characteristic indicator plants—the Joshua tree of the Mohave, the saguaro cactus of the Sonoran, the agave of the Chihuahuan and the sagebrush of the Great Basin. A 36-page appendix lists, with descriptive notes, all the national parks and monuments, national forests, wild-life refuges and other sanctuaries in the United States and Canada; and the book concludes with a well-chosen bibliography.

The depredations of man, Indian and European alike, are duly censured; but the picture painted is less gloomy than some depicted elsewhere. One reads that to-day there are more deer, more moose and more song-birds than when Columbus arrived. Thanks to the National Audubon Society, "the American egret, roseate spoonbill, whooping crane, flamingo and half a dozen other notable species have made the long flight back from near-extinction". "Several decades ago the bison, the elk, the pronghorn, the beaver, the sea otter and many other mammals appeared doomed as species; they are no longer in deep peril to-day."

None of the many excellent illustrations in the volume is in colour—a deficiency which will be deplored by those acquainted with *Arizona Highways* and comparable travel literature—but this is doubtless unavoidable in a work otherwise handsomely produced at a low cost. To the student proposing to emigrate westward, Mr. Farb's book should be obligatory reading. In those fortunate enough to have toured the North American continent it will evoke many pleasing memories. C. F. Davidson

INSPIRATION OF THE MIDLANDS

The Lunar Society of Birmingham

A Social History of Provincial Science and Industry in Eighteenth-Century England. By Robert E. Schofield. Pp. x+491+12 plates. (Oxford: Clarendon Press; London: Oxford University Press, 1963.) 70*s.* net.

Mr. SCHOFIELD, who has in earlier papers analysed the membership of the Lunar Society of Birmingham and the relations between the Society and the Society of Arts, now gives us in this full-scale study of the Society and its members a notable and scholarly contribution to the study of the interactions of science and technology in the late eighteenth century. This is probably the major interest of the book to-day rather than its contribution to the social history of provincial science and industry, which the sub-title suggests. Systematically and thoroughly, Mr. Schofield describes the background in which the Lunar Society arose, the character of the Lunar Circle out of which it was, though still informally, founded, the founding of the Society and its activities in the decade in which it fully flourished, and finally the circumstances which led to its decline and end.

All this is done with a wealth of scholarly detail and precision of reference that must impress the most casual reader with some sense of the immense pains that have gone to the collection of facts so widely scattered, for formal records of the proceedings of this Society do not exist. Much of the material displays the personal qualities and activities of its individual members and their friends—of Small, Wedgwood, Edgeworth, Whitehurst, Watt, Withering, Keir, Boulton, Priestley, Darwin, Day and, finally, Galton, Stokes and Johnson. Here, in so far as it illuminates their relations, the book is fairly described as a contribution on the social history of the times. However, to write this is likely to provoke the comment that there is remarkably little indication of the attitude of manufacturers to the working conditions of their factories, or that, from another angle, we are told too little of Boulton or Wedgwood, for example, as business men. The emphasis is perhaps a little too much on the side of science, insufficiently so on the industrial society.

That seems to be one weakness in the book, for the outstanding feature of the Lunar Society was the way in which it appears to have enlisted the interest of all its members in the discussion and examination of whatever problems were brought to their notice by any one member. At its peak the Society was a remarkable demonstration of the value of discussion by a group of men who could bring to bear on the subject discussed a wide range of outlooks, experience and technique. The Society is of great interest from the point of view of the interaction of science and technology and industry in the late eighteenth century, but in this as in later periods there are still many gaps in our knowledge.

There is one other weakness and that where it might have been hoped the book would make an important contribution. An impressive feature of the Lunar Society is the extent of its links with the Royal Society and with the Society of Arts. Eleven of its members were members of the former and eight of the latter Society: they read papers to them and were able to draw freely on the experience and knowledge to be found among their fellow members. These links are important, and there were other links with local societies—societies which continued long after the Lunar Society ended; for example, the Literary and Philosophical Societies of Manchester and of Newcastle upon Tyne, and the Derby Philosophical Society. Mr. Schofield notes these links, but when in conclusion he discusses "Lunar Descent and Lunar Influence" he has little to add on this aspect, which is far more important than the biographical aspect on which his discussion centres. There are features of the Lunar Society which reappear in the Manchester Literary and Philosophical Society and in the Whalley meetings started by Lyon Playfair about 1843. Both groups included several Fellows of the Royal Society, and some connexion may well exist between the latter meetings and the Chemical Section of the Manchester Society established by the versatile James Young some time after 1839. The exploration of all these links might well be rewarding and throw some light on the development of dyeing and calico printing in the area, and, only a few years later, on the rise of the dyestuffs industry there. Reports prepared for contemporary meetings of the British Association in Manchester and in Newcastle, for example, point to the existence of a lively intermingling of science and technology, with movement from one to the other as well as free discussion.

When all due allowance is made for such shortcomings, this is a welcome and scholarly tribute to a Society the place of which in our industrial and scientific history should not be forgotten. It is a reminder, too, at a time when men are liable to be obsessed with mechanical contrivances in communication and to be separated by over-specialization, of the value of personal contacts and discussion across the different disciplines. The significance of the Lunar Society for innovation and creative advance may well become even clearer if Mr. Schofield's labours induce others to follow his example and patiently strive to fill in some of the further gaps in our knowledge of subsequent interactions of science and technology to which passing reference has here been made.

R. Brightman

ALLOCATION OF FREQUENCIES FOR RADIO ASTRONOMY AND SPACE SCIENCE*

By Dr. R. L. SMITH-ROSE, C.B.E.

Past-President, International Scientific Radio Union, and Secretary-General, Inter-Union Commission on Frequency Allocations for Radio Astronomy and Space Science

DURING the past few years the subjects of radio astronomy and space science have required serious consideration on the part of those, both national and international, organizations responsible for allocating the available spectrum of radio frequencies among the numerous services—including communications, navigation and broadcasting—using radio waves. The present Radio Regulations drawn up by the International Telecommunication Union in 1959 recognize that the radio astronomy service is unique in that it is based solely on the reception of radio waves of cosmic origin. It does not itself originate any radio waves and therefore causes no interference to any other service. It does, however, require to be protected from interference caused by the operation of transmitters in any of these other services, particularly since the radio astronomer has developed receiving techniques of the highest sensitivity.

On the other hand, for the conduct of space research with the aid of unmanned Earth satellites, it is essential to have the use of a number of radio frequencies for transmission and reception. In the first place, once the satellite with its load of instruments has left the launching vehicle, any subsequent and not pre-determined changes to its orbit or flight path must depend on radio control from the ground station. Secondly, radio direction finding and tracking techniques are necessary to determine the position of the satellite throughout its flight. Thirdly, the information collected by the measuring instruments carried in the satellite is, in most cases, transmitted by radio to the observer, either continuously or on command at intervals during the flight.

The frequencies selected for these purposes must be both suitable for transmission over the path from satellite to ground, and selected so as to cause the minimum disturbance to all other services using radio communication. Although general research into the generation and reception of radio waves has been steadily extending the spectrum of frequencies available for practical use, there has also been a corresponding increase in the demand for frequencies by all the other services throughout the world. To avoid what might easily become a state of confusion—and even chaos—in the radio field, international conferences are organized at intervals to discuss, and resolve so far as possible, the technical and administrative problems involved. Since radio astronomers and space scientists are among the latest applicants for the use of portions of the radio frequency spectrum, it will be useful to describe briefly the international organizations concerned with this subject and the manner in which they operate.

International Telecommunication Union

In the early years of the present century it became clear that the new and rapidly developing service of radio communication would need protection at both national and international levels, and rules drawn up to control its use. After various conferences held at intervals of a few years from 1906 onwards, the International Telecommunication Union (ITU) was formed, and later became one of the specialized agencies of the United Nations. The Union has a permanent secretariat in Geneva, Switzerland, together with a specialized staff to deal with technical problems associated with line and radio communications, respectively.

At the Atlantic City Conference of the Union held in 1947, steps were taken to record all frequencies in use by radio stations throughout the world; and a Frequency Allocation Table was drawn up to cover all services then in operation. This Table was used as the basis of negotiation as developments took place in the radio field, and the various new, as well as the existing, services were seeking frequencies for their use.

To assist the ITU in its work, the International Radio Consultative Committee (CCIR) was established in 1927 for the purpose of investigating technical questions of interest to international radio communications. These questions include the technical development of sending and receiving equipment, the propagation of radio waves of all frequencies and under all practical conditions of use, and the interference arising from both natural and man-made disturbances. The Committee also deals with the specification of conditions and tolerances appropriate to all applications of radio. The function of the CCIR is limited to advising the ITU on the various technical problems associated with all the applications of radio, with the object of ensuring that the radio frequency spectrum is divided among the users in the most economical and efficient manner possible; and so facilitate the conduct of the individual services with the minimum of mutual interference.

In 1947, the portion of the radio spectrum in which frequencies were allocated was limited to the range 10 kc/s–10,500 Mc/s (or 10·5 Gc/s). At the next full Administrative Radio Conference held at Geneva in 1959, the range of frequencies covered in the Allocation Table was 10 kc/s–40 Gc/s (corresponding to wave-lengths from 30 km–7·5 mm). The increase was due to the major developments which had occurred in the intervening twelve years in such fields as communication by ionospheric and tropospheric scatter, navigation and surveying techniques, and for scientific research in radio astronomy and the exploration of space. For the first time, the Geneva (1959) conference recognized and defined the Radio Astronomy Service, and also envisaged and made provision for the possibility of radio waves being required for communication with, and control of, man-made vehicles travelling both within and outside the Earth's atmosphere.

International Council of Scientific Unions

Concurrently with these developments in the field of practical radio communications, the International Council of Scientific Unions (ICSU) appreciated the need for advanced and co-operative scientific research in space to increase our general knowledge of the Earth's environment and to provide a basis on which various future expeditions in space could be founded.

As a member body of ICSU, the International Scientific Radio Union (URSI) has since its formation in 1913 been actively engaged in organizing and conducting scientific research in radio throughout the world: and it has encouraged the application of radio measuring techniques to the study of the Earth and its atmosphere, and the influence of the Sun's radiation on the latter. On the practical side, URSI has collaborated very closely with CCIR on many problems of mutual interest; and in recent years these have included communication with, and location of, man-made vehicles in space.

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* Substance of a paper presented at the Seventh Plenary Meeting of the International Committee on Space Research (COSPAR), held in Florence during May 8–20, 1964.

But there are two other member bodies of ICSU very interested in the problems of the use of radio techniques in association with outer space. One of these—the International Astronomical Union (IAU)—is among the oldest of the scientific unions; and it is very much concerned nowadays with the use of radio, as well as light, waves for modern astronomy. Apart from the use of radio for rapid communication between observatories, astronomers have for many years been engaged in an investigation of the relationship between time derived from astronomical observations and that provided by physicists from the atomic frequency standards. The introduction and development of radio receiving techniques to an extremely high degree of sensitivity in radio astronomy has opened up new vistas in our universe which could not be explored by optical techniques alone.

The other, and youngest, member of ICSU is the International Committee on Space Research (COSPAR). Its great and healthy activity is indicated by the fact that, since its formation some five years ago, it has already held five international symposia, the first being in Nice in January 1960, and the fifth has recently been held in Florence, May 1964. The scope of these symposia provides for discussion on a wide international basis of all scientific problems associated with the Earth's atmosphere and outer space, and the radiations and other phenomena which determine the physical conditions associated with the solar system. It is for the direct assistance of all this research in space science that the allocation and protection from interference of a number of suitable bands of radio frequencies is of paramount importance.

Inter-Union Commission on Frequency Allocations for Radio Astronomy and Space Science (IUCAF)

It was at the General Assembly of URSI in London in September 1960 that the subject of frequency allocations for radio research was discussed by the interested commissions of the Union. Afterwards, at an informal meeting of representatives of URSI, IAU and COSPAR, a proposal was formulated to constitute an Inter-Union Commission which should first co-ordinate the future requirements of frequency channels for radio astronomy and space science; and then take appropriate action on behalf of the three constituent bodies to bring suitable proposals before the ITU with the view of securing frequency allocations to meet these requirements. These proposals were afterwards approved by the member bodies of the Commission and endorsed by the Executive Board of ICSU at its meeting in Lisbon in October 1960. The constitution and terms of reference of the Commission have been published elsewhere[1].

While a great deal of the work has been conducted by correspondence, four full meetings of the Commission have been held between October 1961 and May 1963. During this period the activities of the Commission were formally recognized by the ITU, and its representatives participated in both Study Group meetings and in the 1963 Plenary Assembly of the CCIR, at which the technical issues involved in space science and communications were discussed on an international scale. It was following this assembly of CCIR, supported by other representations, that the Administrative Council of the ITU agreed to include Radio Astronomy within the terms of reference of the 1963 Extraordinary Administrative Radio Conference. As a consequence of this action, the IUCAF, with its constituent member bodies, URSI, IAU and COSPAR, was an active participant in the Conference and was able to press through National Administrations for a number of bands of frequencies to be allocated to both radio astronomy and space research, and for the improved protection of these bands from interference by other radio services.

The Extraordinary Administrative Radio Conference (EARC) was held in Geneva during October 7–November 8, 1963. It was attended by more than 350 representatives of 74 National Administrations, and of other interested international organizations. While the main objective of the Conference was to allocate frequency bands for space radio communication purposes, it also dealt with frequencies for space research and radio astronomy[2].

Radio Astronomy

In the publications of the ITU there are now three definitions relating to radio astronomy, as follows: (1) *Radio Astronomy*, astronomy based on the reception of waves of cosmic origin; (2) *Radio Astronomy Service*, a service involving the use of radio astronomy; (3) *Radio Astronomy Station*, a station in the radio astronomy service.

It is to be noted that the Radio Astronomy Service differs from all other radio services in two important respects: (*a*) it does not itself originate any radio waves, and therefore causes no interference to any other service; (*b*) a large proportion of its operation is conducted by the use of reception techniques which are several orders of magnitude more sensitive than those used by other radio services.

Table 1 gives brief details of the twenty bands of frequencies now allocated to the radio astronomy service. In some cases, the allocated frequencies are shared with other services with recommendations that every effort should be made to avoid interference. But it is to be noted that the band 1,400–1,427 Mc/s covering the natural radiation of the hydrogen line is now allocated on a world-wide exclusive basis with no reservations. It was also very opportune and timely that, during the Geneva Conference, the United States delegation was able to announce the identification of two absorption lines—at 1,665 and 1,667 Mc/s—in the radio spectrum of Cassiopeia *A*, due to the presence of the OH radicle[3]. A suitable note has been incorporated in Table 1.

Attention is directed to Annex 12 of the ITU document[2] entitled "Notices Relating to Stations in the Space and Radio Astronomy Services". Section *F* of this Annex describes the "Basic Characteristics to be Furnished in Notices relating to Frequencies to be Received by Radio Astronomy Stations". A suitable tabular form of presentation of these characteristics is illustrated, and this shows that the information sought comprises: the name of the station or observatory; its geographical coordinates and altitude; the central frequency and the width of the frequency band observed; the antenna characteristics and noise temperature; the maximum hours of reception and the class of observation pursued.

It is considered to be in the interests of radio astronomers that they should: (1) supply to their national administrations as much of this information as possible, together with any other relevant details; (2) so far as practicable, concentrate their observations in the frequency bands now allocated for their use as shown in Table 1.

The footnote references relate to the ITU document[2], which should be consulted for their interpretation and for further details of the conditions which apply to the various frequency allocations.

Finally, a recommendation adopted by the EARC provides that: (1) the next Ordinary Administrative Conference should give further consideration to the pro-

Table 1. REVIEW OF FREQUENCIES ALLOCATED TO RADIO ASTRONOMY IN THE "FINAL ACTS OF THE EXTRAORDINARY ADMINISTRATIVE RADIO CONFERENCE", GENEVA, 1963

No.	Frequency band (Mc/s)	Allocations in ITU Radio regulations: Geneva, 1959		Revised allocations, Geneva, 1963, region:		
		Applicable to regions	*Rec.* = Recommendation *F* = Footnote	1	2	3
1	Standard frequency bands: 2·5, 5, 10, 15, 20, 25	1, 2 and 3	*Rec.* No. 31 *F* 204	No change		
2	Within range 37–41 Mc/s (*a*) 38·0 ± 0·25 (*b*) 40·68 ± 0·25	1, 2 and 3	*Rec.* No. 32 *F* 234	37·75–38·25 Mc/s as secondary service with fixed and mobile services as primaries		
3	73·0–74·6	2	*F* 253	—	Exclusive with footnote reservation, 253 *A* and *B*	—
4	79·75–80·25	1 and 3	*F* 261	No change	—	No change
5	150·05–153	1	*F* 286	*F* 286*A* improves position of protection in United Kingdom		—
6	322–329 (deuterium line)	—	*F* 310	No change		
7	404–410	1, 2 and 3	*F* 317	406–410	404–410 Modified footnote 317	406–410
8	606–614	1 and 3	*Rec.* No. 32 *F* 332	606–614 (African broadcasting area exclusive)	608–614 Exclusive until after 1 January, 1974 Modified footnote 332	610–614
9	1,400–1,427 (hydrogen line)	1, 2 and 3	Exclusive, primary allocation, subject to *F* 350	Exclusive world-wide allocation with no reservations		
10	1,664·4–1,668·4 (OH line)	No allocation	*F* 354	Secondary allocation: with meteorological aids and meteorological satellites as primary in all regions. (*Footnote* 353*A*: In view of the successful detection of two spectral lines in the region of 1,665 Mc/s and 1,667 Mc/s by astronomers, administrations are urged to give all practicable protection in the band 1,664·4–1,668·4 Mc/s for future research in radio astronomy)		
11	1,660–1,690 3,165–3,195 4,800–4,810 5,800–5,815 8,680–8,700	No allocation	*F* 354	No change		
12	2,690–2,700	1, 2 and 3	*F* 365	Exclusive world-wide allocation, with reservations in some countries. *F* 363, 364*A*, 364*B*, 365		
13	4,990–5,000	1, 2 and 3	*F* 365	Primary, with fixed and mobile also as primaries: *F* 365 modified	Exclusive with *F* 383 *A*	Primary, with fixed and mobile also as primaries. *F* 365 modified
14 15 16 17	Gc/s 10·68–10·7 15·35–15·4 19·3 –19·4 31·3 –31·5	1, 2 and 3	*F* 405	Exclusive world-wide allocations with some reservations (see *F* 405 *A* and *B*, 409 *C* and *D*, and 412 *A*)		
18	33·0–33·4			Primary with radio-navigation also primary	No allocation for radio astronomy except in Cuba and India *F* 412 *F*	
19	33·4–34			Footnote 412 *G*		
20	36·5–37·5			Footnote 412 *E*		

vision of improved frequency allocations for radio astronomy; (2) in the meantime, administrations should afford all practicable protection to the frequencies now allocated to radio astronomy on a shared basis with other services.

Frequencies for Space Research

The need of the space research scientist for protection of radio frequencies is somewhat different from that of the radio astronomer. While a number of satellites are already in use for direct application to the extension of world-wide radio communications, others are placed in orbit around the Earth or farther afield with the object of extending our knowledge of the upper atmosphere at increasingly greater heights, and of measuring various physical quantities in outer space. In some cases, the frequencies required are for the control of instruments in the satellite or for locating it during its orbit; and here, in some instances, the frequencies can be conveniently shared with the corresponding requirements of, say, communications satellites. But, bearing in mind the inaccessibility of the scientific equipment carried in the space vehicle, and the need to use the measuring apparatus for the maximum proportion of the available time, it is important to ensure that the results of the scientific measurements can be communicated to the ground controlling station or laboratory with the minimum of delay or interference. It is thus desirable that a number of bands of frequencies in appropriate parts of the spectrum should be allocated, without danger of interference, to the needs of scientific research being conducted with the aid of satellites in orbit.

In the Radio Regulations, Geneva 1959, four definitions referred to radio communication services between space stations or between Earth stations and space stations. These are:

No. 70. *Space Service*. A radio communication service between space stations.

No. 71. *Earth–Space Service*. A radio communication service between Earth stations and space stations.

Table 2. Review of Frequencies allocated to Space Research in the "Final Acts of the Extraordinary Administrative Radio Conference", Geneva, 1963

No.	Frequency band (Mc/s)	Allocations in ITU Radio Regulations: Geneva, 1959		Revised allocations, Geneva, 1963, region:		
		Applicable to regions	*Rec.* = Recommendation *F* = Footnote	1	2	3
1	10·003–10·005	1, 2 and 3	*F* 215 †	No change, except the modification of footnotes 215, 221 and 235		
2	19·990–20·010	1, 2 and 3	*F* 221			
3	39·986–40·002	1, 2 and 3	*F* 235			
4	15·762–15·768	No allocation		Secondary allocation (primary in some countries—see *F* 215*A*). Fixed services as primary		
5	18·030–18·036					
6	30·005–30·010	No allocation		Primary allocation, with fixed, mobile and space (satellite identification) also as primary		
7	136–137	1, 2 and 3	Primary service *F* 280	Primary (T and T)*, with fixed and mobile also primary: *F* 281*A* (and recommendation No. 7*A*)	Primary (T and T) and exclusive subject to *F* 281*B*. *F* 281 (and recommendation No. 7*A*)	Primary (T and T) with fixed and mobile also primary: *F* 281*A* (and recommendation No. 7*A*)
8	137–138	No allocation		*(T and T) primary, with meteorological satellite, and space also primary: various footnotes relate to use of this band for other services in some countries. *F* 281*F*		
9	143·6–143·65	No allocation		Primary (T and T), shared with other primary services		
10	183·1–184·1	1, 2 and 3	*F* 294	Secondary allocation in accordance with *F* 294 modified		
11	400·05–401	1, 2 and 3	Primary service *F* 280	Primary (T and T) shared with meteorological aids and meteorological satellite (maintenance telemetering) as primaries		
12	900–960	No allocation		*F* 339*A* specific portions of the band may be used on a secondary basis		
13	1,427–1,429	1, 2 and 3	Primary *F* 280	Assigned to space (telecommand), not research		
14	1,700–1,710	1, 2 and 3	Secondary *F* 280	Primary (T and T) with fixed (primary) and mobile (secondary)	Primary (T and T) exclusive except in Cuba	Primary (T and T) with fixed and mobile also primary
15	2,110–2,120	No allocation		*F* 356 *A* may be used for telecommand in deep space research subject to international co-ordination		
16	2,290–2,300	1, 2 and 3	Secondary *F* 280	Primary (T and T) in deep space) with fixed (primary) and mobile (secondary) *F* 356*C*	Primary (T and T in deep space), exclusive except in Cuba	Primary (T and T in deep space) with fixed and mobile also primary
17	5,250–5,255	1, 2 and 3	Secondary *F* 280	Secondary service, shared with radiolocation as primary		
18	5,670–5,725	No allocation		Secondary service (deep space research), with radiolocation (primary) and amateur (secondary). Primary in some countries (*F* 389*A*)		
19	8,400–8,500	1, 2 and 3	Secondary *F* 280	Primary, with fixed and mobile also primary, subject to *F* 394 *A* and *D*	Primary, exclusive except in Cuba	Primary, with fixed and mobile also primary, subject to *F* 394 *A* and *D*
20 (a)	Gc/s 15·15–15·25	1, 2 and 3	Primary *F* 280	No allocation		
20 (b)	15·25–15·35	No allocation		Primary, exclusive, with reservations in *F* 409 *A* and *B*		
21	31·0–31·3	No allocation		Secondary (primary in some countries, *F* 412*H*), with fixed and mobile as primary		
22	31·5–31·8	1, 2 and 3	Primary *F* 280	Primary, with fixed and mobile as secondary	Primary, exclusive except in Cuba	Primary, with fixed and mobile as secondary
23	31·8–32·3	No allocation		Secondary (primary in some countries, *F* 412*B*), with radionavigation as primary		
24	34·2–35·2	No allocation		Secondary (primary in some countries, *F* 412*C*), with radiolocation as primary		

F = footnote reference: the ITU document should be consulted for interpretation of the various footnotes. * T and T = telemetering and tracking.

No. 72. *Space Station.* A station in the Earth–space service or the space service located on an object which is beyond, or intended to go beyond, the major portion of the Earth's atmosphere and which is not intended for flight between points on the Earth's surface.

No. 73. *Earth Station.* A station in the Earth–space service located either on the Earth's surface or on an object which is limited to flight between points on the Earth's surface.

At the EARC, Geneva 1963, some thirty new definitions were drawn up in relation to 'Space Systems, Services and Stations' and 'Space, Orbits and Types of Objects in Space'. From these may be quoted the following terms and definitions relating to space research.

No. 84 *AM*. *Space Research Service.* A space service in which spacecraft or other objects in space are used for scientific or technological research purposes.

No. 84 *AN*. *Space Research Earth Station.* An Earth station in the space research service.

No. 84 *AO*. *Space Research Space Station.* A space station in the space research service.

No. 84 *AW*. *Space Telemetering.* The use of telemetering for the transmission from a space station of results of measurements made in a spacecraft, including those relating to the functioning of the spacecraft.

No. 84 *AY*. *Space Telecommand.* The use of radio communication for the transmission of signals to a space station to initiate, modify or terminate functions of the equipment on a space object, including the space station.

No. 84 *AZ. Space Tracking.* Determination of the orbit, velocity or instantaneous position of an object in space by means of radiodetermination, excluding primary radar, for the purpose of following the movement of the object.

No. 84 *BA. Deep Space.* Space at distances from the Earth equal to or greater than the distance between the Earth and the Moon.

No. 84 *BH. Spacecraft.* Any type of space-vehicle, including an Earth satellite or a deep-space probe, whether manned or unmanned.

It will be recalled that the main purpose of this EARC was "to allocate frequency bands for space radio communication purposes". During its deliberations, however, the Conference gave full consideration to the need for frequency allocations for space research in addition to those required for communications by means of satellites and for control, meteorological and navigational purposes. While it may not always be necessary to assign separate bands of frequencies for the tracking and reception of data from research satellites as distinct from those used for radio communications, the frequencies now assigned to the space research services are listed in the appended Table 2.

In order to take account of the new conditions resulting from the development of space communications for both practical applications and research purposes, appropriate additional sections were drawn up at the 1963 Conference to Article 7 entitled "Special Rules relating to Particular Services" of the 1959 Radio Regulations. These additions include recommendations as to the choice of sites, frequencies and power limits for Earth stations in the space service, and the use of appropriate devices for terminating radio emissions from space stations when necessary.

In conclusion, attention is directed to the fact already mentioned above that the revised Radio Regulations of the ITU will provide for the notification and recording of frequency assignments to stations in the Space and Radio Astronomy services for inclusion in the Master International Frequency Register. For both Earth and space stations in the Space Research Service, the technical details required include the frequency, power, class of emission and identification signals used for the various purposes of telemetering, tracking, telecommand and transmission of research and other information. Additional orbital information is sought in the case of an Earth satellite; and for a space probe a general indication of its trajectory should be provided. Specimen tables are given in the "Final Acts" of the 1963 Geneva conference indicating in detail the information it is desirable to provide[2].

Table 2 gives details of the bands of frequencies allocated to space research, first in the existing Radio Regulations of the ITU, Geneva, 1959; and secondly, the revised allocations resulting from the Extraordinary Administrative Radio Conference, Geneva, 1963. As was also the case in Table 1, for the purpose of frequency allocations the world has been subdivided into three regions, which are defined in detail in the existing Radio Regulations, 1959. In very broad terms Region 1 incorporates Europe and Africa, but includes all Turkey and the U.S.S.R. if outside these continents; Region 2 comprises the Americas and Greenland; while Region 3 covers the rest of the world, mainly Asia, Australia and New Zealand.

[1] Smith-Rose, R. L., *ICSU Rev.*, 3, 61 (1961).

[2] "Final Acts of the Extraordinary Administrative Radio Conference to allocate Frequency Bands for Space Radio Communication Purposes", I.T.U., Geneva (1963).

[3] Weinreb, S., Barrett, A. H., Meeks, M. L., and Henry, J. C., *Nature*, 200, 829 (1963).

JODRELL BANK *Mark II* RADIO TELESCOPE

By Sir BERNARD LOVELL, O.B.E., F.R.S.

University of Manchester, Nuffield Radio Astronomy Laboratories, Jodrell Bank

SEVEN years ago a description was given in *Nature*[1] of the radio telescope at Jodrell Bank which was then nearing completion. This *Mark I* instrument with its 250-ft. aperture paraboloidal bowl has been popularly associated with *Sputniks* and space probes; but, in fact, 96 per cent of the 30,000 h of research carried out with the telescope have been concerned either with the study of the galactic and extragalactic radio emissions or with radar investigations of the solar system.

The fundamental design characteristics of this telescope were laid down in 1949–51 when there seemed little prospect of effective studies of the radio emission on wave-lengths in the centimetre region. Consequently, the aim was to make a fully steerable telescope as large as possible for work in the metre wave-band. Originally we had a wave-length of about 1 m in mind for the lower working limit, but in practice the instrument has been much used on wave-lengths down to 50 cm, where its performance is still close to the theoretical value. Indeed, important observations (including the only measurement so far made of the interstellar magnetic field[2]) have been carried out on the neutral hydrogen line wave-length of 21 cm. The performance falls off rapidly at shorter wave-lengths, and the telescope cannot be used in the 10-cm band, since neither the bowl profile nor the control system is sufficiently accurate at these short wave-lengths.

Some years ago it became clear that observations in the centimetre range would be essential to a full understanding of the processes of emission of the galactic and extragalactic radio waves, and moreover, the development of low noise maser and parametric amplifiers opened up the possibility of important research work in that region. Application to the Department of Scientific and Industrial Research for a grant to build a large radio telescope for this purpose was made in December 1960, authority to proceed was obtained in the spring of 1962 and the resultant *Mark II* telescope (Fig. 1) is now about to begin its research programmes.

The telescope was designed by Husband and Co. of Sheffield and London, who were responsible for the *Mark I*. Like the *Mark I*, the new telescope is an alt-azimuth instrument, but whereas the former is mounted on bogies moving on a 320-ft. diameter circular railway track to give the motion in azimuth, the *Mark II* is mounted on a single large foundation block. This block carries a 42-ft. diameter roller track ring. The revolving structure is supported on this track by 54 steel rollers each of $11\frac{1}{2}$ in. diameter. The mechanical drive for the azimuth motion is via a driving sprocket to a heavy Renolds chain wrapped round the circular support structure of the upper roller track.

The rotating part of the telescope is a prestressed concrete frame structure, and this forms a platform 85 ft. long and 7 ft. wide at a height of 36 ft. 3 in. above the top roller track. This platform carries three bearings for the elevation motion of the bowl. The means for

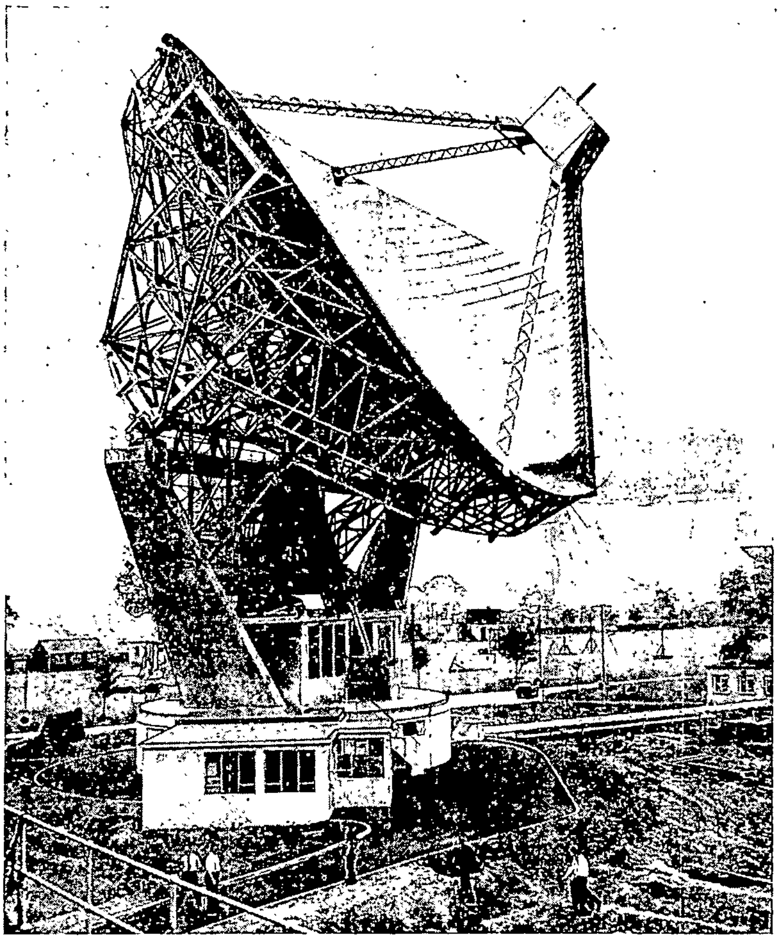

Fig. 1. A general view of the *Mark II* telescope. The 250 ft. *Mark I* instrument is in the background

obtaining motion of the bowl in elevation also differs from that used in the *Mark I*, where two 27 ft. racks were used. In the *Mark II* the motion is obtained by means of a four-start jacking screw 36 ft. 3 in. long by 8½ in. diameter provided with a travelling nut connected to the lower end of a connecting rod which is attached through a pin to the bowl structure at a radius of 40 ft. from its axis of rotation. The elevation motion is from 0° to 95°, which, with a full azimuth motion of 360 deg., gives complete sky coverage.

The driving system in both elevation and azimuth consists of Ward Leonard units with 75-h.p. and 50-h.p. final drive motors respectively, giving an anticipated top speed in still air of 90 deg./min in each motion. The telescope can be operated under full automatic control at wind speeds up to 40 m.p.h. The motion of the telescope will be controlled by a Ferranti *Argus* 104 computer, which will compute and control the azimuth and elevation from a punched tape input to give sidereal or other movement as desired. Once every second the computer converts the sidereal position to azimuth and elevation angles, allowing for precession, and refraction. This occupies 1/4 sec of computer time and is carried out 2 sec in advance of the control. For the control of the telescope, the computer reads and compares the telescope position with the desired position 4 times a second. Voltages representing the speed at which the telescope must move in the next 1/4 sec to correct the differences are then fed out to the servo system. In the analogue system these voltages are compared with voltages representing the actual speeds and the difference is amplified to provide the driving power. Driving accuracies better than 1/3 min of arc are expected.

These functions, together with the production of a complete log of the telescope operations, take only one-half of the computer time. The remaining time is available for other uses, and in some of the research programmes the measurements will be fed into the computer via a data logger for processing in real time.

The reflecting membrane is an elliptical paraboloid with major axis 125 ft. and minor axis 83 ft. 4 in., formed of continuously welded 12 s.w.g. steel sheets. The focal length is 40 ft., and just above the focal point there is an 8-ft. cube laboratory supported on four legs rising from the bowl structure near its edge. The original specification called for a shape tolerance of ± 0·5 in. in the bowl, at all angles of elevation and azimuth positions and, as built, the bowl profile is correct to ± 1/8 in. Within these limits the telescope should work at full efficiency down to wave-lengths of about 1 cm.

The theoretical beam-width and power gain of the *Mark II* as a function of wave-length are shown in Figs. 2 and 3, together with the observed values for the *Mark I* in the overlapping wave-length region of interest. Unexpectedly, the *Mark I* has remained the largest completely steerable telescope in the world. The new instrument has no such claim to be large by world standards, but it will probably be the largest steerable telescope capable of work at wave-lengths of only a few centimetres. (The performance of the Australian 210-ft. Parkes telescope is falling off at wave-lengths below 10 cm and the Russian and American telescopes which remain good down to a few centimetres are smaller than the *Mark II*.) Further, we believe it to be the only large radio telescope under the direct control of a digital computer.

From Fig. 2 it is seen that the anticipated beam-width between half-power points in the azimuth direction is

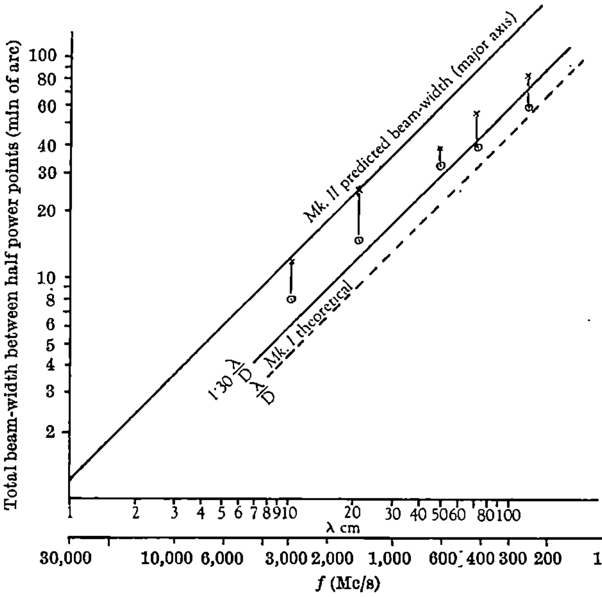

Fig. 2. Theoretical beam-widths of the *Mark I* and *II* telescopes compared with the observed values for the *Mark I*. ×, *E* plane; O, *H* plane

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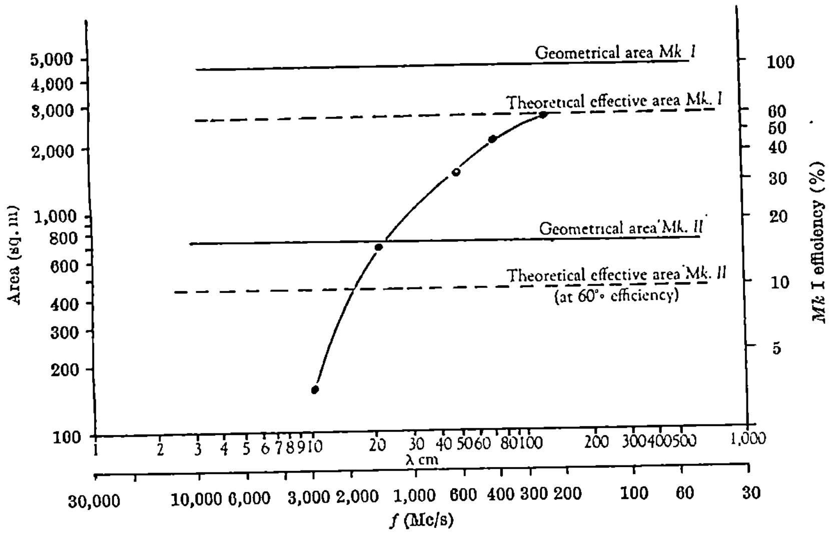

Fig. 3. Effective area and efficiency of the *Mark I* compared with the theoretical effective area of the *Mark II*

6 min of arc at 5 cm, and in the event of the telescope working at full efficiency at 1 cm the beam-width will be only 1·2 min of arc. This presents a considerably greater steering difficulty than in the *Mark I* where at the lowest wave-length on which it is used (21 cm) the beam width is 15 min of arc. The computer itself is capable of driving the telescope with an accuracy of ± 1/3 min of arc and it is necessary to align the elevation and azimuth position pick-offs of the telescope and to determine the collimation errors to this accuracy.

In order to do this initially, and to provide subsequent routine checks, an 8-in. optical telescope aligned along the elevation axis is mounted in a cabin behind the vertex of the bowl. In order that this telescope can be used at all elevations the observer is provided with a pivoted chair in this cabin. Thus the position of a stellar image relative to the axes of the telescope can be determined at any telescope setting. Since the direction of the radio beam depends on the position of the focus box relative to the paraboloid, separate measurements including calibration on known radio sources will be necessary to determine the radio positioning error. The vertex cabin is also equipped with a television camera so that the stellar images can be seen in the control room. Although this arrangement is primarily for the setting up and checking procedures, it is expected to be valuable in future combined optical and radio observations.

When these collimating and alignment tests are completed, the telescope will be used to measure the spectra of several hundred radio sources at wave-lengths of 11 cm and 6 cm. This is a straightforward programming operation which will give experience in the use of the digital computer to drive the telescope, and will extend the spectral measurements of the radio sources already made on the *Mark I* down to 21 cm[3,4]. It is hoped that the variations in spectral shape may give important information about the processes of emission in the distant radio galaxies. Simultaneously performance tests will be made at wave-lengths of 3 cm and lower. Polarization measurements on the planets and the radio sources will follow this programme and later in the year it is hoped to use the *Mark II* and the *Mark I* as an interferometric pair in the 21-cm band, both continuum and *H* line—in the latter case for the examination of the neutral hydrogen structure of extragalactic nebulæ.

The centimetre part of the spectrum has become a region of great interest and importance during the past few years, and the availability of the new telescope in this wave-band coupled with the high performance of the 250-ft. instrument in the metre wave-band provides the United Kingdom with unsurpassed facilities for research in radio astronomy.

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[2] Davies, R. D., Verschuur, G. L., and Wild, P. A. T., *Nature*, 196, 563 (1962).
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A CHEMICALLY SPECIFIED MOLECULAR MECHANISM UNDERLYING EXCITATION IN NERVE: A HYPOTHESIS

By Prof. JULIAN M. TOBIAS
Department of Physiology, University of Chicago

Nerve Excitation

HOW an axon shifts from the resting to the excited state can be discussed in terms of three groups of phenomena. First, there is depolarization. Secondly, there is a complex of sub-molecular, molecular and macromolecular level events which are initiated by the depolarization. Thirdly, if the depolarization is sufficient the second class of events grows to a point where membrane resistance falls and the ion fluxes characteristic of the fully developed excited state[1,2] are permitted to occur. These three sets of changes overlap and interact in time, but can be discussed separately. The word 'excitation' refers to the first and second sets.

Research emphasis for many years has been focused primarily on phases 1 and 3 above, and much is known about their important electrophysiological and electroionic components[1-3], whereas relatively little work has been done on phase 2 which is now so greatly challenging. Nevertheless, enough information is at hand so that it should be fruitful clearly to state a limited but chemically specified working hypothesis about phase 2, to collate the relevant evidence, to verbalize certain questions raised by the hypothesis, and to notice some of the experiments it suggests. Although it is pertinent for other excitable cells and for junctions, and although it is suggestive for recovery phenomena, the hypothesis will, for the sake of simplicity, be presented only in terms of axonal excitation.

Statement of Hypothesis

The early, catelectrotonic, outward, exciting current moves potassium from the axoplasm into the membrane phase. This intruding potassium displaces calcium from polar carboxyl and/or phosphate groups on membrane phosphatidylserine. As one consequence, water content increases in the membrane phase. The combined calcium displacement and increased hydration cause, and are paralleled by, loosening and spreading apart of membrane structural components, phospholipid being the most important. A change in geometry of the membrane protein increases this spatial deformation. The three events: (*a*) catelectrotonic injection of potassium into the membrane phase to displace calcium (ion exchange); (*b*) water moving into the membrane phase (hydrokinesis); (*c*) protein deformation of the membrane (proteokinesis), are responsible for the reversible change in membrane structure which expresses itself as a fall in membrane resistance permitting the ion fluxes of activity to supervene.

Separation of and Evidence for Propositions Explicit or Implicit in the Hypothesis

Proposition 1. Catelectrotonic, outward cation flow, not anelectrotonic inward cation flow, is exciting: (*a*) The evidence that excitation occurs where positive current flows out of the cell cathodally, and not where it flows inward anodally, is too extensively documented to require support here[4,5].

(*b*) The argument that it is an ion displacement current which excites does require clarification, since it is often stated that ". . . in the squid axon the three variables, sodium conductance, potassium conductance and inactivation, are governed solely by membrane potential. . . ."[6]. This can be misinterpreted, and is sometimes considered to mean that ion displacement current has been excluded as a causal factor in the excitation process. In fact, however, when the membrane potential is suddenly reduced (depolarization), an initial pulse of capacity current precedes the large currents carried chiefly by sodium and potassium moving down their own electrochemical gradients, and to account qualitatively for the propagation of an action potential the sequence of events at each point on the nerve fibre is as follows: Current from a neighbouring active region depolarizes the membrane by spreading along the cable structure of the fibre. As a result of this depolarization sodium current is allowed to flow (adapted with minor changes from reference 7). Thus, both in the case of the space clamped membrane action potential and in normal propagation, the ionic currents of the spike proper are preceded by an outward current, a spatial displacement of ions.

The hypothesis deals with the events initiated by this early outward current which lead to the excited state.

Proposition 2. Outward exciting current moves potassium from the axoplasm into the membrane phase. (*a*) Some charged particles must carry the early outward current even if they only move on to and off a high capacity structure of some sort in the membrane phase rather than through the dielectric. The most likely candidate for the role of the particle which normally carries this current into the membrane cathodally is potassium derived from the superficial axoplasm under the membrane.

(*a* 1) Potassium does move toward a cathode in electrically polarized nerve[8,9] and moves there with a mobility close to that in water[9].

(*a* 2) In spite of the relatively high mobility of axoplasmic potassium, mobility alone would favour H^+ (λ_c = 349·8) over K^+ (λ_c = 73·5) ions for carrying the current by a factor close to 5 if the mobilities in water are used for the calculation. However, concentration difference in the axoplasm gives K^+ an advantage over H^+ of about 10^6 times. Therefore, considering only mobility and concentration, potassium will supply an overwhelming number of highly mobile axoplasmic current carriers.

(*a* 3) Potassium efflux at a cathode approximately accounts for the outward current there[10].

(*a* 4) The mean potassium transference number for current flowing out of the squid axon perfused with $(K^{42})_2SO_4$ is about 1·09 (ref. 11).

Proposition 3. There is calcium in the membrane phase: (*a*) There are two pieces of direct evidence.

(*a* 1) Micro-incineration followed by microscopy of myelinated nerve shows the calcium to be in the myelin and not in the axoplasm[12]. To the extent that myelin is a repeating array of cell-membrane material[13], this shows calcium to be present in the cell membrane. Calcium is also found by this technique to be concentrated at the surface of the neurone soma[12].

(*a* 2) Human erythrocyte ghosts contain about 1×10^{-4} m.equiv. of calcium/mg dry material[14]. To the extent that the red cell ghost is cell membrane this also shows calcium to be present there.

(*b*) There is a large amount of indirect evidence. A few examples follow.

(*b* 1) Calcium is needed for repair of membrane rupture in many cells[15] and decline of the injury potential in excised muscle is accelerated by high calcium in the medium[16]. Both effects presumably are due to the role of calcium in reconstituting membrane structure.

(*b* 2) Calcium decreases the width of the dense, double-edged membranes at the axon surface[17] as well as of bimolecular leaflets of phospholipids extracted from neural tissue and dispersed in water[18].

(*b* 3) The numerous effects of calcium on excitability[19], on permeability[15], and on osmotic hæmolysis[20] also indicate that it reacts with membrane components to modify structure.

(*b* 4) Effects of calcium on phospholipid monolayers will be noted in Proposition 5.

Proposition 4. There is phosphatidylserine in the membrane:

(*a*) The erythrocyte ghost contains phosphatidylserine[21–23].

(*b*) Myelin contains phosphatidylserine[24].

Proposition 5. The calcium in the membrane is, in part at least, combined with the phosphate and/or carboxyl groups of membrane phosphatidylserine:

(*a*) There is no direct evidence for the intact cell.

(*b*) There is impressive evidence from studies on monolayers.

(*b* 1) If monolayers of purified phosphatidylserine (PS), phosphatidylcholine (PC), or phosphatidylethanolamine (PE) are made on a Langmuir trough, adding calcium to the aqueous hypophase condenses the PS film, but has no significant effect on the force-area diagram of films made either of lecithin (PC) or of cephalin (PE). This is true over a range of several units on either side of *p*H 7, therefore including physiological *p*H values[25].

(*b* 2) A Geiger–Müller counter suspended over a Langmuir trough containing radioactive calcium in the hypophase solution shows that spreading a monolayer of PS increases calcium-45 in the surface whereas monolayers of PC or PE have no such effect[25].

(*b* 3) A membrane model made of phospholipid impregnated millipore increases its transmembrane resistance on addition of calcium to the ambient medium[26], but only if the phospholipid is PS, not if it is either PC or PE (ref. 27).

These observations are understandable in terms of the acid-base properties of the three phospholipids: PC and PE are nearly electrically neutral around *p*H 7 whereas PS, having three ionogenic groups, a positively charged amino, and two negatively charged acidic groups, phosphate and carboxyl will, at *p*H 7, have at least one negatively charged group available for reaction with cations[28].

Proposition 6. Excitation mobilizes the calcium of the membrane. Potassium, moved into the membrane by the exciting current, is the agent by means of which this mobilization is produced (continued in proposition 7):

(*a*) Excitation increases entry of calcium into and egress from squid axons and muscle[29,30]. Stimulation of the *Elodea* cell permits calcium to move into the vacuole[31].

(*b*) Adding potassium to the outside medium increases movement of calcium into and out of muscle[30].

(*c*) The antagonistic actions of potassium with reference to calcium on electrophysiological properties, permeability, and structure of cells and of various colloidal systems also support the view that potassium can displace calcium.

Proposition 7. The calcium, so displaced by potassium, is displaced from membrane phosphatidylserine polar groups:

(*a*) Here it is possible to say that potassium can displace calcium from myelin and from phosphatidylserine. Direct data on cells are lacking.

(*a* 1) Monovalent cations displace calcium from bovine nerve myelin in the order lithium = sodium < potassium < rubidium < cæsium[32].

(*a* 2) The condensation of a monolayer of PS due to absorption of calcium from the hypophase is prevented by potassium[25].

(*a* 3) Radioactive calcium adsorbed to a PS monolayer is displaced by potassium[25].

(*a* 4) The rise in resistance produced in the phospholipid membrane model by addition of calcium is reversed by potassium chloride[26],[27].

(*a* 5) Direct chemical analysis (flame photometry) shows that calcium taken up by the phospholipid membrane model can be displaced by potassium, and the replacement obeys the rules of ion exchange phenomena[33].

Proposition 8. These events, cathodally produced potassium intrusion and calcium displacement from phosphatidylserine polar groups, are followed by increased water entry into the membrane phase.

(*a*) Many experiments show that water content of whole cells is increased by adding K^+ ions to the medium and is decreased by Ca^{++} (see bibliography in ref. 25). There is also evidence that water content of axons is increased during activity[34,45].

(*b*) In electrically polarized axons the cathodal region swells, increases in transparency, and becomes soft and sticky[35,36], these effects being ascribable to water migrating cathodally.

(*c*) In electrically polarized axons specific gravity of the axoplasm decreases cathodally coincidentally with the swelling. This is also ascribable to water migrating cathodally[37].

(*d*) As mentioned under Proposition 3, X-ray diffraction shows that potassium increases and calcium decreases lamellar spacing of nerve myelin lipid extracts dispersed in water[16], and calcium decreases the spacing of squid axon surface lamellæ[17], both effects presumably being due to hydration changes.

(*e*) A carboxylic acid membrane, made from polyvinylmethylether and polyacrylonitrile plus vinylchloride, shrinks and loses from a half to a third of its water on going from the potassium to the calcium state[38].

(*f*) Recent experiments on the phospholipid membrane model show that potassium increases and calcium decreases water wettability, water content, and water permeability of the model[26,39].

(*g*) The water content of myelin extracted from bovine nerve is increased by potassium and decreased by calcium[32].

(*h*) Myelin in water forms an oil-in-water emulsion in the presence of potassium and a water-in-oil emulsion in the presence of calcium[40].

(*i*) The water content of red cell ghost fragments is increased by potassium and is decreased by calcium[14].

Proposition 9. Membrane protein contributes to the structural deformation of the membrane which is permitted by calcium displacement and started by water intrusion:

(*a*) There is one bit of direct evidence which suggests, though it does not prove, that protein may participate directly in the genesis of the change permitting the ion fluxes of activity. Protease injected into the axoplasm of the squid giant axon destroys excitability without interfering with the resting potential[41]. Such persistence of the resting potential indicates that potassium and sodium segregation and membrane resistance and capacity are maintained. Phospholipases, on the other hand, which also produce inexcitability, *in addition cause the resting potential, resistance and capacity to fall to zero*[37]. These findings suggest that integrity of the phospholipid in the cell membrane is mandatory for the high resistance and capacity which make the ion segregations and resting potential possible, and that protein is somehow involved in a reversible modification of the orientation, packing or structure of the phospholipid to permit the ion fluxes of activity[34,42]. *An interdependence of lipid and protein geometry in the axon membrane is strongly indicated by* electronmicroscopically demonstrable changes in the membrane protein component after phospholipid hydrolysis[42,26b]. This change would, according to the present view, co-operate with calcium displacement and increased hydration in producing the shift of membrane structure from the resting to the active state.

(*b*) Squid axon ATPase activity is concentrated in the sheath region, not in the axoplasm[43]. One wonders if this enzyme may be a contractile protein associated with the membrane and capable of deforming it, since certain nerves have been shown to change surface contour and to shorten reversibly with activity[44–46].

Conclusion

Displacement of membrane calcium by potassium (ion exchange event), increased membrane hydration (hydrokinetic event) and membrane protein deformation (proteokinetic event) underlie the reversible change in membrane structure (primarily phospholipid geometry) which expresses itself as: (1) a fall in resistance with (2) increased transmembrane fluxes of sodium and potassium, both being major signs of the excited state in nerve cells.

Additional Points and Queries

Propositions 2, 6 *and* 7. (*a*) Why does axoplasmic potassium not diffuse into the membrane phase to displace calcium and produce the excited state continuously ? Why must it be driven into the membrane as an outward current ?

To keep potassium out of the membrane phase is probably a function of the resting potential[47]. Assuming that only a few potassium ions need diffuse out of the axoplasm, leaving their non-diffusible anions behind, to produce the resting potential, these few do not displace enough membrane calcium to shift the membrane to the active state, and the resting potential so generated, being close to the potassium equilibrium potential, prevents further movement of axoplasmic potassium into the membrane phase.

This concept is supported by a recent experiment[48]: Normally, both axons and muscle cells become inexcitable if the resting potential is kept low. However, if internal potassium is reduced sufficiently, then axons are excitable even at zero resting potential. In terms of the present hypothesis this would be explained by arguing that when potassium is present internally in normal concentration, then a large resting potential is necessary to keep this potassium out of the membrane (the equilibrium potential is high) so that the high resting impedance of the membrane can be *maintained* until the exciting stimulus is applied. If, however, there is little or no potassium in the axoplasm, then little or no resting potential is needed to keep it out of the membrane phase (the equilibrium potential is low), and in this condition the system can be excited from a low or zero resting potential level.

(*b*) How much potassium is moved into the membrane phase by the early, exciting, outward current ?

An average of about one potassium ion per 45,000 sq. Å of membrane area can be calculated as the amount of potassium which is moved into the membrane catelectrotonically by the time threshold is reached. This is computed from the action current at the frog node of Ranvier[36], from squid axon membrane capacity[10] and from the number of coulombs of outward current per cm^2 needed to excite the squid axon[7]. Since, however, if 1 per cent of the membrane area became freely conducting the resistance as a whole would fall to about 0·002 $\simeq$ cm^2, and since membrane capacity changes little with activity, it can be argued that perhaps as little as 0·001 per cent of the area is involved. Thus, considering the membrane to be 100 Å thick, and if only 0·001 per cent of the area were involved, the concentration change due to the current carrying K into this volume of membrane would be about 37 millimolar by the time threshold is reached. It has been found that something between 10 and 100 millimolar potassium reverses the effect of 1 mM calcium on the phospholipid membrane model and displaces calcium from the phosphatidylserine monolayer[25]. For an internal concentration of potassium of 0·1 M the number of potassium ions needed for the foregoing change could be supplied from a sub-membrane layer about 5 Å thick.

(*c*) If depolarization moves potassium into the membrane phase and this leads to structural dispersion with a fall in resistance, what keeps membrane resistance relatively high during the prolonged plateau of depolarization normally shown by heart muscle[49], and by nerve treated with tetraethylammonium or certain metal ions[50] ?

Here it is suggested that after potassium displaces calcium from phospholipid during excitation in the presence of TEA or nickel, then, during recovery, these unusual ions in turn displace potassium and occupy the sites which would normally be reoccupied by calcium. If they bind to phospholipid more tightly than calcium does, then they would tend to stabilize the membrane structure in the high resistance state, and prevent potassium egress, thus delaying repolarization. As time passes, however, the persisting low potential will eventually permit enough potassium to accumulate in the membrane to displace the binder ion. When this finally occurs the potassium will flow out and repolarize the cell. In this way the persistence of relatively high resistance even in the presence of depolarization could be accounted for. Experiments on the quantity of outflowing current required to excite under these circumstances and on tenacity of TEA and nickel binding to phospholipid as compared to that for calcium could be done fairly easily and would clarify this problem. What substance normally present in heart muscle might produce this effect is not apparent, but the suggestion that such a substance exists may stimulate interesting experiments.

(*d*) Since neither the phospholipid membrane model nor the monolayers used so far show any ability to distinguish potassium from sodium, what protects the phospholipid–calcium complex from external sodium ?

No satisfactory answer is at hand. It has already been suggested here that it is probably the resting potential which protects the resting membrane from potassium, but we do not know what protects it from sodium. Failure to answer this question, however, does not weaken the hypothesis. It does emphasize the fact that one still does not know how cells distinguish sodium from potassium.

(*e*) Perfusion of the squid axon with low-potassium or potassium-free solution does not prevent excitation[48]. How can this be reconciled with the argument of the hypothesis that it is catelectrotonic injection of axoplasmic potassium into the membrane which displaces calcium to start the structural changes which permit the fall in resistance to ion fluxes ?

First of all, as internal potassium is decreased, an increasing amount of depolarization is needed for the membrane resistance to fall to a given value[16]. Since a change in membrane resistance is probably our best evidence of a change in structural state, this finding supports the hypothesis. Thus, in terms of the hypothesis, the less potassium there is inside the axon the more depolarization should be required to permit enough potassium to enter the membrane phase to shift it to the low-resistance state.

Secondly, it is not argued that K^+ is the only cation which can be moved into the membrane phase from inside to displace calcium from negatively charged sites there. We know that sodium is about as effective as potassium in displacing calcium from phosphatidylserine[25]. What is argued is that when potassium is the dominant internal cation, then it is the ion which is moved into the membrane to produce this effect. It is, so to speak, the ion which the cell normally uses to trigger excitation. In the experiments quoted[48] there always had to be high external sodium. This sodium would be expected to leak continuously into the inside perfusate and under these circumstances almost surely would the cation be moved outward by the outwardly directed exciting current to displace calcium from the membrane structure. Thus, the potassium-free perfusion experiments do not pose a serious problem for the hypothesis.

Proposition 3. It becomes ever clearer than an explicit demonstration is required of what calcium is associated within the cell membrane. The evidence for certain reasonable assumptions has been given, and the possibility of combination with nucleic acids has also been investigated[51], but there are no objective experimental data yet available which apportion membrane calcium among the several likely membrane components.

Proposition 8. Since cells are highly permeable to water, that is, if there is a relatively high partition co-efficient for water in the membrane, why should increased membrane hydration increase permeability for ions ?

The fact is that the resting cell membrane may not be significantly hydrated in spite of high water permeability. Thus, the normal red cell surface is relatively hydrophobic[52], the denuded *Arbacia* egg in calcium chloride coalesces more readily with oil than when it is in potassium chloride[53], myelin and red cell ghosts contain less water in the presence of calcium than in potassium[32,14], myelin forms a water-in-oil emulsion in the presence of calcium and an oil-in-water emulsion in the presence of potassium[40], and for many resting cells a high oil : water partition coefficient favours penetration. Therefore it would appear that water can penetrate what may be a water-poor cell membrane phase just as it can to a degree penetrate the hydrophobic part of evaporation retarding monolayers used to conserve water in reservoirs and lakes[54]. Presumably, however, ions could not penetrate significantly by the same pathway and would require increased hydration with formation of water-filled conduits.

Clearly experiments need to be done to determine if axon membranes do in fact become more hydrophilic when excited. Some starts have been made which suggest that water entry into axons increases during activity[34,35,37,45].

Proposition 9. The nature and role of protein in membrane excitation are almost completely unexplored[55,56]. Recent work on a membrane model made of a bimolecular lipid leaflet in water[57] suggests that excitability is conferred on the system only after addition of a specific protein. Even though ATPase was long ago shown to be concentrated in the axonal sheath structure[43], there are not yet any reports of serious attempts to find a contractile protein in nerve cell somata or fibres.

Magnitude of the Transmembrane Potential

It has been argued[36] that the fraction of the resting potential which still exists after threshold is reached at about 15 mV depolarization could do a significant amount of work on membrane components which had been released from mechanical restraint by unbinding of calcium and intrusion of water and which were exposed to this voltage gradient. Thus, it was calculated that a voltage of 50 mV across 100 Å (50,000 V/cm) could move a molecule the size of serum albumin through a distance of 100 Å in about 3×10^{-4} msec. This figure represents maximum speed assuming application of the full voltage and mobility as in water. The velocity is so great, however, compared with the local duration of the nerve impulse, that even with considerable attenuation, say,

1×10^3 or so, such a phenomenon might still play a significant part in altering surface ultrastructure. In addition, it might not be necessary to produce appreciable translation of the liberated membrane component; only a change in orientation as in alignment or disalignment of a dipole might be required. If such a device were operative, then one would add electrokinesis to ion exchange, hydrokinesis and proteokinosis as basic components of the excitation process. The recent finding that a persistent zero resting potential can be tolerated without immediate disastrous consequences[46] de-emphasizes this concept somewhat. Nevertheless, such steep voltage gradients may have important consequences in other contexts.

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HORMONES, AMINO-ACID TRANSPORT AND PROTEIN SYNTHESIS

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CONSIDERATIONS of the availability of metabolic precursors for utilization by intact cells have evoked considerable interest in the process by which small molecules such as sugars and amino-acids penetrate into the intracellular confines of the plasma membrane. With regard to amino-acids, several mammalian systems such as Ehrlich ascites cells[1], human leucocytes[2], intestinal mucosa[3] and kidney cortex slices[4] have been examined to ascertain the characteristics of such a transport system. The process appears to be dependent on aerobic metabolism, has ionic requirements for sodium and potassium, and behaves in a fashion consistent with carrier mediation, that is, the data fit the concept of the presence of a saturatable binding site(s)[5]. The functional result of this process is the concentration of amino-acid within the cell to levels several times greater than that in the fluid in which the cell is immersed.

It is not surprising that attention should be focused on regulatory aspects of concentrative transfer by hormones in animal tissue since increased secretion of hormones or their administration is associated with growth or hypertrophy of tissues known to be target organs. A propelling factor stimulating the interest in hormone-amino-acid transport relationships has been the known effects of insulin[6–8], growth hormone[9], and œstrogen[10], which increase amino-acid incorporation into proteins of certain tissues. Although these hormones may affect the protein-synthesizing mechanism directly, an important question has been raised concerning the dependence of protein synthesis on transmembranous movement of amino-acids and the size of the intracellular amino-acid pool.

Perusal of the published literature on the subject of endocrine control of amino-acid transfer reveals experiments of varied design which report an alteration by a hormone of the intracellular concentration of an amino-acid with regard to the extracellular medium. The purpose of this article is to review these data and evaluate them within the present concepts of amino-acid transport. In addition, an attempt will be made to put the relationship between transport of amino-acids, the size of intracellular pools and protein synthesis into perspective.

Hormonal Effects on Amino-acid Accumulation in Intracellular Fluid

Three basic types of experimental procedures have been employed to obtain the results summarized in Table 1. These are:

(1) *In vivo* administration of a hormone at a suitable time after *in vivo* administration of a radioactive amino-acid followed by analysis of the amino-acid in the tissue water or cellular fluid of various organs. Experiments of this type have been performed for growth hormone, cortisone, and œstrogen[11] and ACTH[12]. A variation of this type has been used where the hormone (cortisone[13]) has been given to the intact animal, and the change in concentration of all natural amino-acids measured in cellular fluid.

Table 1. Hormonal Effects on Amino-acid Accumulation

Hormone	Amino-acid	Type of study		Tissue	Accumulation of amino-acid	Incorporation into protein†
		Hormone	Amino-acid			
Growth	AIB*	*Vivo*	*Vivo*	Muscle, liver, intestine, kidney	Increased (ref. 11)	Increased (ref. 9)
	L-histidine, L-alanine, AIB	*Vitro*	*Vitro*	Muscle	Increased (refs. 18, 41)	
Adrenocorticotrophic (ACTH)	AIB	*Vivo*	*Vivo*	Adrenal	Increased (ref. 12)	Unchanged (ref. 44)
Cortisone	AIB, others	*Vivo*	*Vivo*	Liver, muscle	Increased (refs. 11, 13)	Increased (ref. 42)
	L-histidine	*Vivo*	*Vitro*	Muscle	Decreased (ref. 15)	Decreased (ref. 15)
Thyrotropic (TSH)	AIB	*Vitro*	*Vitro*	Thyroid	Increased (ref. 27)	Increased (ref. 43)
Œstradiol	AIB	*Vivo*	*Vivo*	Uterus	Increased (ref. 11)	Increased (ref. 10)
	AIB	*Vivo*	*Vitro*	Uterus	Increased (ref. 14)	
Insulin	Several	*Vitro*	*Vitro*	Muscle	Increased (refs. 16, 17)	Increased (refs. 6–8)

* α-Aminoisobutyric acid. † Of utilizable amino-acids.

(2) *In vivo* hormonal treatment followed by measurement of amino-acid uptake by excised tissue *in vitro* such as performed for œstrogen[14] and cortisone[15].

(3) The addition of both hormone and the amino-acid to be examined to tissues *in vitro*. Experiments of this type have been performed with insulin[16,17], growth hormone[18], œstrogen[14], follicle-stimulating and interstitial-cell-stimulating hormone[19]. In several instances conflicting results of hormone stimulation of amino-acid accumulation have occurred between experiment of the first or second types and the third, where hormone was supplied with amino-acid *in vitro*. Discrepancies have thus been reported for cortisone with muscle[13], growth hormone with rat kidney or intestine[20], and œstrogen with uterus[14].

Some critical questions must be asked of nearly all these reported experiments. Primarily, have the observed findings actually resulted from an increase in the unidirectional movement or flux of the amino-acid into the intracellular fluid? Have the hormones which cause increased accumulation of amino-acid within the cell actually altered the membrane mechanism for movements of these molecules?

To facilitate answering the first question, a conceptual image of the cell bathed by nutrient medium can be proposed. Amino-acids, by an energy-dependent process, traverse the cell membrane and enter the cellular fluid. Once within the cell, multiple fates may be possible for the molecule. It may be catabolized, incorporated into protein, or egress from the cell back into the surrounding medium. As the active transport process of influx continues, intracellular levels of free amino-acids are built up until the entry is balanced by the combined rates of the processes regulating the fate of the intracellular molecules. Thus, a so-called steady-state concentration of the amino-acid is achieved the value of which is in excess of that in the extracellular fluid. This simplified concept of the extracellular–intracellular amino-acid may be regarded as a two-compartment system[21]. The amino-acids within the cell are believed to be free and unbound to any specific sites[22].

With the foregoing model in mind it becomes obvious that any single observation of an intracellular amino-acid concentration in the steady-state, as has been reported in experiments of the first type, does not present an adequate explanation of how the steady-state level was altered, in most cases increased. It is not known from data of this type whether the actual influx of amino-acid is increased or whether the effect is to decrease the subsequent loss of the amino-acid from the intracellular pool. Since the non-metabolizable amino-acid α-aminoisobutyric acid (AIB)[23] has been used in many experiments, only the efflux of the amino-acid to the extracellular fluid need be considered with regard to the possible fate of the intracellular molecules. A satisfactory explanation for the reported hormone-produced increase in amino-acid levels within the cell could be a blockade of efflux, as well as a stimulation of the inward transport rate, or both. In the experiments of type 2 or 3, where intracellular accumulation with respect to time has been observed (growth hormone[18] and insulin[16]), a clearer picture of the events leading to a new steady state is obtained. Although the data reported for growth hormone and insulin are suggestive of stimulation of influx, definitive analysis remains to be performed. Compartmental analysis of a kidney slice system[24] has shown that experimental alteration of influx rates almost invariably produces changes in efflux rates so that increase or loss of amino-acids from the intracellular pool may be a composite of flux alterations. Kinetic data have recently been presented showing that the remarkable increase in amino-acid accumulation caused by phlorizin in kidney cortex slices is due primarily to a diminution of efflux[25]. The evidence also suggested that phlorizin may be associated with intracellular binding of amino-acids.

With regard to the membrane mechanism for transport of amino-acids, two main features are the coupling of transport to energy metabolism and the apparent role of a saturable binding site(s) in the process. The directional movements and fate of amino-acids are dependent on the energy supplied by the cell. Metabolic inhibitors[26] markedly affect influx and efflux rates and the size of intracellular pools. It is therefore possible that hormonal effects on accumulation may result from changes in metabolism of energy-yielding substrates. For example, the small increase in AIB accumulation caused by thyrotrophic hormone in thyroid gland slices[27] may be a result of the known alterations in glucose metabolism caused by the hormone[28]. Likewise, the increase in AIB uptake in adrenal by ACTH[12] may be a consequence of increased glycogenolysis induced by ACTH[29]. Analysis of hormone effects on the relationship between energy and transport had not been performed to a significant extent.

Most mammalian transport systems of amino-acids conform to the Michaelis–Menten formulation for a saturatable binding site[5]. Experimental determination is possible of an apparent Km giving some indication of alteration of the affinity of the binding site(s) for the amino-acid. Only insulin has been examined in this fashion[17], and although the data are not conclusive they suggest that there is an increased affinity of the binding site caused by insulin. The known binding of insulin to cell membrane is in accord with this. Similar investigations with other hormones on amino-acid transport remain to be performed.

The presently available data, though demonstrating enhanced accumulation of amino-acids induced by hormones in certain tissues, has not clearly delineated the hormonal effects on the parameters of amino-acid transport which control accumulation. Care must be taken in the interpretation of many of the reports cited. These will, no doubt, provide a stimulus for fruitful research.

Relationship of Enhanced Intracellular Amino-acid Levels to Protein Synthesis

Parallel increases in intracellular amino-acid concentration and incorporation of amino-acids into protein are known to be produced by growth hormone[8] and insulin[6–8] in muscle and of œstrogen in the uterus[10]. Does the increased cellular level of free amino-acid arising from hormonal treatment result in increased protein synthesis? The present concept of regulation of protein synthesis places control of the process in the production of messenger RNA or perhaps at the ribosomal level[30]. Recent data suggest that growth hormone affects the aggregation of the protein-synthesizing ribosomes[31] while œstrogen is believed to stimulate formation of messenger RNA[32]. Insulin, on the other hand, increases amino-acid incorporation into protein under conditions where messenger RNA synthesis is inhibited[33]. Besides the control mechanism associated with messenger RNA and the ribosome, does the concentration of the amino-acid itself play a regulatory function in the cell? Can increased protein synthesis be stimulated merely by the increased availability of the intracellular substrate? Do the hormones augment their action at other regulatory sites by providing increased amounts of amino-acid?

Eagle, Piez and Levy[34] assessed the growth rates of human cells in tissue culture as a function of concentration of intracellular amino-acid. They found there was a critical threshold of 0·01–0·05 mM of the amino-acids studied necessary to initiate growth and that the rate of growth reached a nearly maximum value at concentrations only 2–4 times greater than the minimal critical level. Using Ehrlich ascites cells, Riggs and Walker[35] recently found that increasing the intracellular amino-acid level increased incorporation into protein, the rate of increase varying with different amino-acids. A great difficulty in using these results as a model for hormone-induced enhanced intracellular amino-acid levels is the fact that in the foregoing experiments the increase in intracellular concentration was produced by increasing the extracellular levels, whereas with hormone treatment the increase in intracellular level occurs without a similar increase in extracellular concentration.

A very pertinent finding relating intracellular amino-acid pools to protein synthesis is the observation in bacteria[36], rat muscle[37] and rat kidney cortex slices[24] that the kinetics of incorporation of amino-acid into protein are independent of the rate at which an amino-acid enters the intracellular pool. The build-up of an intracellular amino-acid pool is a logarithmic function, while incorporation into protein is a linear one. Theoretically, if protein synthesis is dependent on entry of amino-acid into the extracellular pool, the incorporation into protein should not exhibit linear kinetics until the amino-acid has achieved a steady-state condition, but this is not the case. These types of data have caused speculation that there is compartmentalization of the intracellular amino-acid pools.

In bacteria it is believed that the so-called 'expandable' amino-acid pool is not related to protein synthesis[38]. It has also been observed in a bacterial system that, while cells have stopped growing because of lack of an amino-acid in the medium, the extractable intracellular free amino-acid pool of this deficient amino-acid is at a normal level[39]. In mammalian cells there may be a very small, rapidly turning-over pool which is separate from the general pool and which is directly related to protein synthesis. Kinetically, the incorporation into protein behaves as if the amino-acid was taken directly from the surrounding fluid. Thus the activation of amino-acids for protein synthesis possibly occurs adjacent to the cell membrane[40] and those amino-acids synthesized into protein do not mix with the free intracellular pool. If the latter be true, then experiments correlating amino-acid incorporation with size of the intracellular pool, where the intracellular pool size is increased secondary to higher extracellular levels, may be making an inaccurate correlation.

From the foregoing discussion the following points may be stressed: (1) The terms intracellular accumulation of amino-acid or uptake should not be confused with the term transport of amino-acid, as the former terms represent the summation of movement of amino-acids into and out of cells. (2) Though several hormones have been shown to augment intracellular accumulation of amino-acids, the exact effects on the parameters of transport have not been clearly delineated. (3) Interrelationships of intracellular amino-acid concentration and protein synthesis remain to be clarified. Regulation of protein synthesis by hormonally increased availability of amino-acids remains open for speculation. Judicious attention to the proper design and analysis of experiments should contribute to the understanding of hormone action in this vital area of cell function.

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OBITUARIES

Prof. A. Wormall, F.R.S.

ARTHUR WORMALL died in St. Bartholomew's Hospital on May 9 at the age of sixty-four. He will be greatly missed. The high esteem in which he was universally held is aptly epitomized by a remark made some years ago to one of his junior colleagues: "The one thing we envy you most in your department is your professor".

Wormall received his early education at Leeds, graduating in 1921 and joining the staff of the University in 1922. His work as a lecturer and senior lecturer in biochemistry until 1936 can truly be said to have laid the foundations of the Department of Biochemistry which exists in Leeds to-day.

He early developed an interest in problems of immunology; he helped materially to establish the complex nature of hæmolytic complement, and also described the hitherto unrecorded 'fourth component'. His work on proteins modified by nitration, halogenation and treatment with phenyl isocyanate or mustard gas and its derivatives, particularly his original use of correspondingly modified amino-acids as inhibitors of the precipitin reaction between these protein antigens and their specific antisera, work which he commenced as a Rockefeller Medical Fellow working with Landsteiner at the Rockefeller Institute during 1928–29, did much to clarify our present knowledge of the nature of the groupings which determine the immunological specificity of chemically modified proteins.

In 1930–31 he spent a year in Uganda on behalf of the Colonial Office, investigating chemical and immunological problems connected with trypanosomiasis, and developed methods of measuring the persistence of the drug 'Bayer 205' ('Antrypol', 'Germanin') in the blood stream. Although he later carried out work in widely different fields, these two early interests in immunochemistry and trypanosomiasis remained with him all his life.

In 1936 he was appointed to the newly created chair of biochemistry and chemistry in St. Bartholomew's Hospital Medical College (University of London). His great organizing ability, his continual insistence that preclinical biochemistry teaching must be relevant to medicine, and his deep personal interest in all his students and staff, were responsible for the creation of a flourishing department—and a well-beloved chief.

Shortly after he went to 'Bart's', he recommenced work on mustard gas, and during the War led a research group working in Cambridge on the physiological action of mustard gas. It was at this time also that he commenced his work with artificial radioactive isotopes, and he was the first in Britain to use them in biological investigations. It is a tribute to his farsightedness that when he first envisaged the use of the radioactive sulphur isotope, sulphur-35, in his work on mustard gas, its preparation had been described; but it was stated that its β-particle emission was too soft for it to be of any use in quantitative investigations. At this time, too, the worker with artificial radioactive isotopes not only had to manufacture his own measuring equipment, but his isotopes also! Later, when isotopes became more readily available, he extended his use of them to immunological investigations and to investigations on the metabolism of zinc in normal and neoplastic tissues. In 1953 he organized and conducted the First Latin-American Course in Radioisotope Methodology, and was awarded an honorary doctorate of the University of São Paulo.

He was a governor of the Medical College and of the Hospital, and his wise counsel was greatly valued on the many College and University committees on which he served.

He was always eager to create opportunities for integrating more closely the work of the Hospital and the preclinical departments, and it was mainly due to his endeavours that a research project on the biochemistry of skin was initiated in his Department jointly with the Department of Dermatology in the Hospital.

Wormall had a wide circle of friends, and his gentle courtesy and consideration for others endeared him to all. His great charm of manner readily made the most nervous youngster at ease with him, and no trouble was too great for him to take on behalf of anyone who came to him for help or advice.

Until physical disabilities prevented him, he was a keen golfer, and as a true Yorkshireman had an abiding interest in cricket. His election to the M.C.C. in 1956 probably gave him at least as much pleasure as his election to the Royal Society, which occurred in the same year, and his friends will long remember his account of a day at Lord's with a visiting American scientist when his attempts to explain the rules of the game were frustrated for some time by his visitor's unrealized misconception that the two batsmen were on opposite sides.

In 1925 Arthur Wormall was married to Eva Jackson, who survives him. All his friends would wish to tender to his widow, and his two daughters, their heartfelt sympathy in their loss.

G. E. C. FRANCIS

Mr. H. D. Griffith

THROUGH the sudden death of Mr. Harry D. Griffith on April 11, the University of Aberdeen, and the Natural Philosophy Department in particular, lost a member who had given most loyal service for the long period of forty years. Born in 1898 at Clifton, Bristol, he received his early education at Bristol Grammar School and was awarded an open scholarship in 1916. On account of war conditions then in force, this scholarship was not taken up, but on the cessation of hostilities he entered as a scholar of Trinity College, Cambridge, in 1919 and in due course graduated B.A. Thereafter he continued to work in the Cavendish Laboratory under Sir J. J. Thomson and Sir Ernest Rutherford until 1923. In October of that year he came to Aberdeen on the recommendation of Prof. G. P. Thomson (later Sir George Thomson) with the specific idea of assisting in the teaching of physics to medical students. From this early beginning he was soon engaged in the development not only of the teaching of physics but also in the installation of a radon supply to hospitals in various parts of the country. In order to accommodate the required apparatus, an addition to the Department was built, and there Mr. Griffith installed towards the end of the 1920 decade the apparatus which enabled a supply of radon to be available for hospital work for a period of more than twenty years.

In 1932 he was awarded a Rockefeller Fellowship which enabled him to travel abroad. His first visit was to Berlin. There he studied for some months under Prof. Friedrich at the Institut für Strahlenforschung. Owing, however, to the political upheaval in Germany at that time he found it necessary to leave. From Berlin he went to Stockholm and worked under Prof. Sievert at the Radiumhemmet.

The insight he obtained abroad he put to good use on his return. Soon his services were required by various Departments in the Medical School, and, after the erection of the New Infirmary Buildings at Foresterhill, it was found necessary to provide a small laboratory in the Infirmary for his special use. At the same time the personnel required to meet the calls on the medical physics sections gradually increased, the use and direction of which fell very largely on Mr. Griffith.

After the end of the Second World War it became evident that the laboratory accommodation at Foresterhill was insufficient to meet all requirements. As a result it was decided to erect a laboratory fully equipped with research rooms, workshop and office apartments in close contact with the Medical School at Foresterhill. Much of the work in connexion with this project fell to be carried through by Mr. Griffith.

The development of this laboratory, a considerable undertaking in itself, he carried on along with an ample share in the teaching of both theoretical and practical work in the parent Department of Natural Philosophy. In spite of all, he found time to produce several publications and in consequence was elected a Fellow of the Royal Society of Edinburgh in 1954.

From his entry to the University in 1923 until his death in 1964 he devoted his energies wholeheartedly to the development of his subject. Coming as assistant lecturer, he steadily improved his status to that of reader in medical physics in charge of that division in the University Physics Department. His students held him in the highest regard, and referred affectionately to him always as "Harry". Even those who did not come immediately under his care testify to the same warmth of feeling, and all felt that in his death they had lost one who had been their friend.

Further evidence of this ability to inspire lasting affection is exemplified by the friendship between him and two men of different nationalities—one a German. Dr. Zimmer, whom he met when he first went to Germany and with whom he continued to work for the rest of his life; the other an Englishman, H. Heckstall-Smith, who has recounted in his book, *The Doubtful School-Master*, his first encounter with H. D. Griffith in the Cavendish Laboratory, an encounter which led to a life-long reciprocal exchange of ideas and outlooks. When he came to Aberdeen, he met, in the Natural Philosophy Department, Miss J. M. Jack, herself an honours graduate in physics. A friendship grew up rapidly between them, and in 1927 they were married. They have one daughter who graduated with honours in physics. A. E. M. GEDDES

NEWS and VIEWS

Max Planck Medallists of the German Physical Society: Prof. G. E. Uhlenbeck and Prof. S. A. Goudsmit

PROF. GEORGE E. UHLENBECK and Prof. Samuel A. Goudsmit have been awarded the Max Planck Medal of the German Physical Society. This gold medal, one of the highest awards in international science, was conferred on the 106th anniversary of the birth of Planck, the great pioneer of modern physics. Profs. Uhlenbeck and Goudsmit, in 1925, discovered the 'spin of the electron', a cornerstone of present atomic theory. Dr. Goudsmit is also deputy chairman of the Department of Physics in the Brookhaven National Laboratory and managing editor of the American Physical Society. Both scientists received their education in The Netherlands, and joined the faculty of the University of Michigan in 1927. Prof. Uhlenbeck went to the Rockefeller Institute in 1961. Dr. Goudsmit joined the Brookhaven National Laboratory in 1948 and has been a visiting professor in the Rockefeller Institute since 1957. During the Second World War both were connected with radar research in the Massachusetts Institute of Technology. Dr. Goudsmit, moreover, was the scientific head of a secret intelligence mission which moved in with Allied troops and reported, in the winter of 1944, that German scientists had failed to make any significant progress in their work on atomic explosives.

Chemistry in the University of East Anglia: Prof. S. F. Mason

DR. S. F. MASON has been appointed professor of chemistry in the new University of East Anglia at Norwich. Dr. Mason, who is forty years of age, was educated at the Wyggeston Grammar School, Leicester, and Wadham College, Oxford; he graduated with first-class honours in chemistry in 1945, and obtained his M.A. and D.Phil. in 1947. During 1947–53 Dr. Mason was tutor in chemistry at Wadham College and departmental demonstrator in the Museum for the History of Sciences at Oxford; during this period he published a number of papers on the history of sciences and a successful book, *A History of the Sciences*, which has been translated into French, German and Japanese. In 1953 he was appointed Research Fellow in the Department of Medical Chemistry of the Australian National University, then housed in London, where he worked on the ultra-violet and infra-red spectroscopy of heterocyclic compounds. In 1956 he was appointed lecturer in chemistry in the University of Exeter, where he became a senior lecturer in 1961 and reader in chemical spectroscopy in 1963. During his time in Exeter, Dr. Mason published many papers in the fields of spectroscopy, and its application to structural problems, and in theoretical chemistry; during the past two years he has taken up the investigation of optical rotatory power, particularly circular dichroism and optical rotatory dispersion, in which field, too, he has published a number of important papers. Dr. Mason will take up his new appointment on October 1.

Water Resources of England and Wales

A WATER Resources Board is being set up under the Water Resources Act, 1963, and will have the task of advising the Minister of Housing and Local Government on water policy in England and Wales. Its main practical tasks will be to build up comprehensive information about water resources and demands, to work out action needed to augment resources, to secure the promotion of schemes which it recommends and to commission and supervise research. The Board's permanent offices will be outside London, probably in Reading, although at the outset it will operate from Government offices in London. Twenty-nine new river authorities, also set up under the 1963 Act, will supersede existing river boards and will have important new functions relating to the management of water resources, including a licensing control of abstractions and impoundings of water. Statutory consultations about the size and composition of these authorities, which will have a membership of between 21 and 31, and will be the executive arm of the new system, are now going on.

The following have been appointed members of the newly formed Water Resources Board: *Chairman*, Sir William Goode, until recently Governor and Commander-in-Chief, North Borneo; *Deputy Chairman*, Mr. A. G. McLellan, general manager, Sunderland and South Shields Water Co.; *Members*, Mr. R. A. Banks, until recently a director of Imperial Chemical Industries, Ltd.; Prof. H. C. Darby, professor of geography, University College, London, and until recently a member of the National Parks Commission; Dr. Idris Jones, a former member of the Welsh Advisory Water Committee and lately director-general of research, National Coal Board; Mr. W. A. Muddell, chairman of the Executive Council of the River Boards' Association; Mr. N. A. F. Rowntree, consulting engineer (full-time director of the Board's activities).

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The Bristol College of Science and Technology

In reply to a question in the House of Commons on June 4, Mr. Quintin Hogg, the Secretary of State for Education and Science, said that the Bristol College of Science and Technology, which is one of the ten colleges of advanced technology destined for university status following the Government's acceptance of the recommendations of the Robbins Report, would have to move from its present site and buildings which it shares with the Bristol Technical College. Mr. Hogg said that following a report from the Governors of the Bristol College of Science and Technology it had been decided that it should be moved to Bath, where a site has been made available for future development. Originally the plan had been to provide new buildings for the College in Bristol, but the earmarked site proved too small for the needs of an expanding university institution. Three alternatives were considered: namely, Clevedon, South Gloucestershire just outside Bristol, and Bath. It was felt that the site at Clevedon was unsuitable because of the difficult contours of much of the area, and that in South Gloucestershire did not offer the opportunities afforded by the Bath site of a close association between university and city.

Return of Britain's Scientists

In a written answer in the House of Commons on June 18, the Secretary of State for Education and Science, Mr. Hogg, stated that most of the scientists and engineers who had so far returned from the United States and Canada in 1964 were seen by the Selection Board in 1963, when 146 candidates were recommended. Thirty-seven of those interviewed were known to have returned so far. In 1964, the Board had recommended about 190, but it was too early to say how many offers of employment would be accepted. The Board paid to candidates in North America their travelling expenses to and from the nearest place of interview and, in suitable cases, employing departments and authorities would pay return fares to the United Kingdom of the successfully assigned candidates and their families.

Advisory Committee on Pesticides and Other Toxic Chemicals

In a written answer on June 18, Mr. Hogg stated that following the assurance given on March 24 by the Minister of Agriculture, Fisheries and Food, the Advisory Committee on Pesticides and Toxic Chemicals had been given a new title reflecting its wide responsibilities. It would now be known as the Advisory Committee on Pesticides and Other Toxic Chemicals, and its terms of reference had been extended to keep under review all risks that might arise from the use of pesticides; potentially toxic chemicals on sale to farmers for veterinary use and veterinary medicines prescribed for use by veterinary surgeons; and any other potentially toxic chemicals specifically referred to the Committee by Ministers; and to make recommendations to the Ministers concerned. The industrial and domestic uses of organochloric pesticides were now being reviewed.

Schools and Society

In a written reply in the House of Commons on June 16, Mr. Hogg stated that following the recommendations of the Newsom Committee for an Inter-Departmental Working Party to consider the general social problems, including education, in slum areas, an Inter-Departmental Committee had been established, with terms of reference covering these problems within a wider context. Its task would be to consider plans for, and results of, research and development work on any aspect of the general theme "The Schools and Society", with the view of the better co-ordination of activities and the more efficient use of public funds and other moneys. The Home, Health and Education Departments for England and Wales and Scotland, the Ministry of Housing and Local Government, the Ministry of Labour and the Department of Scientific and Industrial Research were represented.

National Examinations Board in Foremanship and Supervision

In a written answer on June 17, Mr. Hogg stated that after consultation between his Department and representatives of industry and the professional bodies chiefly concerned, including the British Institute of Management, a new National Examinations Board in Foremanship and Supervision had been set up, with as its first task consideration of the early introduction of a qualification to replace the present British Institute of Management Certificate. The Board might introduce other qualifications in foremanship and supervision if it saw the need, and he hoped to see substantially increased numbers taking courses in the technical colleges for the Board's qualifications.

A Decade of Medical Research

In a written answer on June 17, Sir Edward Boyle gave the estimated Government expenditure on medical research in the past ten financial years as follows: 1954–55, £5·5 million; 1955–56, £6·5 million; 1956–57, £7 million; 1957–58, £8 million; 1958–59, £9·5 million; 1959–60, £10·5 million; 1960–61, £12·5 million; 1961–62, £14·5 million; 1962–63, £16 million; and 1963–64, £18·5 million. These figures include the estimated expenditure of the Health Departments, the Medical Research Council, the General Register Office, and the Air Ministry, with the estimated proportion devoted to medical research from the general grants to universities through the University Grants Committee.

U.S. Man-power Requirements in Science, Engineering and Technology

Under the title *Scientists, Engineers and Technicians in the 1960's—Requirements and Supply*, the National Science Foundation has issued a further report presenting the results of the second major investigation undertaken by the Bureau of Labour Statistics to assess these requirements in the 1960's (*NSF* 34. Pp. vii + 68. Washington, D.C.: Government Printing Office, 1964. 45 cents). In engineering, the demand for new personnel over the decade is estimated at more than 700,000, of which 165,000 represents replacement and 550,000 new demands, while the number of new entrants during the period is expected to be about 450,000. The situation will probably be particularly severe in the mid-1960's. By the end of the 1960 decade, if expenditure on the space programme levels off, and the number of engineering graduates increases more than expected, the situation may become somewhat less critical. Over the decade as a whole, the demand for engineers is expected to average about 72,000 a year compared with the projected available supply of about 45,000. In terms of numbers, the man-power situation in the sciences is expected to be less acute, though serious: the anticipated supply of 314,000 scientists, including mathematicians, being in rough balance with a projected demand of 295,000 over the decade. This assumes that the necessary college and university facilities and faculties will be available to handle the expected almost doubled enrolments in science. Scientists in some fields are likely to be in short supply throughout the 1960's, especially chemists, physicists, and medical and biological scientists and scientists with a Ph.D. degree. The demand for technicians is expected to grow at about the same rate as that for scientists and engineers, and on this basis, some 700,000 new technicians will be needed during the decade.

Encouragement for Physics in the United States

The American Association of Physics Teachers and the American Institute of Physics are very conscious of the importance of improvement of the physics teaching in schools, and they have been actively engaged for some years in directing and assisting schools in their programmes and in liaison between them and the national organizations. The "AAPT–AIP Visiting Scientists Programs in Physics", now in their fifth year, provide the stimulus of visits to high schools and institutions of higher learning and furnish valuable feed-back of information and recommendations concerning the chief local educational problems in physics. The Association's Committee on High School Awards recognizes ten high schools each year for excellence in the teaching of physics. The Institute's two booklets, *Physics in Your High School* and *Why Should You Study Physics in High School ?* encourage enrolment in high school physics. A purchase guide has been prepared to assist schools in purchasing equipment under the National Defense Education Act, and a report, entitled *Modern Physics Buildings*, is intended to contribute towards the better design of physics building facilities in schools and colleges. In order that the activities of the Association and Institute should have the greatest impact, the organizations have established the "Regional Counsellor Program" in Physics. The regional counsellors comprise a group of some fifty physicists, chosen for their competence in physics, their interest in the improvement of physics teaching, and their ability to work effectively at local level in co-operation with school and college officials to improve physics teaching. A Regional Counsellor Office has been established at the American Institute of Physics within the Institute Office of Education and Manpower to co-ordinate and support the work of the counsellors. The counsellors are unpaid, but their expenses are reimbursed. The Regional Office ensures that the counsellors receive proper recognition both locally and nationally.

Aspects of Automation

Automation at Low Cost is the title of a series of six booklets being issued during the National Productivity Year by the Information Division of the Department of Scientific and Industrial Research. The first five, dealing with counting and weighing, temperature and humidity, control of liquids, simple automation of machine tools, and control of processes, have already been published; the sixth, *Quality Control and Inspection*, is in the press. In the earlier booklets in the series, a description is given of some of the methods that a manufacturer can use to indicate and control the conditions inside his equipment, for example, the temperature of a furnace, the level of a solution in a boiler, the humidity in a drying chamber, the rate of flow of liquid into a tank, or the weight of material being added to a mixing vessel. Booklet No. 5 deals with processes in which several properties must be measured and controlled, either sequentially from stage to stage or simultaneously (*Control of Processes*. Pp. 16. London: Information Division, Department of Scientific and Industrial Research, 1963). The intention is to show to the small manufacturer some of the ways in which he can obtain the benefits of automation in his batch-production work, and that any capital expenditure on the introduction of automation would be recovered within a few years.

Journal of Medical Entomology

Volume 1, No. 1, of a new journal entitled *Journal of Medical Entomology* made its appearance in April 1964 (Pp. 1–118. Honolulu, Hawaii: Entomology Department, B. P. Bishop Museum, 1964. Published quarterly. Subscription rates: Annual subscription 10 dollars per volume to institutions and dealers; 7 dollars to individuals). It is edited by J. L. Gressitt, supported by an editorial committee widely distributed round the world, and will publish papers dealing with all phases of medical entomology and medical acarology, including systematics of insects and mites of importance in public health and in veterinary medicine. Articles exceeding one page in length are subject to a page charge of 12.50 dollars per printed page. Part 1, which runs to 117 pages, is well printed and produced (in double columns); the contributors are just about as widely distributed geographically as they could be; and the sixteen papers deal with taxonomy of mites, ticks and flies, the physiology of mosquitoes, the isolation of viruses from mosquitoes and ticks, the incidence of filariasis, and methods for mosquito surveys.

Abstracts of Papers on Radio Engineering

With the view of assisting in the general problem of the retrieval of scientific information, the British Institution of Radio Engineers has periodically published abstracts of papers which have appeared in its *Journal*. The first booklet of such abstracts was issued in 1955 (*Nature*, 176, 381; 1955) and the seventh edition has recently become available (*Abstracts of Papers published in the Journal of the British Institution of Radio Engineers*. 1952 to 1963 inclusive. Seventh edition. Pp. viii + 108. London: Institution of Electronic and Radio Engineers. 1963. 10*s*. 6*d*.). This contains 885 abstracts of papers, articles and reports published in the Institution's *Journal* during the twelve-year period 1952–63 inclusive. The abstracts are arranged according to the Universal Decimal Classification System with alphabetical subject and author indexes. Abstracts and references to reports of the Institution on technical and educational matters are included under appropriate headings, as well as editorial articles discussing matters of general and scientific interest. The completion of these abstracts to 1963 is very appropriate, since the name of the publishing body has recently been changed to "The Institution of Electronic and Radio Engineers". This seventh edition of the *Abstracts* is bound in stiff-paper covers with a colour reproduction of the Institution's armorial bearings. In order to avoid confusion in the published literature, the *Journal* of the Institution will retain the title *The Radio and Electronic Engineer*, which was adopted in January 1963.

Blakeney Point and Scolt Head Island

Blakeney Point and Scolt Head Island, edited by J. A. Steers (The National Trust, London, 1964. 5*s*.), is a new guide which replaces one published in 1952. It includes a description of the physical features of the area by Prof. Steers, who also edited the whole work. The botany and plant ecology were dealt with by the late Prof. F. W. Jane and Dr. D. J. B. White. There is an article on the bryophytes by Dr. E. Lodge, and on the insects, marine animals and mammals by Mr. E. A. Ellis. Prof. Jane also wrote the chapter on birds. The book should be invaluable to visitors to these two reserves; it is also a useful introduction to those interested in coastal ecology generally.

Mechanisms of Carcinogenesis

"In spite of all our deficiencies in knowledge, I believe we are now in prospect of a comprehensive synthesis in understanding of the means of action of chemical, physical, viral and indeed purely genetic carcinogenic influences." These brave words were written recently by Prof. Alexander Haddow in his introduction to a recent issue of the *British Medical Bulletin* devoted to "Mechanisms of Carcinogenesis—Chemical, Physical and Viral" (20, No. 2; May, 1964. London: The British Council. 30*s*.). Similar statements in the past have been premature, but the thirteen review articles that he is introducing give Prof. Haddow some justification for his optimism. Because carcinogenic substances are so diverse they must operate by biochemical routes of great variety, but all the agents by their devious routes appear to lead to deletions of genetic material with loss of the in-built controls of the normal cell. He feels that in time these processes can be elucidated with chemical precision to reveal the differences, on the cytogenetic and molecular levels, between normal and cancer cells. Chemical carcinogens have received most attention and there are contributions on the probable modes of action of the biological alkylating agents, the carcinogenic lactones, nitroso compounds, the aromatic amines, the polycyclic hydrocarbons and various natural and metallic substances other than the radioactive metals. Articles on the tumour-producing viruses stress the wide physical, chemical and biological differences among them and indicate that the mechanisms by which they act may be equally varied. The relevance of such investigations to cancer in man has acquired new importance from the recognition that a type of cancer of Africans (the Central African lymphoma, or Burkitt tumour) may well be caused by a virus. Other contributions deal with immunological factors in carcinogenesis and with radiation carcinogenesis.

U.K. Courses in Nuclear Sciences, 1964–66

The Science Department of the British Council has compiled *List* No. 8 (May 1964) of *Nuclear Studies*. The catalogue is primarily a guide for overseas students wishing to undertake specialized formal training in nuclear science in the United Kingdom. Only full-time courses, generally of one week or more in duration, are listed. Information provided includes notes on each of the courses and the institution at which it is held and also the fee and qualifications required for participation. While most of the postgraduate courses listed deal solely with nuclear science, there are a few more general courses with a substantial content of advanced nuclear science. A science or engineering degree is usually necessary for admission, and courses for non-graduates deal solely with nuclear science. The *List* has unavoidably appeared too late for application to be made for many of the courses for the academic year 1964–65. It serves, however, to direct enquiries about future courses to appropriate institutions in time for 1965–66. Copies of the *List* and further information can be obtained from the Science Department, the British Council, 59 New Oxford Street, London, W.C.1.

Royal Statistical Society

At the annual general meeting of the Royal Statistical Society on June 17, Mr. S. P. Chambers was elected president for the ensuing year. Mr. B. P. Emmett, Miss S. V. Cunliffe and Dr. J. A. Heady were elected honorary secretaries and Mr. C. T. Saunders was re-elected honorary treasurer. Prof. G. E. P. Box, professor of statistics in the University of Wisconsin, was awarded the Society's Guy Medal in Silver.

University News: Bristol

The following appointments to lectureships have been announced: Dr. S. M. Anstis (psychology); Dr. A. P. Cox (physical chemistry); Dr. M. Flower (mathematics, with the Computer Unit); Dr. P. L. Goggin (inorganic chemistry); Dr. J. Malos (physics); D. H. Peregrine (applied mathematics); M. J. R. Shave (mathematics, with the Computer Unit).

East Anglia

Dr. S. F. Mason, reader in chemistry at the University of Exeter, has been appointed professor of chemistry in the School of Chemical Sciences.

Leeds

Dr. H. L. Price, senior lecturer in mathematics, has been appointed as professor of mathematics. The following appointments to lectureships have been made: Dr. H. Hassall (biochemistry); T. F. Preece (agricultural botany); Dr. G. H. Smith (agricultural chemistry).

Southampton

Prof. Kenneth Mather, professor of genetics in the University of Birmingham since 1948 and Pro-Vice-Chancellor and Vice-Principal since 1960, has been appointed to succeed Dr. D. G. James on his retirement from the office of Vice-Chancellor of the University at the end of the session 1964–65. He was born in 1911 and was educated at Nantwich and Acton Grammar School and the University of Manchester. From 1934 until 1937 he was a lecturer in the Galton Laboratory in University College, London, and during 1937 and 1938 he was a Rockefeller Research Fellow at the California Institute of Technology and Harvard University. From 1938 until 1948 Prof. Mather was head of the Department of Genetics at the John Innes Horticultural Institution. He was a member of the Agricultural Research Council from 1949 until 1954 and from 1955 until 1960.

Announcements

Prof. Erwin Chargaff, professor of biochemistry in Columbia University, New York, has been awarded the 1963 Charles Léopold Mayer Prize of the Paris Academy of Sciences, for his work on nucleic acids.

Mr. A. Herzka, director of Pressurized Packaging Consultants, Ltd., London, has been elected president of the Society of Cosmetic Chemists of Great Britain for 1964–65.

The third European regional conference on "Electron Microscopy" will be held in Prague during August 26–September 3. Further information can be obtained from the Organizing Committee, Third European Regional Conference on Electron Microscopy, Albertov 4, Prague 2.

A conference on "Recent Advances in Activation Analysis" will be held at the University of Glasgow during August 27–28. Further information can be obtained from Dr. J. M. A. Lenihan, 9–13 West Graham Street, Glasgow, C.4.

A symposium on "High-Energy Electrons", organized by the European Association of Radiology, will be held in Montreux during September 7–10. Further information can be obtained from Dr. P. L. Cova, Casa di Cura S. Ambrogio, Via Faravalli 16, Milan.

The ninth international conference on "Low Temperature Physics", sponsored by the International Union of Pure and Applied Physics, the Ohio State University and the Battelle Memorial Institute, will be held in Columbus during August 31–September 4. Further information can be obtained from Dr. J. G. Daunt, Department of Physics, Ohio State University, Columbus, Ohio.

An international conference on "Microwaves, Circuit Theory and Information Theory" will be held in Tokyo during September 7–11. The programme will include sessions on: microwave theory and techniques and electron devices; microwave antennæ and propagation; microwave communication systems; circuit theory; information theory. Further information can be obtained from Dr. K. Morita, c/o The Institute of Electrical Communication Engineers of Japan, 2–8 Fujimicho, Chiyodaku, Tokyo.

Errata. In the communication entitled "Enolase and Fluorophosphate" by Sir Rudolph Peters, M. Shorthouse and L. R. Murray, which appeared on p. 1331 of the June 27 issue of *Nature*, for hydrolase in line 6 read hydratase; Table 1, lines 7 and 8, for FPO_3^- read $FPO_3^=$; line 2 of the final paragraph, for K_2PO_3 read $K_2F.PO_3$; in ref. 4, for Ochos read Ochoa.

THE AUSTRALIAN DEFENCE STANDARDS LABORATORIES

THE annual report of the Department of Supply of the Australian Defence Standards Laboratories, Maribyrnong, covering the period July 1, 1962–June 30, 1963*, mentions that a joint unit with the U.K. Ministry of Aviation has been set up to investigate the causes of deterioration of materials in tropical environments. The main laboratory for this purpose is near Innisfail, where 'hot-wet' conditions prevail, and a second laboratory is at Cloncurry in western Queensland, where the conditions are 'hot-dry'. Other sites, such as the marine site at Clump Point near Innisfail, will be used as required.

"Operation Blowdown", planned by the Australian Army and the Laboratories in 1961 in order to obtain data for military importance concerning the effects of a large explosion occurring in a tropical rain forest, was expected to culminate in July 1963 by the explosion of some tens of tons of trinitrotoluene at a height of 140 ft. above the ground in the rain forest area at Iron Range, Cape York Peninsula, 320 miles north of Cairns. The scientific tasks undertaken by the Laboratories included: the assembly of the explosive charge and its detonation; measurements of blast wave using pressure gauges with high speeds of response; measurements of tree strain and the investigation of displacement hazards to articulated dummies representing troops; and the assessment of damage to the forest as a whole.

In the Physics Division the work on the detection by an electron spin resonance spectrometer of the defects produced in polymethyl methacrylate by irradiation by γ-rays was extended to include the effect of a red dye, commercially incorporated in 'Perspex', as used in radiation dosimetry. The dye does not affect the free radical spectrum of the substrate, but gives rise to an additional signal. A wide-line nuclear magnetic resonance spectrometer was used to make a general survey of the resonance of nitrogen-14 nuclei in a variety of compounds, including formamide and nitrophenol. Cobalt complexes, irradiated beryllium oxide and resonances in oxygen, aluminium, chlorine, titanium and mercury were also investigated. From measurements of the anisotropy of the electrical conductivity of silicon for high electric fields at lattice temperatures of 77° K and 300° K, the temperatures of the hot electrons in the normally equivalent valleys were deduced as a function of the electric field.

The electronic counting of optical interference fringes is regarded as a promising method for the improvement in high-precision measurements of length, and equipment of this kind for the measurement of lengths up to 2 in. to an accuracy of 1 in 10^6 has been recently installed in the Physics Division. It is to be used for the standardization of about 900 sets of standard gauges. A method for the calibration of analytical weights using the built-in weights of a direct-reading balance as standards has been developed. The uncertainty in the values obtained is less than the precision of the balance, and thus the application of the method will enable the majority of chemical laboratories to standardize their own analytical weights to a high order of accuracy. The Division has taken an active part in the drafting of specifications for the range of 'Radiac' (radiation indicating and computing) instruments for use by the Australian armed services and civil defence organizations. The instruments include pocket quartz fibre dosimeters, β-γ Geiger counter contamination meters, and ionization chamber survey meters.

As part of the Chemistry Division's programme of elucidation of the molecular structure of organic compounds, the nitrogen–hydrogen stretching frequencies in a number of primary and secondary amines and amides have been determined. The results indicate that the apparent splitting of the first overtone symmetric amine stretching band arises from a combination frequency and not, as others have suggested, from the tunnelling through an energy barrier or the presence of rotational isomers. Investigations using electron impact in a mass spectrometer have shown that, while β-bond cleavage is the principal mode of fission in both aliphatic cyanides and iso-cyanides, the iso-cyanides exhibited in addition markedly greater α-bond cleavage and hydrogen rearrangement. The investigation of the effect of activation temperature on the properties of chemically pretreated brown coals has been completed. New methods for the determination of a number of atmospheric contaminants at the maximum allowable concentration for continuous exposure have been developed. The investigation of various factors which could influence the oxygen yield in electro-deposited chromium by the Adcock method has shown that, for material containing oxygen of the order of 0·1 per cent or higher, the preliminary heat treatment as required by the Adcock method significantly reduces the yield by the vacuum fusion method. Research on the photo-conductivity of zinc oxide and zinc oxide–resin mixtures has been carried out in order to explain phenomena associated with the xerographic reproduction. It has been confirmed that the chemisorption of oxygen on the surface of zinc oxide microcrystallites is an important factor in the mechanism of the dark and photo-conductivity of zinc oxide and that photoconductivity decay follows an Elovitch type of chemisorption equation. Many other factors, including the temperature, pressure, and the presence of water vapour and gaseous ions, also affect the conductivity.

The main interests of the Metallurgy Division have been, as formerly, the methods of processing chromium and chromium alloys, and measurements of their ductility, strength and creep; the abrasion of metals, including the examination of magnesium oxide particles suitable for metallographic polishing, and of the dislocation arrays produced in germanium by deformation at room temperature; and the long-term stability of nickel-base thermocouple alloys.

The annual report lists the various personnel of the Laboratories and their status, and gives details of the publications by members of the staff during the year under review. The staff numbered 724 on July 1, 1962, and 730 on June 30, 1963. Six members of the staff were in Great Britain for all or part of the year to gain experience in special aspects of their work, and the superintendent, Dr. F. A. Fox, visited both Great Britain and the United States to study present-day trends in defence and in the management of research and development organizations.

S. WEINTROUB

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* Commonwealth of Australia: Department of Supply. Defence Standards Laboratories Annual Report, 1962–63. Pp. 36. (Maribyrnong, Victoria: Department of Supply, Defence Standards Laboratories, 1963.)

THE AUCKLAND ISLANDS

THE Auckland Islands are a small group about 200 miles south of New Zealand. They were discovered by Captain Bristow in 1806 and visited by American, French and British expeditions in 1840; their flora was studied by Sir Joseph Hooker; and in the days of sailing ships they were the cause of many wrecks, lying as they did on the route between Australia and Cape Horn. After the opening of the Panama Canal, they were left almost deserted—except for a period during the Second World War—until they were visited by an expedition from the Australian Museum during December–January, 1962–63. A preliminary account of this expedition has been published in *Australian Natural History* (14, No. 9; March, 1964).

The main Auckland Island is about 23 miles long, and 15 miles across at its widest part, bordered by precipitous cliffs on the western side while providing fine inlets and harbours on the east. Unlike all other windswept subantarctic islands, its coastal areas are covered with a thick growth of gnarled, stag-headed trees, of southern rata (*Metrosideros umbellata*), which rise to a height of 40 ft. Below these, specimens of the Macquarie Island cabbage (*Stilbocarpa polaris*), with strong antiscorbutic properties, are conspicuous, although they have been much reduced through the introduction of sheep, goats, cattle and rabbits.

Above the rata is a dense growth of scrubland vegetation (chiefly *Dracophyllum* and *Suttonia*) difficult to penetrate, although it is interspersed with lines of tussock grass (*Danthonia* and prostrate *Coprosma*). Both winged and wingless forms of a number of Diptera and Lepidoptera were taken, along with semi-apterous ichneumons, use being made of aerial nets in the ship's rigging. A new wingless cricket (*Raphidophoridae*) was found to be abundant at night.

Along the shores a supra-littoral zone, marked by white lichen, was found to change abruptly into a mid-tidal zone of bare rock, except for scattered barnacles and limpets. In turn, this zone changed into a lower-tidal one of encrusting coralline algae and the giant kelp (*Durvillea antarctica*), to be followed by an extensive zone of red and brown algae in the sub-littoral. In the rock pools, among the cryptic fauna of small gastropods, fish and worms, the expedition discovered large numbers of the small crab *Halicarcinus planatus*, the only intertidal crab on any subantarctic island. A large red spider crab, *Jacquinotia*, dominated the shallow water; two species of nototheneid fish were taken, along with the large crab, *Leptomithrax australis*, and the edible species, *Cancer novaezealandiae*.

Along the beaches, large numbers of Hooker's sea lion (*Neophoca hookeri*) were observed, the sandy beach on Enderby Island being crowded with these animals. Yellow-eyed penguins (*Megadyptes antipodes*) and crested penguins (*Eudyptes*) were plentiful, and among the other birds observed were Auckland Island shags (*Phalacrocorax campbelli colensoi*), red-billed gulls (*Larus novaehollandiae*), Antarctic terns (*Sterna vittata*), giant petrels (*Macronectes giganteus*), the sooty albatross (*Phoebetria palpebrata*), the southern skua (*Stercorarius lonnbergi*), prions (*Pachytila desolata*), diving petrels (*Pelecanoides urinatrix*), sooty shearwaters (*Puffinus griseus*), and white-headed petrels (*Petrodroma lessoni*). Of special ornithological interest were the large numbers of the Auckland Island flightless duck (*Anas aucklandica*) on Ocean and Ewing Islands.

But the expedition's most striking experience was a visit to a breeding colony of the southern race of the royal albatross (*Diomedea epomophora*), which is held to be the largest of all albatrosses, with a wing span of 11·5 ft. and a weight of 25 lb. This bird is known to nest only on Auckland and Campbell Islands, Taiaroa Head on the South Island, New Zealand, and on the Chatham Islands east of New Zealand.

RESEARCH INTO RESEARCH

DURING January–March a seminar was held in Stockholm to stimulate studies on research in social sciences. This seminar was arranged by the Academy of Engineering Sciences and supported by the Social Science and Technical Research Councils. Dr. Stevan Dedijer from the Sociological Institute of the University of Lund led the work. About half the thirty delegates were young social scientists from the universities. The others came from the Ministries and Research Councils, and from certain large industrial firms and research establishments.

The seminar met in Stockholm for two days every other week, making a total of 10 days. During the intervals, each delegate worked on his individual problem. The programme consisted of some twenty lectures, with discussions. The rest of the time was devoted to discussing the delegates' projects and progress reports.

Visitors from overseas who addressed the seminar included: Dr. Charles Kidd, head of the office for International Research at the U.S. National Institutes of Health, who spoke on "Research, Universities and the Government in the United States"; Dr. Stephen Toulmin from the Nuffield Foundation in London, who gave a stimulating lecture on "Science as the Focus of Academic Study"; Chris Freeman from the National Institute of Economic and Social Research in England, who reported his penetrating investigations into the importance of innovations in the plastics industries of different countries; and Jean Jacques Salomon from the Secretariat of the Organization for Economic Co-operation and Development, Paris, who spoke on "Science and Foreign Affairs in Europe". Almost all the leading Swedish research administrators gave lectures to the seminar. Each aired his own views on activities in different areas of research and on the selective approach necessary in a country of Sweden's size.

Subjects which the delegates considered, and which will be included in the collected essays when they are published, are: methodological problems in connexion with research statistics; the Government budget process in the fields of university research and education; an investment calculus on research and development projects in Swedish industry; civilian utilization of results emanating from defence research and development in Sweden; the ageing of research workers; the development of the theory of planning of science.

The seminar was a bold experiment, but one which, in retrospect, appears to have succeeded in its essential aims. The primary object was to pin down linguistic usage in connexion with research, so as to be able to attack clearly defined problems within various disciplines of the social science. A number of the investigations initiated by the seminar may clearly be of immediate relevance to Swedish research policy. The essential gain, however, is that young research workers have had their attention directed to the vital tasks connected with the social relations of science. Increased knowledge is needed in this field in order to weigh the value of different research projects in the industrial countries and to build up a research apparatus in the developing countries.

Social science investigations of numerous aspects of 'research' were started by English scientists in the 'thirties. That these studies did not lead further was due largely to ideological disagreement on the planning of science. To-day the chances of success are better, but occasional reflexions on the vital importance of this field will not suffice. What is needed is much hard work by a great many competent persons over a prolonged period.

SVERKER GUSTAVSSON

ADVANCEMENT OF SCIENTIFIC EDUCATION IN SCHOOLS

THE final report of the Industrial Fund for the Advancement of Scientific Education in Schools* is a brief record of a creditable episode in the history of education in the United Kingdom and Northern Ireland, which has now come to an end. The Fund was established in 1955, mainly on the initiative of Lord Weeks, Sir Alan Wilson, Mr. J. Oriel and Mr. G. Courtauld. Its object was to give a boost to the supply of scientists and technologists. Contributions were received from 141 industrial concerns, and the total sum accumulated amounted to £3,211,035. From this sum grants amounting to £2,640,500 were made to 210 schools for the renovation and extension of buildings for science teaching, and 129 schools received assistance for obtaining apparatus. Among the smaller items, a sum was set aside for the publication of a book on the planning and equipment of laboratories, based on experience acquired through the Fund; seven schools were given an engine test bed; and a special grant was allotted to Malvern College where, through aid provided by the Nuffield Foundation, new approaches are being made to the teaching of physics. Assistance was limited to independent and direct-grant schools for boys and girls, since maintained schools are the concern of the Government and Local Education Authorities; throughout the duration of the scheme interest was concentrated on the 'exact' sciences, so that the teaching of biology benefited only indirectly.

In attempting to assess the success of the scheme it is not possible to arrive at precise conclusions because of the interplay of a variety of factors. But one point not mentioned in the report is that the Fund must have been a great boon to the schools concerned in a post-war period of economic and personal austerities. Since 1955, the number of sixth-form members specializing in mathematics and science has greatly expanded; it is now common practice for all boys to be taught some science before taking the Ordinary Level Examination of the General Certificate of Education, and many schools have organized courses in science for arts specialists.

Only in the fourteen girls' schools which received grants have the results been disappointing. In them, staffing may have been a more difficult problem than lack of facilities; and apart from medicine and its associated services, girls and headmistresses alike do not look favourably on careers in science. Yet the few girls who do take science at the Advanced Level of the General Certificate of Education do well in it and particularly well in biology.

Other questions are posed by the report. In some boys' schools the "express line" of preparation for the Ordinary Level Examination of the General Certificate of Education is producing an increasing number of boys spending a third year in the sixth form, which would seem to be undesirable. Again, in recent years arts specialists have been increasing about twice as fast as specialists in science; in fact, recently in the larger public schools the number of science specialists has tended to fall, suggesting that science is not appealing to some of the most able boys. That in turn may have some relation to the limited amount of science being taught in preparatory schools, although in them there has been some improvement over the past few years. Finally, to the credit of the Fund it can be said that the standard of accommodation for teaching science which it provided was higher than that envisaged by the Ministry of Education in its *Building Bulletin* No. 2A, 1954, while the standard of equipment in maintained schools continues to give grounds for concern in relation to the place of science in the modern world and the extent to which teachers become discouraged and frustrated by present conditions.

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* Report on the Industrial Fund for the Advancement of Scientific Education in Schools. Pp. iii+36. (Barbary, Maresfield Park, Uckfield: Sir Graham Savage (Chief Assessor), 1964.)

ANISOTROPY OF FRICTION IN CRYSTALS

By DR. F. P. BOWDEN, C.B.E., F.R.S., DR. C. A. BROOKES and A. E. HANWELL

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EARLIER experiments in this laboratory have shown that the friction of diamond is dependent on the crystallographic direction of sliding[1-3]. With a diamond slider (of constant orientation) sliding at low speed on the cube face of a polished diamond the coefficient of friction was $\mu = 0{\cdot}15$ when sliding in the [100] direction (parallel to the cube edge) and $\mu = 0{\cdot}05$ in the [110] direction (diagonal to the cube edge). Frictional anisotropy has also been observed on other crystals, for example, sapphire[4] and copper[5].

Magnesium oxide. We have recently examined the frictional behaviour of single crystals of magnesium oxide. This is a convenient crystal since it cleaves readily on the (001) face to give a flat surface and the techniques for examination by chemical polishing, dislocation etching and electron microscopy are well established. With a hemispherical slider moving at low speed (0·1 mm/sec) on a cleaved face in air under a load of 30 g the coefficient of friction was $\mu \simeq 0{\cdot}2$ and appreciable plastic deformation occurred beneath the surface of the crystal. This is illustrated by Fig. 1. There are two major features revealed by this photo-micrograph. First, the processes of fracture which govern the formation of wear debris occur both on and beneath the surface. Secondly, slip has taken place predominantly on those (110) planes which intersect the deformed surface at 45°, although some slip on those (110) planes at 90° to that surface is revealed. The maximum shear stress, under these conditions, is at a point below the surface and it is from this region that the dislocation loops originate and multiply. Similar deformation has been observed under conditions of rolling friction[6].

A crystal of magnesium oxide exhibits a marked anisotropy in its mechanical properties. Measurements of hardness, for example, show that this is very dependent

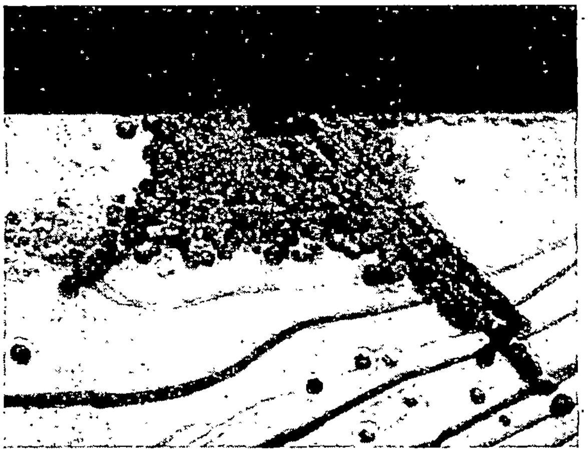

Fig. 1. Cross-section of friction track on magnesium oxide crystal showing extensive bulk plastic deformation and sub-surface cracking (etched. × c. 330)

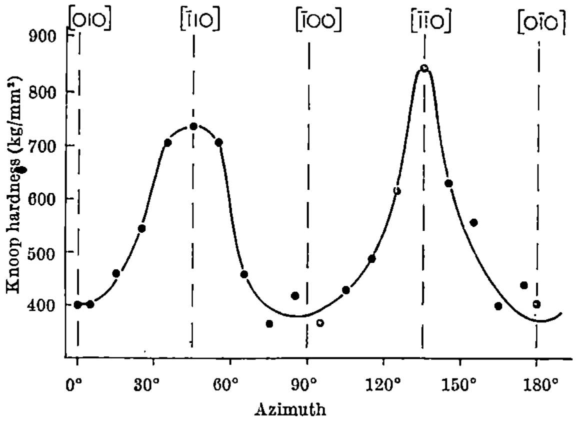

Fig. 2. Knoop hardness as a function of crystallographic orientation on a magnesium oxide cleavage face

on crystallographic direction. The (001) face of a freshly cleaved magnesium oxide crystal was indented with a load of 500 g in a microhardness apparatus using a Knoop indenter. The long axis of the indentation was measured and the results (Fig. 2) show that the hardness of the crystal in the [100] direction is about half that in the [110] direction. In the initial friction experiments a magnesium oxide slider was used. The contacting surface for both slider and crystal was (001). Various measurements were carried out in which the crystallographic orientation of both slider and crystal were varied. Some anisotropy of friction was observed but for a flat slider or one with a large radius of curvature the effect was small and not reproducible.

It is a convenient approximation to regard the frictional force F between sliding solids as being made up of two parts: the force required to shear the adhering junctions in the regions of surface contact, and the force P required to deform the underlying solid. We may write $F = As + P$, where A is the real area of contact, s the shear strength of the contact regions and P is the deformation term[7]. With an elastic solid P may be due mainly to elastic hysteresis loss[8], but if plastic flow occurs it will represent the force to produce bulk deformation or to drag the surface irregularities through the solid and to plough out a track.

In order to determine the significance of the P term, friction experiments were made on magnesium oxide crystals using diamond sliders of different shape. When the diamond slider was a very wide-angled cone (> 120°) it was found that the friction was low (that is, $\mu \simeq 0·2$ over a range of loads between 10 and 350 g), there was little surface damage and the friction was independent of the crystallographic direction of sliding. When cones of smaller angle (that is, 120°, 105°, 90° and 60°) were used, two distinct friction régimes were observed (Fig. 3). Below a critical load the behaviour was similar to that observed with a wide-angled cone, but above this load the friction and surface damage were considerably increased. The friction was then markedly dependent on the crystallographic orientation of the magnesium oxide crystal. It was much higher when sliding in the soft [100] direction ($\mu = 0·55$) than in the hard [110] direction ($\mu = 0·18$) (Fig. 4). The critical load necessary to produce this anisotropy was dependent to some degree on cone angle, decreasing with decreasing cone angle, but usually lay between 20–50 g. Examination of the friction tracks made in the high friction régime showed that they were much wider and deeper in the soft [100] direction than in the hard [110] direction. These observations are illustrated by Figs. 5*a* and *b*. Our experiments show that a critical amount of sub-surface deformation in the magnesium oxide was necessary in order to give anisotropic values of friction, that this anisotropy is associated mainly with the forces necessary to plough out a track in the crystal, and to produce plastic deformation and cracking of the crystal underneath the surface.

Diamond. Having ascertained that well-defined and reproducible frictional anisotropy could readily be observed in magnesium oxide, and that this phenomenon was due primarily to penetration of the surface and to the deformation component of the frictional force, the investigation was extended to diamond surfaces.

Experiments were carried out using the conical diamond sliders of the previous experiments. The lower specimen was a mechanically polished (001) diamond surface. The results in Fig. 6 show that, as with magnesium oxide, the

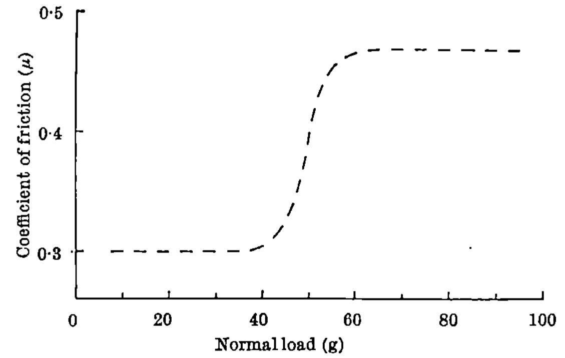

Fig. 3. Variation of coefficient of friction with normal load for a 120° conical slider on a (001) magnesium oxide surface. Anisotropy is only observed in the high friction régime

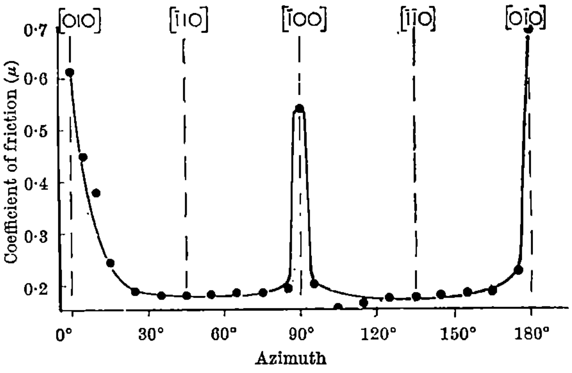

Fig. 4. Relationship between crystallographic direction and sliding friction for a sharp conical slider on (001) magnesium oxide surface

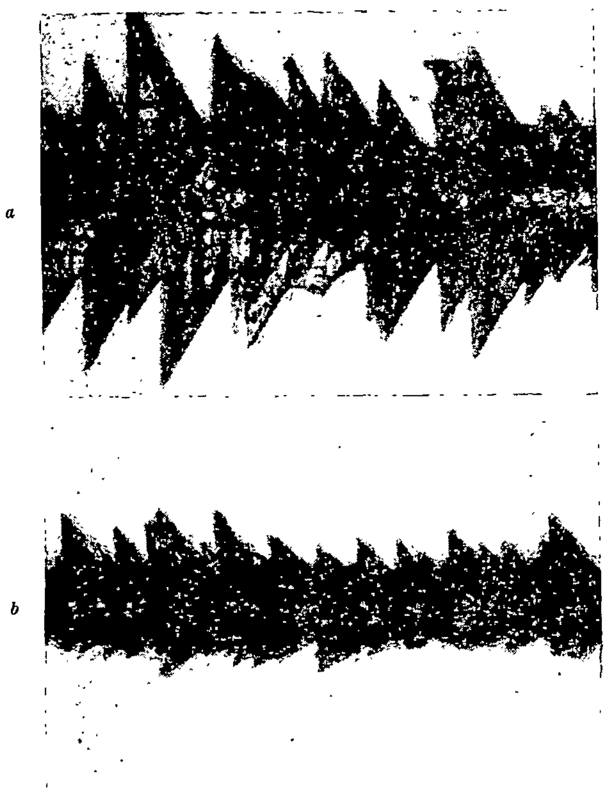

Fig. 5. Damage caused on (001) magnesium oxide by sliding in: (a) high-friction [100] direction; (b) low-friction [110] direction. Direction of sliding right to left (× 400)

amount of frictional anisotropy on this cube face increases with the sharpness of the conical slider. For example, using a 60° cone the friction increased by a factor of 3 when changing from a [110] direction of sliding to [100], and this factor is in agreement with earlier work[1,2]. However, when using a blunter cone (170°) the anisotropy in friction was only just detectable. At the load used in these experiments (130 g) no apparent damage could be detected by optical microscopy. In order to compare the deformation in the high and low friction directions, the experiments were repeated at a load which was sufficient to cause permanent damage to the diamond surface (350 g). The frictional anisotropy was similar to that observed at the lower load and the subsequent damage to the diamond cube surface is shown in Figs. 7a and b. When sliding in the high friction direction [100] considerable cracking was observed and, using the optical microscope, the track depth was estimated as about 4μ. The low-friction direction [110] produced less damage and this was very superficial. If a 'flat' diamond with a rough surface were used as a slider, it behaved in a manner similar to a sharp cone and showed the typical anisotropy of friction and of damage. These observations indicate that, as in the magnesium oxide experiments, the high friction directions are those allowing the deepest penetration by the slider (or the surface irregularities on it) and maximum deformation and cracking of the bulk crystal.

These experiments were carried out in air so that the crystal surfaces are contaminated by adsorbed gas. We have also measured the friction of diamond sliding on itself and of magnesium oxide sliding on itself in very high *vacuo* (about 10^{-10} torr) so that the surfaces are clean[9]. With both crystals the friction under these conditions rises to a high value $\mu \simeq 1$ and the abrasion and sub-surface cracking are greatly increased.

Abrasion of the crystals. Frictional forces play a major part in the abrasion of brittle solids so that we may expect the abrasion to be greatest in the direction where the friction is a maximum. With both magnesium oxide and with diamond sliding in air the high friction direction corresponds to the direction of greatest surface penetration and sub-surface damage. Magnesium oxide is brittle compared with a metal but it is, of course, capable of marked plastic deformation (Fig. 1). It is apparent that the frictional stresses produce brittle fracture due both to easy cleavage on the {100} and to dislocation interaction on the {110}. The relative extent of these will, of course, depend on external conditions such as the temperature of the crystal and the speed of sliding. If the crystal is heated, either by an outside source or by friction, the deformation may become essentially plastic. The effect has already been observed with titanium carbide crystals[10].

We may expect that similar general conclusions will apply to diamond, but because of the different crystallographic properties and because plastic deformation (if it occurs at all) is very small there will be marked differences in the behaviour. However, we see again that sliding in the high-friction direction produced the greatest crystallographic cracking and abrasion of the surface, and this is accompanied by the deeper penetration of the surface by the slider or by the irregularities on it.

A standard method of abrading or 'polishing' a diamond surface is to rub it against a cast-iron lap (or 'scaife') impregnated with diamond dust. The rubbing speed may be about 10 m/sec. It is well known that certain directions along the cube face and dodecahedron surface are easier to abrade than others. (These directions would correspond to our high-friction directions.) Tolkowsky[11] explained this anisotropy of polishing as being due to a mechanical fragmentation of the diamond. He suggested that the abrasion was due to the removal of octahedral and tetrahedral fragments bounded by the {111} cleavage planes. From geometric conclusions, therefore, a blow in a [100] direction from an abrasive particle would be more effective in removing a fragment than one in a [110] direction. More recently, Wilks and Wilks[12] have developed this farther, have confirmed the anisotropy of

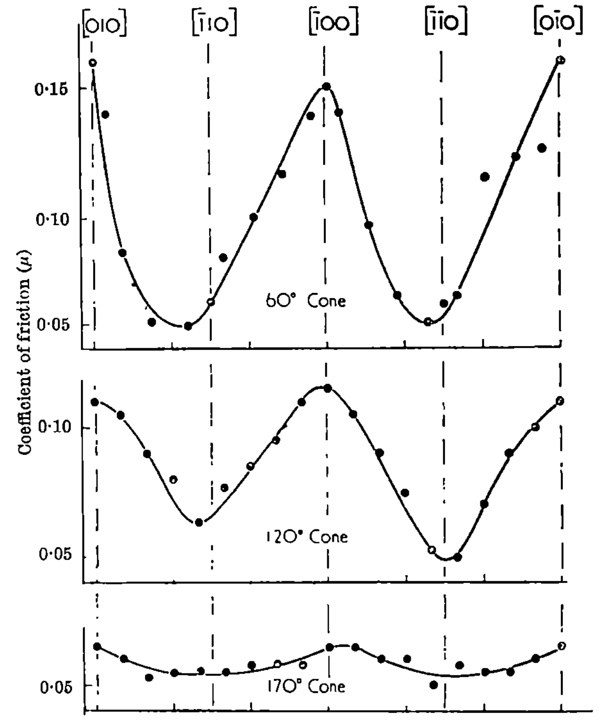

Fig. 6. Coefficient of friction as a function of crystallographic direction of sliding for various diamond sliders on a (001) diamond surface. The degree of anisotropy increases with the sharpness of the conical slider

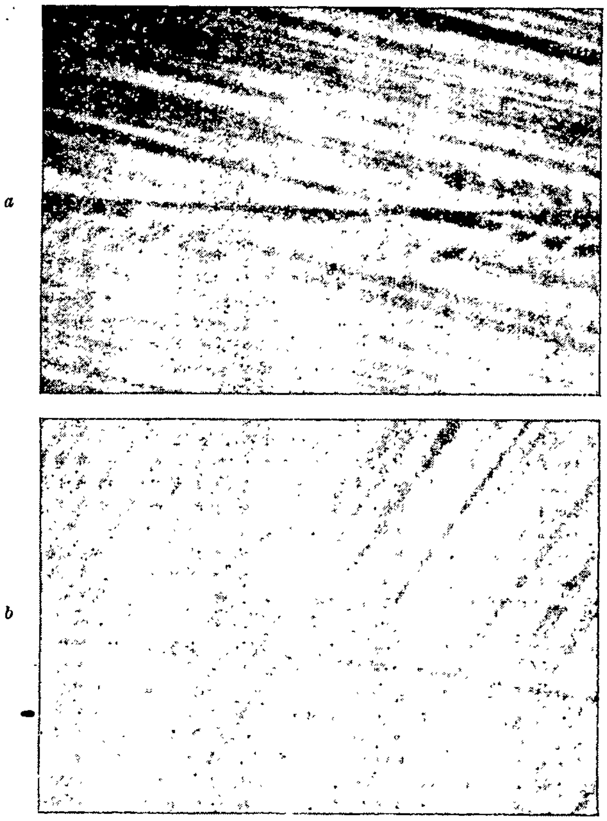

Fig. 7. Friction tracks on (001) diamond surface. (*a*) Considerable damage after sliding in high-friction [100] direction; (*b*) superficial damage after sliding in low-friction [110] direction. Direction of sliding right to left (× 400)

abrasion and have shown that the wear on a surface a few degrees off the cube face of diamond was greatly altered when the direction of rubbing was reversed.

If high sliding speeds are used, other wear mechanisms may prevail. Laboratory experiments carried out at speeds up to 800 m/sec[13,14] show that large amounts of amorphous carbon are produced. At these great speeds the surface temperature of the diamond reaches a high value so that the surface of diamond is transformed to carbon and is removed in this way. This transformation of the diamond surface to carbon occurs readily at temperatures of 1,200° C (refs. 15–17). Amorphous carbon has been observed at rubbing speeds of a few m/sec. The mechanisms of abrasion of the diamond will therefore depend on the experimental conditions and, in particular, on the speed of sliding. Under the low-speed conditions described in this paper the abrasion is primarily due to crystallographic cleavage and deformation, and this is greatest in the direction which corresponds to the greatest penetration and friction.

We thank Industrial Distributors, Ltd., for the diamonds and for a grant to the Laboratory; Owens, Illinois, for a fellowship to one of us (C. A. B.); and S. Smith and Sons (England), Ltd., for support of another author (A. E. H.).

Note added in proof. Since writing this we have seen an account of experiments on the friction of crystals by Steijn (*Wear*, 7, No. 1; 1964) which also provides some evidence that the anisotropy of friction is associated with the ploughing term.

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STRUCTURE AND CATALYTIC ACTIVITY OF ALCOHOL DEHYDROGENASES

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ALCOHOL dehydrogenase (ADH) catalyses the final step in the anaerobic fermentation of glucose to ethanol in yeast. It participates in a coupled enzyme reaction involving glyceraldehyde 3-phosphate dehydrogenase (GPDH) in which the reduced coenzyme NADH formed during the oxidation and phosphorylation of glyceraldehyde 3-phosphate is transferred to yeast alcohol dehydrogenase for the enzyme catalysed reduction of acetaldehyde to ethanol.

There is considerable evidence for the participation of sulphydryl (SH) groups in reactions catalysed by glyceraldehyde 3-phosphate and alcohol dehydrogenases (for references, see reviews by Velick and Furfine[1] and Sund and Theorell[2]), and the inhibition of alcoholic fermentation in yeast and of glycolysis in muscle by halogen acetic acids was attributed to the specific inactivation of an SH enzyme[3–5] even before the enzyme involved, namely, glyceraldehyde 3-phosphate dehydrogenase, was isolated and characterized.

The inhibition of GPDH by halogenacetic acids led to the use of iodoacetic-1[^{14}C] acid for the specific labelling of the catalytic sites in the enzyme, and to the chemical identification of these active sites as the SH groups of four cysteine residues in the primary structure of the enzyme protein. Moreover, the isolation of a unique octadecapeptide fragment containing 1[^{14}C]S-carboxymethylcysteine (^{14}CMCys) from a trypsin digest of the enzyme-inhibitor compound showed that all the reactive sites were identical and that each molecule of active enzyme (assuming a molecular weight of 140,000) was composed of four similar, if not identical, polypeptide chains each with a molecular weight of 35,000–36,000 (refs. 6–8). The active enzyme may thus be pictured as an assembly of four polypeptide chains held together by non-covalent bonds to form a quaternary 'superstructure' within which each individual chain possesses the tertiary structure which is necessary for the manifestation of catalytic activity.

Similar methods have now been applied to an investigation of alcohol dehydrogenases from yeast and from mammalian (horse) liver. The aim of this investigation has been to identify the chemical nature of the reactive sites and to determine the sequence of amino-acids around these reactive sites in the primary structure of the enzyme proteins; to correlate the number of reactive sites with the number and chemical nature of the protein chains in the two alcohol dehydrogenases, and to compare the amino-acid sequences around the reactive sites in alcohol dehydrogenases with the corresponding sequences in glyceraldehyde 3-phosphate dehydrogenases from yeast and mammalian muscle.

Alcohol dehydrogenases have been isolated as pure crystalline proteins from yeast and from horse liver (for review, see Sund and Theorell[2]). Some of the properties of the two enzymes are given in Table 1. Yeast alcohol dehydrogenase has a molecular weight of 150,000 (ref. 9). The active enzyme contains four moles of NAD (ref. 9), four atoms of zinc[10,11] and approximately 36 free SH groups[12]. The horse liver enzyme, on the other hand, appears to be approximately half the size of the yeast enzyme and its molecular weight has been reported to be between 73,000 (ref. 13) and 84,000 (ref. 14); the active enzyme contains two binding sites for NAD (ref. 14), two atoms of zinc[15] and from 24 to 28 free SH groups[16]. The yeast[17] and liver[18] enzymes are both inhibited by iodoacetic acid, but in contrast to glyceraldehyde 3-phosphate dehydrogenases inactivation of the alcohol dehydrogenases does not occur in the presence of coenzyme (NADH).

Table 1. PROPERTIES OF YEAST ALCOHOL (YADH) AND HORSE LIVER ALCOHOL (LADH) DEHYDROGENASES

Enzyme properties	YADH	LADH
Molecular weight	150,000	73,000–84,000
Bound $\overset{+}{\text{NAD}}$/NADH/mole	4	2
Bound zinc (g atoms/mole)	4	2
Total SH groups	36	24–28
Essential SH groups	4	2

Yeast Alcohol Dehydrogenase (YADH)

Reaction with iodoacetic-1[^{14}*C*] *acid.* The enzyme (C. F. Boehringer and Soehne, Mannheim) was dissolved in 0·05 M phosphate buffer (*p*H 7·5), containing 0·01 M mercaptoethanol, kept at 20° for 1 h, and dialysed against 0·05 M phosphate at *p*H 7·5 and 4° for 24 h. The enzyme solution (10 ml., containing 15 mg/ml.; 1 μmole) was incubated with iodoacetic-1[^{14}C] acid (2·7 mg; 15 μmoles) for 8 h at 4°; under these conditions the enzyme lost more than 95 per cent of its activity. Mercaptoethanol (10 μl.) was added to react with excess iodoacetic-1[^{14}C] acid; the enzyme–inhibitor compound was then reacted with an excess of non-radioactive iodoacetic acid in the presence of 8 M urea for 2 h at 25°, after which the reaction mixture was dialysed against several changes of hydrochloric acid at *p*H 3. The carboxymethylated enzyme was digested with trypsin (1 per cent trypsin, at *p*H 8·0 and 25° for 2 h) and the soluble digest was found to contain 3·8–4·4 moles of carbon-14 per mole (150,000*g*) of enzyme protein.

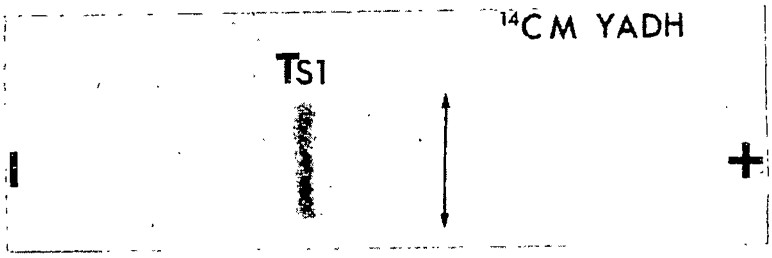

Fig. 1. Autoradiograph of peptides containing carbon-14 produced by trypsin hydrolysis of the iodoacetic 1[^{14}C] acid derivative of yeast alcohol dehydrogenase. Ionophoresis in pyridine–acetic acid buffer at *p*H 6·5

Isolation and structure of peptides containing carbon-14. In order to purify the radioactive peptide(s) a preliminary fractionation of the trypsin digest of the carboxymethylated enzyme was carried out on a column of 'Sephadex' (90 × 1·5 cm, *G*-25 grade); the column was developed with N/20 ammonia and the radioactive component (fraction TS), which emerged as a single peak, was recovered by freeze-drying. Fraction TS was next submitted to ionophoresis on paper (Whatman No. 3) in pyridine–acetic acid buffer at *p*H 6·5; the distribution of radioactivity was revealed by autoradiography and, as shown in Fig. 1, most of the carbon-14 (85–90 per cent of the [^{14}C] introduced into the enzyme protein) was found to be associated with the peptide fraction (TS1) which was 'neutral' at *p*H 6·5. At *p*H 6·2 the radioactive peptide (TS1) proved to be slightly 'basic' (that is, it migrated to the cathode side of the other non-radioactive 'neutral' peptides), and it was obtained in pure form by successive ionophoresis of fraction TS at *p*H's of 6·5 and 6·2, respectively.

Amino-acid composition and sequence of TS1. The acid hydrolysate of TS1 was submitted to amino-acid analysis and the following composition was obtained: ^{14}CMCys (1), Asp (2), Thr (2), Ser (1), Pro (2), Gly (2), Ala (1), Val (1), Leu (2), Tyr (1), His (3), Lys (1); in addition, the unhydrolysed peptide gave a positive Ehrlich test, indicating that it contained at least one residue of tryptophan.

In order to determine the amino-acid sequence of TS1 a sample (1 μmole containing approximately 10^{6} counts of [^{14}C]) was submitted to partial hydrolysis with chymotrypsin. A total of five peptide fragments was isolated by ionophoresis on paper at *p*H 6·5; the amino-acid compositions and end-groups of these peptides were determined and the partial structure of TS1 was established, as shown in Table 2. Peptide TS1 was thus shown to contain two residues of tryptophan and a total of 21 amino-acids.

The sequence around the radioactive 'label' was established by digesting peptide TS1 : C1 with subtilisin; the results of this experiment are summarized in Table 3. The sequence of peptide TS1 : C3 was investigated by stepwise degradation from the N-terminal position by the 'Dansyl–Edman' method as described by Gray and Hartley[19], and by partial acid hydrolysis. In this manner the sequence His.Gly.Asp.*Try.Pro.Leu.Pro.Thr*.Lys was obtained; the sequence in positions 16—20 could not be rigorously established by these methods and must therefore be regarded as provisional.

Table 2. AMINO-ACID COMPOSITION AND PARTIAL SEQUENCE OF PEPTIDES PRODUCED BY PARTIAL HYDROLYSIS OF PEPTIDE TS1 (YADH) WITH CHYMOTRYPSIN

Peptide	Amino-acid composition
TS1 : C1	†Tyr.(Ser Gly Val $\overset{*}{\boldsymbol{Cys}}$ His Thr Asp).Leu**
C1*a*	†Ser.(Gly Val $\overset{*}{\boldsymbol{Cys}}$ His Thr Asp).Leu**
C2	†Tyr.Ser.(Gly Val $\overset{*}{\boldsymbol{Cys}}$ His Thr Asp).Leu.His.Ala.Try**
C3	†His.(Gly Asp Try Pro Leu Pro Thr).Lys**
C4	†His.Ala.Try**
Partial sequence	Tyr.↓Ser.(Gly Val $\overset{*}{\boldsymbol{Cys}}$ His Thr Asp).Leu.↓His.Ala.Try.↓His.(Gly Asp Try Pro Leu Pro Thr).Lys 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

* 1-[^{14}C] S-carboxymethylcysteine. † N-terminal determined by 'Dansyl' procedure (Gray and Hartley) (ref. 19). ** C-terminal; released by carboxypeptidase. ↓ Indicates bonds susceptible to chymotrypsin.

Table 3. AMINO-ACID SEQUENCE OF PEPTIDE TS1 : C1 ISOLATED FROM YEAST ALCOHOL DEHYDROGENASE FOLLOWING ITS REACTION WITH IODO-ACETIC-1[^{14}C] ACID

Peptide	Amino-acid composition and sequence
TS1 : C1 : Su 1	†Tyr.Ser.Gly.Val.*Cys**
Su 3	†Tyr.Ser.Gly.Val
Su 4	*Cys**.His
Su 2	†*Cys**.His.Thr.Asp.Leu
Su 5	†His.Thr.Asp.Leu
Sequence:	Tyr.Ser.Gly.Val.↓*Cys**.↓His.↓Thr.Asp.Leu
	1 2 3 4 5 6 7 8 9

* 1[^{14}C] S-carboxymethylcysteine.

† Sequences determined by 'Dansyl-Edman' procedure according to Gray and Hartley (ref. 19).

↓ Indicate bonds split by subtilisin (Su).

From the results summarized in Tables 2 and 3 the amino-acid sequence of peptide TS1 from yeast alcohol dehydrogenase is established to be:

Tyr.Ser.Gly.Val.^{14}CMCys.His.Thr.Asp.Leu.His.Ala.Try.
1 2 3 4 5 6 7 8 9 10 11 12

His.Gly.Asp.*Try.Pro.Leu.Pro.Thr.*Lys.
13 14 15 16 17 18 19 20 21

Structure of yeast alcohol dehydrogenase. The inhibition of yeast alcohol dehydrogenase by iodoacetic acid has been shown to be due to a specific reaction with four residues of cysteine per mole (150,000*g*) of enzyme. The product of the reaction has been identified as S-carboxymethylcysteine and the replacement of the SH group by the S-carboxymethyl side-chain presumably leads to a conformational change in the tertiary structure which interferes with the spatial orientation of substrate and/or coenzyme molecules at the 'active centre' of the enzyme.

The identity of sequence around the four reactive cysteines shows that each molecule of active enzyme contains four identical reactive sites, and that in all probability it consists of four identical protein chains. If the results of amino-acid analysis are expressed on this basis, the following amino-acid composition may be derived for the protein monomer in YADH; Asp (29), Thr (16), Ser (22), Glu (32), Pro (14), Gly (40), Ala (34), Val (32), Met (6), Ile (19), Leu (25), Tyr (14), Phe (8), Try (6), Cys (8), Lys (24), His (10), Arg (8). This composition corresponds to a total of 346 amino-acids and to a molecular weight of approximately 36,000.

The total number of unique peptide fragments present in a trypsin digest of the carboxymethylated enzyme (approximately 30, as judged by inspection of peptide maps obtained by the fractionation of the peptide mixture by successive ionophoresis at *p*H's of 6·5 and 3·5, followed by chromatography in *n*-butanol–acetic acid–pyridine–water as a 'third dimension') agrees well with the maximum number of peptides to be expected from the number (33) of trypsin sensitive bonds (that is, bonds involving the carboxyl groups of the lysine and arginine residues) present in the protein monomer.

On this basis each individual chain contains one reactive SH group and, by binding one atom of zinc and one mole of NAD/NADH, is presumably capable of forming an independent 'active centre' within the quaternary structure of the active tetramer of molecular weight 150,000.

Kägi and Vallee[11] have already shown that removal of zinc from YADH with *o*-phenanthroline causes the dissociation of the enzyme into inactive sub-units with a molecular weight of 36,000 as determined by physico-chemical methods. On this basis the empirical formula of the enzyme was represented as $[(\text{YADH})_4\,\text{Zn}_4]\,(\text{NAD})_4$. The chemical results which I have described are in complete accord with this view and show in addition that the four sub-units are chemically identical. Removal of zinc and the accompanying dissociation of the enzyme into sub-units in the presence of chelating agents does not occur in the presence of NADH; the coenzyme also protects the reactive cysteines against reaction with iodoacetic acid[17]. These results suggest that zinc atoms are involved in maintaining the quaternary structure, and that in conjunction with the reactive cysteines they may also be concerned with the binding of substrate and/or NAD at each of the 'active centres' in the active tetramer. The chemical nature of the other side-chains which are brought into juxtaposition at the 'active centre' remains to be established. Zinc and NAD are believed to be attached at, or near to, the reactive SH group[11], and in this connexion the presence of two histidines in close proximity to the reactive cysteines in the primary structure could well provide additional ligand groups for binding zinc and/or NAD to the enzyme.

Liver Alcohol Dehydrogenase (LADH)

Reaction with iodoacetic-1[^{14}*C*] *acid.* The liver enzyme (C. F. Boehringer and Soehne, Mannheim) was dissolved in 0·1 M phosphate buffer (*p*H 7·5) containing 0·01 M mercaptoethanol, kept at 20° for 1 h and dialysed against 0·05 M phosphate at *p*H 7·5 and 4° for 24 h. The enzyme solution (8 ml. containing 10 mg/ml.; 1 μmole) was incubated with iodoacetic-1[^{14}C] acid (1·8 mg; 10 μmoles) for 12 h at 4°. The inactive enzyme-inhibitor compound was then reacted with an excess of non-radioactive iodoacetic acid in 8 M urea at 25° for 2 h and the carboxymethylated enzyme was recovered by dialysis against hydrochloric acid at *p*H 3. A sample portion (20 mg) was digested with trypsin (1 per cent trypsin, *p*H 8·0, 25° for 2 h) and was found to contain 1·8 moles of carbon-14 per mole (73,000*g*) of enzyme protein in agreement with the results obtained by Li and Vallee[18]; in a separate experiment another sample (40 mg) of the carboxymethylated enzyme was digested with chymotrypsin (1 per cent chymotrypsin, *p*H 8·0, 25° for 2 h).

Isolation of peptides containing carbon-14. The trypsin and chymotrypsin digests of the carboxymethylated enzyme were fractionated on a column of 'Sephadex' (90 × 1·5 cm, *G*-25); the radioactive components (fractions TS and CS, respectively) were submitted to ionophoresis on paper (Whatman No. 3) in pyridine–acetic acid buffer at *p*H 6·5, and the distribution of radioactive peptides was revealed by autoradiography. Most of the carbon-14 in the trypsin digest (80–90 per cent) was found in the peptide fraction (TS1), which was 'neutral' at *p*H 6·5. In the corresponding chymotrypsin digest the carbon-14 was found to be associated with the two acidic peptide fractions; 80–85 per cent was in fraction CS1 and the remaining 15–20 per cent in fraction CS2 (Fig. 2).

(*Note added in proof.* The structure of peptide TS1 from ^{14}CM-LADH has now been shown to be Met.Val.Ala.Thr. Gly.Ile.^{14}CMCys.Arg. T. K. Li and D. E. Drum (*Fed. Proc. Abst.*, No. 1934; 1964) report that the peptide which they have isolated from a trypsin digest of ^{14}CM-LADH contains Lys, Arg, Cys, Asp, Thr, Ser, Glu, Gly, Ala, Val, Ile.)

Peptide TS1 was heavily contaminated with other 'neutral' non-radioactive peptides, and in order to facilitate the purification of the radioactive moiety the mixture of 'neutral' peptides was digested further with chymotrypsin; the radioactive peptide (TS1 : C1), which was also 'neutral' at *p*H 6·5, was obtained in pure form by successive ionophoreses on paper at *p*H's of 6·5, 3·5 and 1·9. Peptides CS1 and CS2 were purified in a similar way.

Amino-acid composition and sequence of peptides containing carbon-14. The acid hydrolysates of peptides TS1 : C1, CS1 and CS2 were analysed for constituent amino-acids and were found to have the following compositions: TS1 : C1: ^{14}CMCys (1) Gly (1) Ile (1) Arg(1); CS1: ^{14}CMCys (1) Asp (2) Thr (2) Ser (2) Gly (2) Ala (1) Val (2) Leu (1) Ile (1) His (1) Arg (1); CS2: ^{14}CMCys (1) Asp (2) Thr (1) Ser (2) Gly (2) Val (1) Leu (1) Ile (1) His (1) Arg (1).

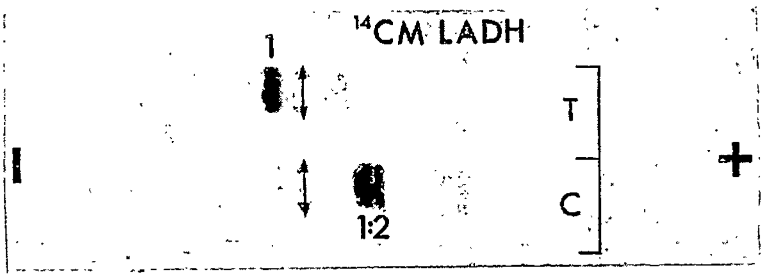

Fig. 2. Autoradiograph of peptides containing carbon-14 produced by trypsin (*T*) and chymotrypsin (*C*) hydrolysis of the iodoacetic 1[^{14}C] acid derivative of horse liver alcohol dehydrogenase. Ionophoresis in pyridine–acetic acid buffer at *p*H 6·5

When peptide CS1 was digested with trypsin it was split into two fragments: a 'neutral' radioactive peptide CS1 : T1, containing ^{14}CMCys (1) Thr (1) Gly (1) Ala (1) Val (1) Ile (1) Arg (1), and an acidic non-radioactive peptide CS1 : T2, containing Asp (2) Thr (1) Ser (2) Gly (1) Val (1) Leu (1) His (1). In a similar manner peptide CS2 was split by trypsin to give CS2 : T1, which was identical with TS1 : C1, and CS2 : T2, which was identical with CS1 : Ts.

The amino-acid sequences of these peptides were established by the 'Dansyl–Edman' procedure, as described by Gray and Hartley[19]. For example, peptides CS1 : T1 and CS1 : T2 (0·1 μmole) were each degraded in a stepwise manner, and the N-terminal amino-acids formed at each successive step were identified as their dimethylaminonaphthalene-sulphonyl ('dansyl') derivatives by ionophoresis on paper at *p*H 4·4. The results obtained are summarized in Table 4.

Structure of horse liver alcohol dehydrogenase. The major radioactive peptides present in trypsin and chymotrypsin digests of the enzyme-inhibitor compound are clearly derived from the same unique sequence of amino-acids. The two reactive sites are identified as the SH groups of two cysteines and these are shown to occur in the same sequence in the primary structure of the enzyme protein. These results show that liver alcohol dehydrogenase contains two structurally equivalent active sites and that in all probability the active enzyme consists of two identical polypeptide chains each with a molecular weight of approximately 36,000 (assuming the lower molecular weight of 73,000 (ref. 13) for the active dimer). By analogy with the yeast enzyme each chain contains a reactive SH group which, in conjunction with zinc and other, as yet unidentified, amino-acid side-chains, appears to be involved in the binding of substrate and/or NAD/NADH to each of the two 'active centres' within the quaternary structure of the active dimer.

Comparative Structures of Dehydrogenases

The amino-acid sequences around the reactive cysteines in yeast and horse liver alcohol dehydrogenases, together with the corresponding sequences in glyceraldehyde 3-phosphate dehydrogenases from yeast and mammalian muscle, are compared in Table 5. In marked contrast to GPDH'S, in which the sequences are identical, significant differences in sequence are found to occur between alcohol dehydrogenases of yeast and mammalian origin. Of particular interest is the replacement of the histidine residue (position 7) which occurs adjacent to the reactive cysteine in the yeast enzyme, by arginine in the horse liver enzyme; the histidine (position 11), five residues away from the reactive cysteine, occurs in the same *relative* position in both enzymes. If the residues in positions 6, 7 and 11 in the sequence (that is, cysteine, histidine/or arginine, and histidine, respectively) are indeed involved in the binding of zinc, then this replacement of histidine by arginine could well account for the observed differences[11] in the zinc-binding properties of the two enzymes.

Mutations which have led to alterations in sequence in the immediate neighbourhood of the reactive cysteines (that is, positions 3–9, Table 5) have clearly involved the replacement of a given amino-acid by another chemically related one; thus serine, valine and threonine in the yeast enzyme have been replaced by threonine, isoleucine and serine, respectively, in the liver enzyme. Outside this heptapeptide segment of the chain (that is, positions 1–2 and 10–16, but with the exception of histidine in position 11) the sequences appear to be entirely unrelated. It may therefore be inferred that the amino-acid side-chains in close proximity to the reactive cysteines in the primary structure cannot be altered to any appreciable extent without at the same time affecting the tertiary structure which is essential for maintaining the structural integrity of the 'active centre' in alcohol dehydrogenases.

Although yeast and liver alcohol dehydrogenases catalyse the same reaction, namely, the reversible oxidation and reduction of alcohols to the corresponding aldehydes (or ketones) in the presence of NAD/NADH, the two enzyme proteins differ considerably in amino-acid

Table 4. AMINO-ACID SEQUENCE OF PEPTIDES ISOLATED FROM HORSE LIVER ALCOHOL DEHYDROGENASE FOLLOWING ITS REACTION WITH IODOACETIC-1(^{14}C) ACID

Peptide	Amino-acid sequence															
TS1 : C1				†Gly	Ile	$\overset{*}{\textit{Cys}}$	Arg									
CS2 : T1				†Gly	Ile	$\overset{*}{\textit{Cys}}$	Arg									
CS2 : T2								†Ser	Asp	Asp	His	Val	Thr	Ser	Gly	Leu
CS2				Gly	Ile	$\overset{*}{\textit{Cys}}$	Arg	Ser	Asp	Asp	His	Val	Thr	Ser	Gly	Leu
CS1 : T1	†Val	Ala	Thr	Gly	Ile	$\overset{*}{\textit{Cys}}$	Arg									
CS1 : T2								†Ser	Asp	Asp	His	Val	Thr	Ser	Gly	Leu
Sequence of CS1	↓C Val	Ala	Thr	↓C Gly	Ile	$\overset{*}{\textit{Cys}}$	Arg	↓T Ser	Asp	Asp	His	Val	Thr	Ser	Gly	Leu ↓C
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

* 1-(^{14}C) S-carboxymethylcysteine.
† Sequences determined by 'Dansyl-Edman' method (Gray and Hartley, 1963). ↓ Peptide bonds split by chymotrypsin (C) and trypsin (T).

Table 5. COMPARISON OF AMINO-ACID SEQUENCES AROUND THE REACTIVE CYSTEINES (*) IN GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASES (1), HORSE LIVER ALCOHOL DEHYDROGENASE (2) AND YEAST ALCOHOL DEHYDROGENASE (3)

(1) ---	Val	Ser	Asn	Ala	Ser	$\overset{*}{\textit{Cys}}$	Thr	Thr	Asn	Cys	Leu	Ala	Pro	Leu	Ala	Lys ---
(2) ---	Val	Ala	Thr	Gly	Ile	$\overset{*}{\textit{Cys}}$	Arg	Ser	Asp	Asp	His	Val	Thr	Ser	Gly	Leu ---
(3) ---	Arg Lys	Tyr	Ser	Gly	Val	$\overset{*}{\textit{Cys}}$	His	Thr	Asp	Leu	His	Ala	Try	His	Gly	Asp ---
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

composition and molecular weight. The yeast enzyme is a tetramer with a molecular weight of 150,000, while the liver enzyme is a dimer with a molecular weight of 73,000–84,000; the former dissociates into sub-units in the presence of metal chelating agents such as *o*-phenanthroline, while the latter does not[11]. The two enzymes also differ in their substrate and co-enzyme specifications; for example, YADH reacts more rapidly with ethanol than with higher alcohols, whereas LADH has a broader specificity, and acts on long-chain primary alcohols such as vitamin A. Thus, although the two enzymes catalyse the same basic chemical reaction, the overall pattern of metabolic events in liver is very different from the process of alcoholic fermentation in yeast. This difference in the biochemical environment and function of the two alcohol dehydrogenases is much more marked than is the case with the glyceraldehyde 3-phosphate dehydrogenases of yeast and mammalian muscle; and the extent of this biochemical adaptation seems to be reflected in the correspondingly greater chemical changes which have occurred in alcohol dehydrogenases during the evolutionary development of the respective enzyme proteins.

In spite of the considerable differences in overall chemical composition and structure which exist between the two alcohol dehydrogenase proteins the fundamental structural unit consists in each case of a protein chain with a molecular weight of about 36,000; this structural unit binds NAD/NADH and zinc and contains a reactive SH-group in a sequence which, although changed, has changed in such a way as to preserve a very similar sequence of amino-acids in the immediate vicinity of the reactive cysteines in the two enzymes.

In this respect alcohol dehydrogenases resemble the glyceraldehyde 3-phosphate[6–8], and lactic[20,21], dehydrogenases, which have also been shown to consist of sub-units with a molecular weight of 35,000–36,000. In glyceraldehyde 3-phosphate and alcohol dehydrogenases the protein chains in each individual enzyme appear to be identical; lactic dehydrogenases, on the other hand, exist in the form of isoenzymes consisting either of four identical chains or of different proportions of two dissimilar chains.

An examination of enzyme-substrate and enzyme-inhibitor compounds by methods of structural organic chemistry has led to the characterization of the protein sub-units in dehydrogenases of the glycolytic cycle. In terms of their chemical morphology dehydrogenases appear to resemble the hæmoglobins[22], and one may hope that future chemical and X-ray crystallographic investigations will lead to the elucidation of the amino-acid sequences, and of the folded three-dimensional structures of their constituent polypeptide chains. Only when this has been achieved will it become possible to provide a chemical basis for their catalytic properties. One may also look forward to tracing the evolutionary development and biochemical adaptation of these enzymes in terms of their chemical structures, and to the discovery of structural interrelationships between enzymes which couple together to promote successive steps in related biochemical processes such as glycolysis and alcoholic fermentation.

I thank Mr. R. J. A. Turner for his assistance throughout the course of this work.

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BIOCHEMICAL PATHOLOGY OF ACUTE HEPATIC ADENOSINETRIPHOSPHATE DEFICIENCY*

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We have been engaged during the past several years in the examination of how some cells and organs respond to the presence of ethionine, an analogue of the amino-acid methionine, so as to manifest disease at both the morphological and biochemical level. The aim was two-fold: (*a*) to investigate a model experimental disease with the hope of understanding the biochemical significance of some alterations in cellular morphology; (*b*) to examine the response of living cells, as working units, to changes in their environment leading to disease. Such information hopefully would give new insight into the mechanisms by which cells integrate some of their reactions. The examination of many such model experimental disease systems, amenable to simultaneous biochemical and morphological analysis, would appear to be essential for the ultimate understanding of the more complex natural diseases. The choice of the amino-acid antagonist, ethionine, for this type of investigation seemed an apt one, since selectivity in its effects on cells might be expected.

Ethionine, the ethyl analogue of methionine, was synthesized by Dyer[1], who observed it to be toxic in the rat. Since then, many well-defined effects have been found in several other animal species as well as in micro-organisms[2,3].

In the rat and in several other species, the oral or parenteral administration of ethionine results in characteristic and reproducible lesions in the liver, pancreas, kidney, testis and certain other organs[2,3]. Most of these changes are acute, occurring within hours or a few days; but others, such as cancer of the liver, appear only after treatment of the animal for several months. One of the main interests in this analogue, from the point of view of disease, stems from the finding that virtually all its effects, both morphological and biochemical, are readily prevented by the administration of adequate amounts of methionine. The relationship between ethionine and methionine appears to be specific, since no other sulphur-containing amino-acid or other amino-acid shows any protective effect.

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* Substance of the Christian Herter Lecture delivered by Prof. E. Farber in New York University Medical School on April 3, 1963.

One of the earliest lesions observed in animals treated with ethionine is the rapid development of a fatty liver[4]. In this choline-resistant lesion, the lipid accumulates first in the liver cells about the periportal region of the liver lobule and afterwards involves the rest of the lobule[5]. The excess lipid is all triglyceride[6] with a fatty-acid pattern resembling that of adipose tissue triglyceride[7,8]. This suggests that the source of the fatty acids for the triglyceride deposited in the liver is the adipose tissue. The fatty liver occurs readily in female rats but either not at all or to a considerably less degree in the male[4,9]. This sex difference in the lipid accumulation appears to be a function predominantly of androgens in the male since: (*a*) ovariectomy has no effect on the occurrence of the fatty liver in the female, while orchidectomy makes the male much more susceptible; (*b*) the administration of testosterone or related compounds, especially those with potent protein sparing actions, completely protect both the female and the castrate male[9,10]. Of the many other hormonal preparations tested, only growth hormone has a similar action[10].

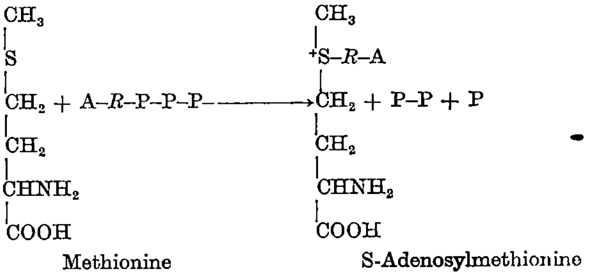
In addition to inducing a fatty liver, ethionine inhibits protein synthesis in the liver when measured both *in vivo* and *in vitro*[11,12]. Many liver enzymes and several plasma proteins have been found to be decreased in the ethionine-treated animal[2,3]. Again, a sex difference is evident in that the inhibition occurs predominantly or exclusively in the mature female but not in the mature male[12]. In contrast to the inhibition of hepatic protein synthesis and the induction of fatty liver, some other effects of ethionine, such as inhibition of transmethylation from methionine to choline precursors[13] or inhibition of the oxidation of the methyl group of methionine to formate and carbon dioxide[14], are manifest equally in both sexes. Thus, a correlation exists between the disturbance in protein metabolism and the accumulation of liver lipid[15–17]. The interrelationships between these two lesions and their dependence on a more basic alteration in ATP metabolism has recently been clarified and is the subject of this report. Some new insight into the integration of some cell functions obtained during this study is discussed briefly.

Induction of ATP Deficiency

The first major clue to our understanding of the biochemical pathology of the effects of ethionine on the liver was derived from the observations by Shull[18] on hepatic glycogen phosphorylase. He found that the activity of this enzyme was decreased in ethionine-treated female rats and that, of the factors known to influence its level, ATP but not 3′-, 5′-cyclic adenylic acid, when added *in vitro*, partially reversed this inhibition. This implied that the decrease in enzyme activity was secondary to some alteration in the liver ATP. This supposition was greatly strengthened by the observation that the enzyme changes could be readily prevented if adenine or ATP were administered to the animal along with ethionine. These compounds proved to be as effective as methionine. Measurement of the liver ATP concentration revealed that ethionine administration is followed by a very striking decrease in the level of hepatic ATP[11] in confirmation of a previous suggestion by Stekol *et al.*[19]. Although the decrease occurred in both male and female rats, it was about twice as large in the female as in the male.

The concentration of ATP begins to decrease within 30 min after the injection of ethionine and reaches a minimum value of about 20 per cent of the control value within 2 h (ref. 20). The magnitude of the decrease is proportional to the dose of ethionine administered up to about 0·5 mg/g body-weight. Because of this, it is possible, by administration of the appropriate amount of ethionine, to obtain a reproducible spectrum of levels of ATP in the liver ranging between the control and the minimal value. The decrease in hepatic ATP concentration is prevented by the administration not only of methionine but also of adenine, ATP, inosine and, to a lesser degree, 5-amino-4-imidazole-carboxamide and nicotinamide, but not by the injection of glycine, sodium formate, glutamine and ribose. Adenine is effective not only when it is administered with ethionine but also when administered 24 h after the ethionine, by which time morphological and biochemical lesions have been obvious for many hours.

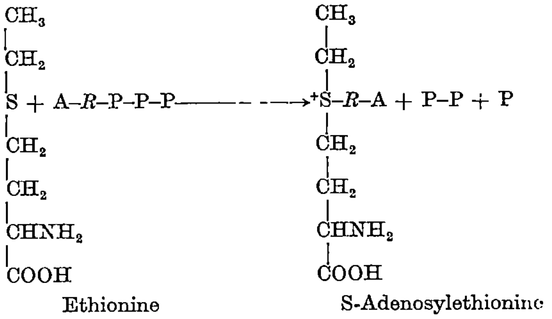
Two suggestions by Stekol *et al.*[19,21] have been proposed to explain the effects of ethionine on ATP. In both of them, the basic explanation depends on the conversion of ethionine to S-adenosylethionine. Through the original work of Cantoni[22] and subsequent investigations of Schlenk *et al.*[23] and of Stekol, it is now well established that methionine reacts with ATP (ARPPP) to form S-adenosylmethionine (SAM) via an activating enzyme[1].



$$\begin{array}{lcl}
\mathrm{CH_3} & & \mathrm{CH_3}\\
| & & |\\
\mathrm{S} & & {}^{+}\mathrm{S{-}}R\mathrm{{-}A}\\
| & & |\\
\mathrm{CH_2} + \mathrm{A{-}}R\mathrm{{-}P{-}P{-}P} & \longrightarrow & \mathrm{CH_2} + \mathrm{P{-}P} + \mathrm{P}\\
| & & |\\
\mathrm{CH_2} & & \mathrm{CH_2}\\
| & & |\\
\mathrm{CHNH_2} & & \mathrm{CHNH_2}\\
| & & |\\
\mathrm{COOH} & & \mathrm{COOH}\\
\text{Methionine} & & \text{S-Adenosylmethionine}
\end{array}$$

I

Ethionine[11] is almost as efficient a substrate for this enzyme as is methionine[22].



$$\begin{array}{lcl}
\mathrm{CH_3} & & \mathrm{CH_3}\\
| & & |\\
\mathrm{CH_2} & & \mathrm{CH_2}\\
| & & |\\
\mathrm{S} + \mathrm{A{-}}R\mathrm{{-}P{-}P{-}P} & \longrightarrow & {}^{+}\mathrm{S{-}}R\mathrm{{-}A} + \mathrm{P{-}P} + \mathrm{P}\\
| & & |\\
\mathrm{CH_2} & & \mathrm{CH_2}\\
| & & |\\
\mathrm{CH_2} & & \mathrm{CH_2}\\
| & & |\\
\mathrm{CHNH_2} & & \mathrm{CHNH_2}\\
| & & |\\
\mathrm{COOH} & & \mathrm{COOH}\\
\text{Ethionine} & & \text{S-Adenosylethionine}
\end{array}$$

II

However, unlike S-adenosylmethionine[23], S-adenosylethionine (SAE) is not readily utilized for many transalkylations and other metabolic reactions[2,3].

(*a*) According to one suggestion of Stekol *et al.*[19], S-adenosylethionine accumulates in the tissue as a result of the activation, causing an inhibition of mitochondrial oxidation and oxidative phosphorylation, and a concomitant decrease in the rate of synthesis of ATP. Evidence in support of this thesis is the observation that S-adenosylethionine inhibits oxidative phosphorylation when added *in vitro* to mitochondrial preparations[19]. Against this interpretation, however, stand the results of several investigations[24–26] showing normal respiration and oxidative phosphorylation in isolated liver mitochondria from animals treated with ethionine in doses which produce pathological changes in the liver and elsewhere. Recent work in this Department has also shown that the administration of adenine or of an adenine-precursor many hours after the induction of severe biochemical and morphological lesions in rats will readily reverse the effect

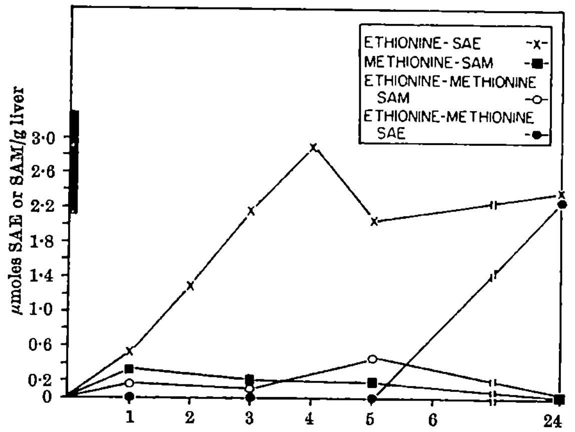


Fig. 1. Hepatic concentrations of S-adenosylethionine (SAE) or S-adenosylmethionine (SAM) following the intraperitoneal administration of ethionine (1 mg/gm body-weight) and/or an equimolar amount of methionine, as indicated in the rectangle. Note the absence of any SAE accumulation in the liver for the first 5 h after the administration of ethionine plus methionine and the high level at 24 h. The highest range of liver ATP concentration as reported in the literature and as observed in this department is indicated by the thick solid bar on the ordinate. SAE and SAM were determined chromatographically on the 15-cm column of the Spinco Moore–Stein–Spackman amino-acid analyzer

on ATP[27], a finding difficult to reconcile with this hypothesis.

(b) According to the other suggestion[21], for which there is increasing evidence, ATP decreases in the liver because it reacts with ethionine to form S-adenosylethionine at a rate faster than the cell can synthesize the nucleotide *de novo* from available precursors. Such an 'adenine-trapping effect' of ethionine was clearly shown in yeast by Schmidt *et al.*[28]. Work in our laboratory has indicated that, shortly after the administration of ethionine, one can detect S-adenosylethionine in the liver[29]. Its concentration increases rapidly with time up to about 5 h and thereafter remains at a high level for at least 24 h (Fig. 1). In the case of methionine administration, the extent of S-adenosylmethionine accumulation is considerably less and the level of this compound in the liver becomes undetectable within 24 h (Fig. 1). These observations are best explained by assuming that S-adenosylmethionine is readily used by the liver for many metabolic reactions and thus does not accumulate in large amounts. This metabolic use of SAM probably releases an adenine derivative[30] which may be re-used for ATP synthesis. In contrast, the poor metabolic utilization of S-adenosylethionine would favour its accumulation in the liver. This trapping action of ethionine would tax the liver beyond its capacity to make the adenine moiety of ATP *de novo* from the usual precursors. The efficacy of adenine in counteracting the drop in ATP would appear to be due to its rapid conversion to ATP, presumably via the adenylate pyrophosphorylase and kinase leading to ADP. and the phosphorylation of this ADP to ATP through oxidative phosphorylation. Since mitochondrial oxidation and oxidative phosphorylation appear to be normal in the liver of the ethionine-treated rat, the generation of ATP from ADP should be sufficiently rapid to maintain adequate ATP levels, even in the presence of an ATP-trapping system, providing sufficient adenine is supplied to form AMP and ADP.

Inhibition of Protein Synthesis

Since ethionine administration is followed by a rapid decrease in the concentration of hepatic ATP the question arose as to whether this decrease might be the underlying basis for the inhibition of protein synthesis[21]. All the available evidence now supports this hypothesis[20,31]. Adenine or ATP administered to an animal counteracts completely the inhibition of protein synthesis induced by ethionine. Most of the measurements of protein synthesis were made on an *in vitro* ribosome-supernatant system in which the incorporation of radioactive leucine into protein was followed. However, similar results were obtained with the incorporation of radioactive amino-acids into liver protein *in vivo* as well as with a non-isotopic method, the increase in hepatic 2-acetylaminofluorene hydroxylase activity following the administration of methylcholanthrene[20]. Furthermore, the examination of the time course of the effects of ethionine shows that the drop in ATP precedes by about 1 h the drop in amino-acid incorporation into the ribosomal system[20]. In addition, the administration of different levels of ethionine induces proportional decreases in the levels of ATP and in the degree of inhibition of protein synthesis.

Assuming then some relationship between ethionine, ATP and protein synthesis, two questions naturally arise: (*a*) How does the drop in ATP inhibit protein synthesis ? (*b*) How is the decrease in ATP related, if at all, to the induction of fatty liver or to the induction of other biochemical or morphological alterations in the liver and other organs ?

Relation of ATP to Protein Synthesis

ATP is known to play a part in protein synthesis in at least three sites: (1) the activation of the amino-acids [AA] for their reaction with transfer RNA (*s*-RNA); (2) the reaction of *s*-RNA–AA with the ribosomal components and the subsequent formation of the peptide bonds of the nascent protein, which appear to require at least two transferases and guanosine-triphosphate (GTP)[32–39], the synthesis of which is dependent on ATP; and (3) the synthesis of messenger RNA (*m*-RNA) (ref. 40) in the nucleus. The attachment of this *m*-RNA to ribosomes appears to play a vital part in the proper assembly of inactive ribosome monomers to form ribosome aggregates or polymers functionally active in protein synthesis in micro-organisms[41–47] as well as in liver[48–51] and in other mammalian cells[52–59].

All the available evidence points to the ribosomes as the major locus for the inhibition of protein synthesis in the ethionine-treated rat: (1) In the *in vitro* assay of amino-acid incorporation into protein, the use of the amino-acid activating and transferring systems and of transfer RNA from normal liver fails to influence the severe degree of inhibition observed when ribosomes from ethionine-treated female animals are used[12,31]. (2) The *p*H 5 fraction from the liver supernatant from ethionine-treated rats is just as active in activating and binding leucine, valine and methionine to transfer RNA as is the fraction from control animals[60]. (3) The presence of ATP and GTP, which are normally used in the assay system for amino-acid incorporation into protein, has no effect on the degree of inhibition[12,31].

Assuming that the ribosome is the major site for the inhibitory action of ethionine, the ATP deficiency could affect the integrity of the ribosome itself or the synthesis or availability of messenger RNA reacting with the ribosome monomers. Since the substrates for RNA synthesis are the four nucleoside triphosphates [ATP, GTP, UTP, CTP] a large decrease in the concentration of one of them, ATP, could seriously reduce the rate of cellular RNA synthesis. There is increasing evidence that the ATP deficiency induced by ethionine does indeed inhibit the synthesis of RNA in the nucleus and that this interference with RNA synthesis may be a major way by which the decrease in ATP concentration affects protein synthesis. This suggestion is based on the following observations: (1) The administration of ethionine is followed by a striking inhibition in the extent of incorporation of ^{14}C-6-orotic acid or of ^{14}C-8-adenine into nuclear RNA[61]. This inhibition is prevented by the administration of methionine along with the ethionine. The time curve of this inhibition coincides with the time

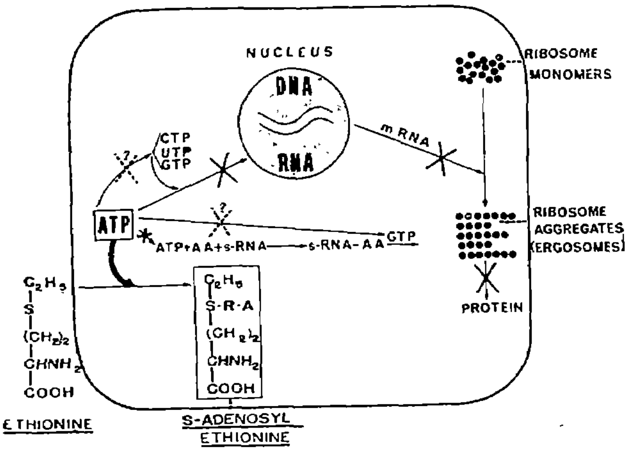


Fig. 2. Diagrammatic representation of the liver cell showing the known sites where ATP is directly involved in protein synthesis and the probable sites of inhibition of protein synthesis in the ethionine-treated female rat (X). Other possible sites of inhibition not yet investigated are indicated by ?✕. CTP, UTP, and GTP are the triphosphates of cytidine, uridine and guanosine respectively

curve of the decrease in liver ATP and precedes by about 1 h the time curve of the decrease in ribosomal protein synthesis. (2) The progressive inhibition of protein synthesis following ethionine administration is accompanied by a decrease in the number of ribosomal aggregates and a corresponding increase in the number of ribosomal monomers as observed on analysis in a sucrose gradient after centrifugation[62]. There is now increasing indication that liver ribosomal protein synthesis is dependent on the continual synthesis and availability of *m*-RNA[49]. The liver ribosomes appear to be active in protein synthesis only when combined with *m*-RNA to form active organized aggregates which may contain from two to twenty or more ribosomes. The liver ribosome pattern from the ethionine-treated female[62] is virtually identical to that observed following actinomycin *D* administration to the rat[49] and is different from that following puromycin injection[62]. Since actinomycin appears to affect ribosome aggregation and protein synthesis by inhibiting DNA-dependent RNA synthesis[63-70], it appears probable that ethionine affects ribosomal protein synthesis and ribosomal assembly basically in a similar manner by interfering with *m*-RNA synthesis (Fig. 2). This is in contrast to puromycin, which seems to act at the level of the ribosome itself[71] and to interfere with the formation of the peptide bonds of the nascent protein[72-75].

The suggestion that the ethionine-induced ATP deficiency acts primarily on protein synthesis at the level of ribosome assembly is considerably strengthened by observations during the reversal of the inhibition of protein synthesis. Unlike the inhibition of protein synthesis by actinomycin *D* or puromycin, the ethionine-induced inhibition can be readily reversed by the administration of adenine, methionine or adenine plus methionine, even many hours after the inhibition has been in force. When this is done, there occurs a rapid progressive reassembly of the ribosome monomers to form large organized aggregates[62] which have also been called ergosomes[48]. In fact, as protein synthesis returns to normal, there occurs an unusual preponderance of the heaviest ergosomes, the significance of which is still not clear. In some animals during the immediate recovery phase, protein synthesis becomes more active than in control animals and it appeared that the presence of the heaviest aggregates correlated with this observation. However, the livers of some animals have been observed to possess a similar pattern of large ergosomes without any apparent acceleration of protein synthesis[62].

Consistent with the suggestion that the main site of interference with protein synthesis in the ethionine-treated rat is in the ribosome are the recent findings on binding of leucine-^{14}C or methionine-^{14}C to liver *s*-RNA *in vivo*[76]. Although some inhibition of formation of *s*-RNA-leucine or *s*-RNA-methionine does occur in the liver of the intact female rat injected with ethionine, this can easily be reversed within 30 min following the administration of adenine without any appreciable reversal of the inhibition of amino-acid incorporation into liver protein at this time-interval.

The present-day concept of the inhibition of protein synthesis by ethionine is portrayed diagrammatically in Fig. 2. In this figure and in the foregoing discussion, only protein synthesis in cytoplasmic ribosomes has been considered. Although amino-acid incorporation into proteins has been demonstrated in other cell organelles such as the nucleus and the mitochondria[77], nothing is yet known about the possible effects of ATP deficiency on these systems.

Relation of ATP to Fatty Liver

Recent work has shown that the administration of adenine or ATP completely prevents the accumulation of excess triglyceride in the liver when measured either chemically or histochemically[78]. The administration of ATP also effectively prevents the decrease in serum lipid levels which is so characteristic a concomitant of several forms of fatty liver[79]. Equally interesting is the observation that the administration of adenine is frequently also effective in rapidly removing the excess liver triglyceride even after it has been accumulating for 24 h (ref. 80). Thus the fatty liver appears to be related in some fashion to ATP.

The interrelationships between hepatic ATP deficiency, inhibition of protein synthesis, and the changes in liver and serum lipids which have been uncovered during these recent investigations on ethionine may offer new clues to the pathogenesis of fatty liver. It has become evident from work initiated by Recknagel, Lombardi and Schotz[79,81,82] that several forms of fatty liver, including that induced by carbon tetrachloride[82,83] and ethionine[79], are probably secondary to a block in the release of liver lipid into the plasma. According to this hypothesis[79,83,84] the liver in the ethionine-treated animal continues to take up free fatty acids (FFA) from the blood and to esterify them to triglycerides but fails to transfer the newly synthesized triglycerides into the plasma[85-88] with a resultant increase in liver triglyceride content.

Based on the correlation between inhibition of protein synthesis and the occurrence of fatty liver, it was suggested several years ago that the development of fatty liver in the ethionine-treated female was secondary to the more fundamental disturbance in protein metabolism[15-17]. Feinberg *et al.*[89] and more recently Lombardi and Recknagel[79,83], Robinson and Harris[90] and Bjornstad and Bremer[91] suggested that the link between the alterations in protein and in lipid metabolism is the synthesis of plasma lipoproteins. Since plasma lipids occur almost wholly as lipoproteins and since the liver is a probable major source for at least some lipoproteins[92-96], an inhibition of protein synthesis in the liver might interfere with the normal transfer of the lipids from the liver to the plasma. Some of these suggested relationships are illustrated in Fig. 3.

The observation that the inhibition of protein synthesis induced by ethionine is in itself secondary to a decrease in hepatic ATP concentration immediately raises the question whether the lipid accumulation and the disturbed protein metabolism might not be independent manifestations of the same basic biochemical disturbance. In this connexion, it should be recalled that Dianzani[97] several years ago suggested that the fatty liver induced by carbon tetrachloride might result from an ATP deficiency. The final answer to these important considerations cannot be

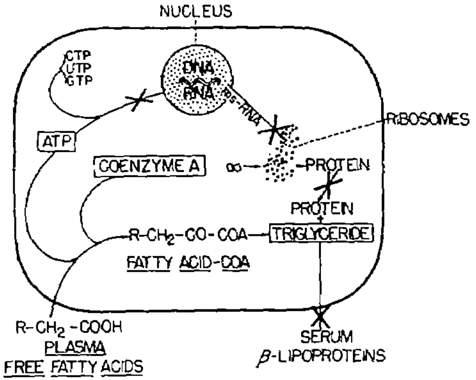


Fig. 3. Diagrammatic representation of a liver cell showing uptake of free fatty acids (FFA) and their conversion to triglyceride. Much of the triglyceride (together with other lipids not shown) is released as serum lipoproteins. The major sites of inhibition by ethionine of protein synthesis and of the release of the lipoproteins are indicated by X

given at this time. However, experiments on ethionine-treated animals given adenine after the liver has become fatty indicates the existence of a better correlation between inhibition of protein synthesis and fatty liver than between hepatic ATP and fatty liver[80]. It is, therefore, probable that the examination of the suggested relation between disturbances in protein and in lipid metabolism offers the best hope for the eventual understanding of the pathogenesis of some types of fatty liver. It is noteworthy that this hypothesis relating the induction of fatty liver to a more basic defect in protein metabolism may not only apply to the animal treated with ethionine but may have wider application to the pathogenesis of fatty liver[78] induced by some other agents such as carbon tetrachloride[98–100] or puromycin[101], as has been suggested also by Robinson and Seakins[101].

Since the accumulation of triglyceride in the liver of the ethionine-treated animal is probably due to an interference with the synthesis of the protein moiety of some lipoproteins, it is possible that the altered lipid metabolism is only a special instance of a more general phenomenon in which the synthesis of several types of conjugated proteins by the liver is interfered with by the ethionine-induced inhibition of protein synthesis. On this basis, it might be expected that cellular components, other than triglyceride, representing prosthetic groups of conjugated proteins such as some complex lipids, polysaccharides, porphyrins, coenzymes, etc., may also show accumulations or other derangements as a consequence of the interference with the synthesis of their associated protein. The recent report of the accumulation of diastase-resistant periodic acid–Schiff-positive material, presumably polysaccharide, in the liver of ethionine-treated rats may indeed be an example of such a derangement[102].

Other Effects of Ethionine

It must be emphasized that the effects of adenine or ATP are specific in that these compounds prevent or reverse only some effects of ethionine. For example, the ethylation of transfer RNA by ethionine or the incorporation of the analogue into liver protein is not affected by adenine[103]. The inhibition by ethionine of methylation of transfer RNA from methionine or of transmethylation to lecithin precursors is not prevented by the administration of adenine or ATP, even though the inhibition of incorporation of methionine into liver protein is completely counteracted in the same animal[104] (Fig. 4). Analogous observations have also been made in respect to morphological changes in the liver induced by ethionine. Although adenine injections protect against fatty liver, other morphological changes are not prevented by this purine[78]. These results with adenine are in contrast to those with methionine, a compound which protects against almost all the inhibitory effects of ethionine (Fig. 4).

It is now evident that ethionine administration is rapidly followed by a striking drop in the hepatic ATP concentration and a concomitant accumulation of S-adenosylethionine and that each of these biochemical effects is, in turn, the initiator of several chemical and morphological consequences leading to characteristic manifestations of disease. Although the rapid induction of an ATP deficiency is one of the major effects of ethionine, as briefly reviewed in this report, there are other effects related to ethionine–methionine interaction (Fig. 4) which are also of potential importance in explaining the biochemical pathology of other acute and chronic changes induced by ethionine. In regard to these changes it should be emphasized that some effects observed when relatively large doses of ethionine are administered, such as the investigations reported here, do not appear to occur when much smaller amounts of ethionine are administered in the diet during the induction of liver cancer. Thus it is possible that phases of ethionine metabolism other than those concerned with ATP deficiency are related to its carcinogenic effect.

General Implications

An obvious by-product of this work is the availability of a technique to investigate several aspects of ATP metabolism under reasonably controlled conditions in an intact animal. Since the S-adenosylethionine molecule appears to be relatively stable metabolically in comparison with the ATP molecule, it is now possible to trap a considerable portion of the adenine of the cell through the reaction of ATP with ethionine and in this way measure approximate minimal rates of *de novo* synthesis of ATP from a variety of precursors. The preliminary results of such an investigation have already suggested that, in the ethionine-treated animal, the ATP molecule can be more readily made from adenine than from other precursors such as glycine, formate and glutamine, which are used in the more complex *de novo* synthesis[27].

It is noteworthy that the results of the work recorded here are consistent with the thesis that there exists a priority system or a compartment system in the intact cell for available ATP[105]. Some ATP-requiring systems such as nucleic acid synthesis, appear to be especially sensitive to a decrease in the ATP level while others, such as the synthesis of triglyceride from fatty acids and glycerophosphate, are not. Whether these differences are a reflexion only of the Km values of the reactions or are dependent also on the existence of intracellular compartments are questions which possibly may be approached with the aid of trapping systems such as that described here with ethionine. The investigations of ethionine

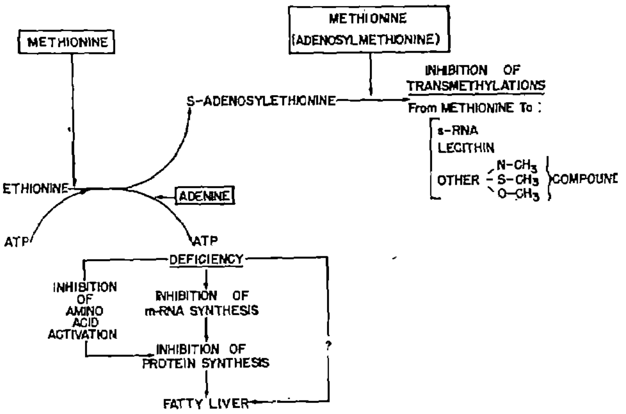

METHIONINE
METHIONINE (ADENOSYLMETHIONINE)
INHIBITION OF TRANSMETHYLATIONS
From METHIONINE To :
s-RNA
LECITHIN
OTHER N-CH3 S-CH3 O-CH3 COMPOUND
S-ADENOSYLETHIONINE
ETHIONINE
ADENINE
ATP
ATP DEFICIENCY
INHIBITION OF AMINO ACID ACTIVATION
INHIBITION OF m-RNA SYNTHESIS
INHIBITION OF PROTEIN SYNTHESIS
?
FATTY LIVER

Fig. 4. A simplified representation of some phases of the metabolism of ethionine in the rat liver, showing two major effects and the probable sites of reversal of these by methionine and adenine. Note that adenine counteracts only those effects due to ATP deficiency while methionine counteracts all effects

also offer further support for the suggestion that protein synthesis in the rat liver[49,51,106–108] resembles more the system in some micro-organisms[65,109,110] and in some other mammalian cells[54–59,66–70,111–115] than in reticulocytes[52] in being dependent on a labile and short-lived messenger RNA.

An interesting facet of this work is the discovery of a 'cure' for an experimental disease, based on a knowledge of pathogenesis. The therapeutic use of adenine and related compounds in the ethionine-treated animal would not have been rational except on the basis of our present understanding of the mechanism of action of ethionine. The experience with this model system strengthens the conviction that a whole new system of therapeutics awaits the detailed knowledge of the biochemical-morphological alterations during the development of each disease process.

Finally, the investigation of some aspects of the biochemical pathology of hepatic ATP deficiency induced by ethionine has given new insight into several facets of cell metabolism and structure in relation to disease. The liver cell within a few hours after ethionine administration is a seriously altered cell with a level of ATP only about 20 per cent of the control and with a residuum of nucleic acid and protein synthesis of only 10–20 per cent. Despite the persistence of these abnormalities for at least 24 h, the liver cell does not die. It is therefore evident that the inhibition of protein synthesis, seen with several hepatotoxic necrotizing agents such as carbon tetrachloride[98–100] or dimethylnitrosamine[116], is not sufficient cause *per se* to induce cell death and necrosis. This strengthens the notion that the death of a cell, like many other reactions of the cell to environmental alterations of both physiological and pathological magnitude, is a highly selective process and that there may exist key metabolic reactions in a cell, the interference with which results in irreversible cell degeneration. A knowledge of such reactions might well help to lay a new foundation for the understanding and control of some diseases.

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INITIATION OF POLYPEPTIDE CHAINS IN THE RETICULOCYTE CELL-FREE SYSTEM

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IN the course of protein synthesis in mammalian cell-free systems, ribosomal monomers are released from the polyribosomes[1-3]. At the same time some monomers become associated with the polyribosome fraction, possibly by attaching to one particular site on each polyribosome[2,4]. Release is more frequent than attachment, so that the net effect of incubation in the cell-free system is the degradation of the polyribosome fraction and the accumulation of ribosomal monomers[1-3]. It has been suggested that the attachment of a ribosomal monomer precedes the initiation of the polypeptide chain and similarly that the completion of the chain is followed by the release of the associated monomer[1,5,6]. Recent studies with HeLa cell and rabbit reticulocyte cell-free systems provide support for this interpretation[2-4].

In the *Escherichia coli* cell-free system polyphenylalanine is synthesized in response to the addition of polyuridylic acid (poly-U)[7]. It will be assumed here that in the reticulocyte cell-free system, too, poly-U-dependent phenylalanine incorporation[8] represents polyphenylalanine synthesis. In essential agreement with this assumption, the incorporated phenylalanine is not rendered soluble in trichloroacetic acid by either trypsin or chymotrypsin.

If the ribosomal monomers which are released from the polyribosome in the course of hæmoglobin synthesis are competent to initiate polypeptide chains, it may be predicted that they will initiate polyphenylalanine chains when poly-U is present. Indirect evidence[9,10] does indeed suggest that ribosomes which are attached to polysome complexes at the outset of incubation in the cell-free system may later become available for polyphenylalanine synthesis. The present studies constitute a more direct approach to this question.

It has been shown elsewhere[10] that rabbit reticulocyte polyribosome (*P*1) and ribosomal monomer (*P*2) fractions can be prepared by differential centrifugation. Fraction *P*1 is contaminated by ribosomal monomers to the extent of about 25 per cent and fraction *P*2 is similarly contaminated by polyribosomes. When titrated with poly-U, fraction *P*2 showed a single maximum of phenylalanine incorporation at about 20 μg poly-U per mg ribosome. Fraction *P*1 showed a slightly higher maximum, at 40 μg poly-U per mg, followed by a sharp minimum and finally by a second region of high phenylalanine incorporation. It was suggested[10] that the phenylalanine incorporation which occurred when the polyribosome fraction (*P*1) was incubated with 40 μg poly-U per mg was partly due to the continuing release of monomers from the polyribosome in the course of protein synthesis. In agreement with this interpretation, Hardesty *et al.*[4] have shown that the addition of poly-A to the reticulocyte cell-free system inhibits the inception of hæmoglobin polypeptide chains. In the experiments reported here, this question is examined further by comparing the capacities of fractions *P*1 and *P*2 to initiate polyphenylalanine and hæmoglobin polypeptide chains in the cell-free system.

Initiation of Polyphenylalanine Chains. It has recently been shown[11] that, in the presence of optimal amounts of poly-U, phenylalanine incorporation with fraction *P*2 occurs in artificial polyribosomes with sedimentation coefficients characteristic of ribosomal dimers and trimers. The artificial polyribosomes may be visualized in another way, by incubating fraction *P*2 with radioactive poly-U (Fig. 1). Before incubation the optical density and radioactivity profiles were similar: the poly-U was apparently non-specifically associated with the ribosomal fraction (Fig. 1*a*). After 5 min incubation a polyribosome peak emerged in the 100–240*S* region (Fig. 1*b*). At this time a considerable amount of poly-U was also associated with the ribosome monomer fraction. After incubation for 10 min the polyribosome peak was still evident but had subsided (Fig. 1*c*).

Calculation of the total amount of ^{14}C-poly-U recovered from the gradients suggested that the emergence of the polyribosome peak was due to the preferential degradation of poly-U not bound to ribosomes[14]. Degradation of poly-U is further illustrated in Table 1. Approximately 1·4 μg of poly-U was rendered TCA-insoluble in 10 min at 37° C by 1 mg of ribosome fraction *P*2. One mg of the enzyme fraction similarly degraded about 1 μg of poly-U. Degradation by ribosome and enzyme fractions was approximately additive. The level of poly-U used in the experiments of Fig. 1 was sub-optimal for phenylalanine incorporation. When greater amounts were used, the polyribosome peak failed to emerge, presumably because

Table 1. DEGRADATION OF ^{14}C-POLY-U IN THE CELL-FREE SYSTEM

Incubation conditions	c.p.m. TCA-insoluble	% degradation
No incubation	664	0
Complete system	404	39
Complete minus ribosomes	550	17
Complete minus enzyme	494	25

One mg of fraction *P*2 was incubated for 10 min at 37° with 5·75 μg ^{14}C-poly-U in a total volume of 0·4 ml. containing the additives described in the legend to Fig. 1. The mixtures were chilled and precipitated with 1 ml. of cold 10 per cent TCA, washed and counted.

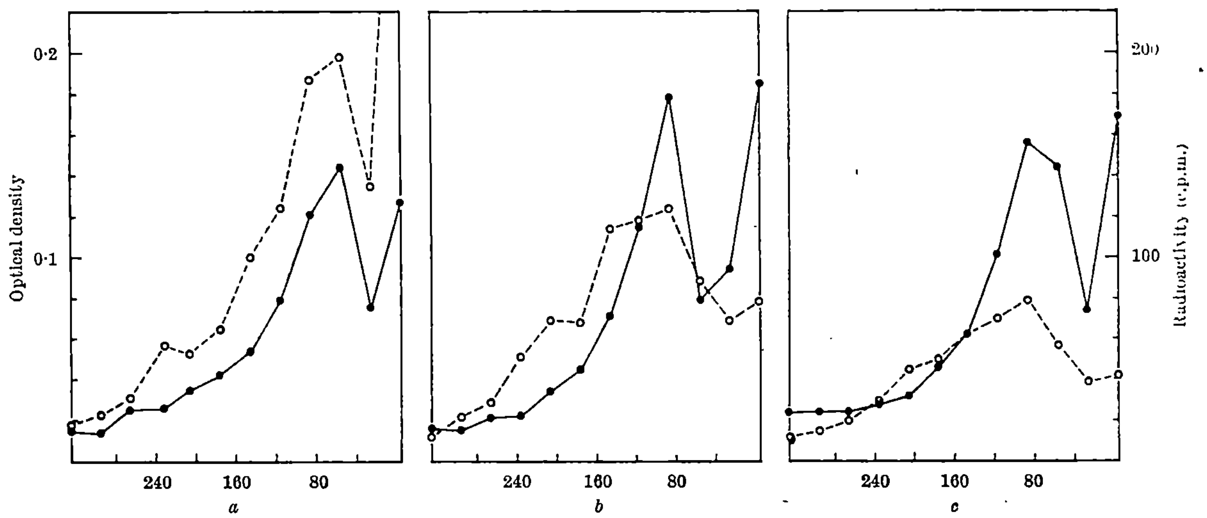

Fig. 1. Sedimentation analysis of the interaction between ribosome fraction *P*2 and ^{14}C-poly-U. (*a*) 10 min at 0° C; (*b*) after 5 min; (*c*) 10 min incubation at 37° C. Direction of sedimentation from right to left. Solid lines, optical density at 260 mμ; broken lines, radioactivity; abscissa, approximate sedimentation coefficients. 2 mg of fraction *P*2 was incubated with 11·5 μg poly-U (1,370 c.p.m.) in a total volume of 0·4 ml., containing in μmoles: ATP, 0·28; GTP, 0·08; creatine phosphate, 2·8; $MgCl_2$, 2; KCl, 16; *tris*-HCl, *p*H 7·2, 16; glutathione, 6; 18 *l*-amino-acids, 0·01 each; 5 μg creatine kinase, 30 μg yeast *s*RNA and 1 mg of enzyme fraction prepared by precipitating the ribosomal supernatant between 40 and 70 per cent ammonium sulphate saturation (refs. 10, 12). The mixtures were chilled, layered on a nearly linear sucrose gradient (5–20 per cent in 1·5 mM $MgCl_2$, 0·01 M KCl, 0·01 M *tris*, *p*H 8·1) and centrifuged for 1 h at 30,000 r.p.m. in the Spinco *SW* 39 rotor at 2° C. The bottoms of the tubes were pierced (ref. 13) and 20-drop fractions collected in the cold through a gauge 20 hypodermic needle. 0·05 ml. withdrawn from each fraction was diluted with 3 ml. water for the optical density measurements. 0·1 ml. of 1 per cent serum albumin was added to the remainder, and each was precipitated with 1 ml. of cold 10 per cent TCA for 30 min, collected on a 2 cm diameter Oxoid membrane filter, washed with 5 per cent TCA, dried with ethanol and ether and counted in a Packard 'Tricarb' liquid scintillation counter

the radioactivity not associated with the ribosomes was not degraded rapidly enough to reveal the transient polyribosome peak. These experiments suggest, however, that the extent of complex formation between poly-U and fraction *P*2 reaches a maximum within the first 5 min of incubation in the cell-free system.

Based on this finding, and the hypothesis that polyribosomes release free ribosomes to poly-U in the course of protein synthesis, predictions can be made regarding the time-course of the initiation of polyphenylalanine chains in the cell-free system. In the case of ribosomal monomers, all chains should be initiated during the first 5 min of incubation, and the remainder of the incubation period should be occupied by chain growth. Chain initiation would continue after the first 5 min only if ribosomes could complete one polyphenylalanine chain and initiate another in the course of the incubation. In the case of polyribosomes, on the other hand, the initiation of polyphenylalanine chains should continue so long as the completion of polypeptide chains and the release of free ribosomes from the polyribosome continues.

In view of the extensive cross-contamination of fractions *P*1 and *P*2, only quantitative predictions can be made about them. Fraction *P*2, rich in free ribosomes, should show the greater initial rise in polyphenylalanine chain inception, while fraction *P*1, rich in polyribosomes, should show the greater rate of chain inception after the initial period.

The rate of chain initiation was examined using a modification of the fluorodinitrobenzene technique. The total TCA-insoluble fraction of the incubation mixture was washed, treated with fluorodinitrobenzene, hydrolysed and extracted as previously described[15]. After drying, the ether extract was counted in a liquid scintillation counter with 10 ml. of a toluene-based counting fluid (0·5 per cent w/v PPO, 0·03 per cent w/v dimethyl POPOP in toluene). The acid phase was freed of ether and 1 ml. dried over potassium hydroxide and phosphorus pentoxide under reduced pressure. The residue was dissolved in 0·3 ml of water and counted with 15 ml of a dioxane counting fluid (8·6 g PPO, 70 mg dimethyl POPOP, 88 g naphthalene, 200 ml. xylene, 1 l. dioxane). Under these conditions quenching was negligible: the ether extracts counted with 55 per cent efficiency (^{14}C-benzoic acid standard) and the acid phases with 60 per cent efficiency. About 70 per cent of the radioactivity of the sample was recovered in the combined acid and ether extracts. In the case of polyphenylalanine synthesis with ^{14}C-phenylalanine, paper chromatography showed all the radioactivity of the acid phase to coincide with the position of free phenylalanine, indicating that acid hydrolysis was complete.

When either ribosome fraction was incubated with 30 μg poly-U per mg ribosome, the number of *N*-terminal phenylalanine residues rose abruptly within the first 5 min (Fig. 2*a*). Fraction *P*2 showed a slightly greater initial rise than fraction *P*1 (in similar experiments this difference has usually been greater). With both fractions the initiation of *N*-termini continued at a steadily decreasing rate during the remaining 35 min of incubation. Throughout this time the rate of chain initiation was about twice as great with fraction *P*1 as with *P*2. The levels of chain initiation by the two fractions after 5 min incubation are more similar than perhaps might have been expected. However, from the time-course of hæmoglobin synthesis in the cell-free system[12] and the linear relationship between incorporation and polyribosome breakdown[3], it may be concluded that the release of free ribosomes from the polyribosome is greatest in the early minutes of incubation. This would tend to equalize the extent of chain initiation by the two ribosome fractions, measured after 5 min. These results thus bear out the predictions made, and confirm that the polyribosome releases free ribosomes to poly-U in the course of incubation in the cell-free system.

Analyses of the time-course of total incorporation and of chain growth are consistent with the same interpretation. As previously shown[10], the rate of total incorporation with fraction *P*2 decayed during the 40-min incubation, while the rate of incorporation with fraction *P*1 was more nearly linear (Fig. 2*b*). In the early minutes of incubation fraction *P*2 incorporated phenylalanine twice as rapidly as fraction *P*1. The rate of growth of the polyphenylalanine chains is shown in Fig. 2*c*. From 5 to 40 min of incubation the average chain-length synthesized with fraction *P*2 increased from 65 to 222 residues. The comparable values for fraction *P*1 were 39 to 188 residues. The lesser chain-length throughout

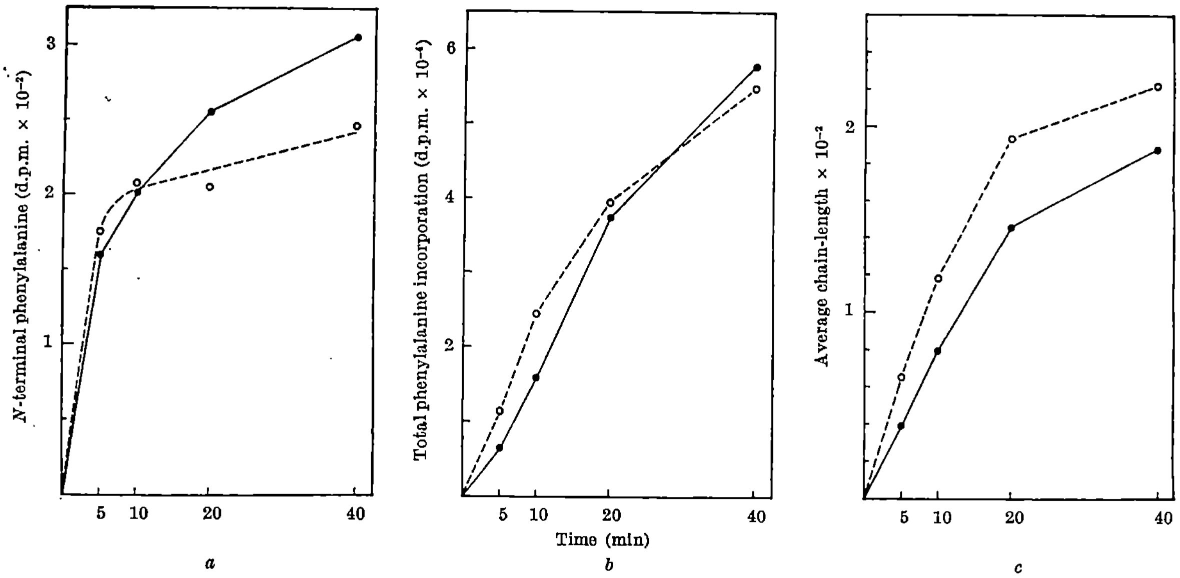

Fig. 2. Incorporation of ^{14}C-phenylalanine by ribosome fractions in the presence of poly-U. (*a*) *N*-Terminal phenylalanine; (*b*) total phenylalanine incorporated; (*c*) average chain-length of polyphenylalanine. Solid lines, fraction *P*1; broken lines, fraction *P*2. 1 mg of the appropriate ribosome fraction was incubated at 37° C in a total volume of 0·4 ml. containing the additives described in the legend to Fig. 1 except that 30 μg of unlabelled poly-U was added and 0·02 μmole ^{14}C-phenylalanine (22×10^6 d.p.m./μmole) was substituted for 0·01 μmole ^{12}C-phenylalanine. Following incubation the tubes were chilled, 10 mg hæmoglobin was added, and the mixtures were precipitated with TCA and analysed as described in the text

the incubation with fraction *P*1 may be attributed to the continuing initiation of polyphenylalanine chains by the free ribosomes released from the polyribosomes during cell-free incubation.

Assuming a coding ratio of three, an average polyphenylalanine chain-length of 220 residues corresponds to an average poly-U chain-length of 660 residues. This is in good agreement with the demonstration that the optimum chain-length for phenylalanine incorporation in a bacterial cell-free system is between 500 and 750 residues[16]. The poly-U used in the experiments described here was synthesized using *Micrococcus lysodeikticus* polynucleotide phosphorylase, partially purified to stage III of Singer and Guss[17] and heated for 5 min at 60° (ref. 18) before storage at –15° C. The polymer was isolated by extraction with phenol, passage through 'Sephadex *G*-25', ethanol precipitation and dialysis. Ultracentrifugation results are not available, but the average chain-length measured by the release of inorganic phosphate during digestion with alkaline phosphatase was of the order of 50 residues. Since the average polyphenylalanine chain-length was 5 times greater, the existence of a selective mechanism in the interaction between ribosome and poly-U is implied. To account for the 2·5 mμmoles phenylalanine incorporated, 7·5 mμmoles UMP would be required; that is, about 2¼ μg out of a total of 30 μg added. Selection is evidently not ruled out on a quantitative basis. Using a different method, Gilbert[19] has found an average chain-length of only 40 residues when *E. coli* ribosomes were incubated with unfractionated poly-U. This suggests that reticulocyte ribosomes may discriminate more efficiently against shorter polymers than *E. coli* ribosomes, and provides an explanation of the higher optimum poly-U level observed with reticulocyte ribosomes.

Initiation of Hæmoglobin Polypeptide Chains. Isolated reticulocyte polyribosomes contain incomplete polypeptide chains with an average chain-length approximately one-half that of complete hæmoglobin polypeptide chains[15]. In the cell-free system a proportion of these chains is completed and released to the soluble fraction, and at the same time free ribosomes are released from the polyribosome[3]. If all the completed polypeptide chains were released, and all the ribosomes made available in this way initiated fresh polypeptide chains, effective uniform labelling would be observed in the cell-free system. Analysis of the total TCA-precipitable fraction of the incubation mixture after labelling with ^{14}C-valine would show the same relationship between *N*-terminal and total radioactivity as found with authentic hæmoglobin; that is, 8·4 per cent of the valine incorporated would be in the *N*-terminal position[15]. Deviations from this value represent a failure either of the release of ribosomes from the polyribosome at the completion of chain synthesis, or of the inception of fresh polypeptide chains by the released ribosomes. The latter interpretation is strongly favoured by the demonstration[3] of a linear relationship between incorporation and polyribosome breakdown in the presence of poly-A.

In cell-free experiments with fraction *P*1, 1·4–2·2 per cent of ^{14}C-valine incorporation was *N*-terminal (Table 2). Thus only 16 to 25 per cent of the ribosomes which had completed polypeptide chains succeeded in initiating new ones. Since fraction *P*1 is contaminated by free ribosomes, the true proportions were probably smaller. This is illustrated by an experiment in which fractions *P*1 and *P*2 were compared (Table 2, experiment 3). The percentage of the total valine incorporation which was *N*-terminal was twice as great in the case of fraction *P*2, due presumably to the preponderance of free ribosomes in this fraction. Hardesty *et al.*[4] report the value of 3·4 per cent *N*-terminal valine in hæmoglobin synthesized in the cell-free system, showing that the probability of a free ribosome initiating a fresh polypeptide chain is greater under their conditions of incubation.

In parallel experiments with and without added poly-U we can compare the absolute amounts of *N*-terminal phenylalanine and *N*-terminal valine in polyphenylalanine and hæmoglobin respectively. Up to twice as

Table 2. CHAIN INITIATION IN THE CELL-FREE SYSTEM

Experiment	1	2	3
Fraction *P*1, *N*-terminal valine	9·4 (1·7%)	5·3 (2·2%)	14·2 (1·4%)
Fraction *P*2, *N*-terminal valine			13·6 (2·7%)
Fraction *P*1 + poly-U, *N*-terminal phenylalanine	11·8	13·9	15·2

3 mg of the ribosome fraction was incubated for 40 min at 37° C in 3 volumes of the incubation mixture described in the legend to Fig. 2. Either ^{14}C-valine alone, or ^{14}C-phenylalanine and 30 μg poly-U per mg ribosome, was added. *N*-terminal analysis was carried out as for Fig. 2. *N*-terminal incorporation is expressed as μμmoles of radioactive amino-acid per mg ribosome.

much phenylalanine as valine was found in the *N*-terminal position (Table 2). Thus the probability that a free ribosome will initiate a polyphenylalanine chain in the cell-free system is as much as twice its probability of initiating a hæmoglobin chain, that is, something less than 0·5.

A current model of protein synthesis suggests that a cycle of ribosomal events occurs during the synthesis of a polypeptide chain[1,3,6]. The initiation of chain synthesis is thought to follow the attachment of a ribosomal monomer to the informational RNA moiety of a polyribosome. As chain synthesis proceeds, the monomer is thought to travel along the strand of RNA, and when chain synthesis is complete, polypeptide and ribosome are thought to become detached from the informational RNA and thus from the polyribosome. Investigations with cell-free systems have shown a correlation between chain initiation and monomer attachment[4] and a similar correlation between chain completion and the release of monomers from the polyribosomes[2,3]. However, protein synthesis in cell-free systems is a limited reaction. Furthermore, incubation in the cell-free system produces a *net* degradation of the polyribosomes and an accumulation of free ribosomes[1–3]. This increase in the number of ribosomal monomers, however, was not accompanied by an increase in polyphenylalanine synthesis at the poly-U optimum[10]. Thus it may reasonably be asked whether the lesion in protein synthesis in the cell-free system might not be this very release of ribosomal monomers from the polyribosome.

Ribosomes are conserved during protein synthesis in living cells. Therefore if polyribosome breakdown occurs in the cell, the ribosomal monomers released must be capable of reattachment to the polyribosomes. An alternative possibility would be that free ribosomes are not released from polyribosomes or are only released as the capacity of the cell for protein synthesis is exhausted. However, the evidence presented here, and in a separate publication[10], indicates that a proportion of the monomers released from polyribosomes in the cell-free system is able to initiate polyphenylalanine chains in conjunction with poly-U. The possibility remains that these ribosomes are nevertheless deficient in a special function required for attachment to the endogenous informational RNA but not for attachment to poly-U. In the absence of any evidence for the existence of such a function, the present work supports the hypothesis that ribosomal monomers recurrently become detached from the polyribosome during protein synthesis.

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FRACTIONATION OF TRYPSIN BY PAPER ELECTROPHORESIS

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PREVIOUS work from this laboratory[1–3] has demonstrated the heterogeneity of crystalline trypsin by paper and starch column electrophoresis. This article presents an electrophoretic method of greater resolution. Using this technique it was possible to demonstrate in a sample of crystalline trypsin the occurrence of fractions showing different ratios of proteolytic to amidasic activity.

In the experiments recorded here a buffer of pyridine acetate of *p*H 4·9 (0·48 : 0·56 : 98·96 v/v of acetic acid : pyridine : water) was used for electrophoresis in a Durrum-type apparatus. To a strip of Whatman 3 filter paper of 12 × 50 cm, 0·12 ml. of an 8 per cent trypsin solution in 0·001 N hydrochloric acid were applied. An electrical tension of 450 V was used and the experiment was allowed to run for 5 h in the cold room. In these conditions at the end of the experiment the temperature of the buffer was 9° C. The strip, while still wet, was cut in 1-cm wide transversal segments and transferred individually to test-tubes containing 3 ml. of a 0·25 M pyridine formate buffer of *p*H 2·7. This extraction was allowed to continue for about 20 h in the cold room. The extract of each tube was then analysed for protein content, proteolytic activity against azo casein and amidasic activity towards α-benzoyl-L-arginine amide (BAA).

The protein content was determined by the Folin–Lowry[4] method as applied to the 'auto analyser' system[5]. Proteolytic activity against azo casein was measured by the method of Charney and Tomarelli[6]. The hydrolysis of BAA was followed colorimetrically in the auto analyser[7]. Chromatography of trypsin in carboxy methyl cellulose was carried out according to the method of Liener[8].

The trypsin samples used in this work were purchased from the Worthington Biochemical Corporation ('Cryst. Trypsin, lyophilized, lot *TL* 747–58'), Freehold, New Jersey, and from Novo Industri ('Cryst. Trypsin Novo, batch 114–3'), Copenhagen, Denmark.

The results obtained by the fractionation of the Worthington trypsin are shown in Fig. 1. The presence of three distinct fractions is clearly shown, and all of them are active against azo casein. In the conditions of these experiments, however, the slower fraction (F_1) showed no activity towards BAA, while the faster fractions (F_2 and F_3) are active. The activity of each separated fraction is

Table 1

	Worthington trypsin				Novo trypsin			
	T*	F_1	F_2	F_3	T*	F_1	F_2	F_3
Proteolytic activity† (azo casein)	4·70	6·85	3·90	1·10	7·4	8·35	2·4	1·0
Amidasic activity‡ (α-benzoyl-L-arginine amide)	0·62	0·71	0·10	0·0	1·10	1·20	0·35	0·15
Ratio (proteolytic to amidasic)	7·8	9·5	39	—	6·7	6·9	6·8	6·7

* Unfractionated trypsin.

† The specific activity is given as the slope, multiplied by 10^3, of the straight line obtained by plotting the absorbance at 440 mμ against trypsin concentration in micrograms per millilitre.

‡ Same as above but plotting absorbance at 620 mμ.

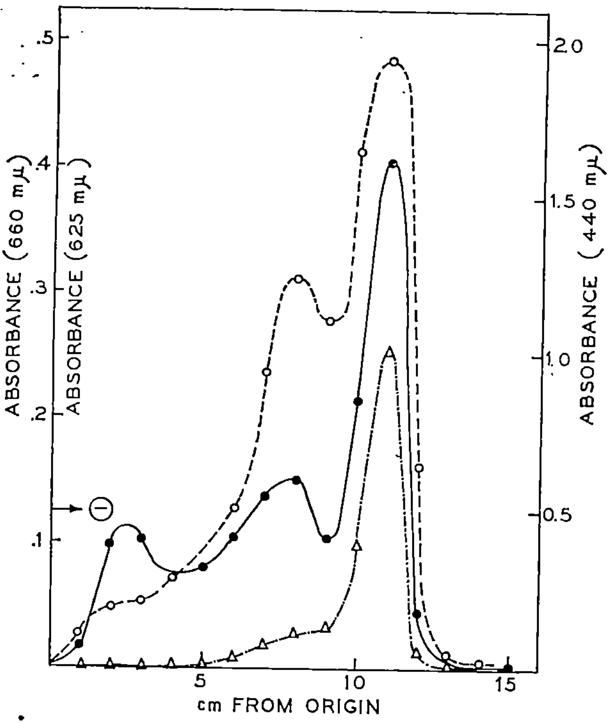

Fig. 1. Paper electrophoresis of a sample of Worthington trypsin. ●, Protein value (absorbance at 660 mμ); ○, proteolytic activity (absorbance at 440 mμ); △, amidasic activity (absorbance at 625 mμ)

given in Table 1 and shows that the specific activity of F_3 for both substrates is higher than that of the unfractionated enzyme.

The fact should be noted that the ratio obtained by dividing the proteolytic specific activity by the amidasic activity is different for each fraction. These results were observed only with the sample of Worthington trypsin.

These findings are consistent with those obtained by the electrophoretic fractionation of this same sample of trypsin in starch columns at *p*H 2·7 (ref. 3) which showed that the peaks representing the maximum of proteolytic activity and amidasic activity are not coincident.

The fractionation of Novo trypsin is presented in Fig. 2 and shows the appearance of only two fractions while the presence of a third slower fraction is only indicated. The specific activity of these three fractions is also given in Table 1 together with the calculated ratios of proteolytic activity to amidasic activity. These ratios for the sample of Novo trypsin examined are the same for all fractions obtained.

Electrophoresis of both samples of trypsin were also carried out in the presence of stabilizing concentrations of calcium. In this case calcium chloride was added to the *p*H 2·7 buffer to obtain a 0·05 M concentration of Ca^{++}. The trypsin samples were dissolved in this calcium-containing buffer and incubated for 2 h at 37° C before electrophoresis. The fractionation diagrams obtained by electrophoresis of the calcium-treated samples of trypsin were in all respects similar to those recorded in Figs. 1 and 2.

In order to test a possible degradation of trypsin during the electrophoretic run the main fraction (F_3) was extracted, freeze dried and again submitted to the same electrophoretic fractionation process. The results of this experiment are recorded in Fig. 3 and show the presence of only one fraction which was found to be active against both substrates.

A search for degradation products was carried out by treating with ninhydrin the paper strip after the electrophoretic run. This test was also negative and showed that no autolysis products reacting with ninhydrin were formed during electrophoresis.

These experiments tend to demonstrate that no degradation or formation of artefacts takes place during electrophoresis in the conditions employed in this investigation.

The appearance in the Worthington trypsin of fractions giving different ratios of proteolytic to amidasic activity could be explained by gross contamination of this sample with some other proteolytic enzyme such as chymotrypsin. However, the possibility of contamination with chymo-

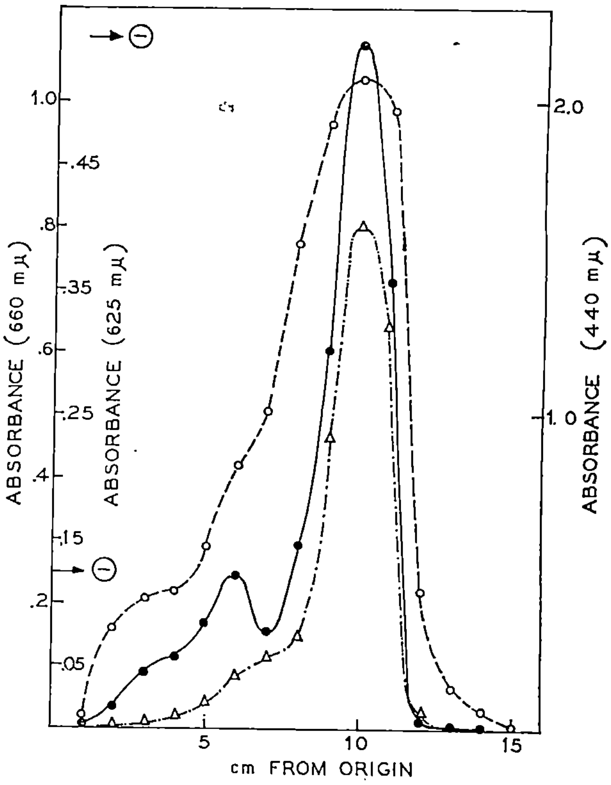

Fig. 2. Paper electrophoresis of a sample of Novo trypsin. ●, Protein value (absorbance at 660 mμ); ○, proteolytic activity (absorbance at 440 mμ); △, amidasic activity (absorbance at 625 mμ)

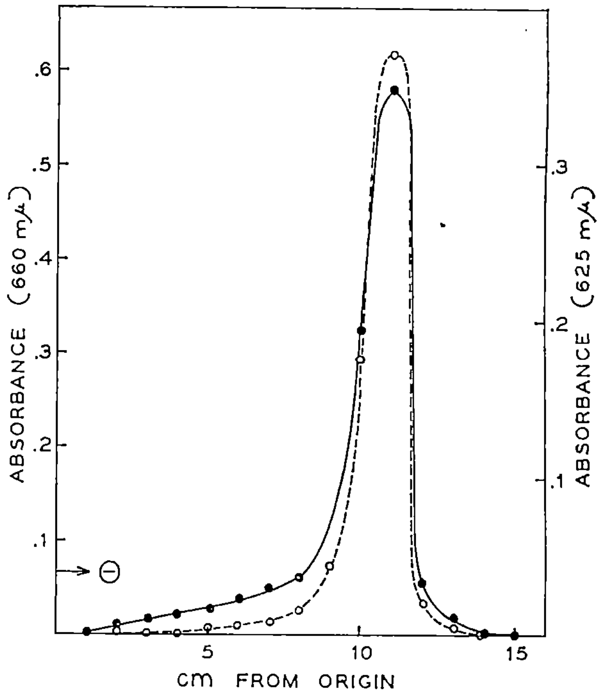

Fig. 3. Re-electrophoresis of the main fraction from the sample of Worthington trypsin. ●, Protein value (absorbance at 660 mμ); ○, amidasic activity (absorbance at 625 mμ)

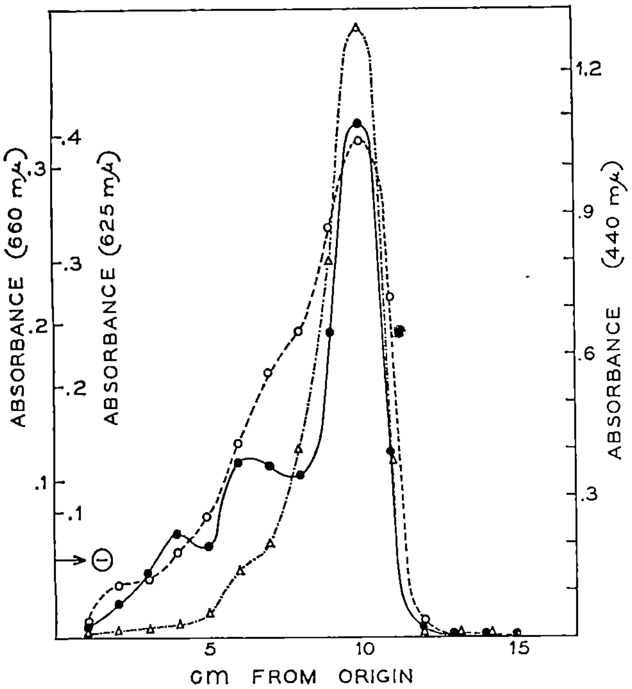

Fig. 4. Paper electrophoresis of the main trypsin fraction obtained by chromatography in carboxymethylcellulose. ●, Protein value (absorbance at 660 mμ); ○, proteolytic activity (absorbance at 440 mμ); △, amidasic activity (absorbance at 625 mμ)

trypsin was ruled out by paper electrophoresis at *p*H 2·7 (ref. 1). At this *p*H, chymotrypsin remains at the application point while trypsin migrates towards the anode, and when the Worthington trypsin was analysed by this method no proteolytic activity was found at the origin.

In order to correlate the results obtained with the present method of fractionation with those of Liener[8] using chromatography in carboxymethyl cellulose (CMC) the main fraction obtained from the Novo trypsin by this method was investigated by paper electrophoresis at *p*H 4·9. Prior to electrophoresis the main chromatographic fraction was dialysed in the cold against *p*H 2·7 pyridine formate buffer and freeze dried. The data obtained from this fractionation are recorded in Fig. 4 and show that the main fraction resulting from the chromatography of trypsin in CMC is heterogeneous when analysed by paper electrophoresis at *p*H 4·9. The fractionation diagram presented in Fig. 4 is similar to those obtained in the same conditions with the unfractionated enzyme.

The presence in crystalline trypsin of fractions each one showing a different ratio of proteolytic activity to amidasic activity was unexpected. One possible explanation for the observation would be the occurrence of limited autolysis of the enzyme during isolation. Changes in the specificity of trypsin following autolysis were observed by Bresler *et al.*[10]. Hakim *et al.*[11] have also obtained experimental evidence indicating that changes can occur in the specificity of trypsin following limited autolysis of this enzyme. The work of Viswanatha and Liener[12] is also pertinent to this question in showing that the specificity of the active fragment resulting from the peptic activation of acetyl trypsinogen was different from that of trypsin.

We have suggested in a previous article[3], based on the observations of Liener[8] and Gladner and Folk[9], that limited autolysis of trypsin resulting in the cleavage of lysine bonds near the trypsin C-terminal residue could account for the occurrence of the observed heterogeneity.

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FRACTIONATION OF RIBONUCLEIC ACIDS

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THE fractionation of ribonucleic acids (RNA) on a new type of exchanger in which guanine residues were linked to cellulose has been previously reported[1]. The results indicated that the RNA was attached to the exchanger by means of hydrogen bonds. Other workers have since described similar systems[2]. This work has now been extended to the study of an exchanger in which a derivative of uracil was attached to cellulose. This was made by treating partially acetylated cellulose with 5-hydroxymethyluracil[3] in the presence of dilute acid, and then removing the acetyl groups by mild alkaline hydrolysis. The procedure was as follows:

Cellulose acetate (British Celanese, Ltd.; 2·4 acetyl groups per glucose unit; 125 g) was allowed to stand in 7·5 per cent nitric acid (1 l.) at room temperature for 19 h with occasional stirring. The product was filtered off, washed with water until free from acid and dried in the air at 90°–100°. It contained 1·8 acetyl groups per glucose unit. This partially acetylated cellulose (13 g) and 5-hydroxymethyluracil (8 g) were suspended in dimethylformamide (80 ml.) containing 2 per cent (w/v) of dry hydrogen chloride. The mixture was shaken at room temperature until complete solution was obtained and then this was allowed to stand at 50° for 40 h. The solution was cooled, poured into sodium bicarbonate solution (200 ml.) and the resulting precipitate filtered off, and washed alternately with hot water and hot ethanol until the washings contained no material absorbing in the region 230–300 mμ. The product at this stage contained one 5-hydroxymethyluracil (HMU) residue per 5 glucose units (determined by hydrolysis with boiling N hydrochloric acid and determination of the 5-hydroxymethyluracil spectrophotometrically). The material was suspended in acetone (200 ml.) and water (about 10 ml.) added until a homogeneous solution was obtained and then 2 N sodium hydroxide (50 ml.) added. This hydrolysed off the acetyl groups. The resulting gelatinous precipitate was washed with 0·5 M sodium chloride and stored in suspension. The yield was 40 per cent, and the product contained one 5-hydroxymethyluracil residue per

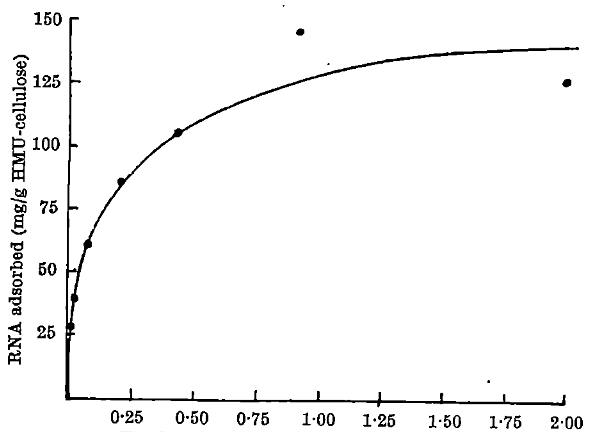
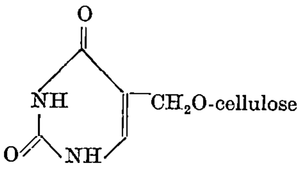


Fig. 1. Adsorption of yeast *m*RNA on HMU-cellulose (1 mg/ml. of RNA soln.) at 0° in 0·5 M NaCl. Solution allowed to equilibrate for 18 h

9 glucose units. It appeared that the more highly substituted molecules were soluble in water and were removed by the washing procedure. Several batches of this exchanger were prepared, and the procedure was found to be reproducible.

The exchanger (HMU-cellulose; I) adsorbed yeast microsomal RNA (*m*RNA)[4] from solution in 0·5 M sodium chloride and the amount adsorbed depended on the concentration of the RNA (Fig. 1). Material which still contained the acetyl groups did not adsorb the *m*RNA, neither did nylon nor polyacrylamide gel. The RNA could be eluted from the exchanger by lowering the ionic strength and/or increasing the temperature.



Attempts were made to fractionate yeast *m*RNA by the use of HMU-cellulose at 0° by elution with a gradient of decreasing salt concentration, but the time taken to reach equilibrium at this temperature was too long.

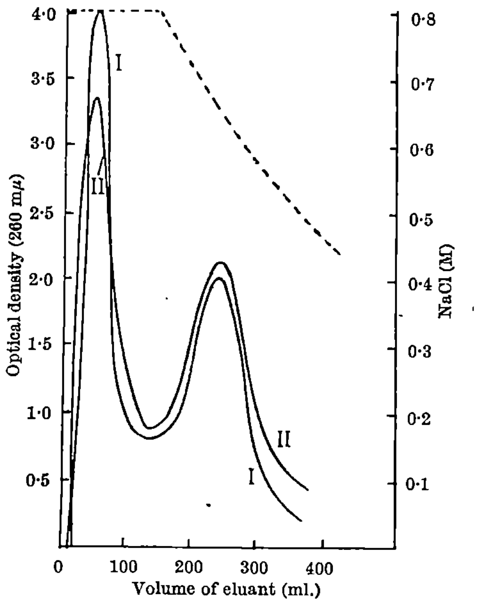


Fig. 2. Fractionation of *m*RNA on HMU-cellulose at 26°. The *m*RNA (30 mg) in 0·8 M NaCl was adsorbed on the column (18 cm × 1·8 cm diam.). Eluted at 7·5 ml./h 10 ml. fractions collected. I = yeast *m*RNA; II = pig liver *m*RNA (separate fractionations); ------, salt gradient

Fractionations of yeast *m*RNA and pig liver *m*RNA were carried out at 26°, but there was no appreciable difference between the nucleic acids from the two sources (Fig. 2). When the fractionation was carried out at 16°, however, there was a considerable difference (Fig. 3). The fractionations were also carried out by elution at constant ionic strength (0·35 M sodium chloride); small differences were found (Fig. 4), but it did not appear to be possible to separate the two ribonucleic acids from each other. The amount of RNA retained was apparently dependent on the amount of salt passed down the column as well as on the concentration. The ribonucleic acids were fractionated by this process into a strongly retained and a weakly retained fraction. This was a true fractionation as shown by the fact that the same proportion of the RNA was retained when different amounts of yeast *m*RNA were

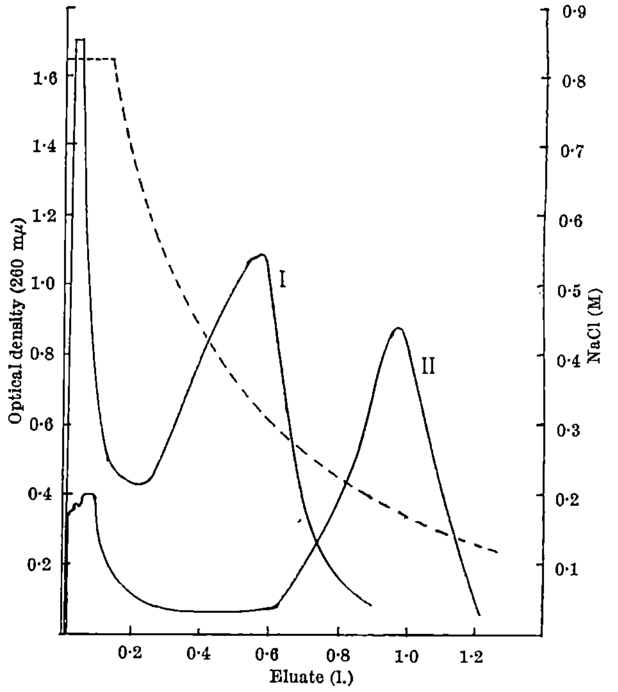


Fig. 3. As for Fig. 2. Temp. 16°

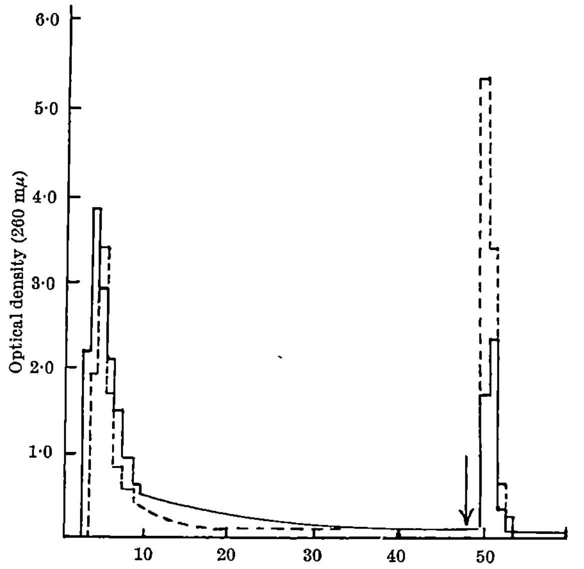


Fig. 4. Fractionation of *m*RNA on HMU-cellulose. 12 mg of *m*RNA adsorbed on the column (18 cm. × 1·8 cm diam.), eluted with 0·35 M NaCl and then with water (the arrow shows the start of elution with water) at 16°. 10 ml. fractions collected. Flow rate 7·5 ml./h. ——, yeast *m*RNA; - - -, pig liver *m*RNA (separate fractionations)

Table 1. COMPOSITIONS OF FRACTIONS OF MICROSOMAL RNA ELUTED FROM HMU-CELLULOSE

	Moles of base per 100 nucleotides																
	Yeast *m*RNA							Pig liver *m*RNA					*Serratia marcescens* *m*RNA				
	Original	F_1*			F_2*			Original	F_1*		F_2*		Original	F_1*		F_2*	
Guanine	26·5	26·8	25·6	27·7	24·3	25·5	26·5	34·5	33·6	34·0	35·2	33·5	30·2	30·0	30·6	30·2	28·9
Adenine	26·4	23·7	24·7	23·5	27·4	28·0	27·1	19·0	18·6	18·7	17·4	18·9	26·6	25·3	24·8	29·2	27·4
Cytosine	21·2	22·3	22·3	21·9	22·1	21·0	20·4	29·7	30·6	30·9	30·5	30·8	22·0	23·2	23·3	21·2	22·5
Uracil	26·0	27·2	27·4	26·8	26·2	25·7	26·0	16·8	17·3	16·4	16·9	17·3	21·2	21·5	21·3	19·3	21·1

For each fractionation, 25 mg of *m*RNA was adsorbed on a column of HMU-cellulose (18 cm × 1·8 cm diam.) and eluted with 0·35 M NaCl at 10°–11° (except second fractionation of *S. marcescens* *m*RNA when temp. was 16°). Flow rate 7·5 ml./h.

* F_1 = the first 37 per cent of the RNA eluted; F_2 = remainder of the RNA (eluted with water). Each column indicates an independent fractionation. For the base analysis one hydrolysis was carried out on each fraction and three chromatograms run for each hydrolysis.

fractionated on the same column and that when one tube of a fractionation was removed and refractionated it was far more homogeneous than the original *m*RNA (Fig. 5).

Samples of *m*RNA from yeast, pig liver and *Serratia marcescens* were fractionated on HMU-cellulose into a fraction eluted with 0·35 M sodium chloride and a fraction retained by 0·35 M sodium chloride but eluted by water. The temperature was kept constant (± 1°) by means of tap water; but there was a variation in the actual temperature (10°–16°) according to the time of year. The fractions so obtained were analysed for purines and pyrimidimes by hydrolysis with N hydrochloric acid, and separation of the nucleotides and bases by paper chromatography[5].

Table 2. STATISTICAL ANALYSIS OF THE RESULTS SHOWN IN TABLE 1

Significance of the differences in base content of the 1st and 2nd fractions.

(*a*) Yeast *m*RNA
  (1) Student's *t* test.
    Adenine, $t = 24{\cdot}3$ (2 deg. freedom) $P < 0{\cdot}002$.
    Uracil $t = 3{\cdot}83$ (2 deg. freedom), $P < 0{\cdot}1$.
  (2) Variance ratio test.
    Adenine, $F = 24{\cdot}0$ (5 and 12 deg. freedom) $P < 0{\cdot}001$.
    Uracil $F = 7{\cdot}4$ (5 and 12 deg. freedom) $P < 0{\cdot}05$.
No significant difference for guanine and cytosine contents.

(*b*) *S. marcescens* *m*RNA. Variance ratio test.
  (1) 1st fractionation (at 11°).
    Adenine $F = 81{\cdot}6$ (1 and 4 deg. freedom) $P < 0{\cdot}001$.
  (2) 2nd fractionation (at 16°).
    Adenine, $F = 87{\cdot}2$ (1 and 4 deg. freedom) $P < 0{\cdot}001$.
No significant difference for guanine, cytosine or uracil contents.

(*c*) Pig liver *m*RNA. No significant differences.

The results (Table 1) showed that in the case of yeast and *Serratia marcescens* *m*RNA there was a difference in the adenine contents of the first and second fractions (for statistical analysis see Table 2) but not in the case of pig liver *m*RNA. As was expected, with a column consisting of uracil residues the fractions containing the higher amount of adenine were the more strongly retained, indicating that the uracil residues were interacting with the adenine residues in the RNA. In support of this it was found that polyadenylic acid was retained strongly by HMU-cellulose and that polyuridylic acid was only weakly retained (Fig. 6). On this basis, however, it would have been expected that pig liver *m*RNA would have been retained less strongly than yeast *m*RNA when actually the opposite was the case. It appeared that the secondary structure of the *m*RNA was of importance, because if the *m*RNA was heated to 100° and slowly cooled before fractionation there was a change in the elution pattern; the pig liver *m*RNA was more strongly retained and the yeast *m*RNA less strongly retained by the HMU-cellulose. In the case of the yeast *m*RNA also no significant difference in the composition of the fractions could be detected; this showed that the fractionation depended on the method of isolation and storage of the *m*RNA.

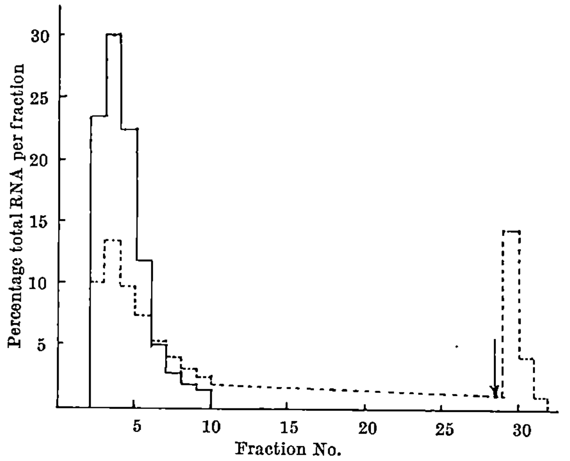


Fig. 5. Refractionation of yeast *m*RNA 25 mg of *m*RNA adsorbed on HMU-cellulose column (18 cm × 1·8 cm diam.) and eluted at 16° with 0·35 M NaCl and then with water (arrow shows start of elution with water). 10 ml. fractions collected. Flow rate 7·5 ml./h. Fraction 4 isolated and refractionated under the same conditions. - - -, original *m*RNA; ——, refractionation of fraction 4

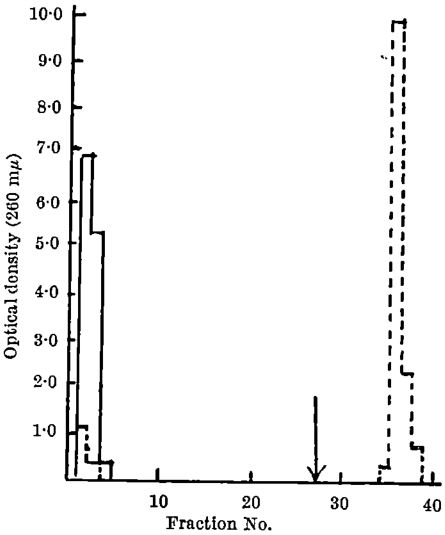


Fig. 6. Adsorption of polyribonucleotides on HMU-cellulose. - - -, polyadenylic acid (11 mg) adsorbed on the column (18 cm × 1·8 cm diam.) and eluted with 0·35 M NaCl and then with water (arrow shows start of elution with water). ——, polyuridylic acid (5 mg) adsorbed as above and eluted with 0·35 M NaCl. Temp. 13° in both cases. 10-ml. fractions collected. Flow rate 7·5 ml./h

Fractionations were also carried out on commercial yeast RNA under the same conditions except that M sodium chloride was used for the elution. In this case it appeared that the preparation would be more heterogeneous and that secondary structure would be of less importance. The results (Table 3) show that there were large differences in the composition of the fractions

Table 3. FRACTIONATION OF COMMERCIAL YEAST RNA ON HMU-CELLULOSE

	Moles of base/100 nucleotides			
	Original RNA	F_1	F_2	F_3
Guanine	27·18 ± 0·45	24·19 ± 0·24	26·84 ± 0·76	28·43 ± 0·37
Adenine	26·20 ± 1·85	21·10 ± 0·96	29·28 ± 0·44	30·56 ± 0·30
Cytosine	20·16 ± 0·46	22·85 ± 1·41	19·48 ± 0·13	18·82 ± 0·60
Uracil	26·24 ± 1·53	31·85 ± 1·24	24·39 ± 0·07	22·20 ± 0·67

Conditions for the fractionation were the same as described in Table 1 except that M sodium chloride was used for the elution. The commercial yeast RNA was supplied by Boehringer and Soehne, Mannheim.

F_1 = first 5 per cent of the RNA eluted: F_3 = last 5 per cent eluted (eluted with water); F_2 = 18 per cent of the total RNA eluted (eluted with M NaCl) before the last 5 per cent. The fractionation pattern was similar to Fig. 4 except that 95 per cent of the material was eluted with the M sodium chloride.

obtained, so that it can be concluded that polyribonucleotides can be fractionated on HMU-cellulose. In this case the adenine-rich fractions were retained as expected.

We thank Prof. M. Stacey for his advice and the British Empire Cancer Campaign (Birmingham Branch) for financial support.

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SELECTIVE EXTRACTION OF A PROTEIN FRACTION FROM WOOL KERATIN

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THE reaction of tetrakis (hydroxymethyl) phosphonium chloride (THPC) with keratin has recently been examined by several authors[1-4]. We have now extended the scope of the previous work and have also investigated the reactions of a number of other organophosphorus compounds with the disulphide bonds of wool keratin. Since it has been shown previously that reduction in a solvent system which swells the wool fibre and thus promotes the diffusion of a reagent offers a method for the almost complete removal of disulphide bonds, we have investigated the reaction of several organophosphorus reducing agents in anhydrous formamide.

Samples of conditioned wool top (20° C, 65 per cent relative humidity) were treated for 24 h at 20° C and a 50/1 liquor/wool ratio with solutions or suspensions containing 5 per cent w/w (large excess) of either THPC, *tris* (diethylaminomethyl) phosphine (DEAMP), triethylphosphite (TEP), triphenylphosphine or tetraphenylphosphonium chloride in anhydrous formamide. The percentage disulphide reduction of each sample was estimated amperometrically[6] and found to be 70, 98–99, 97–98, 64–66 and 17–20 per cent respectively. The reductions with DEAMP and TEP were afterwards examined in greater detail.

The most interesting feature of this work is that we have been able to extract preferentially a protein located between the cuticular cells of the wool fibre. This protein has a distinctive amino-acid composition, and a brief description of its isolation and properties are of interest. After reduction of the wool with DEAMP in formamide, the liquor containing the extract was diluted with water and the solution afterwards treated with potassium iodoacetate at *p*H 8 to prevent the re-oxidation of sulphydryl groups. The extract, after dialysis against distilled water and freeze-drying, gave a protein residue amounting to 2–3 per cent of the initial weight of wool. The S-carboxymethyl protein was only completely soluble in aqueous solution above *p*H 10.

Amino-acid analysis of the hydrolysate shows the protein to be unusual in containing abnormally high percentages of glycine (27 per cent)*, tyrosine (11 per cent), phenylalanine (6·7 per cent) and tryptophan (1·7 per cent) and a low content of S-carboxymethyl cysteine. It is apparent from electron microscopic studies of the reduced fibre that this protein is located in the membrane complex between the cuticular cells of the wool fibre (Fig. 1). In fact, there is a tendency for these cells to be released from the fibre as is evident in Fig. 1. Furthermore, the method offers possibilities worth exploring for the isolation of cuticular cells for subsequent analysis. From the structural point of view the extraction appears to be selective and quite reproducible while the microfibrillar texture remains intact (Fig. 2). However, even after complete reduction, the extraction is incomplete, for in a few regions the membrane system is not completely removed.

A very heterogeneous protein fraction of similar composition[7] has also been isolated from wool by total extraction with urea-2-mercaptoethanol and fractionation by DEAE-chromatography. Moreover, Gillespie and Harrap[8,9] obtained a protein fraction which had a high tyrosine and glycine content and was insoluble in aqueous

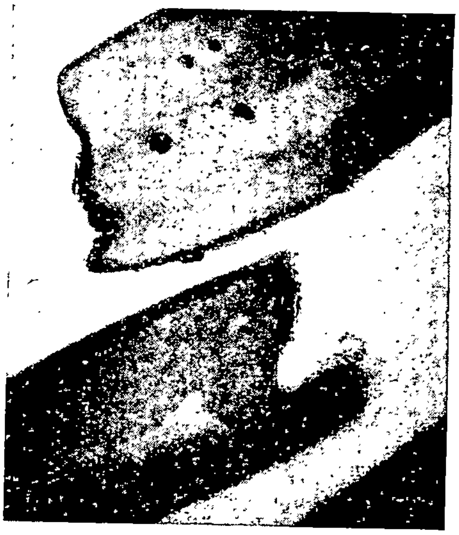

Fig. 1. Transverse section of merino wool cuticle treated with DEAMP in formamide and subsequently stained in silver nitrate (× 48,000)

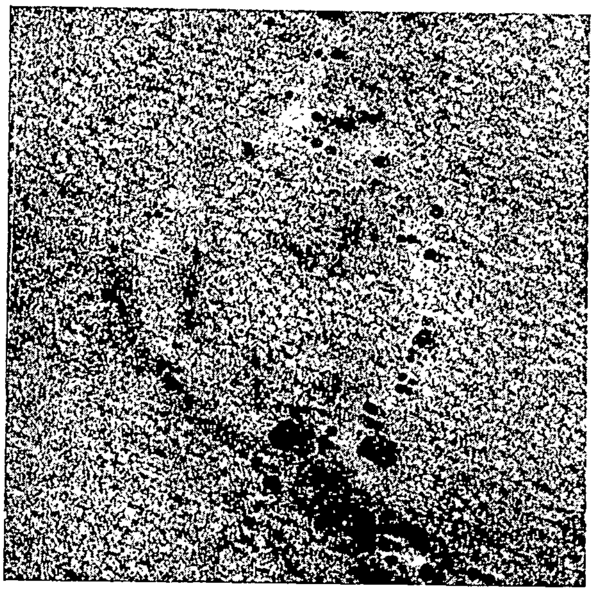

Fig. 2. Transverse section of merino wool treated as in Fig. 1 (× 200,000)

solution except at high pH values. It is therefore tempting to suggest that these proteins all have a common origin within the wool fibre.

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* Percentages are based on the total nitrogen content of the protein hydrolysate.

It has been suggested[10] that tryptophan and tyrosine degradation may be responsible for the yellowing of wool under the action of sunlight. Our treatment, which removes a protein fraction containing a relatively high amount of these amino-acids, may therefore reduce the yellowing of wool by sunlight.

We have also investigated the reduction of disulphide bonds in wool with 0·3 M aqueous solutions of THPC and DEAMP at 40° in the range pH 1–9. Contrary to the results published by Jenkins and Wolfram[2] it was found that the rate of reduction increases with pH up to pH 5 and thereafter remains approximately constant. Using the foregoing conditions (pH 3–5), virtually quantitative reduction can be achieved in approximately 4 h. The trialkyl phosphines thus appear to be the most effective reducing agents so far described for reduction of disulphide groups in acid media.

It had previously been assumed[3] that reduction by aqueous THPC was associated with the absorption of the phosphonium ion on the fibre. However, we have been unable to detect any phosphorus above a level of about 0·02 per cent in samples reduced either in aqueous[2] or formamide media.

Reduction of model disulphides with tertiary phosphines can give rise to either thiol[11,12] or lanthionine-type[13] derivatives, depending on whether reductions are carried out in aqueous or non-aqueous media respectively. Under our experimental conditions we have found that DEAMP or TEP reductions of wool in formamide solution of disulphides in wool produce 30–64 μM lanthionine/g wool, while reduction in aqueous solution at pH 1–8 gives almost no lanthionine. However, Zahn and Vassiliadis[1] have observed lanthionine formation in aqueous media and at higher temperatures. We thank Mr. L. M. Dowling for determinations of lanthionine.

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CRYPTORCHISM IN AUSTRALIAN MERINO SHEEP

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DURING an investigation of the inheritance of cryptorchism in medium wool Australian merinos, rams with only one or neither testicle descended into the scrotum, or with descended testicles of unequal size, were observed in several flocks.

Several workers have quoted the incidence of bilateral and unilateral cryptorchids in sheep. Glembockii and Moiseev[1] reported that approximately 8 per cent of cryptorchid lambs observed in the Valahian and the Précoce merino were unilateral, the left testicle being descended in each case. The number of unilateral cryptorchids was 14; it is likely that these were included in a total of 23 reported later[2], in 18 of which the left testicle had descended. Kochetkova[3] reported that approximately 10 per cent of the cryptorchids in another Précoce flock were unilateral, but did not record which testicle had descended. Wendt, Pohl and Mrosk[4] reported that most cases of cryptorchism which they observed in the mutton merino were bilateral.

The definition of cryptorchism used here is that of Sisson and Grossman[5], that is, retention of one or both testicles in the abdominal cavity or inguinal canal. In the 6 unilateral and 2 bilateral cryptorchids which have been slaughtered the missing testicles have been located in the abdominal cavity. Other animals have been scored by palpation of the scrotum, and any testicle not descended has been assumed retained in the abdominal cavity.

Cryptorchid rams have been inspected in medium wool flocks at Cunnamulla, Queensland, and in the New England district of New South Wales. These rams came from both horned and polled flocks (Table 1, *a*, *b* and *c*). Each polled ram would have been either homozygous or heterozygous for the dominant gene for polledness[6]. The numbers quoted cannot be used to estimate the relative frequencies of abnormal rams among those of the two horn types, because a random sample of flocks was not taken.

In the Cunnamulla flocks, 41 cryptorchids (85 per cent of the total) were unilateral, and in 40 of these the left testicle had descended. The ratio 40 : 1 differs significantly ($P < 0{\cdot}0001$) (ref. 7) from the 1 : 1 ratio expected if the side of descent were determined at random.

Table 1. NUMBERS OF MEDIUM WOOL MERINO RAMS OBSERVED WITH TESTICULAR ABNORMALITIES

Abnormality	No. of rams					
	Cunnamulla, Queensland			New England, N.S.W.*		
	Horned	Polled	Total	Horned	Polled	Total
No testicle in scrotum						
(*a*) Neither testicle descended to scrotum or palpable	1	7	8	0	20	20
One testicle in scrotum						
(*b*) Left testicle descended to scrotum, right testicle not palpable	8	32	40	0	3	3
(*c*) Right testicle descended to scrotum, left testicle not palpable	0	1	1	0	3	3
(*b*) or (*c*) One testicle descended to scrotum, other testicle not palpable	—	—	—	2	7	9
Two testicles in scrotum						
(*d*) Left testicle larger than right	8	2	10	N.R.†	N.R.	N.R.
(*e*) Right testicle larger than left	10	2	12	N.R.	N.R.	N.R.

* In the New England flock, groups (*b*) and (*c*) were scored as one group except at one lambing.

† N.R., not recorded, but may have been present.

Table 2. Location of Testicles in Normal Male Merino Fœtuses

Interval, service to slaughter (days)	Male fœtuses						
	No. observed	Testicles		Right kidney		Right testicle	
		Internal to inguinal ring	External to inguinal ring	Anterior* to left	Level with left	Anterior§ to left	Level with left
50	2††	2	0	0	0	0	0
60	3††	3	0	1	0	0	1
70	2†	2	0	1	0	1	0
75	1	1	0	1	0	1	0
80	2	0	2	2	0	—	—
85	5	0	5	5	0	—	—
90	2	0	2	2	0	—	—
100	3	0	3	3	0	—	—
105	4	0	4	4	0	—	—

* Right kidney is not completely anterior to left, but anterior surface of right kidney is anterior to anterior surface of left kidney.

§ Right testicle is not completely anterior to left, but anterior surface of right testicle is anterior to anterior surface of left testicle.

No observations were made on the symmetry of kidneys or testicles of unfrozen fœtuses or of those twisted slightly during freezing. The number of daggers indicates the number of such fœtuses in a group.

In the New England flock, 15 cryptorchids (43 per cent of the total) were unilateral. The 6 unilateral cryptorchids of one lambing only were examined in detail. In 3 lambs the left testicle had descended, in 3 the right.

To determine the fœtal age at which testicles migrate from the abdominal cavity to the scrotum in normal merinos, ewes bearing fœtuses of known ages were slaughtered. Each ewe had been removed from the mating pen 24 h after her first service was recorded. To prevent movement of organs during dissection, most fœtuses were frozen on removal and dissected while thawing.

Details of the observations are given in Table 2, the indication being that testicles pass through the inguinal ring between 75 and 80 days after conception.

Only one case has been reported in which a testicle might have descended after birth. In the flock of a co-operating breeder, a polled ram recorded by him as a bilateral cryptorchid at several weeks of age was observed with the left testicle descended at 1·5 years. No case has been observed of the withdrawal of a testicle after birth. Unless withdrawal takes place *in utero* between 80 days and full term, cryptorchism in merino rams probably results from the failure of one or both testicles to descend at 75–80 days of fœtal age.

The reproductive organs of vertebrates develop in association with the kidneys and their ducts[8]. In 8 adult merino rams slaughtered, the right kidney has been completely anterior to the left in each case; this is in general agreement with the situation described by Sisson and Grossman for adult sheep. An asymmetry was observed also in the kidneys of 19 male merino fœtuses (Table 2).

In the 3 of these which were aged 75 days or younger, the relative positions of the testicles were observed. In the youngest the two testicles were level, but in the other 2 the right was partly anterior to the left.

Asymmetry of testicles in the fœtus could be associated with the tendency of the left testicle to descend in unilateral cryptorchids in the Cunnamulla flocks. The left testicle probably begins to migrate before the right. The resulting asymmetry prior to actual descent to the scrotum would give the right testicle farther to travel during descent. As a consequence it may meet a mechanical or physiological block, such as the occlusion of the inguinal canal or a hormonal interference, which the left testicle evades. Retention of both testicles could be due to such a block acting on both sides, or to some other cause.

Among rams in which both testicles have descended but which are different in size, there is no indication that one is consistently larger than the other (Table 1, *d* and *e*). Factors producing asymmetry in testicle size thus seem to differ from those producing cryptorchism in Cunnamulla flocks.

As the investigation of the inheritance of cryptorchism progresses, it is hoped to investigate the migration of testicles in male fœtuses which have a known probability of developing into cryptorchids.

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IMMUNOLOGICAL CONTROL OF FERTILITY IN FEMALE MICE

By Dr. R. G. EDWARDS

Physiological Laboratory, University of Cambridge

THE advantages and possibilities of an immunological method for the control of fertility have been discussed repeatedly[1]. The recent publication by Dr. Anne McLaren[2] on the immunological control of fertility in mice prompts the present communication of my results in this field and on the same species. I have examined fertilization in female mice immunized with homologous spermatozoa. Various schedules of immunization have been used, from a few subcutaneous or intraperitoneal injections of spermatozoa to two successive immunizations with spermatozoa emulsified in complete Freund's adjuvant followed by many subsequent immunizations with spermatozoa. The major intention has been to find out whether prolonging the immunization course causes progressive decline in fertilization rates.

For immunization, spermatozoa were stripped from the vas deferens of outbred adult mice, and between 10 and 50 × 10^6 fresh unwashed spermatozoa were injected at each immunization into outbred female mice. Three schedules of immunization were carried out. (1) Nine mice were given 3–5 subcutaneous and intraperitoneal injections of spermatozoa in saline at weekly intervals. (2) Twenty-one mice were given an initial subcutaneous injection of spermatozoa in Freund's adjuvant, followed by 2–5 subcutaneous and intraperitoneal injections of spermatozoa in saline at weekly intervals. (3) Twenty-one mice were given two courses of spermatozoa in Freund's adjuvant at an interval of two weeks, and after a further two weeks were injected intraperitoneally with spermatozoa in saline every four or five days. These mice received 3–11 subsequent intraperitoneal injections of spermatozoa, excluding the two initial injections with adjuvant. In groups 2 and 3, the adjuvant/spermatozoa emulsion was injected into four separate sites on the back at each immunization. Control mice were given identical courses of treatment, but substituting saline for sperm suspensions.

Between 6 and 36 days after the end of the immunization schedule, the female mice were paired with males of proved ability, and examined next morning for mating (judged by the presence of a vaginal plug). Immunization did not affect mating performance, the proportion mating on the first night of pairing being similar in the immunized and control groups. Females which mated were killed by ether between 12 noon and 5 p.m. on the same day, bled from the heart and the uterus and Fallopian tubes removed. The uterine contents were examined, special attention being given to any spermatozoa therein. Living eggs liberated from the ampulla of the Fallopian tubes were examined for evidence of fertilization under a phase-contrast microscope[3]. In all mice except six, the eggs could be classified as fertilized or unfertilized without difficulty. In the remaining six (two immunized, four controls) several eggs were unpenetrated but living spermatozoa were present in the perivitelline space or between the cumulus cells, and the remaining eggs had only just been penetrated. Most or all of the eggs would presumably have been fertilized in these mice. A few mice were autopsied 2–3 h after mating for detailed inspection of the uterine contents.

Table 1. DETAILS OF THE IMMUNIZED AND CONTROL MICE

	Group 1*		Group 2*		Group 3*	
	Immunized	Control	Immunized	Control	Immunized	Control
Total No. of mice	9	17	20	13	21	16
No. which mated	8	14	20	13	19	12
No. with all or most eggs fertilized	3+2†	10+2†	9	11+2†	11	8
No. of eggs fertilized	38/48	83/91	74/76	88/105	85/88	54/57
No. with many eggs unfertilized ('partially fertile')	2	1	6	0	5	3
No. of eggs fertilized	3/20	2/7	22/50	–	13/39	8/20
No. with all eggs unfertilized ('sterile')	1	1‡	2	0	3	0
No. of eggs fertilized	0/11	0/8	0/17	–	0/25	–
No. failing to ovulate	0	0	2	0	0	1
No. with eggs activated parthenogenetically	0	0	1	0	0	0

* See text.

† In these mice, active spermatozoa were present among the cumulus cells or in the perivitelline space of many unfertilized eggs, and the penetrated eggs were in the early stages of fertilization. All or most eggs would presumably have been fertilized in these mice.

‡ Spermatozoa were recovered from the uterus of this female. The male concerned was under test at the time of mating, and it was afterwards excluded from the experiment.

Sera obtained from the mice at the time of killing were examined for the titres of spermagglutinins and spermimmobilisins. For spermagglutination, the medium used consisted of equal amounts of medium 199 and phosphate-buffered Hanks's saline (pH 7·1) with a final concentration of 0·1 per cent glucose. For spermimmobilization the same medium plus fresh guinea pig serum as a source of complement was employed. Fresh complement was necessary for this test, the complement being previously titred against mouse spermatozoa to avoid the effects of fresh serum on spermatozoa[4,5]. All tests were read twice. at intervals of approximately 15–30 min and 60–90 min. All sera were coded before the tests, read as unknowns, and decoded after the final titration had been decided.

Of the 47 immunized mice which mated, six ('sterile mice') had unfertilized eggs only, and in 13 others ('partially fertile') more than half of the eggs were unfertilized and living spermatozoa were not found in the perivitelline space of these eggs or between the cumulus cells. No eggs were found in two mice, and one mouse had eggs which had evidently been activated parthenogenetically. Unfertilized eggs were also found in control mice, but more rarely than after immunization (Table 1). The infertility after immunization was not correlated

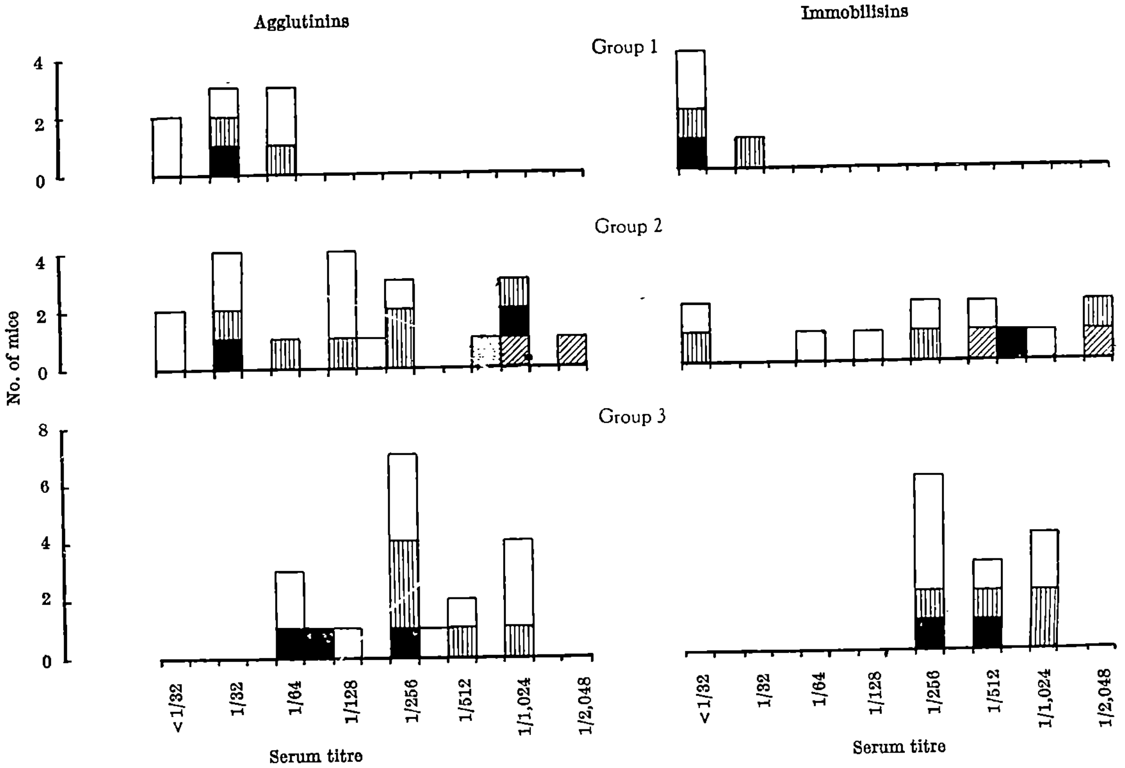

Fig. 1. Relationship between serum titres of spermagglutinins and spermimmobilisins and fertility in the three groups of immunized mice. Black, sterile; vertically hatched, partially fertile; obliquely hatched, failed to ovulate; stippled, eggs activated parthenogenetically; white, most or all eggs fertilized

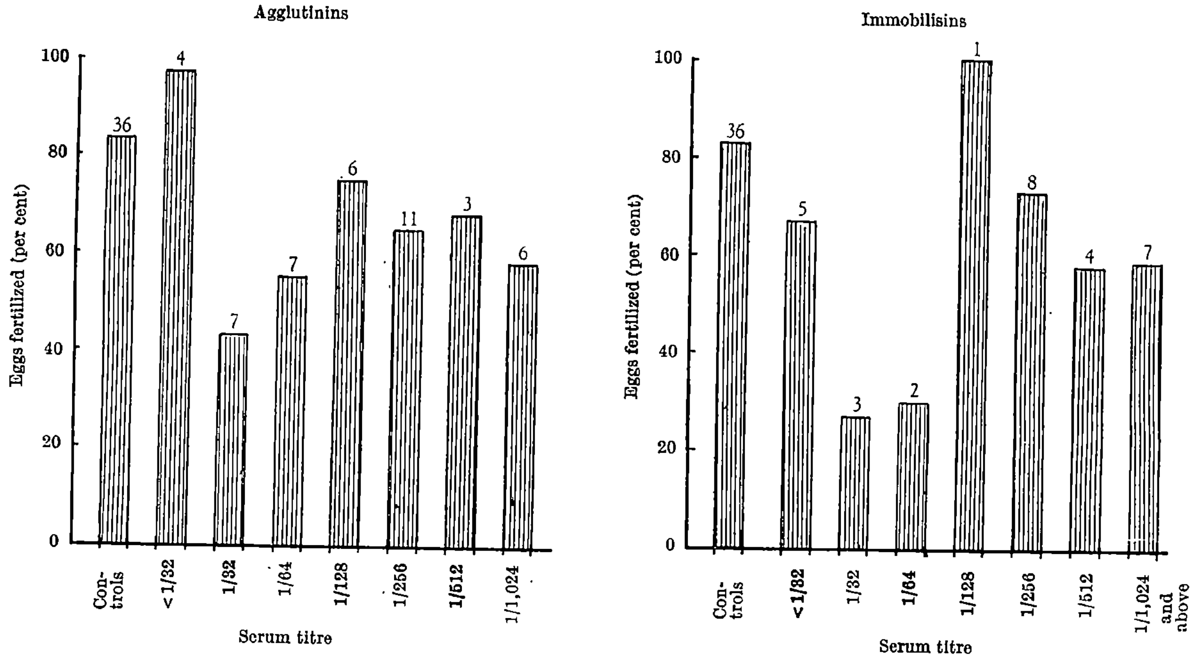

Fig. 2. Relationship between the serum titres of spermagglutinins and spermimmobilisins and the incidence of fertilized eggs. Data from the groups of immunized mice are combined, the numbers of mice contributing to the data being shown in each column

primarily with the titres of circulating antibodies. With one exception, the sterile mice were among those with lower titres (Fig. 1); the exception had high titres, and was the only one of the sterile mice with spermatozoa in the uterus (see following). The partially fertile mice were, in general, among those with higher titres, as were the non-ovulators and the mouse with eggs activated parthenogenetically. But a high serum titre did not necessarily lead to infertility, for all eggs were fertilized in several mice with high titres. The clearest relationship between circulating antibody titres and infertility was found in group 2 (Fig. 1); but this was not confirmed in group 3. When results from all three groups were combined, no consistent trend in the incidence of unfertilized eggs with increasing amounts of circulating antibody could be found (Fig. 2).

Examination of uterine spermatozoa supported the conclusion that circulating antibodies were not necessarily involved in the cause of the infertility. Typical immune spermagglutination was found in none of 36 immunized mice (serum titres between <1/32 and 1/1,024) with uterine spermatozoa. The spermatozoa were clearly separate—more separate indeed than in *in vitro* samples of epididymal spermatozoa—except for occasional large clumps attached by their heads, which is not typical of immune spermagglutination. The absence of agglutination could be especially seen when the uterine spermatozoa were still motile. It is known that antagglutinins in the female tract abolish non-immune head agglutination[6], and agglutination caused by heteroantibodies[7], but we have failed to find such abolition using sperm autoantibodies *in vitro*. Mice with high serum titres of spermimmobilisins often possessed active uterine spermatozoa and/or fertilized eggs, and even the addition of guinea pig complement failed to inactivate the spermatozoa. Serum antibodies thus either failed to reach the uterus or did so in small amounts[8]. The relative absence of serum proteins from the uterus could also be inferred from the remarkable immune-like reactions between spermatozoa and fresh serum from adult animals, which often result in lysis and inactivation of spermatozoa[5].

McLaren's evidence[2] that the numbers of spermatozoa reaching the ampulla are correlated with serum spermagglutinin titre, taken with our evidence of the absence of these antibodies from the uterus, suggests that any effect of these antibodies must be expressed in the Fallopian tubes. Whether the antibodies are secreted into the Fallopian tubes, infiltrate from the peritoneum, or enter with the follicular fluid is not known. Small amounts of antibody in the Fallopian tubes would presumably have a greater effect on fertility than similar amounts in the uterus because the volume of the tubes is considerably less and fewer spermatozoa are found in them. Such a mechanism could have accounted for the infertility in nine of the partially fertile and one of the sterile mice, each of which had high serum titres, many spermatozoa in the uterus, and few or no spermatozoa round the eggs. It seems unlikely that spermimmobilisins are involved, for mouse complement was considerably less efficient than guinea pig complement in immobilization tests *in vitro*. Spermimmobilization and spermagglutination tests evidently measured different antigens on spermatozoa[9], for mice with high serum titres in one test did not necessarily have high titres in the other.

A further cause of infertility was indicated from the examination of the uterine contents of the mice. In our experiments, infertility was evidently associated with the more rapid removal of spermatozoa from the uterus. When uterine spermatozoa were classified as present or absent, and if present as large, medium or small in number, differences became apparent between mice of differing fertility (Table 2). Five of the six sterile mice had no uterine spermatozoa. The somewhat arbitrary classification of mice into those with motile and non-motile uterine spermatozoa also hinted that differences existed between the groups (Table 2). Whether this loss and/or inactivation of spermatozoa was due to induced uterine anaphylaxis[10], to an increased rate of phagocytosis, or some other reason is not known; these effects were certainly not correlated with the titres of circulating antibody. It may not be coincidental that the degree of testicular destruction in guinea pigs and mice after immunization with testis or spermatozoa also bears little or no relationship to the amounts of circulating antibodies[11].

In our experiments, immunization of mice with spermatozoa and adjuvant has not led to drastic reductions in infertility as found by other workers[12] in guinea pigs. It is difficult to make comparisons between species when the

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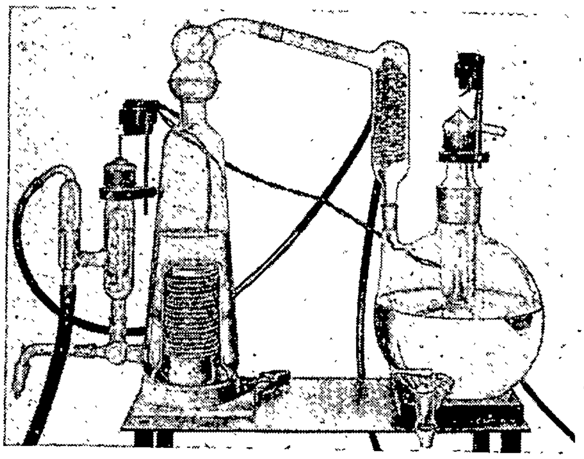

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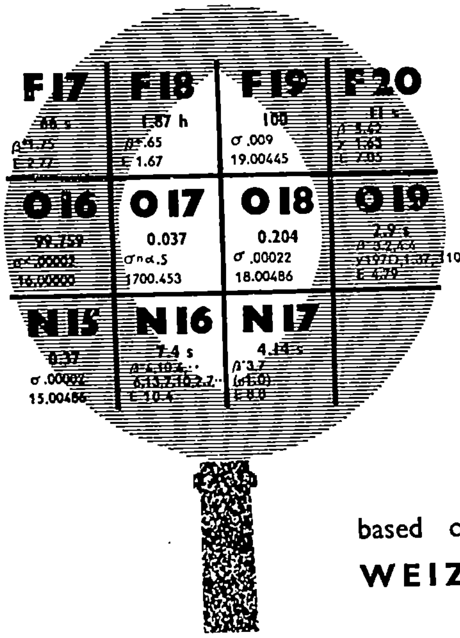

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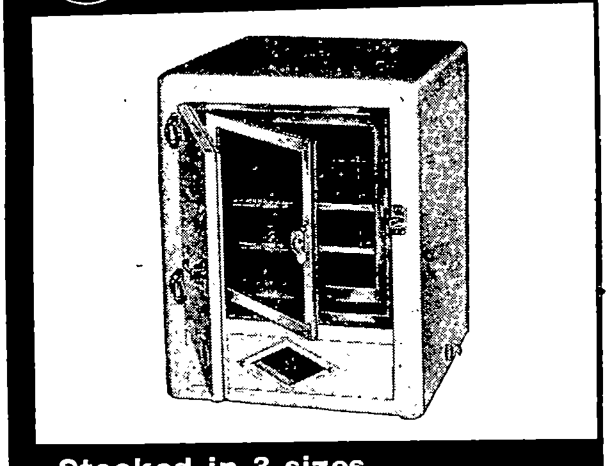

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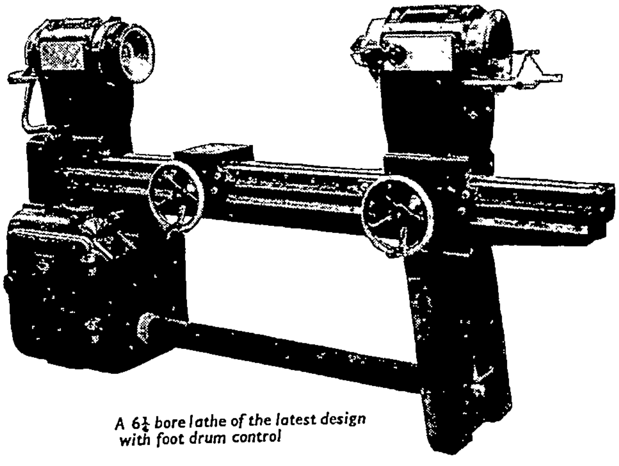

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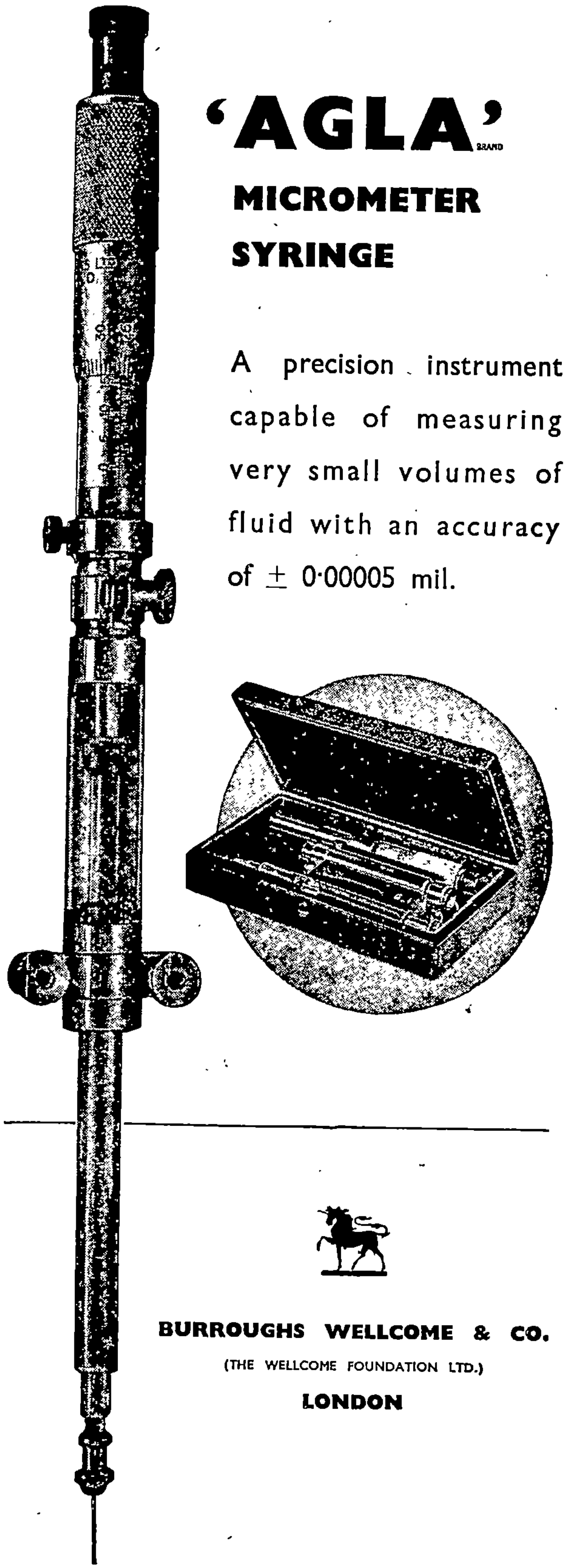

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Table 2. No. and Motility of Uterine Spermatozoa in the Immunized and Control Mice

	Control mice	Immunized mice		
		With most eggs fertilized	Partially fertile	Sterile
No. of mice	33	25	13	6
Mice with no uterine spermatozoa	7	2	4	5
No. of uterine spermatozoa in remaining mice				
Large	17	15	3	1
Medium	5	3	4	0
Small	4	5	2	0
No. of mice with motile or immotile uterine spermatozoa*				
Motile	12	10	1	0
Immotile	8	12	8	0

* Some mice were not classified.

fertilization rate in the guinea pigs was unknown. The incidence of fertilization in our work was higher and more independent of the amount of circulating antibody than was the incidence of infertility found in mice by McLaren[2]. Differences existed between the two investigations in the immunization procedure, and it is possible that a more effective immune response was produced in McLaren's work by the prolonged intraperitoneal immunization. The high incidence of fertility remaining in mice after immunization indicates that an immunological method using spermatozoa for the control of fertility has not yet been realized.

This work was supported by the Ford Foundation.

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CÆSIUM-137 FALL-OUT BODY BURDENS, TIME VARIATION AND FREQUENCY DISTRIBUTION

By WILLIAM H. ELLETT and DR. GORDON L. BROWNELL

Physics Research Laboratory, Massachusetts General Hospital, Boston, Mass.

Any prediction of the hazard associated with fall-out depends on an assessment of both the average level of radioactivity in the exposed population and the variation in body burden between individual members[1,2]. Although results are regularly published on average body burdens[3], the difficulty of providing a sufficiently large sample size has precluded meaningful estimates of body burden variations. We have measured the cæsium-137 content of skeletal muscle samples from 878 persons who died between January 1959 and August 1963 and used the results to test for the likelihood of various frequency distributions.

Of the fission fall-out products, cæsium-137 is an ideal isotope for a study of this type. Because the turnover of cæsium in the body is relatively fast, 50–150 days[4], the adult population is essentially in equilibrium with the radioactivity of its environment. Skeletal muscle contains approximately 60–80 per cent of the body cæsium and is among the organs of highest cæsium concentration[5,6]. Estimates of cæsium-137 body burdens based on measurements of muscle activity agree with those made by means of total body γ-counting[7]. The convenience of counting a γ-emitting isotope allowed us to prepare a large number of samples with minimum handling and attendant risk of sample contamination. γ Spectroscopy on the 662 KeV γ from the barium-137 daughter was used to separate the different radioisotopes in the samples.

Muscle samples weighing 90–140 g were dissected from the thigh or lower abdomen during routine post-mortem operations at the Massachusetts General Hospital. Samples were not collected from patients who had received isotopes for medical purposes or died of highly infectious disease. Frozen tissue samples were dehydrated in a vacuum oven and packed in plastic tubes for counting in a 5 in. × 5·5 in. sodium iodide (Tl) well counter. A technical description of this apparatus has been published elsewhere[6]. For a typical sample the counting error was less than 15 per cent. Following counting, samples were defatted in a 2–1 mixture of chloroform and methyl alcohol and dried to constant weight at 102° C. Final counting data are expressed in picocuries (10^{-12} c.)/g of defatted dry muscle.

To determine how the average level of cæsium-137 activity varied throughout the period of sample collection, the results were averaged in serial sub-sets of 25 samples each. The average sample activity of each subset is shown by the crosses in Fig. 1. The mean sample activity as a function of time, as determined by fitting a fourth order polynomial to the 878 data points, is shown by the solid curve (increasing the order of the approximating polynomial did not significantly change this curve or the subsequent data analysis). The results were also analysed separately for each sex. The mean sample activity per gram of muscle for men was 10 per cent greater than that of women—a difference similar to that previously reported[9]. Since this difference is much smaller than the variability between individuals of the same sex, data for the two groups are combined in Fig. 1.

Following the bomb test moratorium in the autumn of 1958, the body burden of cæsium-137 as reflected in samples collected throughout 1959 was relatively constant. Starting in early 1960 there was a decrease in sample

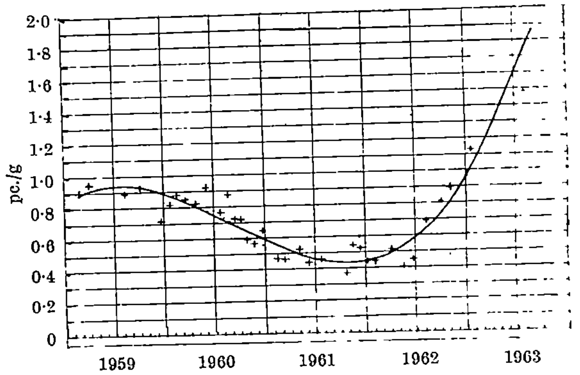

Fig. 1. Time variation of cæsium-137 in muscle. The crosses are average values for serial 25 sample sub-sets. The solid curve is a fourth-order polynomial fitted to the 878 data points

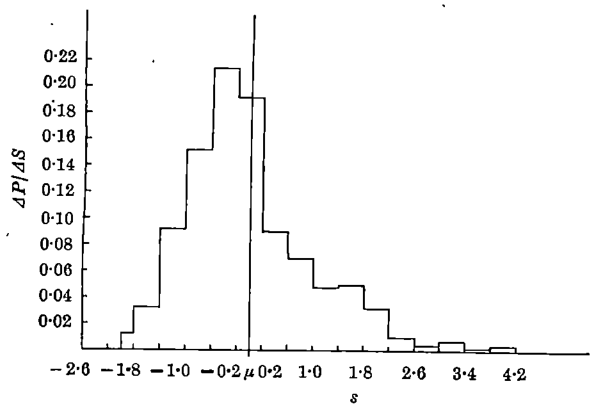

Fig. 2. Probability histogram of the first 670 samples plotted as a function of *s*, the relative standard deviation of the sample. For a normal distribution, the histogram would be symmetric about *μ*, the sample mean

activity which continued until January of 1961. During January 1, 1961–June 1, 1962, the mean levels of cæsium-137 activity in our samples was constant and at a minimum. During this period the average cæsium-137 concentration in muscle, based on 325 samples, was 0·47 (± 0·02) pc./g (dry defatted). This average concentration is probably close to the steady-state value for the amount of fission products, 62 megatons[10], introduced into the environment prior to the test moratorium.

Although atmospheric testing was resumed on September 1, 1961, a rising trend in sample activity was not seen until the summer of 1962. The delay was probably due to a variety of factors such as altitude, at which the nuclear debris was injected, the fact that most crops and fodder had been gathered by September and the time-lag in foodstuff distribution and use. Since mid-1962, the increase in activity has been quite rapid (Fig. 1). This increase will eventually be followed by another decrease as in 1960. If the geographical distribution of the 101 megatons[10] of fission products from the last period of atmospheric testing is not too different from that of tests prior to 1961, the final mean steady-state cæsium-137 concentration in muscle will be about 1·2 pc./g (dry defatted). This will result in an average total body burden of about 8 nc. It is expected that this level will be reached after a transient maximum in 1964–65.

J. Rundo has reported average cæsium-137 body burdens in Great Britain based on total body measurements of potassium-40/cæsium-137 ratios[4]. His results are compared with ours in Table 1. It is seen that the time variation in the two countries was quite similar while the absolute levels in Rundo's group were somewhat higher. Although there is some evidence that cæsium-137 body burdens are higher in Europe than the United States[10a], the difference seen in Table 1 may be due to differences in the two methods of measurement.

Table 1. Comparison of Time Variation of Average Cæsium-137 Body Burdens in Great Britain and North-eastern United States during 1959–63 (nanocuries)

	Rundo U.K.	U.S.A. calculated*		Rundo U.K.	U.S.A. calculated*
Jan. 1959	8·0	5·9	July 1961	5·5	3·0
July 1959	9·5	6·1	Jan. 1962	4·0	2·9
Jan. 1960	8·7	5·7	July 1962	4·5	3·9
July 1960	7·5	4·8	Jan. 1963	7·5	6·3
Jan. 1961	6·0	3·8	July 1963	—	10·4

* Assuming 63 per cent of the body cæsium-137 is in muscle (ref. 11): both sets of data are for a 70 kg standard man.

Before determining the frequency distribution of the samples, it is necessary to eliminate the time variation by normalizing the data in terms of standard scores. This was done by analysing the data in serial sub-sets of 50 samples each. The variance of each sub-set was calculated about a mean given by the polynomial in Fig. 1, and a histogram of the sub-set prepared in terms of relative standard deviation intervals, *s*. A grand histogram of the first 14 sub-sets, prepared by summing their histograms in terms of *s*, is shown in Fig. 2. The asymmetry of this distribution is obvious; in particular, the number of samples above the $+3s$-level and the lack of samples below the $-2s$-level indicate that these results are not part of a normally distributed parent population. A χ^2 test shows there is essentially zero probability that this sample set was derived from a normally distributed population ($\chi^2 = 200$ for 13 degrees of freedom).

In 1962, Yamagata[11] reported a log normal distribution of natural cæsium-133 in muscle. His results are based on 78 samples, and a visual analysis of a log transformed histogram. We find that most of the asymmetry of the distribution shown in Fig. 2 is removed by a log transformation. However, a χ^2 test on the transformed data gives a probability of less than 1 per cent that the parent population is log normal[12].

Another simple frequency distribution that can be used to describe an asymmetric unimodal data population is the Γ distribution. It has the limits of zero and infinity and is expressed by the function:

$$P(X) = \frac{1}{\beta^{\alpha+1}\Gamma(\alpha+1)} X^{\alpha} e^{-X/\beta}$$

where $P(X)$ is the probability density of a cæsium-137 concentration of X pc./g and α and β are the two parameters which describe a particular distribution. The parameter β serves mainly as a scale factor while α determines the skewness of the distribution[13].

Values of α for each of the 50 sample sub-sets have been calculated from the mean sample activity and subset variance[14]. The results, Table 2, indicate that the values of α are relatively constant and independent of the absolute level of sample activity. The solid curve in Fig. 3 is a histogram of the sample data scaled for comparison with a histogram of a Γ distributed parent population having the same α (ref. 15). A χ^2 test gives a probability of 0·25 that the data are from an infinite set having the same Γ distribution. In Table 2, it is seen that the mean α value for females is less than that for males, and the range of α values is reduced by a factor of 2 when the sexes are analysed separately. Although a *t* test for difference between means gives a 0·15 probability that this difference is due to chance, χ^2 tests show that the results are more adequately described by an appropriate Γ distribution when each sex is considered separately. A χ^2 test on the male data gives a 0·55 probability that it was taken from a parent population with $\alpha = 3·9$. The same test on the female data gives a 0·70 probability that it was taken from a parent population with $\alpha = 3·3$. Since the histogram shown in Fig. 3 ($\alpha = 3·5$) is a summation of two histograms, male and female, normalized to an average mean, the χ^2 probability increases when each set of data is normalized to its own mean.

Table 2. Calculated α Values for Serial 50 Sample Sub-sets

Both sexes and unknowns 878 samples				Males 500 samples		Females 345 samples	
Median time (days)	α	Median time (days)	α	Median time (days)	α♂	Median time (days)	α♀
131	3·6	878	3·6	165	4·0	284	3·8
292	4·3	966	3·5	441	5·5	518	3·4
428	4·5	1,105	2·2	549	4·1	716	2·9
491	3·8	1,145	1·2	668	3·3	859	4·2
558	2·7	1,229	3·7	775	2·1	1,109	2·0
638	3·7	1,312	7·2	917	3·0	1,264	4·0
692	5·6	1,385	3·0	1,109	3·1	1,476	2·9
738	2·1	1,481	2·6	1,228	4·2		
801	2·4			1,358	4·4		
				1,532	5·1		
	$\bar{\alpha} = 3·5 \pm 0·35$			$\bar{\alpha}$♂ $= 3·9 \pm 0·33$		$\bar{\alpha}$♀ $= 3·3 \pm 0·29$	

We believe the γ distribution can serve as a useful model for the distribution of cæsium-137 body burdens. It has reasonable limits and adequately describes the sample data over the range of observed values. Of particular importance is the observation that α was relatively constant throughout a five-year period in which the mean sample activity varied by as much as 300 per cent. This

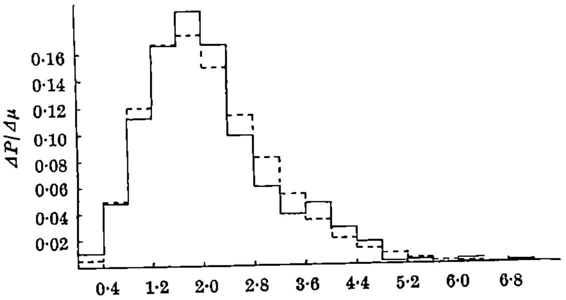

Fig. 3. The solid curve is a histogram of the sample data scaled for comparison with the γ distribution ($\alpha = 3·5$) shown by the dotted lines. The sample mean is at $2·12\mu$. $\mu = X/\beta\sqrt{\alpha+1}$

leads us to believe the Γ distribution can be used to predict what fraction of the population will have N times the mean activity, relatively independent of how this mean varies.

The U.S. Federal Radiation Council has suggested that a factor of three be used in determining permissible total population exposure risks, to allow for variations in individual body burdens[1]. Langham and Anderson[6] have reported that cæsium-137 body burdens are normally distributed, with a standard deviation of 36 per cent, and based hazard predictions on this model. Such a model can seriously underestimate the number of people receiving several times the average body burden. For example, using the model of Langham and Anderson approximately one person per fifty million would have a cæsium-137 body burden which exceeds 3 times the mean. For the asymmetric distribution of body burdens found in this investigation, approximately 75,000 persons per 50 million would exceed this criterion. At the present time, the average body burden of cæsium-137 is small enough that the asymmetry of the frequency distribution is not a critical factor. However, other fission fall-out products, notably strontium-90 (refs. 16 and 17), have similar asymmetric distributions. Depending on the average level of body burdens and the skewness of its frequency distribution, the factor of 3 allowance for population inhomogeneity may or may not be an adequate safeguard for all fall-out products.

We thank Prof. G. Wadsworth, Department of Mathematics, Massachusetts Institute of Technology, for his contributions to the statistical analysis, the Department of Pathology, Massachusetts General Hospital, for supplying the tissue samples, and the U.S. Public Health Service for financial support under grants *Rad C*–2187 and *AM* 05423–03.

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COMPARATIVE ROLE OF NITRATE AND MOLECULAR OXYGEN IN THE DISSIMILATION OF GLUCOSE

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MICRO-ORGANISMS may utilize nitrate via two markedly different biochemical pathways[1]. For assimilatory purposes, nitrate is routed through reduction to ammonia and appropriate amination reactions to cell protein synthesis. A vast spectrum of micro-organisms is capable of carrying out the foregoing assimilation reaction sequence. However, the dissimilatory route of nitrate utilization involves use of nitrate oxygen as the terminal acceptor of hydrogen and hence acts as the physiological oxidant by which organic substrates are oxidized to produce utilizable cell energy[2]. Thus, the dissimilation of nitrate is termed 'nitrate respiration'.

The quantitative or stoichiometric expression of the participation of NO_3^- oxygen in the catabolism of organic carbon remains to be clarified. Similarly the dual role of molecular and nitrate oxygen as electron acceptors is not understood. It is the purpose of this article to present results which clarify several aspects of the biochemistry of nitrate and molecular oxygen utilization by a typical denitrifier, *Pseudomonas stutzeri*.

A strain of *Pseudomonas stutzeri* isolated from soil was used throughout the work. The culture was maintained by weekly transfers on agar slants consisting of 0·5 per cent potassium nitrate, 1 per cent trypticase (Difco), 0·1 per cent dried yeast extract (Difco), 1·5 per cent Bacto agar (Difco) and distilled water. In all experiments involving glucose utilization the following basal medium was used: yeast extract (Difco), 100 mg; K_2HPO_4, 50 mg; $MgSO_4$ (anhydrous), 3·5 mg; $FeCl_3.6H_2O$ (0·0075 per cent), 1 ml.; MoO_3 (0·0075 per cent), 1 ml.; distilled water, 14 ml. Glucose was added at the rate of 140–180 mg/flask and nitrate at concentrations ranging between 250–1,000 p.p.m. nitrogen.

For obtaining cell yields, the culture was grown on bottle slants at 28° C using the foregoing growth medium. After growth for 36 h the surface cells were removed, washed in phosphate buffer and re-suspended in the basal medium. A uniform cell suspension was obtained by adjusting the cell mass to an optical density of 0·8 using a Bausch and Lomb Spectronic 20 colorimeter at the 630 mμ wave-length.

The electrolytic respirometer assembly described by McGarity *et al.*[3] was used. 100 g of washed sand (20 mesh) was evenly distributed in each 300 ml. respiration flask. The flasks, along with an empty cup in the centre well, were sterilized overnight at 15 lb. pressure. These were then inoculated with 14 ml. of sterilized basal medium containing the required cell mass. 1 ml. of sterile glucose solution brought the sand-medium mixture to 60 per cent of the moisture-holding capacity. 10 ml. of 20 per cent sodium hydroxide was transferred to the empty cup in the centre well of each flask. Then the top joint of the flask was greased and the head assembly put in place. Precautions to avoid contamination were followed, even though the concentrated cell mass minimized this danger. The aerobic sets were attached to the oxygen-generating electrolytic respirometer and flushed with a helium–oxygen (80–20 per cent) mixture for 15–30 min; whereas helium alone was used for anaerobic experiments. All experiments were carried out at a temperature of 28° ± 1° C. In the aerobic experiments oxygen uptake was calculated by noting the volume (ml.) of hydrogen generated and then dividing the latter values by the correction factor of 2. At various time-intervals flasks were taken out to test for nitrogen gas production. Gases evolved were detected using a Beckman *GC*-2 gas chromatograph.

At the end of each experiment the centre well containing the sodium hydroxide was removed and diluted to 100 ml. with carbon-dioxide-free distilled water. Carbon dioxide was determined by precipitation as barium carbonate. After removal of the cup from the centre well, sufficient distilled water was added to each flask to give a 2 : 1 ratio of water to sand. Then the flask was shaken on a New Brunswick type rotary shaker for 30 min at 240 r.p.m. After allowing sufficient time for the sand to settle, the liquid was centrifuged at 2,000–3,000 r.p.m. for 20 min. The supernatant was removed and analysed for residual glucose, nitrite–nitrogen, and nitrate–nitrogen according to standard methods[4–7]. Proper precautions were exercised in all analyses to eliminate the effect of any interfering ions.

The utilization of nitrate oxygen as the terminal acceptor of hydrogen by nitrate reducing and denitrifying micro-organisms represents a rather unique form of bacterial respiration. In such instances, oxidation of the carbon substrate may occur with nitrate as the [O] donor either in the presence or absence of molecular oxygen. However, as is shown in Figs. 1 and 2, increasing the concentration of NO_3^- from 250 p.p.m. to 1,000 p.p.m. results in a progressive decrease in the capacity of the test organism to utilize molecular oxygen. Experiments conducted with 1,000 p.p.m. NO_3^- gave no uptake of molecular oxygen until 36 h had passed. With 500 p.p.m. NO_3^-, a 10–12 h lag in oxygen uptake is apparent, whereas with 250 p.p.m. NO_3^- very little lag is obtained. It is clear that in such experiments we are observing both the simultaneous utilization of nitrate oxygen and molecular oxygen as terminal acceptors of substrate hydrogen; an observation which attests to the probable operation of dual electron carrier systems. In addition, it is evident that high concentrations of NO_3^- either via specific toxicity side-effects or via the preferential use of nitrate oxygen as the terminal acceptor can bring about a complete cessation of molecular oxygen uptake.

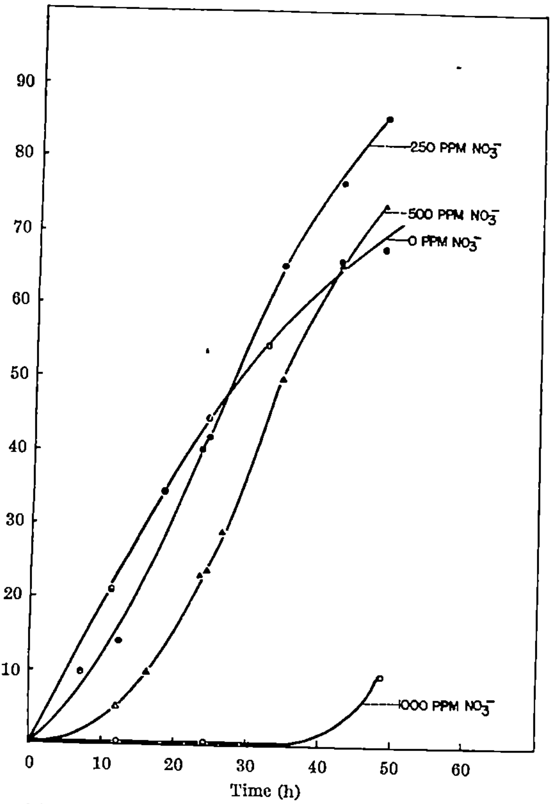

Fig. 1. Oxygen uptake in presence and absence of nitrate

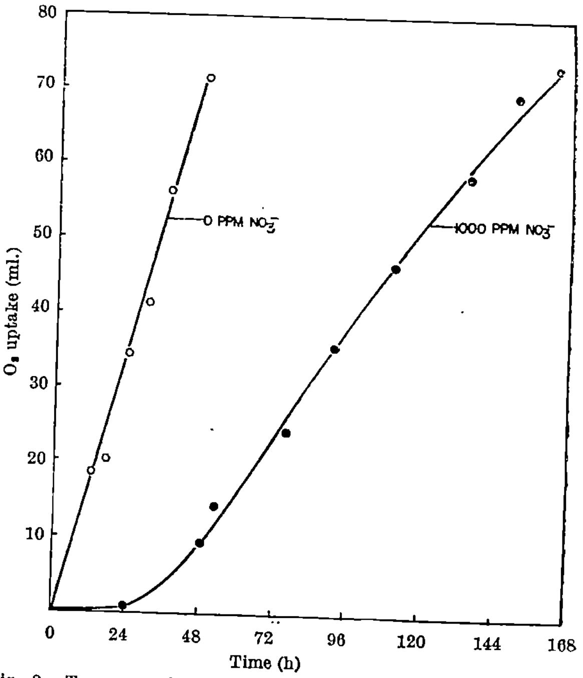

Fig. 2. Temporary inhibitory effect of nitrate on oxygen uptake

The results given in Table 1 show that, as with the decrease in oxygen uptake, there is also a concomitant decrease in carbon dioxide evolution on the addition of nitrate. Here the effect is also reversible, the length of the lag in evolution of carbon dioxide being determined by the concentration of nitrate. Aerobically, at the 48-h interval, we observe a two-fold decrease in millimoles carbon dioxide evolved when comparing 1,000 p.p.m. nitrate with the minus nitrate control sets. On the other hand, at 12 h under aerobic conditions, the 500 and 1,000 p.p.m. NO_3^- sets gave almost identical millimole values of carbon dioxide evolved. As was expected with no nitrate added to the system, the anaerobic examinations showed very low carbon dioxide evolution. A further point of significance of the data given in Table 1 may be obtained by comparing the carbon dioxide values at 250 p.p.m. NO_3^- under aerobic and anaerobic conditions. At the 12- and 48-h time-periods, the millimoles carbon dioxide evolved under anaerobic conditions is approximately half that obtained under aerobic conditions. This observation reflects the dual utilization of NO_3^- and molecular oxygen in the aerobic experiments and the resulting more complete oxidation of the glucose substrate. The lower values obtained for the anaerobic experiments suggests a greater accumulation of metabolic end products or increased assimilation of substrate carbon. Fig. 3 provides added evidence for the latter idea as illustrated by the percentage carbon dioxide produced from glucose at the 12- 24-, 36- and 48-h time-intervals. It is apparent that, with molecular oxygen as the terminal acceptor and no NO_3^- present, the percentage conversion of glucose to

Table 1. MILLIMOLES CARBON DIOXIDE EVOLVED PER MILLIMOLE GLUCOSE UTILIZED UNDER AEROBIC AND ANAEROBIC CONDITIONS IN PRESENCE OF NITRATE

Time (h)	NO_3^- (p.p.m.)	Aerobic	Anaerobic
12	0	3·76	0·57
	250	2·07	1·35
	500	1·70	—
	1,000	1·75	1·70
24	0	4·03	1·33
	250	2·42	—
	500	2·64	—
	1,000	1·87	1·60
48	0	4·37	1·72
	250	4·56	2·05
	500	3·44	—
	1,000	2·23	1·15

Table 2. TOTAL MILLIMOLES (O) USED AND CARBON DIOXIDE EVOLVED PER MILLIMOLE GLUCOSE UTILIZED IN PRESENCE OF NITRATE AND MOLECULAR OXYGEN

Time (h)	NO_3^- (p.p.m.)	Molecular (O)	NO_3^- (O)	Total (O)	m.moles CO_2	% contribution of NO_3^- (O)
12	0	3·90	—	3·90	3·76	0
	250	2·67	1·08	3·75	2·07	28·8
	500	1·07	1·05	2·12	1·70	49·3
	1,000	0	0·53	0·53	1·75	100·0
24	0	6·47	—	6·47	4·03	0
	250	4·48	0·90	5·38	2·42	16·7
	500	2·58	0·94	3·52	2·64	2 ·7
	1,000	0	0·65	0·65	1·87	100·0
48	0	7·96	—	7·96	4·37	0
	250	7·61	0·78	8·39	4·56	9·3
	500	6·58	0·85	7·43	3·44	11·4
	1,000	2·12	0·67	2·79	2·23	24·0

carbon dioxide is much higher than is indicated for 1,000 p.p.m. NO_3^- and molecular oxygen.

At this point in the investigation, it became necessary to ascertain the quantitative use of molecular and/or NO_3^- [O] for the oxidation of each millimole of glucose. Previous data had shown the dual utilization of both sources of acceptor oxygen. It remained to determine the percentage contribution of each oxygen source. An examination of the data in Table 2 provides evidence for the observation that the percentage contribution of NO_3^- oxygen is a linear effect of the degree of suppression of free oxygen utilization by *P. stutzeri*. For example, at 1,000, 500 and 250 p.p.m. nitrate the linear percentage contribution of [O] by NO_3^- is approximately 100, 50 and 25 per cent respectively, at the 12-h period. Between 24 and 48 h, the suppression of molecular [O] uptake is progressively decreased and the linear effect disappears. The lower percentage use of NO_3^- as a terminal acceptor at 48 h for each nitrate-level reflects the removal of the initial nitrate suppression of molecular oxygen uptake and the increased use of free oxygen as the electron acceptor. These data thereby supply further confirmation for the reversibility of the nitrate inhibitory effect on free oxygen utilization.

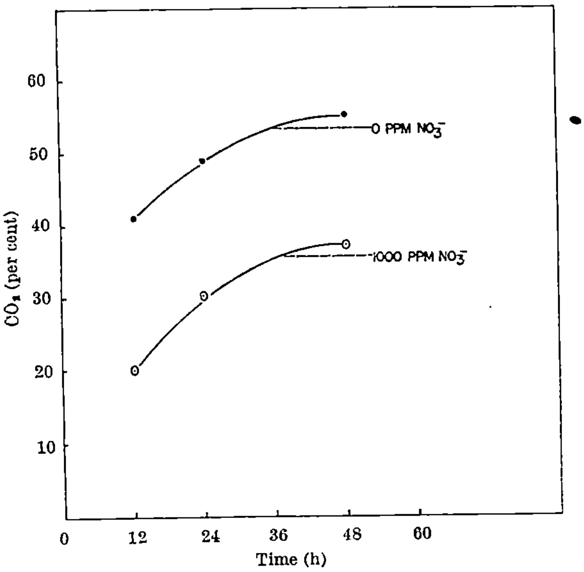

Fig. 3. Percentage conversion of glucose to carbon dioxide

An additional point illustrated by the data in Table 2 (total [O] versus total CO_2) relates to the observed values obtained for total oxygen required for the oxidation of carbon to CO_2; $C + 2\,[O] \rightarrow CO_2$. At 12 h the aerobic system minus nitrate gave an approximate 1 : 1 ratio of [O] to CO_2, thereby falling far short of the theoretical 2 : 1 ratio. It appeared probable that at 12 h the steady state of glucose oxidation had not been attained. However, at 24 h, the aerobic minus nitrate set gave an [O] to CO_2 ratio of 1·6–1 and at 48 h a ratio of approximately 1·8–1. These latter values approach the theoretical ratio of 2 : 1, indicating that in the absence of nitrate *P. stutzeri* quantitatively oxidizes glucose carbon to carbon dioxide and water with little accumulation of other metabolic end products. Similarly at 250 p.p.m. nitrate under aerobic conditions the 2 : 1 theoretical ratio is approached at all time-intervals. However, at 500 p.p.m. nitrate, an approximately 1 : 1 ratio held for 24 h; at 48 h the 2 : 1 ratio is regained. At 1,000 p.p.m. NO_3^-, the aerobic system gave extremely low observed [O] values at all time-intervals. It would appear, therefore, that with the higher (500 and 1,000 p.p.m.) NO_3^- levels a shift in glucose catabolism has occurred in the direction of a more incomplete oxidation of the substrate carbon.

When the data given in Table 2 are calculated so as to equate the total oxygen contributed by nitrate and free oxygen on the basis of glucose carbon oxidized to carbon dioxide, it is then possible to note the quantitative contribution of nitrate according to the following generalized equation:

$$C_6H_{12}O_6 + NO_3^- + O_2 \rightarrow (CH_2O)_n + CO_2 + H_2O$$

These data are shown in Table 3. At 12 h, the total millimoles [O] used gradually decreased from 1·84 at 250 p.p.m. nitrate to 0·32 at 1,000 p.p.m. nitrate. Likewise, at 24 h the total [O] value decreased from 2·32 at 250 p.p.m. nitrate to 0·35 at 1,000 p.p.m. nitrate. As shown previously there was no utilization of molecular oxygen at 12 and 24 h with 1,000 p.p.m. nitrate. It is also evident that the oxygen contributed at the 12- and 24-h periods by the added nitrate is approximately the same, 0·32 and 0·35 millimoles, respectively. It appeared, therefore, that the average value of 0·33 millimoles [O] represented the net contribution of nitrate nitrogen for the production of 1·0 millimole of carbon dioxide. To test this hypothesis, the 0·33 theoretical [O] value was added to the observed molecular [O] value and compared with the actual totals given in Table 3. It became clear that although some variation in the theoretical versus actual observed values was encountered, a remarkably close correlation was obtained; particularly during the steady state of glucose utilization 12–24 h. During the first 12 h the system was somewhat sluggish, and during the 24–48 h period glucose

Table 3. TOTAL MILLIMOLES (O) USED PER MILLIMOLE CARBON OXIDIZED TO CARBON DIOXIDE IN PRESENCE OF NITRATE AND MOLECULAR OXYGEN

Time (h)	NO_3^- (p.p.m.)	Molecular (O)	NO_3^- – (O)	Total (O)	% (O) contribution from NO_3^-–N
12	0	1·03	—	1·03	0
	250	1·31	0·53	1·84	28·8
	500	0·66	0·64	1·30	49·2
	1,000	0	0·32	0·32	100·0
24	0	1·60	—	1·60	0
	250	1·85	0·47	2·32	20·2
	500	0·98	0·37	1·35	27·4
	1,000	0	0·35	0·35	100·0
48	0	1·82	—	1·82	0
	250	1·67	0·18	1·85	9·7
	500	1·90	0·25	2·15	11·6
	1,000	0·98	0·33	1·31	25·6

was depleted with the 250 and 500 p.p.m. sets. Thus it appears that the 0·33 mean millimole [O] value does represent a usable index for *P. stutzeri*. The theoretical relationship can be written as follows:

$$\begin{tikzcd} & \text{Source} \arrow[dl] \arrow[dr] & \\ O_2 \arrow[d] & & NO_3^- \arrow[d] \\ C + 2/3\,[O] & + & 1/3\,[O] \rightarrow 1 \text{ millimole } CO_2 \end{tikzcd}$$

The role of NO_3^- as a donor of [O] in the terminal respiration of facultative anaerobes raises many questions. These relate primarily to the nature of the stepwise reduction of nitrate, the factors influencing nitrate reduction and the fate of the carbon undergoing oxidation. In the work recorded here an attempt is made to clarify certain aspects of the foregoing problem areas.

An initial point involves the dual utilization of NO_3^- [O] and molecular [O]. Since facultative anaerobes such as *P. stutzeri* can grow over a rather wide range in *Eh*, it is considered important to ascertain the extent of utilization of both hydrogen acceptors. The results of the present study establish the point that at low concentrations of NO_3^- both molecular [O] and nitrate [O] are used as final electron acceptors. However, with a concentration of 500–1,000 p.p.m. NO_3^-, there is a progressive decrease in the utilization of molecular [O]. The latter inhibition of molecular [O] uptake becomes a striking characteristic of the entire respiration system and is not easily explained. Toxicity of the formed NO_2^- does not appear to be the answer. Since the inhibition by NO_3^- of both oxygen uptake and carbon dioxide evolution disappears after approximately 48 h, it may be that the inhibition effect centres at some point or sites in the cell enzyme complex. However, as is shown in this investigation, under aerobic conditions NO_3^- [O] is used as the sole acceptor only when high concentrations are used. As the concentration of NO_3^- decreases from 500 to 250 p.p.m., a greater utilization of molecular oxygen occurs. It is evident that if a sufficiently high concentration of NO_3^- is used then a preferential utilization of this acceptor occurs. It may be that the explanation lies in the greater availability to the cell of NO_3^- oxygen versus the rather low concentration of dissolved molecular oxygen. This would clarify the progressive inhibition of oxygen uptake with increasing concentrations of nitrate and would also make plausible any accompanying shift in carbohydrate metabolism. This aspect of the work is now being investigated.

A further point worthy of discussion relates to the stoichiometric expression of 'nitrate respiration'. Over the years investigators have attempted to equate nitrate reduction and carbon oxidation. No definitive results were obtained, particularly in systems containing both molecular and bound NO_3^- oxygen. Yet with facultative anaerobes such as *P. stutzeri* both acceptors must be considered. In this regard, the electrolytic respirometer and companion closed flask assembly used in the present investigation provides an ideal apparatus to examine the aforementioned question. Two questions may be posed: What is the percentage contribution of NO_3^- [O] to the over-all oxidation of the carbohydrate in the presence and absence of molecular [O]? Secondly, what is the quantitative expression of the amount of molecular oxygen or NO_3^- [O] required for the production of one millimole of carbon dioxide? On a strictly theoretical basis, two atoms of oxygen are required to give one millimole of carbon dioxide. For the complete oxidation of one mole of glucose 12 atoms of oxygen are used to give six moles of carbon dioxide (a 2 : 1 ratio). The results given here make clear that *P. stutzeri*, under aerobic conditions, quantitatively oxidizes glucose to carbon dioxide and water with little or no accumulation of other end-products. However, with increasing levels of NO_3^- a progressive decrease in the theoretical [O] to CO_2 ratio occurs, indicating that under conditions of 'nitrate respiration' carbon dioxide plus other end-products accumulate. Here it is possible to observe a shift in carbohydrate breakdown peculiar to the use of NO_3^- as the final electron acceptor.

Of equal interest is the dual operation of NO_3^- and molecular oxygen. Although NO_3^- undergoes only a one-step reduction to NO_2^- in the presence of molecular [O], a considerable contribution is made by NO_3^- to the over-all oxidation of carbon. It appears evident that the one reductive step provides 0·33 millimoles of [O] for each millimole of carbon dioxide evolved. For each millimole of carbon dioxide produced, two-thirds of the total oxygen involved is derived from molecular [O] and one-third from NO_3^- [O]. It is clear, therefore, that with the organism under investigation the quantitative relationship of molecular to NO_3^- oxygen's role as dual acceptors is clarified.

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THERMAL EXPANSION OF PYROLYTIC GRAPHITE AND ITS VARIATION DUE TO NON-ALIGNMENT OF THE CRYSTALLITES

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PYROLYTIC graphite, normally prepared at about 2,200° C, shows a high degree of preferred orientation. However, subsequent heat treatment improves the alignment until, at 3,600° C, the material resembles a mosaic single crystal[1]. Now the thermal expansion of pyrolytic graphite is a property of some interest, since it permits a check to be made of values of the graphite lattice expansion deduced from X-ray diffraction data. It is the purpose of this article to demonstrate, first, that the thermal expansion coefficient of pyrolytic graphite depends markedly on the degree of crystallite alignment, and secondly, that it can be estimated from a knowledge of the degree of alignment and the graphite lattice expansion coefficient.

The material investigated was supplied by High Temperature Materials Inc., U.S.A., in the form of plates 4 in. × 3 in. × 0·25 in. It is understood that these samples

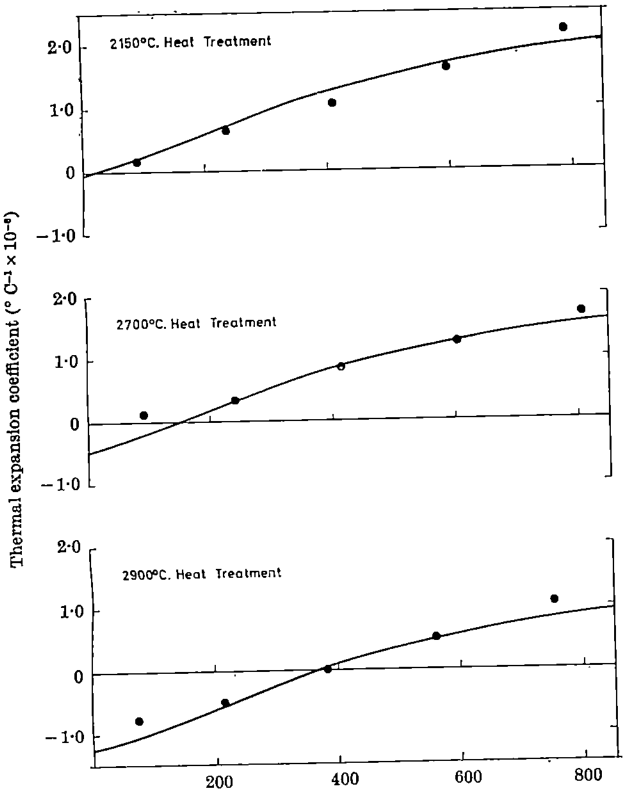

Fig. 1. Variation with temperature of the thermal expansion of pyrolytic graphite heat treated to 2,150° C, 2,700° C and 2,900° C. Measurements made parallel to the planes of deposition
●, Experimental; ——, theoretical

were manufactured at 2,150° C. Specimens were cut and then heat treated to 2,700° C and 2,900° C in an argon atmosphere, the final temperature being maintained for half an hour in each case. Expansion measurements were made over the temperature range 20°–1,000° C using the well-established technique employing tube and push rod, both being made of graphite. Displacements of the push rod were measured using a 'Precimeter' dial gauge. The degree of crystallite alignment was established by the method of X-ray diffraction, these measurements being made by Mr. E. A. Kellett and Mr. C. A. Wallace at the General Electric Co., Hirst Research Centre, Wembley. Measurements were made from forward-reflexion photographs of the tilt angle φ_0 defined as the limiting angular displacement of the crystallite c axes from the normal to the plane of deposition. The full angular distribution was not measured but was estimated in a manner to be indicated later.

The thermal expansion coefficient of a polycrystalline aggregate may, in certain circumstances, be described in terms of the orientation distribution of the crystallites[2]. For a layered structure such as pyrographite, the coefficients relating to directions parallel and perpendicular to the layer planes may then be written:

$$a_{/\!/} = a_a + \left(\frac{a_c - a_a}{2}\right) \cdot \frac{\int_0^{\pi/2} I(\varphi) \cdot \sin^3\varphi \, d\varphi}{\int_0^{\pi/2} I(\varphi) \cdot \sin\varphi \, d\varphi} \tag{1}$$

$$a_{\perp} = \frac{a_c \cdot \int_0^{\pi/2} I(\varphi)\cos^2\varphi \sin\varphi \, d\varphi + a_a \cdot \int_0^{\pi/2} I(\varphi)\sin^3\varphi \, d\varphi}{\int_0^{\pi/2} I(\varphi) \cdot \sin \cdot \varphi \, d\varphi} \tag{2}$$

where a_a and a_c are the graphite lattice expansion coefficients along the a and c axes; the angle φ is measured relative to the normal to the plane of deposition. and $I(\varphi)$ is the number of crystallite axes, per unit solid angle, lying at an angle φ. Use of these functions is justified only if each crystallite contributes fully to the behaviour of the polycrystal, and if there is no interaction between crystallites. It was asumed that pyrolytic graphite satisfied these conditions in view of its near single crystal character.

The distribution function $I(\varphi)$ can be closely approximated for this type of material by $\cos^n\varphi$, where n is an arbitrary parameter[3]. Integration of equations (1) and (2) then give:

$$a_{/\!/} = \left(\frac{n+2}{n+3}\right) a_a + \left(\frac{1}{n+3}\right) a_c \tag{3}$$

$$a_{\perp} = \left(\frac{n+1}{n+3}\right) a_c + \left(\frac{2}{n+3}\right) a_a \tag{4}$$

As n becomes large, equations (3) and (4) simplify to $a_{/\!/} = a_a$ and $a_{\perp} = a_c$, as would be expected for perfect crystal alignment. Values of n were estimated from the values of the tilt angle, φ_0, by adopting the convention that $\cos^n \varphi$ falls to 1 per cent of its peak value when $\varphi = \varphi_0$. The underlying assumption here is that the measured values of φ_0, which can be estimated to within about 1°, represent the true limit of the actual distribution curve. Values of n deduced in this way are given in Table 1, together with values of the tilt angle φ_0.

Table 1. ANGLE OF TILT FOR PYROGRAPHITE OF VARYING DEGREE OF PERFECTION

Treatment temperature (°C)	Tilt angle (φ_0)	n
2,150	41°	19
2,700	30°	32
2,900	8°	475

On substituting known values of a_a and a_c (refs. 4 and 5), $a_{/\!/}$ and $a_{\perp}$ can be calculated using equations (3) and (4). In Figs. 1 and 2 are shown the experimental values of

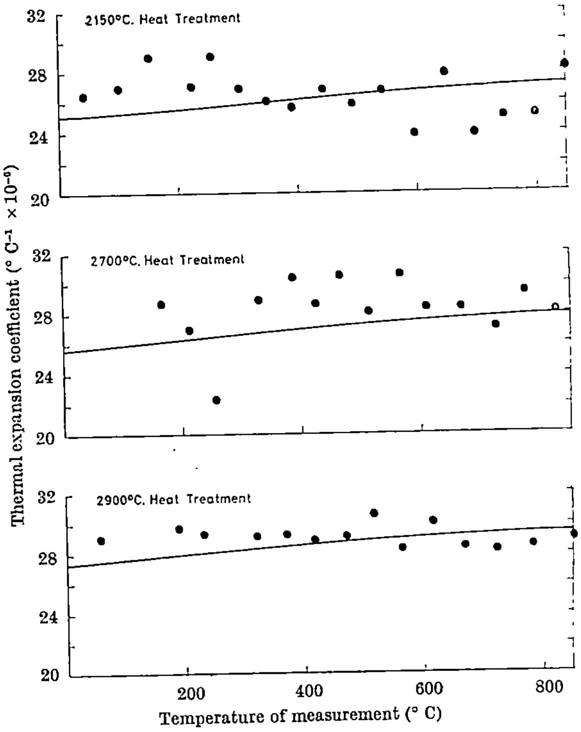

Fig. 2. Variation with temperature of the thermal expansion of pyrolytic graphite heat treated to 2,150° C, 2,700° C and 2,900° C. Measurement made perpendicular to the planes of deposition
●, Experimental; ——, theoretical

the expansion coefficients as a function of temperature, together with curves representing the expected behaviour of the coefficients as derived from equations (3) and (4). It will be seen from the values of φ_0 in Table 1 that the crystallite alignment does not improve rapidly until the treatment temperature exceeds 2,700° C. These changes are reflected in the thermal expansion of the material as illustrated in Figs. 1 and 2, and particularly as measured in the direction along the layer planes. In this respect the 2,900° C material is indistinguishable from a single crystal.

It is concluded that the good agreement obtained between the 'calculated' curves and the experimental values justifies calculations of expansion coefficients of highly oriented materials, based purely on the geometrical distribution or crystallites.

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EFFECT OF MANGANESE ON WATER CONTENT OF 'SOLAR' SALT

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SODIUM chloride produced by solar evaporation of sea-water at various localities contains varying amounts of included water, ranging from almost none to as much as 20 per cent by weight. We have observed that, in addition to such factors as temperature and rate of crystallization, the presence of manganese in the brine has an important effect on the amount of mother-liquor included in solar salt.

Manganese (II) has been used as an additive to obtain transparent sodium chloride crystals from aqueous solution[1-3] and to produce high-quality salt from sea-water by solar evaporation[4,5]. The addition of 40–500 p.p.m. Mn(II) to brine is sufficient for these purposes. Manganese below this concentration range has been reported to have no effect on the crystallization of sodium chloride[2]. Lead, mercury, cadmium and tin (II) also increase the transparency of sodium chloride[2].

Our observations differ from some reported in the literature. A saturated brine prepared from analytical-grade sodium chloride gave clear crystals even at fairly high rates of evaporation (temperatures up to 50° C), provided that the brine was well stirred; at a moderate rate of crystallization, stirring is not essential. The presence of divalent manganese (0·02–50 p.p.m.), lead (0·4–8 p.p.m.), divalent mercury (2–200 p.p.m.), cadmium (4–100 p.p.m.) and divalent tin (5–600 p.p.m.) in the brine resulted in the formation of mother-liquor inclusions in the crystals. Sodium chloride solutions containing these metals in concentrations outside these ranges gave clear crystals, as did solutions containing other metal ions, except iron (II, III), cobalt, and copper (II), which gave clear crystals in the range 0–5 mM (or up to about 250 p.p.m.).

The liquid inclusions due to manganese, mercury and tin form an hour-glass pattern, the axes of which coincide with the diagonals of the cubes (Fig. 1), indicating adsorption on the 111 planes. The pattern becomes obscured as the number of inclusions increase or the growth rate is high. The same inclusion pattern is found in some solar salt crystals. The inclusions formed in the presence of lead and cadmium are confined to the centres of the crystals, because these ions are so strongly adsorbed by the growing crystals that they are rapidly depleted in the solution[6].

Among the metal ions causing liquid inclusions in sodium chloride crystals, only manganese will be present in sufficient amounts in sea brines to produce a possible effect. It can be derived from the soil of the crystallizing

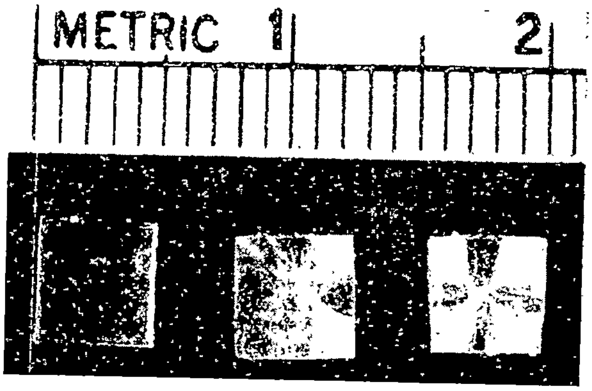

Fig. 1. Sodium chloride crystals grown from well-stirred brines containing 0, 0·02 and 0·4 p.p.m. of manganese (II)

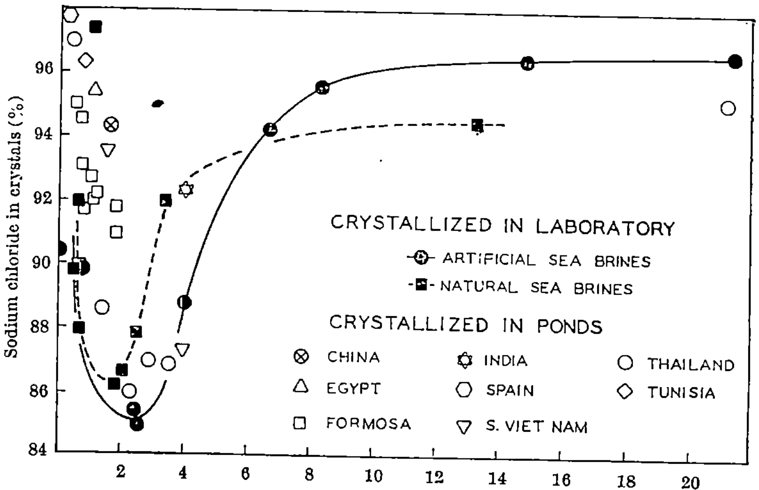

Fig. 2. Relation between manganese content and sodium chloride content of moist sodium chloride crystals obtained by evaporation of artificial and natural sea brines in the laboratory at 50° C and of sea brines in crystallizing ponds under natural conditions

ponds and bio-material as well as from the sea-water. It appears that in the presence of decaying plant material sea-water can extract appreciable amounts of manganese from soil, and this may be the major source of manganese in the brines of crystallizing ponds. As much as 25 and 140 p.p.m. manganese have been found in solar salt and sea brine respectively. Since the quality of solar salt is determined mainly by the amount of mother-liquor retained by the crystals, the manganese content of the brines is an important (but not sole) factor in the quality of the salt they yield.

The effect of trace quantities of manganese on the inclusion of mother-liquor by sodium chloride was studied in the laboratory by evaporating artificial and natural sea brines of varying manganese content at 50° C in 125-ml. Erlenmeyer flasks. The initial volume of saturated brine was 80 ml. and evaporation was continued until 20–30 ml. of water had been lost. The rate of evaporation was approximately 0·3 ml./h. Approximately 10 g of the crystallized salt was washed twice by decantation with 25-ml. portions of a saturated solution of pure sodium chloride and transferred to a Buchner funnel fitted with a filter paper disk. Suction was applied for 5 min to remove as much as possible of the adhering water. The sodium chloride content of the moist crystals was obtained by titrating chloride with silver nitrate and applying a correction for the magnesium chloride present. The percentage of sodium chloride thus found is a measure of the quality of the salt. The deficit, from 100 per cent, represents vacuole water and capillary water between intergrown crystals, as well as water which may be loosely called adhering water. This method is admittedly crude, but it is useful in practice. Manganese was determined colorimetrically with formaldoxime.

Manganese and sodium chloride were also determined in a number of solar salts (first washed with pure saturated sodium chloride solution as described) from a number of countries. The results are plotted in Fig. 2.

Laboratory-crystallized salt from artificial and natural sea brines shows a minimum sodium chloride content when the manganese content of the crystals is 2–3 p.p.m., corresponding to a manganese concentration of 1–4 p.p.m. in the brine. Above and below this value there is a definite decrease in the water content. Solar salts show a similar trend, but, for the same concentration of manganese in the crystals, the included water in solar salt is generally less than in laboratory crystallized sodium chloride. This difference is due to other factors, especially temperature and rate of crystallization, which also affect formation of vacuoles and other imperfections.

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THERMIONIC EMISSION AND HEAT OF HYDRATION OF LIME

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THERMIONIC emission and hydration are two important physical and chemical properties of lime (calcium oxide, a semiconducting oxide). Thermionic emission from lime depends on the position of Fermi-level in it. If hydration of lime were a semiconductor surface reaction, hydration would also depend on the position of Fermi-level and then there would be a connexion between thermionic emission and hydration of lime. It would then be possible to explain the observed peculiarities of hydration of lime, as well as of the effects of impurities, temperature of firing and of particle size on the hydration of lime on a single principle.

Experiments described in this article show that the lower the temperature at which thermionic emission starts for a given sample of lime, the higher is its heat of hydration. Since thermionic emission may occur at a lower temperature if the thermionic work function decreases, the above observation means that the lower the thermionic work function of lime the higher is its reactivity with water. Reactivity of lime with water thus depends on the position of Fermi-level in the lime crystal.

Lime of the following composition (per cent) was used in these experiments: CaO, 89·76; MgO, 0·47; SiO_2 and insolubles, 0·64; Fe_2O_3 and Al_2O_3, 1·67; loss on ignition, 7·56; arsenic was absent. Lime was heated at 850° C for 4 h (1) as it is and (2) with admixture of the following oxides individually: ZnO, Cr_2O_3, NiO, CoO and oxides of molybdenum and manganese. Percentage of CoO mixed was 0·5 and the amount of other oxides mixed was 1 per cent. (Ammonium molybdate and manganous acetate were mixed with lime in calculated amounts and heated and it is presumed that MoO and MnO were formed.) A part of each sample was used for measurement of heat of hydration and a part for electrical resistivity measurement.

Heat of hydration of lime was determined from the difference in the heats of solution of unhydrated and hydrated samples in 6 N hydrochloric acid by the method for the determination of hydration of cements[1,2]. Two determinations of heats of solution of each sample, hydrated or unhydrated, were made and the mean taken. For hydration, a weighed quantity of a sample was mixed thoroughly with 40 per cent by weight of distilled water and was kept in sealed tubes for 7 days at ordinary temperatures. Results are given in Table 1. As lime from the same lot was used and as no sintering was observed on heating with or without oxides, it is presumed that the surface area of lime remained the same in all the samples.

Resistivities of the samples prepared were determined at ordinary and at higher temperatures. For this, about 3 g of a sample was made into a tablet by compressing under a total pressure of 1,000 lb. in a steel mould. Dimensions of the tablets were noted and their resistances at ordinary and at high temperatures were measured, both during heating and cooling, in the manner described elsewhere[3]. No conducting layer was applied on the tablets. Heating rate was 20° C/min, the average cooling rate was 30° C/min, and the specimens were heated up to 950° C. Results of resistivity measurements are given in Figs. 1 (*a*, *b*) (heating) and 2 (*a*, *b*) (cooling), in which $\log_e \rho$ (ρ = resistivity ohm-cm) has been plotted against $10^5/T$ (T in deg. K). Inflexion temperatures in the $\log_e \rho \sim 10^5/T$ curves during cooling are given in Table 1.

Table 1. Heat of Hydration and Temperature of Inflexion of Lime

Serial No.	Sample	Heat of solution of lime (cal./g)	Heat of solution of hydrated lime (cal./g)	Heat of hydration (cal./g)	$1/T$ (inflexion)	Temperature of inflexion (° K)
1	Lime original	805·50	624·20	181·30	0·00119	840
2	Lime heated with 1% ZnO	940·30	651·90	288·40	0·00124	807
3	Lime heated alone	953·50	662·80	290·70	0·00129	775
4	,, ,, with 0·5% CoO	940·57	648·40	292·20	0·00136	735
5	,, ,, ,, 1% Cr_2O_3	936·87	638·74	300·13	0·00145	690
6	,, ,, ,, 1% MoO	942·70	642·37	300·33	0·00148	676
7	,, ,, ,, 1% MnO	945·00	644·65	300·35	0·00145	690
8	,, ,, ,, 1% NiO	970·97	632·76	338·21	0·00160	625

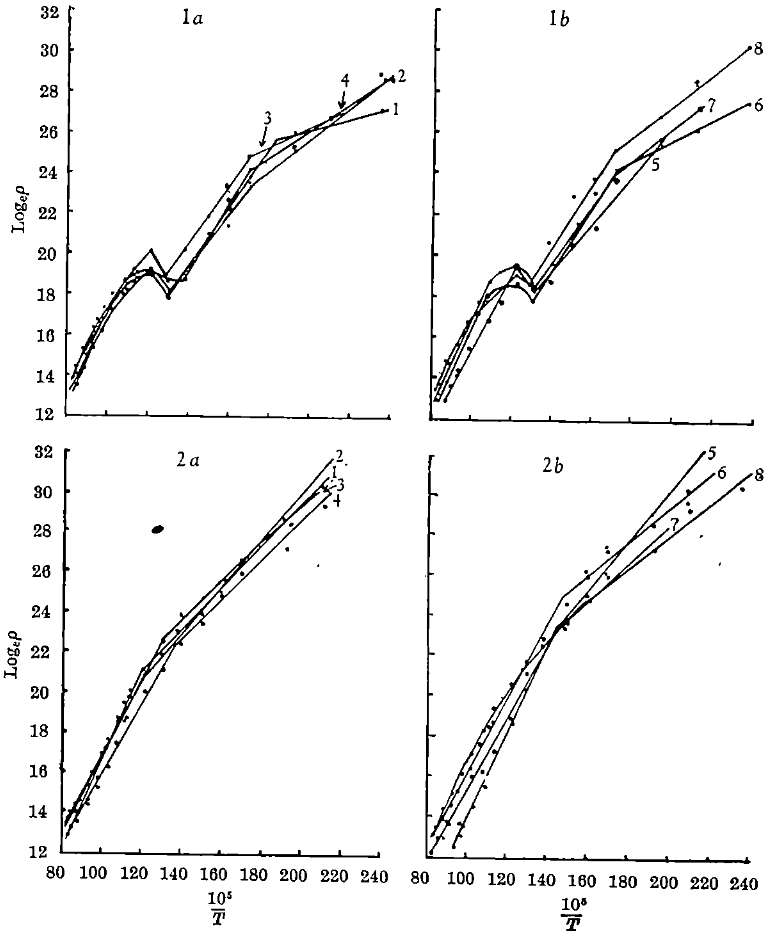
Heat of hydration for lime is given as 276 cal/g (ref. 4). Heat of hydration of the lime as received (lime original, Table 1) is fairly low, indicating that some hydration had already occurred. This is corroborated by the loss of ignition and the break in the resistivity temperature curve (Fig. 1*a*, *b*) at about 527° C during heating. ($Ca(OH)_2$ loses the (OH) groups at about this temperature.) The break in the curves at about 527° C is observed in the heating curves (Fig. 1*a*, *b*) for every sample. This indicates that a certain amount of hydration had taken place during the experiments. A sharp change in slope is observed in some of the curves (Fig. 1*a*, *b*) at about 315° C. This may be the temperature at which adsorbed carbon dioxide escapes completely and so resistivities measured below this temperature are not reliable[5].

Curves in Fig. 2 which represent the variation of resistivity with temperature during cooling show that in general the plots of $\log_e \rho \sim 10^5/T$ are straight lines with a sharp point of inflexion at which there is an increase of slope. This indicates an abrupt additional decrease in resistivity at temperatures higher than represented by the inflexion point. Such plots have been obtained for CaO (refs. 5, 6) and for BaO and BaO–SrO mixtures[7]. Explanation given for such a variation of resistivity with temperature is that, at low temperatures, semiconductivity of the crystallites is responsible for conductivity and at high temperatures the additional conductivity is due to the electron gas in the pores which originates due to thermionic emission from the crystallites[7]. Obviously, the temperature at which the inflexion of the curves occurs represents the temperature at which thermionic emission commences.

Such measurements are taken under vacuum conditions, whereas present measurements were taken in ordinary atmosphere. To see if the explanation offered for the additional increase in conductivity above the inflexion point were applicable in the present case, it is to be noted that the emission of electrons taking place inside the pores is being considered and the external surface of the specimen does not come into the picture, and that the inflexion points, similar to those on cooling, were not observed here during heating. The specimens were prepared under compression and the diameter of the pores must have been very small. The specimens were heated up to 1,223° K. Gas inside the pores had escaped during heating and, at such a high temperature, the probability of escape of gas molecules from the orifice of tube of small diameter is much higher than that of entering it, and the pores, at high temperatures, had been depleted of gas molecules, and adequate vacuum conditions had been obtained and retained during cooling. This may be the reason for the present resistivity–temperature curves (cooling) being similar to those observed by others.

Inflexion temperature for CaO reported in the literature is 625° K[5] and 700° K[6]. Taking the heat of hydration of lime as 276 cal/g, the value closest to this in Table 1 is 288·40 and the corresponding inflexion temperature is 807° K. This means that in the present experiments thermionic emission from the crystallites has started at a slightly higher temperature. That is, some poisoning of the system had occurred.

Table 1 shows that the heat of hydration increases as the temperature of inflexion, temperature at which thermionic emission starts, is lowered. The effect is more



Figs. 1 and 2. $\text{Log}_e\rho \sim 10^5/T$ plots. Figs. 1*a* and *b*, during heating; Figs. 2*a* and *b*, during cooling. Curves 1 and 3 are for lime original and lime heated: curves 2, 4, 5, 6, 7 and 8 for lime heated with ZnO, CoO, Cr_2O_3, MoO, MnO and NiO respectively pronounced as the acidity of the added oxide increases. Lowering of the temperature at which the thermionic emission starts means that the thermionic work function is lowered, that is, the Fermi-level moves closer to the energy level just outside the crystal. Thus the reactivity of calcium oxide with water depends on the position of Fermi-level inside the CaO crystal. The higher the Fermi-level (less the thermionic work function), the greater is the reactivity of lime.

Since calcium oxide is an '*n*' type semi-conductor and since its reactivity with water increases as its Fermi-level is raised, the reaction between calcium oxide and water is an '*n*' type semi-conductor surface reaction. The reaction then takes place through the capture of free electrons from the CaO crystal by the adsorbed water molecules. The nature of this reaction is thus similar to that suggested for the reaction between Portland cement clinker and water[3].

Impurities (SiO_2, Fe_2O_3, Al_2O_3, etc.) as solid solution in lime may raise or lower the Fermi-level of lime and thus affect its reactivity with water. Groups of chemicals which accelerate or retard the hydration of lime[8] may do so by changing the position of Fermi-level by altering the surface charge on being chemisorbed (strong chemisorption) on the lime crystal. The very reaction of lime with water should lower the Fermi-level of lime and hence after the initiation of the reaction it should slow down, that is, there should be an induction period[8]. The effect of firing temperature of lime on its hydration should be governed by the effects produced on Fermi-level of lime as the latter is fired at different temperatures. The effect of particle size of lime crystals on its hydration will be governed by how far the linear size of the crystal affects its Fermi-level.

If the Fermi-level is lowered, heat of hydration of lime becomes less; on the other hand, dehydration of $Ca(OH)_2$ (obtained from CaO with lowered Fermi-level) will be accelerated as the lowering of Fermi-level favours dehydration. Thus, lime of low heat of hydration should, on hydration, dehydrate at lower temperature and vice versa.

It is concluded that: (*a*) The reaction between calcium oxide and water is an '*n*' type semi-conductor surface reaction. (*b*) Reactivity of lime with water depends on the position of Fermi-level inside the calcium oxide crystal—and thus on the thermionic work function of lime. The higher the Fermi-level, that is, the lower the thermionic work function, the greater is the reactivity. Positioning of the Fermi-level in lime crystal is an essential factor in hydration of lime and may be responsible for some of the observed peculiarities of the phenomenon of hydration of lime.

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A CASE OF AN INAPPROPRIATE MODEL

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DURING the early part of this century, three analyses of battle situations were propounded: one by Colonel (afterwards Marshal) F. Foch[1] and two by F. W. Lanchester[2]. The latter have been reprinted by Newman[3], and one of them has recently been given very wide publicity by Hoyle[4]. These models which may be called the N, N^2 and F models, respectively, are of interest, particularly because of the errors they include and exemplify. As such sorts of errors are always likely to occur when mathematical models of complex social situations are constructed, it may be worth while to examine them. Each of these models sought, in effect, to find a measure for the 'value' of a force on a battlefield. Let us state each in turn, and then discuss them critically.

The N model. This, due to Foch, is the least interesting and least sophisticated of the three. Paraphrasing the argument, Foch reasoned that if a force of numerical strength x, the individuals of which had a rate of fire F_x missiles/unit time, attacked a force of strength y, and individual rate of fire F_y, then, if $x > y$, and (as was probable) $F_x = F_y = F$, the 'missile balance' in favour of the attackers in unit time would be $F\ (x - y)$. He concluded that any increase in F must add to the 'balance'; and hence add to the power of numbers, and of the offensive. The 'value' v, of a force, would be shown by:

$$v = kFN \tag{1}$$

where N is its numerical strength and k a constant.

The N^2 model. Lanchester's argument is much more elegant. He supposed that "... each man will, in a given time, score, on an average, a given number of hits that are effective; consequently the number of men knocked out per unit time will be directly proportional to the numerical strength of the opposing forces". Thus, if we express the 'efficiency' (that is, accuracy, etc.) of each unit of the opposing forces by the constant terms a_x and a_y, we have:

$$\left.\begin{aligned} \frac{dx}{dt} &= -\ F_y\, a_y\, y \\ \frac{dy}{dt} &= -\ F_x\, a_x\, x \end{aligned}\right\} \tag{2}$$

Giving, for the reasonably likely case $F_y = F_x$, $a_x = a_y$:

$$x^2 - y^2 = \text{constant} \tag{3}$$

and thence, more generally, that

$$v = k'\ F\ N^2 \tag{4}$$

The F model. Lanchester recognized that his model supposed that individual units were firing at one another, and that this was not always the case. Sometimes enemies simply fired 'into the brown'. In this situation, he reasoned that the rate of loss would be proportional to the rate of arrival of missiles, and to the density of troops on the ground. Thus, introducing another constant term b, being the ratio of the exposed area of a soldier to the area being plastered, the equations derived are:

$$\left.\begin{aligned} \frac{dx}{dt} &= -\ bxF_y\, y \\ \frac{dy}{dt} &= -\ byF_x\, x \end{aligned}\right\} \tag{5}$$

Whence the rate of loss is proportional to the fire-power of the component units, and is independent of the numbers involved. Otherwise:

$$v = k''\ F \tag{6}$$

The model-makers' own assessments. Neither Foch nor Lanchester had any serious doubts of the validity of their theoretical models. In Foch's case this is scarcely surprising; for the argument seems to have been advanced only to support a conclusion already adopted as an article of faith. Lanchester, however, was no less sure that his N^2 model correctly described the contemporary situation. The F model, he rather surprisingly declared, approximated "more closely to the conditions of ancient warfare"; while the N^2 model truly described battle in the twentieth century. He wrote: "Macaulay's lines:

> 'In yon strait place a thousand
> May well be stopped by three'

belong intrinsically to the methods and conditions of the past".

Discussion

(1) *Fighting on land.* If we examine the history of war from 1860 until 1919 (to which period the N and N^2 models were clearly intended to apply) one thing appears to me to stand out: the models are not appropriate. Increases in fire-power overwhelmingly favoured the defence; superiority of numbers on the battlefield displayed nothing like the advantage predicted; it frequently happened that handfuls stopped multitudes. Neither was there anything obscure in this process: it had been quite clear from the happenings of the American Civil War (1861–65), the Franco-Prussian War (1870–71), the Russo-Turkish War (1877–78), the Boer War (1899–1902) and the Russo-Japanese War (1904–5) before the First World War started—and confirmed it. In the first of these struggles it was reckoned that odds of 3 : 1 were often inadequate for victory. In the Boer War, the series of British reverses in 1899 were suffered in spite of favourable odds of more than 2 : 1. The grisly series of western front battles in the First World War bloodily underlined the lessons of the previous decades: throughout 1917, for example, the Allies in France had an overall superiority of about 3 : 2, and often more on the actual battlefield[5]. Since the mathematics is sound, then, where did the assumptions err?

Perhaps, instead of sniping at Macaulay, Lanchester should have remembered the line from a well-known, though rarely printed, limerick:

> "Who does what? And with which? And to whom?"

For a built-in assumption is that both parties are doing the same sort of thing. This is very rare indeed in war. Suppose that, still in the range of ultra-simple models, we remedy this. Let us imagine a 'Boer War' situation: force x is attacking force y, the men of which are snugged down among rocks and entrenchments. Then the riflemen of force x are under fire as individuals, as in the first equation of (2); but the y position is merely being plastered, as in the second equation of (5). The loss rates are then (again supposing $F_x = F_y = F$)

$$\left.\begin{aligned}\frac{\mathrm{d}x}{\mathrm{d}t} &= -\,Fa_y y\\ \frac{\mathrm{d}y}{\mathrm{d}t} &= -\,Fbyx\end{aligned}\right\} \tag{7}$$

whence

$$bx^2 - 2ay = \text{constant} \tag{8}$$

It is immediately evident, without even noting that, for example, $a \gg b$, that no 'value' term can be put on a force without first asking: what is it trying to do? And even this slightly more realistic expression leads to some very odd conclusions (the reader can play with them himself) as x and y become very large. These arise from two more built-in assumptions, namely, (i) (as in (2)) every component unit of x can shoot at any component unit of y; and (ii) (as in (5)) there is no limit to the density of force in a given space. It is obvious that both these assumptions will, in general, be false. (Parenthetically, it may be remarked that the F model, though it looks a trifle odd, is not a bad one in some special cases.)

(2) *Fighting at sea.* The built-in assumptions which we have noted in the N^2 model were most nearly satisfied by naval fighting during the heyday of the battleship, say from 1890 to 1917. Indeed, the valid application of the N^2 law was, perhaps, never better demonstrated than at Tsu-Shima (1905). It also explains many of the manœuvres at Jutland (1916)[6]. However, even at sea, the assumptions are not generally valid. For example, throughout the age of sail, gun ranges were so short that it was not possible to bring the fire of more than a few ships to bear on a single enemy ship, however many were available. This brings us to the historical case cited by Lanchester.

(3) *Trafalgar.* Lanchester claimed that Nelson showed an intuitive understanding of the N^2 law by his tactics at Trafalgar. In particular, he cited the famous memorandum, in which Nelson discussed the hypothetical case of a British fleet of 40 sail tackling a Franco-Spanish fleet of 46. Here he devised a plan to bring 32 British ships against 23 enemies, while the remainder were held in play. But in the actual battle, what did Nelson do? In the event, his lee division of 15 ships under Collingwood took on 15 of the enemy; and he was pleased enough with this to call it 'magnificent'. His own weather division of 12 ships also tackled roughly equal numbers. Before he sailed, he told a friend that he wished to bring on "a pell-mell battle". He was ready and anxious to do this because he was justly confident that, so superior were his crews in gunnery and seamanship, that they could be relied on to win single-ship actions, so long as the enemy could be made to stay and fight it out. His great problem was not to secure a fight at numerical advantage; it was to compel a fight to a finish. This was difficult, and his solution brilliant, because all naval actions in the age of sail were fought in an effectively anisotropic space. Because of the wind, the time taken to get from A to B was not, generally, the same as that taken to get from B to A. This, however, is quite another story[7].

Nothing that has been written here contradicts in any way the common-sense notion that superior numbers are, as a rule, advantageous and sometimes decisive. (Not always: they may be more difficult to control, or to supply, or they may offer too dense a target, and so on.) Neither does it bear on the utility of the whole-versus-part stratagem, which has been successfully used on scores of occasions throughout history. We have, however, underlined how misleading, and potentially disastrous, it can be if a mathematical model is applied to a real situation which it does not truly represent. All mathematical models must simplify: that is their strength. They may, in over-simplifying, distort: that is their danger.

Perhaps the moral was stated—as so often—by the Jeffreys[8]: "One often sees a statement that some result has been rigorously proved, unaccompanied by any verification that the conditions postulated in the proof are satisfied in the actual problem—and very often they are not. This misuse of mathematics is to be found in most branches of Science"; and, I may add, it is especially seductive in the sciences which deal with human behaviour. I may also add: exercise extreme care when citing historical examples.

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LETTERS TO THE EDITOR

RADIOPHYSICS

OH Absorption Spectra in Sagittarius

We have measured the 18-cm OH absorption spectrum of Sagittarius *A* over a range of 6·4 Mc/s using the 60-ft. radio telescope of Harvard College Observatory. This frequency range covers both the 1,667 Mc/s $F(2 \rightarrow 2)$ and the 1,665 Mc/s $F(1 \rightarrow 1)$ transitions. The spectrum is dominated by two features at +40 km/sec and −120 km/sec with respect to the local standard of rest. Parts of this spectrum have been previously reported[1–3], notably the feature at +40 km/sec[1]. We have also obtained an absorption spectrum for $l^{II} = 4°$, $b^{II} = 0°$. The characteristics of the background continuum radiation near 18 cm and this absorption spectrum allow us to establish an upper limit on the state temperature of interstellar OH.

The spectrum shown in Fig. 1 covering a range of velocity of several hundred km/sec has been obtained in the direction of the strong radio source Sagittarius *A* with 80 kc/s filters. The antenna temperature of Sagittarius *A* as determined by declination scans is 45° K. We find two broad lines at radial velocities of +40 km/sec and −120 km/sec. The characteristics of these lines are given in Table 1. No attempt has been made to investigate the previously detected narrow lines at 0 km/sec and −50 km/sec[4]. We find an apparent intensity ratio of the $F(2 \rightarrow 2)$ transition to the $F(1 \rightarrow 1)$ transition of 1·2 ± 0·1 for the +40 km/sec and of 1·5 ± 0·1 for the −120 km/sec line. The ratio for the +40 km/sec line agrees with that obtained by Robinson *et al.*[4].

For both +40 km/sec and −120 km/sec lines we have determined a mean separation between the $F(2 \rightarrow 2)$ and $F(1 \rightarrow 1)$ components and find a value of 1·96 ± 0·04 Mc/s. This experimentally observed separation is in good agreement with the difference in rest frequencies of 1·955 Mc/s as determined by Weinreb *et al.*[5]. The velocity range of the OH absorption measurement in Sagittarius *A* lies within the range where hydrogen has been detected and which has been assigned to regions near the galactic centre by Oort and Rougoor[6].

In order to convert the apparent depths of the absorption lines to optical depths, it is necessary to make some assumptions about the background radiation and the angular size of the absorbing region. Robinson *et al.*[4] have suggested that there is evidence that the OH cloud at +40 km/sec has an angular size less than 4 min of arc. We evaluate the partial contribution to the total antenna temperature due to the core source behind the OH by scaling the value quoted by Robinson for the 210-ft. antenna (135° K) to the value applicable to the 60-ft. antenna. Based on a measured collecting area of 95 m² for the 60-ft. antenna and an assumed cross-section of 1·6 × 10³ m² for the 210-ft. antenna, we adopt a value of 8° K for the contribution of the core.

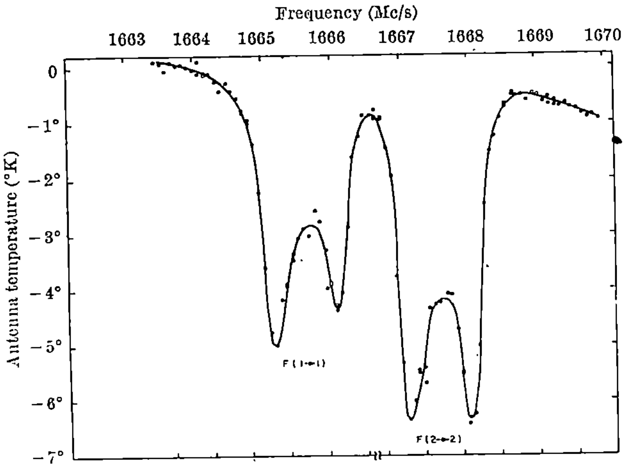

Fig. 1. OH absorption spectrum of Sagittarius *A* showing both transitions. Velocities are relative to the local standard of rest for each transition. The zero point of the temperature scale is arbitrary. The line is a free-hand fit to the data points. Each point represents an integration time of 480 sec

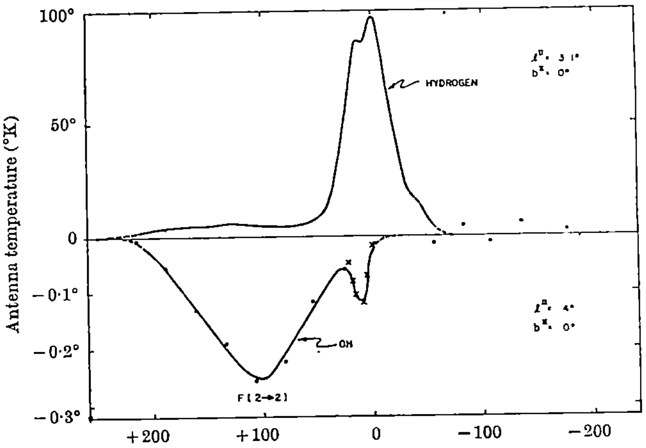

Fig. 2. A comparison of the Leiden HI emission profile for $l^{II} = 3·1°$, $b^{II} = 0°$ with the OH absorption profile for $l^{II} = 4°$, $b^{II} = 0°$ on the same velocity scale. Note the difference in temperature scales. The open circles are measurements made with 150 kc/s filters and an integration time of 1,400 sec; the crosses are measurements made with 25 kc/s filters and an integration time of 25,000 sec

For the +40 km/sec component we then obtain a τ of 1·4 for the $F(2 \rightarrow 2)$ transition and 0·95 for the $F(1 \rightarrow 1)$ transition, giving a ratio of 1·5. Considering the difficulty of assigning an exact value to the antenna temperature due to the core source, the difference between the computed value of 1·5 and the predicted value of 1·8 may not be significant. In view of the evidence of a peculiar spatial distribution of the OH in the +40 km/sec cloud, and recognizing that a similar situation may exist at −120 km/sec, we have not attempted to obtain a solution for the opacity at −120 km/sec.

The limits in velocity published by Robinson *et al.* for the +40 km/sec line[4] are −60 to +100 km/sec. They did not report the −120 km/sec line. It is possible that the −120 km/sec feature lies outside the beam of the 210-ft. antenna when directed toward Sagittarius *A*.

Table 1

Velocity (km/sec)	ΔT (° K) 1,667	1,665	Half-width (km/sec)
+40	6·0 ± 0·2	4·9 ± 0·2	86 ± 6
−120	6·0 ± 0·2	4·1 ± 0·2	76 ± 6

The spectrum at $l^{II} = 4°$ shown in Fig. 2 was made with filters having band-widths (to half-power points) of 150-kc/s spaced 150 kc/s. Two features are evident. One is a broad absorption line with a width of 100 km/sec, a depth of 0·25 ± 0·1° K and a shift of +110 km/sec with respect to the local standard of rest. A narrow absorption line having a width of 15 km/sec, a depth of 0·09 ± 0·03° K superimposed on the broad feature, and a shift of +10 km/sec was observed with 25-kc filters spaced 25 kc. The broad line coincides in velocity with the long wing of the hydrogen emission profile at nearby positions[7,8]. We have checked these results by observing the $F(2\rightarrow2)$ and the $F(1\rightarrow1)$ transitions for both features.

We measured the 18-cm continuum radiation at $l^{II}=4°$, $b^{II}=0°$ with respect to the points about 10° above and below the plane. We obtain $8° \pm 2°$ K for the increase in antenna temperature. The position $l^{II}=4°$, $b^{II}=0°$ was selected because it is a broad local minimum of continuum emission in the galactic plane, and provides an approximately uniform background brightness temperature over our antenna beam (52′)[9-11]. The occurrence of absorption lines allows us to place an upper limit on the state temperature of about 10° K; this is a direct result not dependent on assumptions about the optical depth as is the case with all previous estimates.

The radiometer used compares the available power from the antenna to that from a liquid-helium cooled resistor in ten frequency bands at the same time. The first amplifier is a cavity maser similar to the 21-cm maser[12] which has been used for hydrogen-line observations at Harvard since 1960. The system noise temperature neglecting the contribution due to the source is 110° K when the bandwidth is 2·8 Mc/s.

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Lightning Flash Counts and Radio Noise Fields in the Tropics

IT is now generally accepted that the source of atmospheric radio noise is the natural lightning discharge associated with thunderstorms, the discharges possessing energy components throughout the radio-frequency spectrum[1]. It is, therefore, to be expected that a correlation exists between the measured level of noise fields and the frequency of occurrence of lightning discharges in a given area. A standard lightning flash counter has been adopted by the International Radio Consultative Committee (C.C.I.R.) for obtaining statistical data about lightning flashes at various locations throughout the world[2]. The Central Radio Propagation Laboratories of the United States (C.R.P.L.) and the Radio Research Board of the Department of Scientific and Industrial Research of Great Britain (D.S.I.R.) have designed two separate equipments for the measurement of atmospheric noise-levels, and these have been installed at chosen sites over the world. Fortunately, a standard lightning flash counter and one each of the noise recording equipments were kindly made available at Ibadan, Nigeria (7° 26′ N, 3° 54′ E.), in the tropical belt during and after the International Geophysical Year, thus facilitating the collection of a fairly large amount of statistical data for lightning flash counts and median values of noise power.

The effective range of the lightning flash counter is 30 km with a probability of 90 per cent; and the triggering is at a step function voltage of 3 V, the equivalent field strength at the aerial 7 m high being about 3 V/m. The flash counter was operated at very low frequencies, but since both ground and cloud flashes are considered, its response is over a wide bandwidth extending from 1 kc/s to over 30 kc/s. The median values of noise power referred to 1 kc/s bandwidth for seasonal blocks (one season and one of six 4-h time periods of day as recommended by the C.C.I.R.)[3] have been compared with the mean values of number of lightning flash counts per h for corresponding seasonal blocks. The comparison has been based on long-term rather than hour-by-hour comparison since the world-wide predictions of atmospheric noise levels[3] are made for seasonal blocks only. The field values used in the comparison have been restricted to 24 kc/s and 51 kc/s, being the lowest frequencies available on the D.S.I.R. and C.R.P.L. equipments, respectively. Fig. 1 presents the variation of measured noise field at 51 kc/s in F_a, expressed in db. above kTB (the thermal noise power in watts in a passive resistance) on linear scale where: k = Boltzmann's constant = $1{\cdot}38 \times 10^{-23}$ joule/°K; T = absolute temperature in deg. K (290° K); B = effective noise bandwidth (c/sec); with the mean counts per h (on logarithmic scale). The values plotted here are the medians of noise fields for all the seasons of the year and the count per h has been averaged for all the seasons. Fig. 2 presents similar results at 24 kc/s for the spring season only.

The 'best fit' lines have been drawn on Figs. 1 and 2 and the derived equations which best suit the relationships are:

$$F_a = 130 + 17{\cdot}5 \log_{10} n \ (24 \text{ kc/s})$$

and

$$F_a = 113 + 18 \log_{10} n \ (51 \text{ kc/s})$$

Apart from the expected difference in the magnitude of field values at the two frequencies, the two relationships are similar in that they have the same exponential form.

An attempt has already been made to compare the noise field and mean counts per hour from a similar 3 V, 7-m aerial, C.C.I.R. lightning flash counter at Singapore, Malaya (1·3° N., 103·8° E.), also in the tropics[4]. A similar C.R.P.L. noise-recording equipment was used to obtain noise fields at a low frequency of 13 kc/s. In the Report of Study Group VI of the C.C.I.R. Tenth Plenary Assembly in 1961, the relationship that closely agreed with observed results at Singapore is that:

$$F_a = 28 + 10 \log_{10} (nW/f^2)$$

where W = total energy in a single atmospheric in a speci-

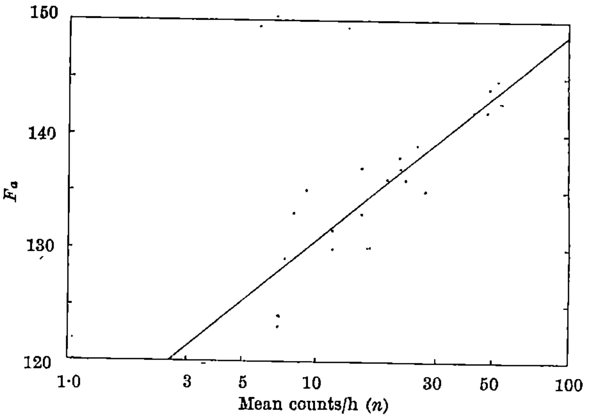

Fig. 1. Lightning flash counts and noise fields at 51 kc/s for all seasons

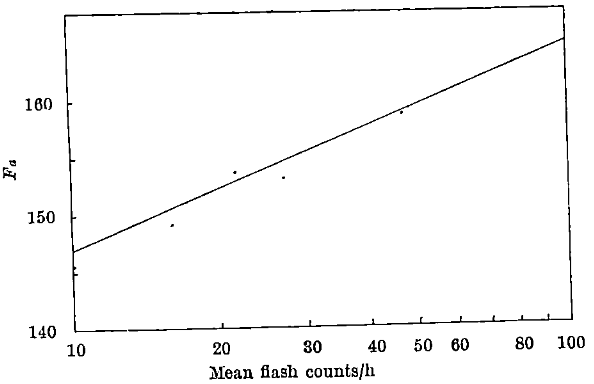

Fig. 2. Lightning flash counts and noise fields at 24 kc/s for spring only

fied bandwidth and taken as 3×10^8 $(\mu V/m)^2$ sec in a bandwidth of 1 kc/s. (The value was that obtained in Slough, England, in a temperate area and assumed here for the tropical areas.) f = frequency (Mc/s).

For direct comparison between the results from observations at Singapore and Ibadan, correction has been made at Ibadan for differences between the tuning frequency of the flash counter and 24 kc/s and 51 kc/s, respectively.

Hill[5] discussed the theory of the generation of low-frequency electromagnetic radiation by cloud-to-ground lightning strokes and predicted the spectral intensity of total radiation from the return stroke as:

$$R = 8 \times 10\ F(\gamma)\ \text{joules/(kc)}$$

where $\gamma = 0{\cdot}19\, f_{\text{kc/s}}$.

The function $F(\gamma)$ has the form:

$$F(\gamma) = \gamma^{-4}\ (\sin\gamma - \gamma\cos\gamma)^2$$

The maximum point of the spectral distribution lies at 11·2 kc/s, and the total width at half maximum is 12 kc/s. From the power attenuation with respect to maximum at the frequencies considered and the response curve of the flash counter, appropriate corrections are applied to the relationship of the Study Group which reduces to:

$$F_a' \simeq 130 + 10\log_{10} n\ (24\ \text{kc/s})$$

$$F_a' \simeq 110 + 10\log_{10} n\ (51\ \text{kc/s})$$

which show closer similarity to the suggested relationships for Ibadan. A significant difference, however, lies in the exponential part. Whereas the results from Singapore suggest that F_a varies as $\log_{10} n$, the results at Ibadan indicate variation with $\log_{10} n^{(1{\cdot}75-1{\cdot}80)}$. This leads to the suggestion that a more accurate law of variation in a tropical area should be of the form:

$$F_a = 28 + 10\log_{10}(n^{\alpha}\ W/f^2)$$

and at Ibadan, a value of approximately 2·0 is suggested for the exponent α. The plots for 51 kc/s (Fig. 1) at Ibadan have been averaged over all seasons of the year whereas those for 24 kc/s (Fig. 2) have been averaged over one spring season. I have already indicated that the spring season is the most intense for lightning activity at Ibadan[6]. The relatively wide scatter in Fig. 1 indicates that the relationship between lightning intensity and noise field should preferably be expressed over one seasonal period only; and that the exponent α has seasonal variations probably arising from slight seasonal variations in the structure of lightning discharges and seasonal changes in the relative contribution of local flashes to the total noise intensity.

As already pointed out[7], the meteorological thunderstorm day suffers from a number of deficiencies when used as a symbol of spherics activity; and cannot be used as indication of noise-levels. The investigation described here confirms that in areas where storms are prevalent, the noise power at low frequencies bears a direct relation to the density of lightning flashes, and at these frequencies the rate of occurrence of lightning discharges will assist in more accurate predictions of atmospheric noise levels for aiding radio communication from one place to the other on the Earth's surface. Further investigation, however, is necessary at many more locations, especially in tropical areas, to ascertain the dependence of the exponent α on time of day, season of year, geographic location and frequency.

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GEOPHYSICS

The Satellite Geoid and the Structure of the Earth

COOK[1] has shown that, in the Earth's gravitational potential when developed into a series of zonal spherical harmonics, the coefficients are influenced to the fourth harmonic by the inhomogeneities of the inner core; the inhomogeneities of the outer core will appear at first in the coefficients of $n = 5$–11.

Shortly after this communication, Izsák's communication appeared[2] giving the details of a satellite geoid determined by the coefficients of zonal and tesseral harmonics up to an order of 4.

Cook's results suggest, however, that the general form of this fourth-order geoid must have a source in the inhomogeneities near the core of the Earth.

Vogel[3] has shown on the basis of *PcP*, *ScS*, *PcS* and *ScP* waves that on the surface of the outer core undulations with amplitudes up to 200 km exist.

Vogel's results plotted in a simplified way on the map of a satellite geoid (namely, denoting the spots at normal depth by 0, below the average depth by −, above the average depth by +) show the surprising result that the undulations of the core (where data exist) are followed by the undulations of the satellite geoid.

As a generalization of Cook's theorem, it may be concluded that the undulations of the geoid are caused by undulations on the surface of the outer core.

It may be shown that the gravitational effect of mass inhomogeneities observed in the form of undulations of the core yields the undulations of the satellite geoid with a right order of magnitude.

The core undulation below a certain geoid undulation can be approximated by a sphere of a diameter of 200 km, if it is supposed that the effect of the other core undulations are compensated by each other. The undulation caused on the geoid may be computed by the quotient of the sphere's gravitational potential and gravity acceleration on the surface: $h = \dfrac{W}{g}$.

Denoting by $R = 10^7$ cm the radius of the sphere approximating the core undulations, $\rho_1 = 5{\cdot}7$ g cm^{-3} the density on the bottom of the mantle, $\rho_2 = 9{\cdot}7$ g cm^{-3} the density on the top of the core, $r = 2{\cdot}8 \times 10^8$ cm the average distance of the sphere approximating the core undulation from the surface, and $g \cong 10^3$ cm sec^{-2} the gravity, then

$$h = \frac{4\pi f R^3(\rho_2 - \rho_1)}{3rg} \cong 40\ m$$

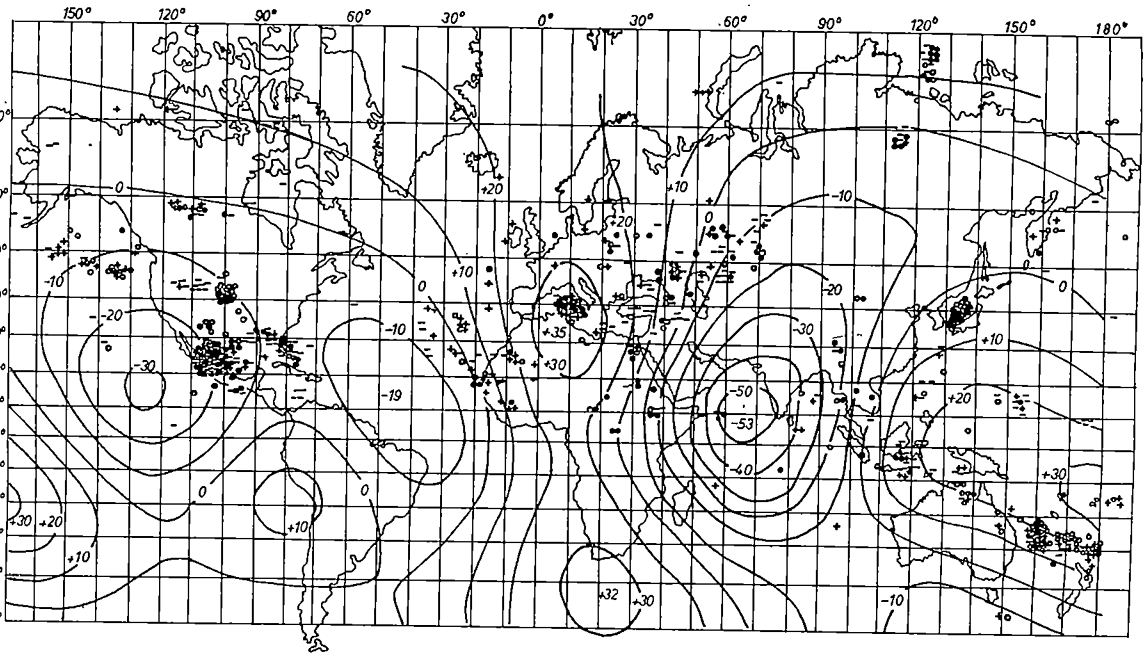

Fig. 1

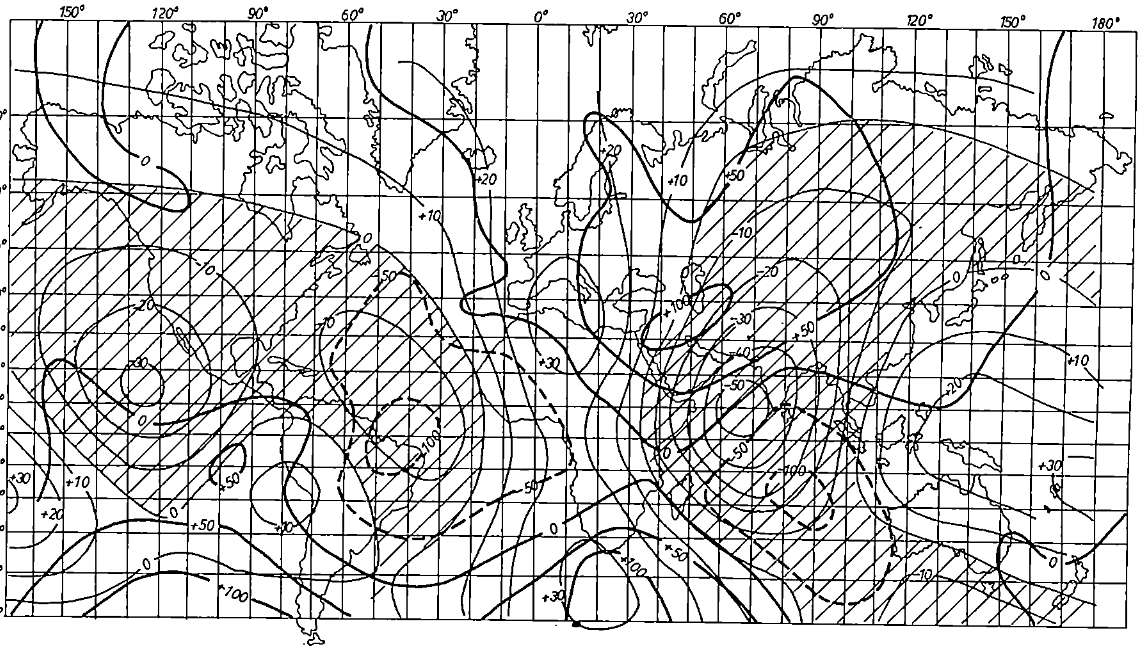

Fig. 2

a value in very good agreement with the average maximum values of geoid undulations observed.

The close connexion between geoid undulations and core undulations suggests a comparison also with geomagnetic maps.

Fig. 2 represents the isopores of vertical magnetic intensity for the year 1942·5 on the map of the satellite geoid.

There is a fair coincidence in their location, sign and trend in comparison with the undulations of the satellite geoid. It seems very probable that the coincidences of these two sets of results would be more complete by use of recent magnetic values.

If this correlation may be regarded as valid, then the gravity field must possess a secular westward drift. Moreover, the correlation points towards the importance of the core in the origin of the magnetic field. Core undulations must also show a westward drift running together with the gravity and magnetic fields, as was also suggested by Vogel[3]. The core undulations are resulting also in a variation of the momentum of inertiæ, which must be reflected in the nutational motion of the Earth, according to the formula $T_E = \frac{A}{C-A}$. The variation of the length of the Chandler nutation, between 404 and 456 days, supports this conclusion. Moreover, the close connexion between secular variation in the amplitude of nutation, geomagnetic field and angular velocity of the Earth strengthens this inference.

The high-rate core undulation and its secular variation diminish the probability of a material change at the mantle-core boundary. On the other hand, the undulations can be explained easily by the hypothesis of Ramsey, explaining the mantle-core boundary by a phase-transition of material.

A curious correlation was shown also between geoid undulations and heat-flow distribution[4], though this correlation is less tight. While the former correlations refer to deep connexions, the latter may be based only on shallow effects.

The authors[4] suggest for this correlation an explanation by convection currents. Convection currents have, however, also tectonical effects, which must be reflected in the major tectonic features of the Earth. But it is hard to discover any relation between heat-flow 'undulations' and the tectonic zones of the Earth.

I wish first of all to direct attention to the connexions between core and geoid undulations probably being the keys of problems far from being solved.

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Periodic Precipitation of the Particle Flux in the Auroral Zone

EVIDENCE of periodic and quasi-periodic precipitation of the particle flux in the auroral zone is often observed in the riometer absorption[1], 5577 Å photometer records, visual observation of pulsating aurora (also seen in the all-sky camera records) and other related geophysical phenomena. Further, the recent satellite observations have also shown periodic variation in the measured flux[2]. In this communication a possible mechanism for the periodic precipitation of the particle flux is proposed and its validity and limitations are discussed.

It is well known from the work of Dessler[3], Piddington[4] and of Dessler and Parker[5] that the compressional magnetic disturbance travels along the magnetic field as a transverse hydromagnetic wave. During this period longitudinal acoustic wave propagates perpendicular to the magnetic field[6]. Herlofson[7] pointed out that in the presence of a magnetic field the longitudinal acoustic wave propagates with hybrid velocity and is known as magnetic-acoustic wave[8]. Its velocity is:

$$V_{\mathrm{ma}} = \sqrt{V_{\mathrm{a}}^2 + V_{\mathrm{hm}}^2} \tag{1}$$

where V_{ma}, V_{a} and V_{hm} are magneto-acoustic, acoustic and hydromagnetic velocities.

The incident solar plasma excites a longitudinal magneto-acoustic wave in the finite equatorial cross-section of the Van Allen belt, causing a compression and rarefaction in the intervening plasma and in the magnetic field. The magneto-acoustic waves propagating along the equatorial belt are heavily damped at high frequencies and are refracted away from the equatorial region. However, under idealized conditions such propagation at low frequencies (smaller than the proton gyro-frequency) is a possibility, and was pointed out by MacDonald[9]. The compression and the rarefaction in the magnetic field result in a one-dimensional compression and rarefaction of the intervening plasma. During compression, diffusion of the plasma along the magnetic field is comparatively more than the diffusion perpendicular to the magnetic field. Consequently, a larger number of particles will have required pitch angle ($\leqslant 2°$) to be precipitated into the auroral zone. On the other hand during the rarefaction of the magnetic field, lesser numbers of particles will have required pitch angle to be precipitated into the auroral zone. The periodicity thus produced will have the period of the magneto-acoustic wave propagating along the equatorial Van Allen belt.

The phenomena may further be complicated if there exists a coupling of the transverse hydromagnetic waves along the field lines and the longitudinal magneto-acoustic wave along the equatorial belt. The resonance between the two modes propagating perpendicular to each other will occur only when the fundamental period of one is equal to, a multiple of, or sub-multiple of the other. In this situation, particle flux heading to the lower ionosphere (having required pitch angle) is greatly affected. The period of the incident flux on the ionosphere in this case is governed by the frequency of resonance between the transverse hydromagnetic wave and longitudinal magneto-acoustic wave propagating approximately perpendicular to each other.

An approximate estimate of the fundamental period of the longitudinal magneto-acoustic wave along the equatorial belt in the presence of the magnetic field is given by:

$$T = \frac{2\pi R_E}{\sqrt{V_{\mathrm{a}}^2 + V_{\mathrm{hm}}^2}} \tag{2}$$

Taking average value of the proton mass density obtained from nose whistler data[10]:

$$\rho = 1{\cdot}2 \times 10^{-2} \frac{B_0\, e\, m_i}{2\pi e\, m_e}$$

the period

$$T = 0{\cdot}28\pi R_E^{5/2} \left(\frac{e m_i}{a\, m_e}\right)^{1/2}$$

where m_i, m_e are the proton and electron mass respectively, e is the electron charge, $R_E = 5{\cdot}4$ equatorial radial distance and $a = 8{\cdot}1 \times 10^{25}$ gauss cm^3 is the dipole moment of the Earth. The fundamental period from equation (2) becomes approximately 300 sec, which is equal to the most probable period reported by Parthasarathy and Hessler[1]. This period mainly depends on the exact knowledge of the proton mass density in the region of magneto-acoustic propagation. The periods of the transverse hydro-magnetic waves obtained from the micro-pulsations at mid-latitude[11] and from the spectral analysis of telluric currents[12] have shown the composite nature of the observed period. At higher latitude, periods anywhere between 100–500 sec having different power may exist. Under this situation there are better chances for the magneto-acoustic wave to become coupled to a suitable transverse hydromagnetic wave and give rise to a resonance effect.

The narrow latitude coverage of the observed periodicity and its simultaneous and similar appearance seen in Macquarie Island, the riometer records from which are approximately magnetically conjugate to College, is in accordance with the proposed mechanism.

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Electron Microprobe Analysis of the Metallic Phase in Basic Achondrites

There are no modern analyses in the literature of the nickel and cobalt contents of the rare metallic phase in those achondritic meteorites which have gross chemical compositions similar to terrestrial basic igneous rocks. I have called these achondrites the basic achondrites and both the eucrites and the howardites are included in this group.

The question of the composition of the metallic grains in basic achondrites has an important bearing on the evolution of these meteorites but the main problem is that their rarity and small sizes have made accurate conventional chemical analysis difficult. In the work recorded here an Applied Research Laboratories electron probe X-ray microanalyser was used to determine iron, nickel and cobalt abundances in a number of metallic grains in the Macibini eucrite and the Binda howardite. The analytical method and the standards used have been described previously[1].

Table 1. Analyses of Metallic Grains in Basic Achondrites

Meteorite	Metallic grain identification	Fe (%)	Ni (%)	Co (%)	Ni/Co
Macibini eucrite	1	97·3	2·15	0·54	4·0
	2	93·2	2·21	0·54	4·1
	3	97·3	2·00	0·64	3·1
	4	97·2	2·53	0·29	8·7
	5	95·2	3·85	0·92	4·2
Binda howardite	1	95·8	1·95	2·19	0·89
	2	98·2	0·44	1·38	0·32
	3	95·9	1·93	2·20	0·88
	4	95·8	2·00	2·18	0·92
	5	95·7	2·10	2·19	0·96
	6	95·7	2·06	2·22	0·93

The results indicate that although the nickel and cobalt contents measured at various places on individual grains seem to be constant within the precision of the method (approximately ± 3 per cent), there is considerable variability between different grains in each meteorite. In the Macibini eucrite the nickel content of various metallic grains ranged from 2·00 to 3·85 per cent and cobalt ranged from 0·29 per cent to 0·92 per cent (nickel/cobalt = 4·0–8·7). In the metallic grains in the Binda howardite, nickel ranged from 0·44 to 2·1 per cent and cobalt from 1·38 to 2·22 per cent (nickel/cobalt = 0·32 – 0·96) (Table 1). Both these meteorites show evidence of severe brecciation so that the variation observed between grains within each achondrite may well result from slight differences in the environments in which individual metallic grains were formed.

The low nickel/cobalt ratios measured in the metallic grains of the basic achondrites are quite different from the ratios observed in the metallic phase of other meteorites. For example, the average nickel/cobalt ratio in the metallic phase of the hypersthene–olivine, bronzite–olivine and enstatite chondrites is close to 20. Similarly the average nickel/cobalt ratio in the metallic phase of pallasites, which has previously been suggested to be the parent material from which the iron meteorites have evolved[2], is also 20 (Table 2).

Table 2. Composition of Metallic Phase in Meteoritic and Terrestrial Materials

Material	Fe (%)	Ni (%)	Co (%)	Ni/Co
Average metallic phase of hypersthene–olivine chondrites (ref. 3)	79·7	19·4	0·88	22·2
Average metallic phase of bronzite–olivine chondrites (ref. 3)	88·0	11·4	0·55	20·6
Average metallic phase of enstatite chondrites*	92·5	7·1	0·35	20·2
Average metallic phase of pallasites (ref. 3)	88·4	11·0	0·55	20·0
Metallic phase in Disko Island Basalt (carbon free) (ref. 1)	97·6	1·76	0·68	2·6

* Average of five superior analyses: Hvittis, Indarch, Saint Sauveur (ref. 4) and Abee (matrix), Abee (fragment) (ref. 5).

On the other hand, the metallic masses occurring in the basic igneous rocks at Uifaq, Disko Island, West Greenland, have nickel and cobalt abundances and nickel/cobalt ratios almost indistinguishable from the metallic phase in the Macibini eucrite and very similar to that in the Binda howardite (Tables 1 and 2). The metallic masses in the Disko Island rocks are generally considered to have been formed by the virtually complete reduction of oxidized nickel and cobalt, and the partial reduction of oxidized iron, in a basic silicate magma as a result of reactions with carbonaceous matter derived from surrounding coal measures or carbonaceous shales which these rocks are observed to intrude[1]. In view of the close similarity between the metallic phase in the Disko Island basalts and the basic achondrites, it is suggested that the metallic phase in the basic achondrites has formed from partial reduction of the parent basic magmas from which these achondrites have evolved.

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PHYSICS

Chromium Oxide–Chromium Cermets

The preparation and properties of chromic oxide–chromium cermets have been examined as part of a general investigation into the possibility of preparing metal–ceramic mixtures ('cermets') by partial reduction of refractory oxides. The method consists of pressing a mixture of chromic oxide and carbon, both in the finely divided state, into compacts and sintering to temperatures in the range 1,400°–1,600° C in a vacuum of circa 10^{-4} mm mercury. The amount of carbon is calculated to give 1–10 per cent metallic chromium in the sintered body.

Under the foregoing conditions, considerable shrinkage of the compact occurs, resulting in an increase of density in a typical case from 3·2 to 4·7 g/cc. This can be compared with an increase in density from 3·2 to 3·4 for pure oxide sintered to the same temperatures. The increase in sintered density is accompanied by an increase in compressive strength from 3 tons/in.^{2} for the pure oxide to approximately 20 tons/in.^{2} for the cermets.

Provided that the temperature of sintering is sufficient to cause the reduction to proceed to completion, the most significant variable affecting sintered density is the proportion of carbon. Less than a threshold addition produces no effect, whereas more than the optimum causes the density and compressive strength to fall off, though less markedly. The latter deterioration is presumably due to an expansion effect of the carbon monoxide involved.

The mechanism responsible for this unusual shrinkage is being investigated.

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GEOLOGY

Age of the Newgale Beds

The Newgale Beds of Pembrokeshire (Fig. 1) have not previously yielded any fossil evidence about their age, and consequently there has been considerable doubt on this matter. On the earliest map of the area[1] they are shown as Carboniferous, an interpretation that is retained on the map published by Jones[2]. On the Geological Survey Map of 1917[3] they are more specifically identified as Millstone Grit.

In recent years their Carboniferous age has been questioned and Jenkins[4] considered them, without positive evidence, to be Lower Palaeozoic. Owen, in the discussion of this paper, stated they may be either Cambrian or a facies variant of the Llandovery. Jones, in discussing the same paper, said that if they are Cambrian they most likely are equivalents of the Middle Cambrian, Solva Series.

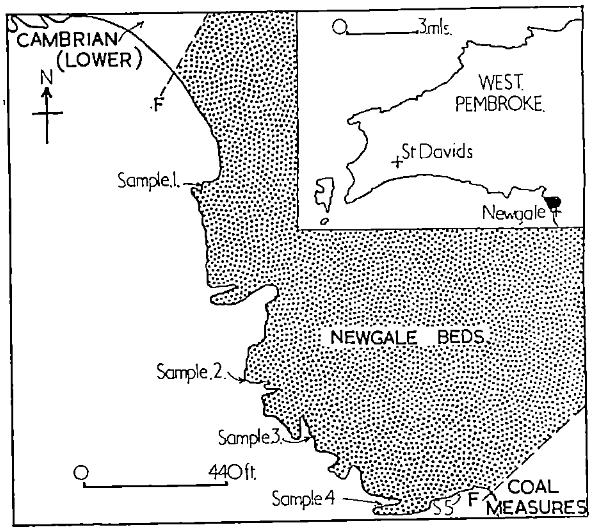

Fig. 1. Map showing the location of the Newgale Beds and the samples examined

Table 1. Range of Comparable Species in the U.S.S.R.

	Pre-Cambrian	Cambrian			Tremadoc	Arenig
		L	*M*	*U*		
Trachyoligotriletum minutum	+	+	+	+	+	+
Ocridoligotriletum kryshtofovichi	–	+	+	+	–	–
Protoleiosphaeridium conglutinatum	+	+	+	+	+	+
Trachyoligotriletum rugosum	–	+	+	+	–	–
Tyloligotriletum asperum	–	–	+	+	–	–
Stenozonoligotriletum sokolovi	+	+	+	+	+	+
Archaeohystrichosphaeridium strictum	–	–	+	–	–	–
A. operculatum	–	–	+	+	–	–
A. minor	–	–	+	–	–	–
A. oblongum	–	+	–	–	–	–
Acanthodiacrodium barbullatum	–	–	+	–	–	–
A. secundarium	–	–	+	–	–	–
Ooidium sablincaense	–	–	+	+	–	–
	3	6	12	8	3	3

One of us (H. G. D.) while mapping the area collected a number of samples which were processed in the Micropalæontology Laboratory, Geology Department, University of Sheffield. The samples were selected dark grey mudstones occurring as thin bands among the sandstones and grits which form the greater part of the Newgale Beds. Five samples were treated, Nos. 3–5 yielding a large number of microfossils of the type once known as hystrichospheres but now called acritarchs[5]; the others yielded small numbers of similar types. These assemblages, typical members of which are illustrated in Fig. 2, are clearly Cambrian in age. An earlier date is eliminated by the presence of *Acanthodiacrodium* (Fig. 2*n*, *o*) and *Archaeohystrichosphaeridium* (Fig. 2*i*–*m*). An Ordovician or Silurian age can be discounted because of the absence of *Veryhachium*, *Tasmanites*, *Leiofusa*, certain species of *Baltisphaeridium*, and chitinozoa, and because of the presence of *Acanthodiacrodium*, which is typically Cambrian. The absence of spores and plant debris excludes any younger age.

Dating within the Cambrian is less certain because no systematic work has been done with fossils of this type in the British Cambrian. One of us (C. D.) has observed acritarchs in Lower and Upper Cambrian rocks of Wales and Scotland which differ in type from those discussed here, but the Middle Cambrian has not yet been investigated. In these circumstances the only comparative material is that from the Baltic regions of U.S.S.R. described by Timofeyev[6]. Counterparts of all the species found in the Newgale Beds can be found among those described by Timofeyev but none is so closely similar that specific identification is certain. In each case the Welsh species is compared with that most closely resembling it in Russia. It should be noted that no triradiate marks have been observed in any of the species from Wales. The stratigraphic range of these Russian counterparts is shown in the table, which clearly indicates a Middle Cambrian age. Complete confirmation of this must wait until detailed work on the main sequences of Cambrian rocks in Pembroke has been carried out.

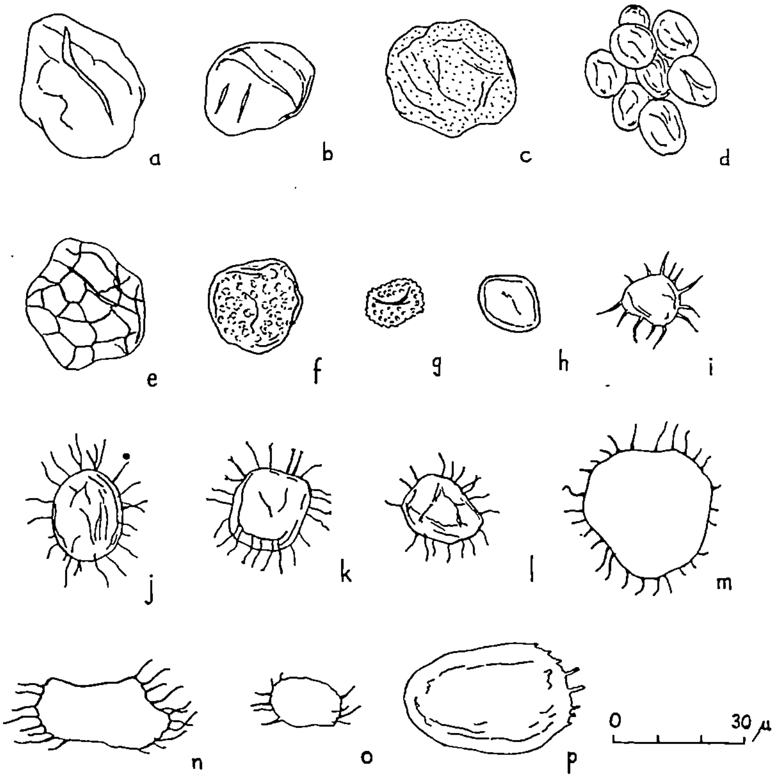

Fig. 2. Camera lucida drawings of the more common acritarchs in the Newgale Beds. *a and b*, cf. *Trachyoligotriletum minutum*; *c*, cf. *Ocridoligotriletum kryshtofovichi*; *d*, *Protoleiosphaeridium* sp. cf. *conglutinatum*; *e*, *Protoleiosphaeridium* sp.; *f*, cf. *Trachyoligotriletum rugosum*; *g*, cf. *Tyloligotriletum asperum*; *h*, cf. *Stenozonoligotriletum sokolovi*; *i*, *Archaeohystrichosphaeridium* sp. *cf. strictum*; *j* and *l*, *Archaeohystrichosphaeridium* cf. *operculatum*; *k*, *Archaeohystrichosphaeridium* cf. *minor*; *m*, *Archaeohystrichosphaeridium* cf. *oblongum*; *n*, *Acanthodiacrodium* sp. cf. *barbullatum*; *o*, *Acanthodiacrodium* sp. *cf. secundarium*; *p*, cf. *Ooidium sablincaense*

Meanwhile the palæontological evidence proves a Cambrian age and is strongly suggestive that it is Middle Cambrian. This being so the Newgale Beds, as Jones stated, must be equated with the Solva Series, the Menevian Series of the Middle Cambrian being a most improbable equivalent because of its lithology.

We thank Mr. P. Shepherd for making the preparations and Prof. L. R. Moore for the use of the facilities of the Department of Geology at Sheffield.

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Coal Ranks and Geothermal Gradients in High-volatile Bituminous Coalfields

FORTY samples of coal from oil-prospecting wells drilled by Shell BP Todd Oil Services, Ltd., in the Taranaki area of New Zealand have been analysed at the Chemistry Division of the New Zealand Department of Scientific and Industrial Research. Full analytical details and a discussion of the depth/rank relation are given by us[1], and we emphasize that direct comparison of the analyses is not satisfactory because the coal types and sulphur contents vary widely. It was necessary to make some allowance for these variations, the method used being that of Suggate[2]. Within the high-volatile bituminous range of rank penetrated in the wells, the most satisfactory parameter of rank is the calorific value.

All four wells penetrated coals ranging through stratigraphic intervals of between 2,100 and 2,500 ft., the deepest coals being from depths ranging between 12,900 and 14,700 ft. The coal measures are of Upper Eocene or Lower Oligocene age[3], and above them sediments from Oligocene to early Pleistocene in age were deposited without major breaks in sedimentation. Slight uplift and erosion followed, and for some wells it has been found possible to make reasonable estimates of the thicknesses eroded, and consequently of the original depths of burial. From a composite column over the 12,000–17,000 ft. range of depth of burial, an increase of 280 B.T.U./lb. per 1,000 ft. increase of depth is estimated; individual well sequences, based on comparatively few analyses, tend to show rather lower rates of increase of calorific value with depth.

The proximate analysis (air-dried basis) of the most deeply buried coal is as follows: M., 1·2 per cent; V.M., 44·1 per cent; F.C., 48·4 per cent; A., 6·3 per cent; S., 1·2 per cent; B.T.U./lb., 14,380; Sw. No., 6½. If it is assumed that no great changes in geothermal gradient have occurred since the early Pleistocene, this coal was metamorphosed at a temperature of 280°–300° F., judged by temperature measurements in the wells.

The rate of increase of calorific value with increasing depth is only a little more than half the 500 B.T.U./lb / 1,000 ft. found in Carboniferous coals in the Pie Rough Bore in England[4] and in bores in the Saar and Upper Silesia[5]. The most obvious explanation is that of a nearly two to one difference in geothermal gradient. Comparison of data from the Carboniferous coalfields of the Netherlands with analyses of single samples of Jurassic coal from Mississippi at a depth of 16,193 ft. and of Eocene coal from Venezuela at a depth of 11,200 ft. has already led Kuyl and Patijn[6] to suggest that the geothermal gradient in the Carboniferous was much greater than in Mesozoic and Tertiary times. The present geothermal gradient in the Taranaki coal measures is about 19° F./1,000 ft., and if that in the European Carboniferous were greater in proportion to the difference between the rank gradients, it would have been about 34° F./1,000 ft. Such a high gradient, however, is greater than that now found at depth almost anywhere except in hydrothermal areas. Before this gradient is accepted for the Carboniferous, the effects of possible lower pressure should be considered[7], if depth of burial was less and the temperature gradient only moderately higher.

We thank the geologists of Shell BP Todd Oil Services, Ltd., for their assistance.

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CRYSTALLOGRAPHY

Production of Dislocation Etch Pits on Calcite using Optically Active Etchants

SEVERAL etchants, usually consisting of aqueous solutions of organic acids, have been shown[1-5] to produce etch pits at the points of emergence of dislocations on the rhombohedral cleavage faces of calcite crystals. Moreover, if the pits are particularly well defined it is often possible to deduce, from their internal structure, the plane and the direction in which the dislocation line runs through the crystal, a possibility of considerable interest to us since we are endeavouring to assess the role of line imperfections in the chemical reactivity of calcite.

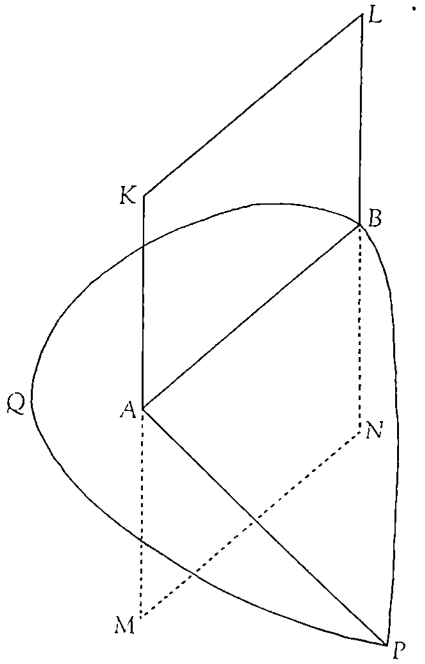

Fig. 1. Schematic representation of etch-pit produced by D- and L-tartaric acids showing plane in which the dislocations lie. Dotted lines indicate the section of the plane below the plane of the paper

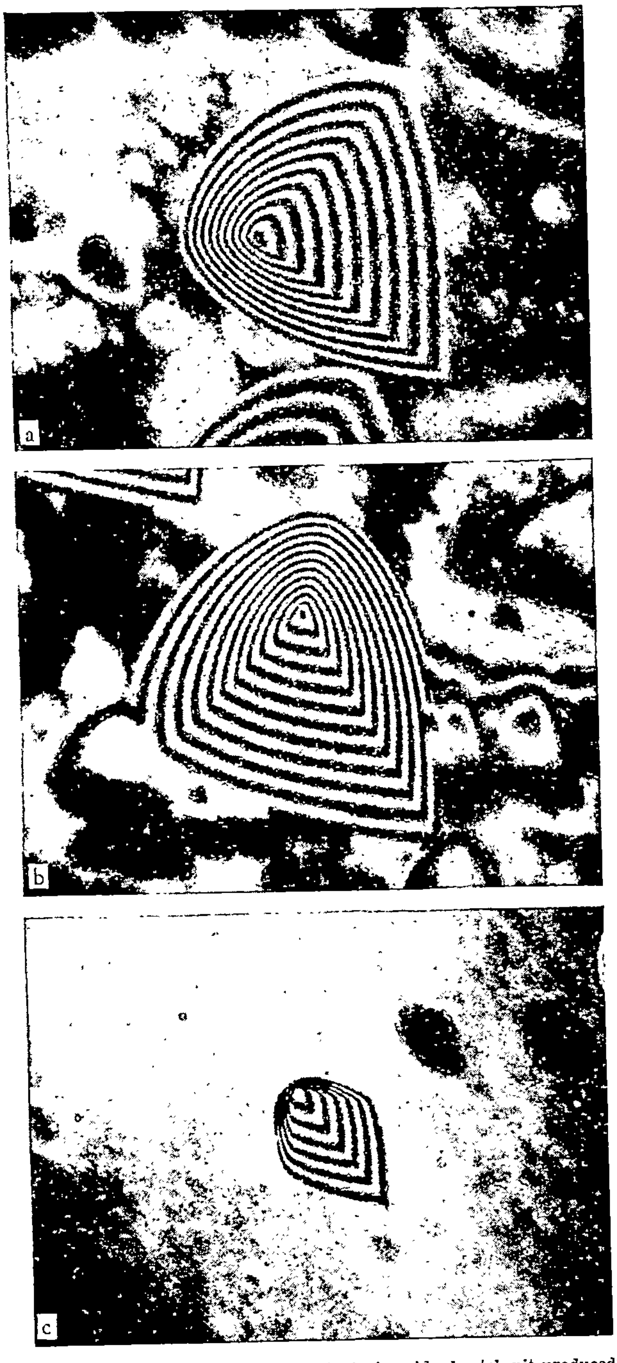

Fig. 2. *a*, Etch pit produced by D-tartaric acid; *b*, etch pit produced by L-tartaric acid; *c*, etch pit produced by *meso*-tartaric acid. Interferograms (× *c*. 425)

It has been shown by Gilman and Keith[4] that the nature of the anion of the organic acid used as etchant is important in deciding such characteristics as the orientation and internal structure of the dislocation etch pits; and since these are the characteristics which are used to arrive at the crystallographic directions of the dislocation lines, we decided to devise a simple test to ascertain whether it is possible to obtain reliable information concerning the direction of the dislocation line from the differently shaped pits produced by different etchants.

Bengus *et al.*[2] state that, for a pyramidal pit such as that shown diagrammatically in Fig. 1, *PQB*, the plane in which the dislocation line lies, is that which is constructed by taking the pit edge (*AB*) opposite the steepest face and projecting this edge on to the base of the pit. Thus, the relevant plane is *KLMN* in Fig. 1. The steepest faces of the etch pits described by Bengus and in this communication were determined using optical interference microscopy[6].

Figs. 2*a* and *b* are interference photomicrographs (sodium light illumination) of separate parts of the same 'as-cleaved' surface of a calcite rhomb, one end of which was immersed, with gentle agitation, for 60 sec in 10 per cent aqueous solution of D-tartaric acid at 22° C, and the other in L-tartaric acid under identical conditions. Immediately following etching the crystals were washed, successively, in running water, ethanol, ether and finally dried in air. In all the photomicrographs shown here the top edges of the micrographs lie along the [100] direction.

It is to be noted that a change in pit orientation for D- and L-tartaric acids occurs, and that the angle through which the pit is re-oriented is, to within experimental error, the same as the obtuse angle in calcite, 102°. This reveals that the etching process is stereospecific, a fact recorded prior to the advent of dislocation theory in the investigations of Honess and Jones[7], who found that the pits produced by the D- and L-acids were mirror images.

Micrographs such as those shown in Figs. 2*a* and *b* verify that the test of Bengus *et al.*[2] holds good because, even though marked re-orientation of the pits takes place, the plane in which the dislocations lie, determined as described here, remains unaltered when either the D- or the L-tartaric acid is used.

Quite apart from substantiating Bengus's method, this work, which is continuing, opens up the possibility that stereoisomeric etchants, such as D- and L-tartaric acids, may be able to distinguish right-handed from left-handed screw dislocations or positive from negative edge dislocations. Some caution is, however, required for we have found that, using an aqueous solution of *meso*-tartaric acid as etchant, under the conditions described above, it is extremely difficult to evaluate the plane in which the dislocation lies from the internal structure of the etch pit (Fig. 2*c*).

We thank Prof. S. Peat for his advice and the Department of Scientific and Industrial Research for a maintenance grant to one of us (C. R.).

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CHEMISTRY

Nitrous Oxide as a Scavenger for Electrons in the Radiolysis of Propane

Recent investigations of the radiation chemistry of liquid hydrocarbons indicate that in these systems N_2O reacts rapidly with electrons but relatively slowly with hydrogen atoms[1]. We have investigated the effect of N_2O in the radiolysis of propane gas, and the results obtained have led to information about the mechanism of hydrogen formation.

The gas mixtures (760 mm mercury) were irradiated in 'Pyrex' vessels (~400 ml.) using a cobalt-60 γ-ray source. The dose-rate in propane was 5×10^{14} eV ml.$^{-1}$ min^{-1} (nitrous oxide dosimeter: assuming $G(N_2) = 12{\cdot}5$ molecules/100 eV[2] and using an experimentally determined value of 1·42 for the stopping power of propane relative to N_2O). Total doses were 10^{16}–10^{17} eV ml.$^{-1}$. The results, taken from linear yield-dose plots, are given in Table 1.

The yield of hydrogen from pure propane was found to be $G(H_2) = 6·5$ and was in agreement with previously reported values[3,4]. Addition of N_2O decreased the yield of hydrogen and led to the formation of nitrogen. The yields of H_2 and of N_2 were independent of the N_2O concentration between 2 and 4 mole per cent.

In propane, containing N_2O and propylene at equal concentrations ($\sim$ 3 mole per cent), the yield of N_2 was the same as in the absence of propylene. It is known[5,6] that the ratio of the rate constants $k_{(H+C_3H_6)}/k_{(H+N_2O)} \gg 10^5$, and it may be concluded that hydrogen atoms will react exclusively with propylene under these conditions. The formation of N_2, therefore, cannot be due to the reaction of hydrogen atoms with N_2O.

Table 1. RADIOLYSIS OF PROPANE (760 MM MERCURY, 20° C). EFFECT OF ADDED N_2O, SF_6, CCl_4 AND PROPYLENE

Additive	Yield (molecules/100 eV) Hydrogen	Yield (molecules/100 eV) Nitrogen
None	6·5	—
N_2O (2–4 mole %)	4·2	7·5
C_3H_6 (3 mole %)	1·75	—
C_3H_6 (3 mole %) + N_2O (3 mole %)	1·75	7·5
SF_6 (5×10^{-3} to 3 mole %) + N_2O (3 mole %)	4·2	0
CCl_4 (3 mole %) + N_2O (3 mole %)	4·2	0

Our results can be interpreted by assuming that, in irradiated propane + N_2O mixtures, N_2 is formed by reaction of electrons with N_2O, an assumption which would be consistent with the reactions of N_2O in irradiated liquid systems[1,5,7,8]. In electron beam experiments it has in fact been shown that the capture process:

$$e^- + N_2O \rightarrow N_2 + O^- \qquad (1)$$

can occur with electrons of thermal energies[9].

On the basis of this interpretation, SF_6 and CCl_4, which have large cross-sections for electron capture[10], should be able to compete with N_2O for electrons. We have found that this is the case and that the cross section for electron capture by SF_6 is at least 10^4 times as great as that of N_2O.

Since the energy for the formation of an ion pair in propane is 26·3 eV[11], the yield of electrons $G_e = 3·8$. The value of $G(N_2) = 7·5$ obtained in our experiments suggests that electron capture by N_2O in the gas-phase leads to the production of two molecules of N_2. It is possible that, under these conditions, reaction (1) is followed by:

$$O^- + N_2O \rightarrow N_2 + O_2^-$$

which would be at least 3 eV exothermic.

The decrease in $G(H_2)$ when an electron scavenger (N_2O, SF_6, CCl_4) is added to propane may be attributed to a change in the process by which the positive ions are neutralized. In the absence of an electron scavenger, the ion will be neutralized by an electron; with an electron scavenger present, however, the neutralization will involve a negative ion. If it is assumed that neutralization by negative ions does not lead to hydrogen formation, then the decrease in $G(H_2)$ of 2·3 found on adding N_2O, SF_6, or CCl_4 corresponds to the hydrogen yield resulting from electron-ion neutralization in pure propane. This value is less than G_e, suggesting that only approximately 61 per cent of the neutralization events in propane lead to formation of H_2.

The decrease in the hydrogen yield to $G(H_2) = 1·75$ when propylene is present has been observed previously and is attributable to the scavenging of hydrogen atoms by the olefin[3]. The hydrogen yield in the presence of propylene is not influenced by N_2O. It would appear, therefore, that this 'molecular' H_2 is not produced as a result of ion neutralization, a finding that is in agreement with evidence discussed previously by Back[3].

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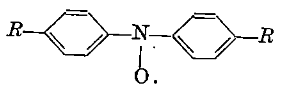
Stable Nitroxide Radicals

THE stability of organic free radicals is generally attributed to electron delocalization, steric inhibition of dimerization, or both (for example, 2,4,6-tri-*t*-butylphenoxyl), and the nitroxide radicals appear to accord well with these views. In the well-known diaryl nitroxides (I)[1,2] the >N—O group is attached to two conjugated systems, in (II)[3] and (III)[4] and related radicals[5] it is highly hindered, while in (IV)[6] and (V)[7] both conjugative and steric factors may contribute to radical stabilization.



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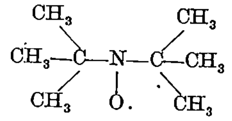

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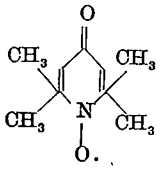

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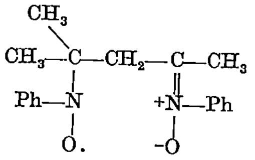

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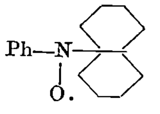

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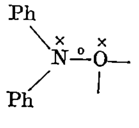

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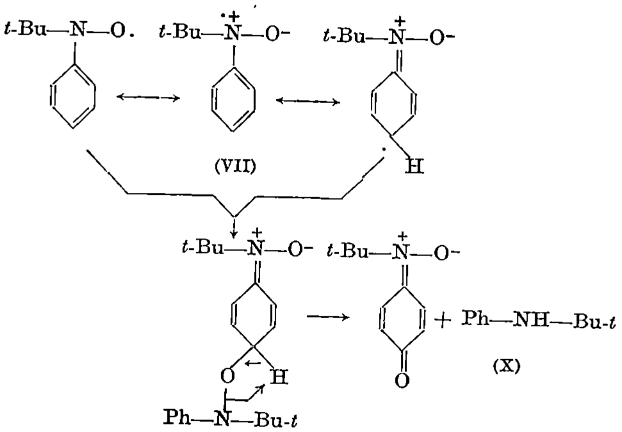

(VII)

(VIII) (IX) (X)

According to Linnett[8], however, the stability of nitric oxide and the organic nitroxides can be explained by the double quartet hypothesis; on this basis diphenyl nitroxide (VI) (Linnett's notation) is resistant to dimerization because the total number of electrons involved in bonding would not thereby increase, whereas electron repulsion would. Conjugative and other factors are disregarded.

t-Butyl phenyl nitroxide (VII)[9-10], which we have prepared by oxidation of the corresponding hydroxylamine (from *t*-nitrosobutane and phenyl magnesium bromide) with silver oxide, undergoes continuous decomposition in air over a period of several days to form *t*-butylaniline (X) and N-*t*-butyl-*p*-benzoquinonimine N-oxide (IX). The production of these compounds can be accounted for by the coupling of the radical at the *para* position of the benzene ring as shown. Diphenyl nitroxide (I; $R = \text{H}$) decomposes similarly in ether solution to diphenylamine and N-phenyl-*p*-benzoquinonimine N-oxide[2]. This mechanism is analogous to that proposed for the oxidation of phenols to quinones by the nitroxide, Fremy's salt[11].

It appears, therefore, that the relatively low stability of diphenyl- and *t*-butyl phenyl nitroxides can be attributed, at least in part, to their tendency to dimerize to unstable adducts of the type (VIII), which rapidly decompose. These results, together with the observed hyperfine structure of the electron spin resonance spectra of (I)[12] and (VII)[9] and related radicals[13], and the fact that the dianisyl (I; $R = \text{OCH}_3$)[14,15] and the 4,4′-dinitrodiphenyl (I; $R = \text{NO}_2$) nitroxides are much more stable than the parent radical (I; $R = \text{H}$), show that the odd electron is not confined to the $>$N–O bond in these radicals.

We are investigating the possible use of stable nitroxide radicals as replacements for Fremy's salt in oxidation reactions; in general we find that they will abstract hydrogen from N—H and S—H bonds but they do not oxidize monohydric phenols to quinones.

Note added in proof. Since this communication was submitted the preparation of the hydroxylamine and the nitroxide has been reported by A. K. Hoffmann, A. M. Feldman and E. Gelblum, *J. Amer. Chem. Soc.*, 86, 646 (1964).

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Redox Cleavage of S—S Linkage in Diaryl and Cyclic Alkyl Disulphides

THE rupture of S—S linkages in organic disulphides by either ionic or free radical processes is well known. On the other hand, redox cleavage of such S—S bonds has never been demonstrated, although Kolthoff[1] postulated that ferrous citrate complex with organic disulphide underwent redox transformation analogous to that with hydrogen peroxide. However, this observation was made under quite different conditions in their polarographic investigations.

I wish to report that diaryl disulphides, such as phenyl disulphide, and cyclic disulphides, such as 1,2-dithiolane-3-valeric acid (lipoic acid), interact with organometallics such as ferrocene and vanadium acetylacetonate to yield the corresponding thiyl radicals, mercaptide ions and the concomitantly oxidized metal ions. In a typical run. 10 mg (0·05 mmole) of phenyl disulphide (Eastman. m.p. 59°–60°) and 4 mg (0·02 mmole) of ferrocene (Aldrich Chem. Co., m.p. 174°–176°) in 10 g (70 mmole) of purified *n*-butyl methacrylate at room temperature in the dark produced 150 mg of polymers over a period of 20 h, while phenyl disulphide or ferrocene alone in the same monomer failed to yield any polymer under the same conditions. The oxidation of ferrocene is evidenced by the appearance of the faint blue colour of ferrocenium ion. When the reaction was carried out in ethanol instead of vinyl monomers with an appreciably higher concentration (2 mmole of each of phenyl disulphide and ferrocene in 100 ml. of ethanol) under the same conditions as in the previous experiment, the reaction mixture furnished a few per cent of lead mercaptide, after treating the mixture with lead acetate, and much of the unchanged disulphide and ferrocene. It was also noticed that ferrocenium ion can be reduced by phenyl mercaptide ion, hence this reaction is a truly reversible one. The same result was obtained with lipoic acid and vanadium acetylacetonate. However, alkyl disulphides, such as benzyl disulphide, butyl disulphide and heptyl disulphide, failed to react with either ferrocene and acetylacetonate. It is possible that these disulphides may be responsive to other specific reductants.

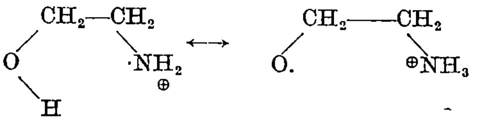
In addition to the metal induced redox cleavage of S—S bond in aryl and cyclic alkyl disulphides, we also have noticed that certain basic nitrogen compounds, such as *N*,*N*-dimethylaniline, trialkylamines, ethanolamine and substituted pyridines are also effective in cleaving the same kind of disulphide linkages, as evidenced by initiation of polymerization of acrylonitrile. For example, a solution of 50 mg (0·25 mmole) of phenyl disulphide and 60 mg (0·5 mmole) of *N*,*N*-dimethylaniline in 10 g (190 mmole) of acrylonitrile in the dark at room temperature became completely solidified within 5 h. Other reactive but less-effective amines are triethylamine, tributylamine, 2,4,6-trimethylpyridine and ethanolamine; while aniline, pyridine, hydrazobenzene and tribenzylamine failed to react with phenyl disulphide. In the case of lipoic acid, the same result was obtained with *N*,*N*-dimethylaniline, ethanolamine, 3-hydroxypropylamine and 2-amino-2-methyl-1,3-propandiol, while other amines were not effective.

Ionic scission of sulphur–sulphur bonds on attack of nucleophilic reagents is known[2-4], but the disulphides reported capable of interacting with ammonia and amines are limited to only 2-benzothiazyl disulphide[5] and ethyl xanthic acid disulphide[6]. Since ionic scission of S–S bonds by amines appears to be limited to a few specific disulphides, I propose that the series of reactions between diaryl and cyclic disulphides with various amines examined in our laboratory proceeds by a redox free radical mechanism.

The amine and disulphide reaction may parallel to the known free radical process exhibited by certain organic peroxides and amines[7-9], wherein an ionic intermediate formation was suggested to be the rate-controlling step. However, I have observed no correlation between the basicity of nitrogen compounds and their activity toward cleavage of the sulphur–sulphur linkage in lipoic acid. It might be possible that in the redox cleavage of disulphide linkage with organic base, two concurrent reactions are taking place, one following the ionic intermediate route

$$R_3\text{N}{:} + R'\text{SS}R' \longrightarrow [R_3\text{N–S}R']^+ + R'\text{S}^-$$

and the other involving electron transfer process as demonstrated in the disulphide–organometallic interaction. The former mechanism is preferred in the case of diaryl disulphides, since reactivity toward these parallels the basicity of the bases, whereas the latter mechanism is preferred in the case of lipoic acid since reactivity toward this molecule is apparently exhibited only by bases which can form stabilized cationic radicals. Of course, it is also possible that the low reactivity of stronger bases, such as triethylamine, in this case may be attributed to salt formation with the lipoic acid. The reactivity of ethanolamine in cleaving S—S bond in lipoic acid is an interesting case. We may speculate that the amine cationic radical resulting from a direct electron transfer mechanism can be stabilized by tautomerism between the following forms:



In line with this hypothesis, I found 1-amino-3-propanol to be equally effective. In this case the geometry of the molecule still permits such tautomeric transformation, while other amino alcohols showed no effect.

The redox cleavage of disulphides with basic organic molecules and organometallics may have implications in certain biological systems.

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Role of Grinding of Titanium Trichloride in Natta's Polymerization

THE kinetic behaviour in the initial stages of the polymerization of propylene with titanium trichloride–triethyl aluminium can be divided into two types: the build-up type (the rate increases to the stationary value) and the decay type (the rate approaches decreasingly to the value). Natta and Pasquon[1] observed that the build-up type changed into the other type as grinding the titanium trichloride, whereas the stationary rate did not change, and supposed that the produced polymer adjusted the particle size of titanium trichloride into the stationary value appropriate for the stationary polymerization. This supposition is based on the experimental result that the same stationary rate per unit weight of titanium trichloride was obtained with the use of the titanium trichloride catalysts in various particle sizes.

We have carried out the polymerization with the titanium trichloride catalysts ground in various degrees and confirmed the change in the types of the rate-time curve, excepting the different values of the stationary rate, as follows.

The titanium trichloride was obtained from Stauffer Co., Ltd. (prepared from titanium tetrachloride by reduction with hydrogen), of which the surface area is 3·5 m²/g. Grinding the titanium trichloride with a small ball mill in nitrogen atmosphere 30–100 h, we have the ground catalysts. Using the BET method with nitrogen adsorption, the surface areas of the ground catalysts have been determined as shown in Table 1.

Table 1. GRINDING TIME AND SURFACE AREA

Hour	0	36	50	70	100
m²/g	3·5	10·3	18·9	22·1	24·1

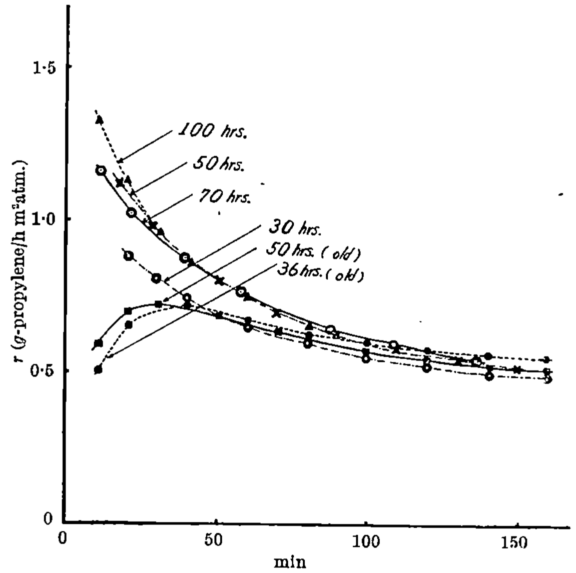

Fig. 1. Polymerization rate and time

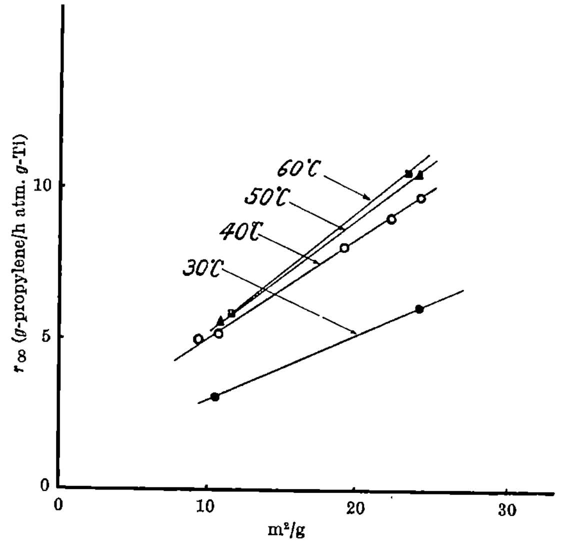

Fig. 2. Stationary rate and surface area

Carrying out the polymerization with these catalysts under the condition that aluminium/titanium 1, n-heptane 250 ml. and 30°–60° C, the rate-time curves have been obtained as illustrated in Fig. 1, where the rate is expressed by g propylene/h atm m². The decay type of the curve can be represented by the following equation[2]:

$$r = B \exp(-kt) + r_\infty$$

with $$r_\infty = A \exp(-10\,\text{kcal}/RT)\,P$$

where t is the polymerization time, P the pressure of propylene and B, k, A are the constants. Applying the equation for the experimental data, we have the calculated value of r_∞ which corresponds to the stationary rate. The calculated value per unit weight of the catalyst is approximately proportional to the surface area as shown in Fig. 2.

From these results we have arrived at the conclusion that the stationary rate is proportional to the surface area of the catalysts used and the effect of the grinding may be divided into two effects: the expansion of the surface area and the promotion of the initial activity. The decay type obtained with the ground catalysts changed to the build-up type with the same catalysts maintained for about a month in an atmosphere of nitrogen, which shows the initial activity made to appear by the grinding is unstable.

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Some Interferometric Observations on the Water/Solids Relation of Activated Sludge Flocs, using a Compression Technique

As part of a fundamental investigation of the water/solids relation of activated sewage sludge an attempt was made to apply interference microscopy[1] to the measurement of the solids content of the sludge floc.

The gelatinous consistency and irregular shape and size of the zooglœal flocs have so far prevented any reliable estimate of the solids content of the flocs; such knowledge is of importance when investigating the behaviour of different sludges and may have a practical value in treatment plant design.

Since the calculations involved in the interferometric method required precise measurement of the thickness of the particles it was thought desirable to introduce some uniformity to the particles by compression. A cell compressor for the measurement of mass and concentration by interference microscopy has been described by Goldacre *et al.*[2]. We have found it more convenient to modify a Rousselets compressor to provide a compressed field by making a sandwich of the particles between two rubber cushioned cover-slips. For the type of material being examined this compressor has been found to be quite adequate, there being a relatively large area of compression. The compressor is also suitable for immersion refractometry.

The glass disk is removed from the base of the Rousselets compressor and the cover-slip and its retaining clips removed from the upper plate. Two small strips of rubber are cemented to the bottom of the upper plate on either side of the aperture and a strip of cover-slip 7 mm wide is placed across the aperture and cemented to the strips of rubber. The hole in the base plate is enlarged and four strips of rubber are cemented to form a square about the hole.

A drop of suspension to be examined is placed on a hæmacytometer cover-slip supported by the strips of rubber on the base plate and the upper plate racked down. Initially the cover-slips are held together by the fluid between them. Further careful racking down of the upper plate results in the formation of the sandwich between the rubber cushions on the upper and lower plates.

The thickness of the compressed field can be found by conventional focusing methods making allowance for any slight variation across the width of the cover-slip.

Alternatively the thickness of the particle may be found by using the interferometric air-bubble technique[3] or by calculating thickness from phase changes in immersion media of different refractive indices[1].

Particles can be kept under observation during the compression process and they can usually be seen to flatten and spread out. The microscope adjustment is checked and adjusted when compression is complete.

Measurements are taken using a Baker half-shade eyepiece, and monochromatic light of 5420 Å from a Didymium and Green filter.

In calculating the results a value of 0·0018 has been assumed for α as the particles contain a large amount of protein.

The results obtained for three samples of activated sludge taken from a sewage works at different times are given in Table 1.

Table 1

Sample No.	(1)	(2)	(3)
Mean concentration of solids (g/100 ml. floc.)	12·02	14·65	9·65
Mean concentration of water (g/100 ml. floc.)	90·99	89·01	92·76
Mean density	1·030	1·036	1·024

Unlike most cytological materials, activated sludge cannot be represented in terms of a typical structure and average particles dimensions, consequently it is not an easy material to examine. So far as is known this is the first time information about the *in situ* density and solids content of sludge floc has been presented.

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BIOCHEMISTRY

α-Glycerolphosphate Production by Rat Hepatomas

IT has been reported previously[1] that the α-glycerolphosphate dehydrogenase (L-glycerol-3-phosphate: NAD^+ oxido-reductase, E.C. 1.1.1.8), either the crystalline form of the enzyme (Boehringer, U.S.) or that present in the post-mitochondrial fraction of rat liver and hepatomas, could be activated by potassium cyanide. At that time it was also found, although published only recently[2], that potassium cyanide increased the anaerobic production of α-glycerolphosphate (GP) from glucose-6-phosphate by the fortified post-mitochondrial fraction of three rat hepatomas.

Pette and Ruge[3] have very recently demonstrated that potassium cyanide causes the dismutation of methylglyoxal, which contaminates the triosephosphate esters used as substrate in the enzyme assay, to pyruvate, which is then reduced by added $NADH_2$ and the lactic dehydrogenase present in the post-mitochondrial fraction. These authors also obtained evidence for a non-enzymatic oxidation of $NADH_2$ in mixtures of the triosephosphate esters, ethylenediamine tetraacetic acid and potassium cyanide.

Experiments carried out shortly after our first report[1] was made cast doubt on the identity of the potassium cyanide effects obtained with the crystalline and post-mitochondrial enzymes. Both the activity of the crystalline enzyme and the effect of potassium cyanide thereupon varied, the two being inversely related; stimulation by potassium cyanide (5×10^{-4} M) ranged from 30 to 200 per cent. In the latter cases the $NADH_2$ oxidation by the crystalline enzyme was stimulated by EDTA (10^{-3} M) < cysteamine (10 μmoles) = potassium cyanide = cysteamine plus potassium cyanide < serum albumin (0·1 per cent) = hæmoglobin (250 μg) = these proteins plus potassium cyanide. Using the post-mitochondrial liver fraction as enzyme source, cysteamine, EDTA and protein showed only a small effect (20–30 per cent increase of activity). However, the marked stimulation[1] obtained with potassium cyanide and the post-mitochondrial liver and hepatoma enzymes was abolished by addition of cysteamine (even 0·5 μmole was effective). Another difference was apparent in the presence of *p*-chloromercuribenzoate (PCMB, 5×10^{-5} and 10^{-4} M). The activity of the crystalline enzyme both in the absence and presence of potassium cyanide was reduced to zero shortly after addition of PCMB. The activities of the post-mitochondrial liver and *B Y* 484 hepatoma enzymes in the absence of potassium cyanide were likewise affected by PCMB, but the potassium cyanide-mediated increase in $NADH_2$ oxidation remained largely unaffected in these cases. Oleic acid (5 μg) inhibited the crystalline enzyme, the inhibition being reversed equally well by potassium cyanide, cysteamine or EDTA. The diminished activity of aged enzyme (300-fold dilution in water allowed to stand for some hours) could be restored with either potassium cyanide or cysteamine. When, instead of the triosephosphate esters, fructose diphosphate plus aldolase was used as substrate for the crystalline enzyme, potassium cyanide caused also an increase (30–50 per cent) in the rate of $NADH_2$ oxidation.

In view of the findings of Pette and Ruge[3] we have repeated very recently some of our experiments. Now, using the crystalline enzyme, no potassium cyanide effect could be observed. However, after addition of lactic dehydrogenase, potassium cyanide did stimulate the rate of $NADH_2$ oxidation using the triosephosphate esters, but no such effect was obtained using fructose diphosphate plus aldolase. The non-enzymatic oxidation[3] of $NADH_2$ in the presence of potassium cyanide did not operate under our assay conditions unless the concentration[1] of the triosephosphate esters was increased 3-fold. The sensitivity of the α-glycerolphosphate dehydrogenase for heavy metal ions is well known[1], and the foregoing results lead us to the conclusion that the potassium cyanide effect observed in our previous experiments with the crystalline enzyme must have been due to the presence of some sort of heavy metal ions inhibitory to the enzyme and removed by potassium cyanide. (Oleic acid may have been inhibitory by introducing such ions.) However, we agree with Pette and Ruge[3] that the potassium cyanide-mediated increase of the $NADH_2$ oxidation by the post-mitochondrial enzymes, as reported in our original paper[1], is largely due to the above-mentioned side-reaction.

Table 1. α-GLYCEROLPHOSPHATE PRODUCTION FROM GLUCOSE-6-PHOSPHATE BY LIVER AND HEPATOMA POST-MITOCHONDRIAL FRACTIONS

Tissue	mμmoles GP/mg protein/30 min KF absent	KF present
Liver (2)	222 ± 30	425 ± 67
Primary hepatomas (2)	468 ± 63	828 ± 31
Hepatoma *BY* 252 (2)	107 ± 26	285 ± 55
Hepatoma *BY* 448 (2)	170 ± 30	342 ± 39
Hepatoma *BY* 484 (5)	453 ± 51	862 ± 91

Conditions as described previously (ref. 2). The enzymatic measurement of GP was carried out according to the method of Bublitz and Kennedy (ref. 4), the results of which were found in agreement with those obtained by the method of Ciaccio (ref. 5). GP added directly to the enzyme assay mixture or to the post-mitochondrial fraction, before (not incubated) or after incubation with glucose-6-phosphate, was recovered quantitatively. Lactic acid did not affect the assay

Also in contrast to our previous report[2], the stimulatory effect of potassium cyanide on the anaerobic production of GP by the hepatoma fractions could not be confirmed in our recent experiments. Now the incubation of the fractions in the absence of potassium cyanide led to the same high amounts of GP (Table 1) as previously obtained in the presence of potassium cyanide; as before, fluoride (10^{-2} M) increased GP production. In these experiments GP was measured enzymatically[2] with the α-glycerolphosphate dehydrogenase operating in the reverse direction of that in the above experiments. It was found that added GP could not be recovered in terms of $NADH_2$ at enzyme concentrations lower than 0·1 unit/flask, unless potassium cyanide (5×10^{-4} M) or EDTA (10^{-2} M) was also added. It is suggested that the difference between our recent and previous results on GP production may also have been due to the presence of heavy metal contamination under the previous experimental conditions. If the production of GP by the post-mitochondrial fractions (Table 1) is compared with the GP dehydrogenase activities, a correlation is found in the case of the transplanted hepatomas, but not in that of liver. In the absence of potassium cyanide the specific activities of the *B Y* 252 and *B Y* 448 enzymes amounted to 30–70 mμmoles $NADH_2$ oxidized/mg protein/min. Those of hepatoma *B Y* 448 and liver amounted to 140–200 and 200–400 mμmoles $NADH_2$ oxidized, respectively. The much lower or zero activities of the hepatoma enzymes, which may also be found[1], could, in some of our experiments, be increased to the former activities by addition of cysteamine or EDTA.

In summary, our results show that rat hepatomas may contain the soluble NAD-linked α-glycerolphosphate dehydrogenase and that these tumours produce appreciable quantities of GP from glycolytic intermediates. These results are contrary to those obtained by Boxer *et al.*[6–8]. It should, however, be noted that, using the Ehrlich-Lettré ascites tumour, Boxer *et al.* were unable to demonstrate GP production, whereas Wenner and Cereijo-Santalo[9] have been successful.

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Low Molecular Weight γ-Globulins of Urine and Plasma and their Relation to 7S γ_2-Globulin

IN the work recorded here, 1–3*S* γ-globulins from human urine[1], human plasma 3*S* γ-globulins[2], human plasma 7*S* γ_2-globulin and the *S* and *F* fragments obtained by papain hydrolysis of the 7*S* γ_2-globulin[3] were compared immunochemically and by means of peptide patterns.

Human 7*S* γ_2-globulin (lot No. 330–2, Squibb) was dissolved in 0·1 M potassium chloride containing 0·01 M cysteine and 0·002 M ethylenediamine tetraacetate and hydrolysed with twice crystallized papain at *p*H 7·0 in an enzyme to globulin ratio of 1 : 1,000 by weight for 30 min in a *p*H-stat at 37° C[4]. The papain hydrolysate was resolved into three protein peaks, I, II and III, by DEAE-cellulose chromatography using gradient elution from 0·01 M phosphate to 1·0 M phosphate concentration at *p*H 7·6. Protein peak I was further resolved into two peaks I–1 and I–2 by stepwise DEAE-cellulose chromatography at *p*H 7·6, first using 0·01 M phosphate and then 1·0 M phosphate; with columns of 'Sephadex *G*–100' (Pharmacia, Uppsala, Sweden), the *S* fragment in I–1 was separated from a protein that appeared to be relatively undigested 7*S* γ_2-globulin. Peak II was rechromatographed in DEAE-cellulose columns by stepwise elution at *p*H 7·6, first with 0·01 M phosphate and then with 1·0 M phosphate: two components were obtained, II–1 and II–2. Using columns of 'Sephadex *G*–100', another *S* fragment (*S*′) and the *F* polypeptide fragment were isolated from a pool of I–2 and II–2. As previously reported[5], a crystalline polypeptide fragment appeared during dialysis of the original 7*S* γ_2-globulin hydrolysate at *p*H 5·5; using 'Sephadex *G*–100', the crystalline polypeptide was separated into two components: an *F* fragment and a smaller amount of the *S*′ fragment.

1–3*S* γ-globulins were isolated from pooled human urine by pervaporation and lyophilization of the urine and chromatography through columns of DEAE-cellulose using 0·0175 M phosphate at *p*H 6·3 for elution; the first protein peak was then separated into 4 protein peaks using 'Sephadex *G*–100': the 1–3*S* urine γ-globulins were isolated in the third peak. A crude preparation of human 3*S* plasma γ-globulins was obtained from pooled Cohn Fraction VI as described by Takahashi and Schmid[2]: filtration of this preparation through a column of 'Sephadex *G*–75' layered over 'Sephadex *G*–100' yielded five protein peaks and the second peak contained purified 3*S* plasma γ-globulins. The sedimentation coefficients of the low molecular weight urine and plasma γ-globulins were verified by analytical ultracentrifugation. Since the γ-globulins used in this study were from pooled plasma or pooled urine, all of the preparations contained myeloma type I and type II antigenic determinants as revealed with specific rabbit antisera.

Electrophoresis of the different γ-globulins and polypeptide fragments on paper and in acrylamide gel is shown in Fig. 1; it will be noted that the mobilities of the different γ-globulins differed one from the other and that the human plasma 3*S* γ-globulins could be resolved into 2 proteins of different mobilities by paper electrophoresis. Two-dimensional agar diffusion with rabbit antisera specific for 7*S* γ_2-globulin revealed that the 1–3*S* urine and 3*S* plasma γ-globulins had antigenic sites similar to, but not identical with, those of *S* fragment and 7*S* γ_2-globulin (Fig. 2*A*). No antigenic similarity was demonstrated between the *F* polypeptide from 7*S* γ_2-globulin and the low molecular weight plasma and urine γ-globulins. It will be noted that the anti-7*S* γ_2-globulin antisera revealed several low molecular weight urine γ-globulins, which was also recently reported by Takatsuki and Osserman[6]. Rabbit antiserum against urine γ-globulins revealed the presence of four γ-globulins in the 1–3*S* urine γ-globulins as well as an antigenic constituent which was immunochemically unrelated to the γ-globulins of either urine or plasma; adsorption of this antiserum with serum from a child with agammaglobulinæmia removed all of the antibodies against this unrelated component but not those antibodies against the remaining 1–3*S* urine γ-globulins (Fig. 2*C*). With this anti-urine γ-globulin antiserum, there was antigenic identity between one of the 1–3*S* urine γ-globulins and the 3*S* plasma γ-globulins (Fig. 2*B*), and confirmation was obtained of the similarity but non-identity between the low molecular weight γ-globulins, 7*S* γ_2-globulin and the *S* fragment. Urine myeloma γ-globulin has been found[7] to consist of *L* chains, and the *L* chains of all the γ-globulins have been reported to be the same[7]; however, it will be noted from Fig. 2*B* that low molecular weight normal urine γ-globulins have antigenic determinants not present in myeloma urine γ-globulin. With this same antiserum, the antigenic determinants of the plasma 3*S* γ-globulins were the same as those in myeloma urine γ-globulin.

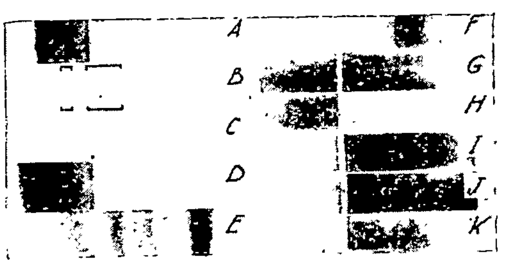

Fig. 1. *A–E*, Electrophoresis in barbital, *p*H 8·6, on paper; *F–K*, electrophoresis in glycine-*tris*-HCl, *p*H 8·3, in acrylamide gel; *A*, 7*S* γ_2-globulin; *B*, 3*S* plasma γ-globulins; bars demarcate zones of the two 3*S* γ-globulins; *C*, urine 1–3*S* γ-globulins; *D*, *S* fragment; *E*, normal serum; *F*, *F* fragment from crystallized component; *G*, papain hydrolysate of 7*S* γ_2-globulin; *H*, *S* fragment; *I*, urine 1–3*S* γ-globulins; six bands could be discerned; *J*, crude plasma 3*S* γ-globulin; *K*, fraction II-2 containing *F* fragment and *S'* fragment. Anode to the right of figure

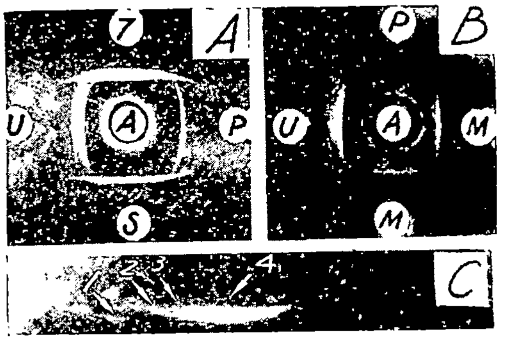

Fig. 2. *A*, Double gel diffusion using rabbit anti-7*S* γ_2-globulin in central well (*A*); *B*, double gel diffusion using adsorbed rabbit anti-urine 1–3*S* γ-globulins in central well (*A*); *C*, immunoelectrophoresis in 0·1 M borate *p*H 8·6 of urine 1–3*S* γ-globulins, reacted with adsorbed rabbit anti-urine γ-globulin antiserum; arrows indicate the four specific urine γ-globulins. Symbols: *U*, 1–3*S* urine γ-globulins; *P*, plasma 3*S* γ-globulins; 7, 7*S* γ_2-globulins; *S*, *S* fragment; *M*, myeloma urine γ-globulin

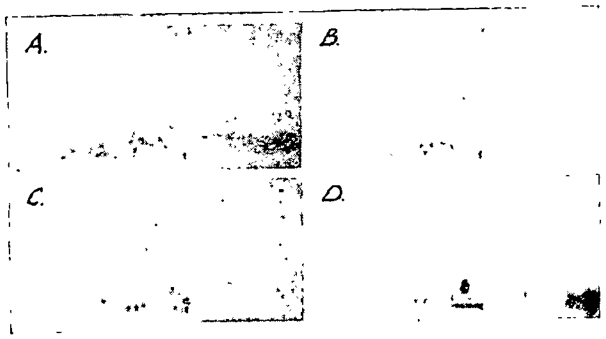

Fig. 3. Peptide patterns of trypsin hydrolysates of 7*S* γ_2-globulin (*A*), *S* fragment (*B*), 1–3*S* urine γ-globulins (*C*), and 3*S* plasma γ-globulins (*D*)

In Fig. 3, the ninhydrin stained peptide patterns of trypsin hydrolysates of the different γ-globulins are compared: these patterns were obtained with high-voltage electrophoresis in the horizontal direction followed by ascending chromatography in the second direction[8]. The peptides in the patterns of the urine γ-globulins could not be superimposed upon those of the other γ-globulins and similarly the patterns for 3*S* plasma γ-globulins were different from those of the other γ-globulins: if the 3*S* plasma γ-globulins were identical to the urine γ-globulins, then the peptides of one should be identified in the peptide patterns of the other even in the presence of contaminating proteins in one or both protein preparations, and this could not be done. Virtually all of the peptides in the *S* fragment were identified in the 7*S* γ_2-globulin peptide pattern as expected.

Recently it has been suggested that the low molecular weight γ-globulins of urine and plasma are free *L* chains and that they are the normal counterparts to Bence-Jones protein. The work recorded here has demonstrated at least four low molecular weight urine γ-globulins and two low molecular weight plasma γ-globulins. The antigenic similarity between these low molecular weight γ-globulins and 7*S* γ_2-globulin was confirmed; however, immunochemical differences between the low molecular weight γ-globulins of normal urine and those of myeloma urine and those of normal plasma were also found. In addition, since *S* fragments contain *L* chains, if the low molecular weight γ-globulins contained identical *L* chains, the peptide patterns should have areas of similarity; there was no apparent similarity between the peptide patterns of *S* fragment and those of normal low molecular weight γ-globulins. The evidence as to the antigenic and peptide structure of the low molecular weight plasma and urine γ-globulins is by no means conclusive, but it would suggest that they may not be derived from nor represent components of plasma $7S$ γ_2-globulin.

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Effects of 17-β-œstradiol on the Production of Acid Mucopolysaccharides by Cell Cultures of Human Fibroblasts

FIBROBLASTS from various tissues of origin have been shown to produce acid mucopolysaccharides (AMPS) *in vitro* for long periods of time up to several years[1]. It has also been pointed out that cultured fibroblasts, regardless of their original derivation, secrete predominantly hyaluronate and only small amounts of sulphated AMPS[2]. Only 'fibroblasts' growing from explants of beef embryo cartilage have been found to produce mainly chondroitin sulphate (*A* and *C*) *in vitro*[3]. This is somewhat at variance with what happens *in vivo* where different spectra of AMPS typify different tissues[4]. Nevertheless, in spite of lack of qualitative differences in AMPS secreted *in vitro*, fibroblasts grown from different tissues of origin have been found to produce different amounts of AMPS[5]. The experiments recorded here are intended to show that fibroblasts of different origin produce different amounts of AMPS *in vitro* in response to œstrogenic stimulation.

Primary cell lines were prepared from skin and soft tissues of the trunk and extremities of a 9-week-old human embryo and from foreskin of human new-borns. The cells were grown on plasma from primary explants until a good outgrowth developed. Cell suspensions were then obtained by trypsinization and used for replicate cultures. For each experiment equal aliquots of cells suspended in the medium were dispensed into several Earle *T*–30 flasks previously coated with an extremely thin layer of chicken plasma. Some of the flasks were kept as control and fed with a medium composed of Eagle HeLa medium (supplemented with glutamine) 50 per cent; whole egg ultrafiltrate 20 per cent; human placental serum 20 per cent; medium from the previous feeding period 10 per cent. The other flasks were used as experimental cultures and fed with the above medium to which 17-β-œstradiol was added in various concentrations (0·01γ, 0·05γ, 0·1γ, 0·4γ/ml.). In these circumstances, the addition of 0·01γ of 17-β-œstradiol per ml. of medium represents approximately a 5-fold increase over the amount contributed to the medium by the placental serum. The cultures of each experiment were maintained for 10 days, during which time they were washed and fed twice. The growth increment (GI) was evaluated by relating the number of cells at the beginning and at the end of each experiment. The medium from each flask was pooled separately and the AMPS extracted using the method described by Prince and Castor[6]. No attempt was made to separate sulphated and non-sulphated AMPS. Their amount was estimated as uronic acid (UA–AMPS) with the method of Dische[7]. The mean of the values for the various flasks was given as the final result of that experiment and was expressed in γ per ml. of medium. These values were then adjusted to their GI = 1. To estimate the production of UA–AMPS per cell, the total amount of UA-AMPS produced in 10 days by a culture was divided by the number of cells of that culture at 5 days. For the latter value, the mean between the total number of cells in the original inoculum and that at the end of the experiment was accepted as the closest approximation. Several experiments were carried out per each concentration of hormone. The amount of UA–AMPS present in fresh medium averaged 0·866 γ/ml. (± 0·179).

The cells from both embryonal and neonatal tissues grew rapidly and maintained the morphology of fibroblast-like cells throughout this study. Cultures which exhibited degenerative changes were excluded.

The results are summarized in Table 1. Preliminary results of some of these experiments have already been reported[8]. The control cultures of both embryonal and neonatal fibroblasts exhibited approximately the same GI and produced AMPS at about the same rate. The differences were so small as to be insignificant. The cultures of embryonal fibroblasts were exposed to 0·1 γ/ml. and 0·4 γ/ml. of 17-β-œstradiol. With both concentrations the GI was consistently smaller than that of the controls to indicate that the hormone had exerted an inhibitory effect on the growth rate of these fibroblasts. The numerical reduction of the cells in these cultures appeared to be related to decreased mitotic activity inasmuch as no cell necrosis was demonstrable. In contrast, the production of AMPS was significantly stepped up. To this end, the concentration of 0·1 γ/ml. seemed to be slightly more effective. In this connexion, it is interesting to observe that under ordinary conditions larger amounts of AMPS are produced by cultures with higher growth rate[1]. 17-β-œstradiol (0·01γ, 0·05γ, 0·1 γ/ml.) did not appear to exert any significant influence on either the growth rate

Table 1

17-β-Œstradiol (γ/ml.)	Control cultures			Experimental cultures		
	GI	UA-AMPS (γ/ml. per unit of GI)	Total UA-AMPS (γ/cell × 10^5)	GI	UA-AMPS (γ/ml. per unit of GI)	Total UA-AMPS (γ/cell × 10^5)
Embryonal fibroblasts						
0·1	1·69 (1·506–1·908)	1·676 (1·5–2·136)	0·849 (0·72–0·978)	0·906 (0·763–1·049)	4·566 (4·331–4·802)	2·402 (2·266–2·538)
0·4	1·69 (1·506–1·908)	1·676 (1·5–2·136)	0·849 (0·72–0·978)	0·975 (0·95–1·00)	3·66 (3·277–4·043)	1·861 (1·596–2·127)
Neonatal fibroblasts						
0·01	1·455 (1·238–1·673)	1·533 (1·06–2·007)	1·152 (0·838–1·466)	1·392 (1·047–1·74)	1·36 (0·891–2·277)	0·97 (0·561–1·619)
0·05	1·711 (1·164–2·288)	1·56 (1·051–2·528)	1·341 (0·862–1·727)	1·673 (0·822–2·482)	1·637 (1·053–2·138)	1·16 (0·837–1·631)
0·1	1·59 (1·5–1·68)	1·172 (1·147–1·198)	1·009 (0·862–1·157)	1·451 (0·911–1·94)	1·348 (0·97–1·715)	0·898 (0·831–1·088)

of the neonatal fibroblasts or their AMPS production. Indeed, the production of UA–AMPS per cell in the experimental cultures appeared to be less than in the respective control cultures. However, it is difficult to say whether œstradiol in effect inhibited the production of AMPS by these neonatal fibroblasts since the differences between control and experimental cultures were very small.

These results indicate a definite difference between these two groups of fibroblasts in their response to stimulation by 17-β-œstradiol. The differences in GI and AMPS production when these cells are exposed to œstradiol are even better appreciated if one considers that the control cultures of both embryonal and neonatal fibroblasts have behaved in essentially the same fashion in regard to GI and AMPS production for several months of experimentation.

It should be kept in mind that these two groups of cells differ from each other not only in developmental age but also in that they are derived from different regions of the body. However, whether the difference in AMPS production *in vitro* by fibroblasts exposed to œstrogenic stimulation is attributable to the difference in their age, or in their topographical derivation or in both, is difficult to say at the present time and must wait for further investigation.

In our opinion the results reported here lend support to the contention that, functionally, there are different kinds of fibroblasts[9] which exhibit different functional properties in the face of striking morphological similarities. In addition, they indicate that functional differences among fibroblasts exist *in vitro* as well as *in vivo*.

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Off-flavour of Dehydrated Carrot stored in Oxygen

WHEN dehydrated carrot is stored in the presence of oxygen its colour, due to β-carotene, is destroyed[1] and simultaneously an off-flavour develops which has been described as having an odour similar to that of violets[2]. Although it was earlier suggested that the off-flavour was due to the formation of β-ionone[2], no unequivocal proof of the nature of the product or products responsible has yet been put forward.

The substances responsible for the off-flavour of oxygen-stored carrot dice, dehydrated by the accelerated freeze drying technique[3], can be extracted with ether, and separated from the non-volatile fraction by high vacuum distillation. This fraction, on gas chromatography, gives twenty-four components only nine of which have a violet-like odour (Table 1). A further twenty-three components are present in a low-boiling fraction obtained by head-space analysis[4], but none of these smelled like violets. Of the compounds in Table 1, five, which account for more than 90 per cent of the total, have been isolated by large-scale gas chromatography and their structures investigated.

Table 1. COMPONENTS WITH A VIOLET-LIKE ODOUR ISOLATED FROM OXIDIZED DEHYDRATED CARROT

No.	Percentage total violet-like substances	Retention index at 150° PEG *A*	Identity
1	4	1,872	—
2	11	1,907	—
3	12	1,927	α-ionone
4	17	1,996	β-ionone
5	2	2,034	—
6	21	2,059	—
7	30	2,075	β-ionone 5,6-epoxide
8	2	2,204	—
9	1	2,310	—

Fraction 2 had a molecular weight (Rast) and elemental analyses corresponding to $C_{13}H_{20}O$ with 2·1 methyl groups and 2·6 double bonds. From ultra-violet and infra-red spectroscopic data it contained a methyl ketone group isolated from two conjugated double bonds one of which was alkyl substituted. The infra-red spectrum also indicated the presence of an isopropyl or gem-dimethyl group. The nature of the carbonyl group was confirmed by the properties of the 2,4-dinitrophenylhydrazine (DNP) derivatives. Gas chromatographic retention data on fraction 2 and both its catalytic hydrogenation and lithium aluminium hydride reduction products confirmed the absence of polar groups other than the ketone and the conjugated double bonds. The substance did not correspond with any known compound but probably has an ionone-like structure.

Using similar criteria, fraction 3 proved to be α-ionone; its infra-red spectrum was identical with that of a purified specimen of α-ionone and it was not separable from the authentic substance by gas chromatography using two different stationary phases at two temperatures. Lithium aluminium hydride and catalytic hydrogenation products were identical with those of the authentic specimen, as was the 2,4-dinitrophenylhydrazine derivative.

Fraction 4 was similarly shown to be β-ionone. The infra-red spectrum, gas chromatographic properties, lithium aluminium hydride and catalytic reduction products and its 2,4-dinitrophenylhydrazine derivative were identical with those of an authentic sample of β-ionone.

The data for fraction 6 corresponded best to $C_{13}H_{20}O_2$ with 1·6 methyl groups and 0·7 double bonds. The molecular weight, however, was high (253). The infra-red spectral data indicated the presence of an aldehyde, a substituted vinyl group and an isopropyl or gem-dimethyl group in the molecule. The ultra-violet absorption (λ_{max} 275 mμ ε 252) appears to indicate conjugation of the aldehyde and vinyl groups; but the infra-red evidence does not support this. The second oxygen atom is probably present as an epoxide.

By the criteria above, fraction 7 was found to be identical, with a sample of β-ionone-5,6-epoxide synthesized by the method of Karrer and Stürzinger[5].

Fractions 1, 5, 8 and 9 were not isolated in sufficient quantities for full investigation of their structures.

It would appear that the violet-like aroma of oxygen-stored dehydrated carrot is due to a number of compounds with ionone-like structures. The three of these substances which have been positively identified account for 60 per cent of the total substances with a violet-like aroma. These substances can be presumed to arise from the oxidation of carotenoids. Indeed, we have shown that the oxidation of β-carotene and β-ionone under similar conditions yields β-ionone-5,6-epoxide. α-ionone, on the other hand, may be formed from α-carotene present in the carrot. The fact that the two ionone isomers are present in similar amounts, whereas the corresponding carotenoids occur in quite different amounts (75 and 20 mg/100 g dry weight of β- and α-carotenes, respectively), probably indicates a difference in the stability of the α- and β-ionone rings.

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Purification of Pinguinain by Gel Filtration

The separation and characterization of macromolecules have been recently simplified by the introduction of a useful technique which has been variously termed gel filtration[1], molecular sieve chromatography[2], restricted diffusion chromatography[3], or exclusion chromatography[4]. The formulation of the mechanism of operation of these columns has been discussed by Ackers[5]. Since the presence of more than one protein component in proteolytic enzymes isolated from plant tissue is not an unusual finding, this method should be useful in their separation. Recently, based on filtration experiments, alcohol fractionation techniques and free electrophoresis, Messing *et al.*[6,7] have identified two proteolytic components in the juice obtained from the Cuban variety of the fruit of *Bromelia pinguin* L.

We were interested in the fractionation and purification of the enzyme or enzymes present in the Puerto Rican variety of the same fruit. For this purpose, we used this type of macromolecule fractionation technique.

The juice was extracted from the fruit by squeezing the pulp in a cheesecloth. The debris was removed by centrifugation at 25,000 r.p.m. in a Spinco model *L* preparative ultracentrifuge using a swinging bucket rotor *S.W.* 25.1. Macromolecular constituents were concentrated as follows: the *p*H of the juice (which has a *p*H range of 3–4) was brought to 7·2 with 1·0 M sodium hydroxide after addition of 3 g cysteine/l. juice. A dark precipitate containing high-molecular-weight impurities, mainly carbohydrates, was formed. After centrifugation for 5 min at 2,000 r.p.m., the precipitate was discarded, the supernatant removed and brought to 0° C. Enough acetone was added to the supernatant to bring the proportion of acetone to juice to a 2:1 ratio. The precipitate formed, which contains the bulk of the enzymatic activity found in the juice, was allowed to settle for 15 min. The supernatant was discarded after centrifugation in the cold. The precipitate was redissolved in distilled water and lyophilized.

The material used for gel filtration was 'Sephadex *G*–100' obtained from Pharmacia Fine Chemicals, Uppsala (lot No. *To* 2594, particle size 140–400 mesh, water regain 10 ± 1 g/g). Columns prepared had bed dimensions 1·9 cm × 25 cm. Acetate buffer (*p*H 4·6, 0·1 M) was used as eluent. The flow of these columns was 30 ml./h.

Two-ml. samples were collected with the aid of an automatic fraction collector and analysed for protein contents and assayed for enzymatic activity. Protein contents were determined by measuring the absorption at 280 mμ in a Beckman *DU* spectrophotometer. Enzymatic assays were performed as described previously[8]. Material for the assays, iodine-131 labelled human serum albumin and normal human serum albumin (25 per cent solutions), was obtained from Volk Radiochemical Co., Skokie, Illinois, and E. R. Squibb and Sons, New York, respectively.

Experimental results indicate the presence of two macromolecules. One component appears with the void volume indicating a very high molecular weight. This component is not precipitable by trichloroacetic acid, is not rendered insoluble by heat and does not show any enzymatic activity (Fig. 1). Although absorbing at 280 mμ, its absorption maximum occurs at 330 mμ. The most abundant macromolecule shows the properties of a protein; shows absorption maximum at 280; does not absorb at 330; is rendered insoluble by heat; is precipitable by trichloroacetic acid and shows enzymatic activity proportional to protein concentration (Fig. 1). A third peak obtained contains low molecular weight peptides and amino-acids, but shows no enzymatic activity.

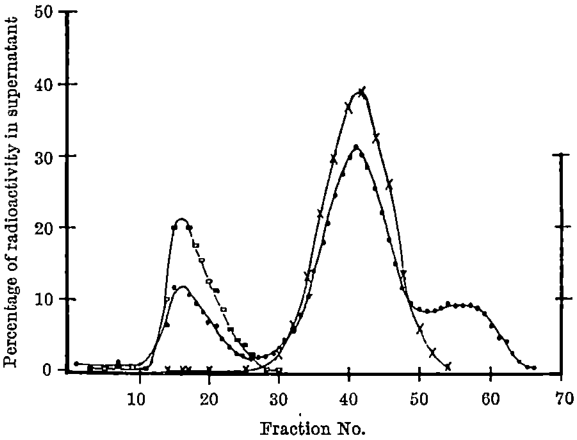

Fig. 1. Separation of pinguinain by gel filtration. Proteolytic activity was found in fractions 30–52, with a maximum in fraction 40. ×, Enzymatic activity; □, absorbancy at 330 mμ; ●, absorbancy at 280 mμ

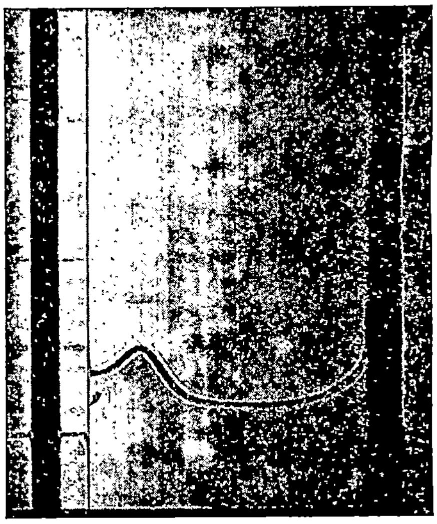

Fig. 2. Ultracentrifuge pattern of purified pinguinain. Run performed at 59,780 r.p.m. Picture taken 80 min after obtaining full speed. Direction of migration from left to right

The most abundant macromolecule migrates as a single component in paper electrophoresis in acetate buffer (*p*H 4·6, 0·05 M). Ultra-centrifugal analysis of this component, performed in a Spinco model *E* ultracentrifuge at 59,780 r.p.m. using 0·1 M phosphate buffer *p*H 7·3 as solvent, showed a single component with an $S_{20,w}$ of 2·1 *S* (Fig. 2). The diffusion coefficient ($D_{20,w}$) calculated for this purified preparation from steady state diffusion experiments using a Northrop–Anson cell was 14 ± 1·0 cm^2 sec^{-1}. The calculated partial specific volume for purified pinguinain was 0·746 ml./g. On the basis of these results and Svedberg's equation[9] a molecular weight of 14,700 ± 1,000 was calculated.

These results indicate that the Puerto Rican variety of the maya fruit contains just one proteolytic component.

It also shows that pinguinain is a protein having a small molecular weight.

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Inhibitory Effect of Progesterone on Activity of Human Myometrial Adenosine Triphosphatase

During the previous investigations[1] in this laboratory human myometrial strips were exposed to progesterone until all spontaneous activity ceased and the changes in the concentration of adenosine triphosphate (ATP) and phosphocreatine (PC) measured. Until such paralysis had existed for at least 40 min, no significant change in high-energy phosphate bonds (ATP and PC) could be documented. It was, therefore, concluded that in the earlier stages of the myometrial inhibition progesterone might be exerting its effect in the utilization of ATP rather than in its synthesis. As a continuation of the investigations on this problem, the effect of progesterone was then determined on the ATPase activity of the human myometrial strips. This communication deals with the results obtained.

The method of DuBois and Potter[2] was used to determine the ATPase activity in which human non-pregnant myometrial tissue homogenate was incubated in the presence of calcium chloride, diethyl barbiturate (buffer) and ATP. The amount of inorganic phosphorus liberated in homogenate corresponding to 6 mg and 9 mg wet weight tissue in 15 min at 37° C as determined by the procedure of Rockstein and Herron[3] was taken as a measure of ATPase activity. One microgram of phosphorus liberated was interpreted as one unit of ATPase activity. Ten to 300 μg of progesterone dissolved in 30λ of propylene glycol was added to the homogenate to determine its effect.

The two requirements: (*a*) that the rate of reaction is linear during the time of incubation; (*b*) that the rate is directly proportional to the amount of enzyme present in the test system, should be fulfilled if any enzyme assay is to be found satisfactory.

Propylene glycol used as a vehicle (30λ) did not alter the ATPase activity. Progesterone in concentrations of more than 10 μg (and up to 50γ tested) per mg wet weight human myometrial tissue was found to inhibit the human myometrial ATPase activity on an average of 27·3 per cent ($P < 0{\cdot}001$) based on 15 experiments as shown in Table 1.

It is interesting to note that such high concentrations of progesterone were needed to inhibit the human myometrial ATPase activity. It may be that the smaller concentrations of progesterone might inhibit the ATPase activity when exposed to spontaneously contracting myometrial strip in muscle-bath preparation over a prolonged period of time. Such an investigation is in progress and the results will be reported later.

Table 1

No. of cases	Average ATPase activity in units of human myometrial tissue/mg wet weight* Control	Progesterone treated (90–300 μg)	Average reduction in ATPase activity by progesterone
15	1·98	1·44	0·54 (27·3%)†

* 1 ATPase unit = 1 μg phosphorus liberated.
† $t = 7{\cdot}09$.
$P < 0{\cdot}001$.

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Stimulation of Glucose Utilization by Insulin in Human Adipose Tissue Homogenates

To date no effect of insulin on utilization of glucose in a cell-free preparation has been described. Indeed, the apparent ineffectiveness of insulin in cell-free preparations was a major clue to the discovery of the insulin stimulation of trans-membrane transport. We have been examining utilization of glucose in human adipose tissue and have observed a distinct stimulation of utilization of glucose by insulin added to homogenates of omentum.

Omentum was obtained from subjects undergoing abdominal surgery being performed for reasons other than malignancy. Homogenization was achieved within 15 sec, using a 'Vir Tis 23' homogenizer in a Krebs–Ringer bicarbonate buffer[1] gassed with $O_2 : CO_2$(95 : 5) to *p*H 7·4. The potassium chloride concentration of the buffer was doubled at the expense of the sodium chloride. A 'defatted' homogenate was obtained by removing the supernatant layer of congealed fat formed during centrifugation in the cold at 500*g* for 10 min. The homogenate was then supplemented to give final concentrations of 0·001 M ATP, 0·001 M TPN$^+$, 0·005 M Mg^{++} and 5·6 mM glucose. Incubation vessels containing 1 ml. of homogenate were incubated for 1 h at 37° C with agitation at 60 cycles/min. Insulin was added to give a concentration of 0·1 unit/ml. homogenate. Glucose was measured by a micro adaptation of the glucose oxidase method of Sunderman and Sunderman[2].

Table 1. Stimulation of Glucose Utilization by Insulin in Human Adipose Tissue Homogenates

Glucose utilization μg/mg tissue N_2/h	Without insulin	With insulin	Δ Insulin
	279 ± 75	466 ± 246	187 ± 105*

Values are means ± *S.D.* in 11 experiments
* $P < 0{\cdot}01$.

As seen in Table 1, insulin produced a 67 per cent increase in glucose disappearance. Though there is indirect evidence of an intracellular action of insulin on both carbohydrate[3] and protein metabolism[4], we believe that this is a direct demonstration of an intracellular insulin effect on utilization of glucose. Previous inability to show such an effect may have been due to the choice of tissue and incubation conditions.

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Presence of Carbonate in a Low-density Fraction of Diaphyseal Bone

USING a method of centrifugation in liquids progressively increasing in specific gravity[1], it is possible to separate, from a sample of bone powder, fractions at various degrees of mineralization[2]. These fractions, which can be recovered quantitatively, can then be analysed by appropriate chemical and biophysical techniques. We used infra-red spectroscopy for the examination of the carbonate present in bone, comparatively for a low specific gravity fraction (1·7 g/c.c.) and a pool of the high specific gravity fractions (1·8–2·3 g/c.c.) derived from diaphyseal bone samples of normal rats. It must be pointed out that less than 0·5 per cent of the samples belonged to the 1·7 gravity fraction. We used a Perkin–Elmer 221 infra-red spectrophotometer equipped with a sodium chloride prism. Potassium bromide pellets were prepared with various concentrations of whole unashed diaphyseal bone after separation.

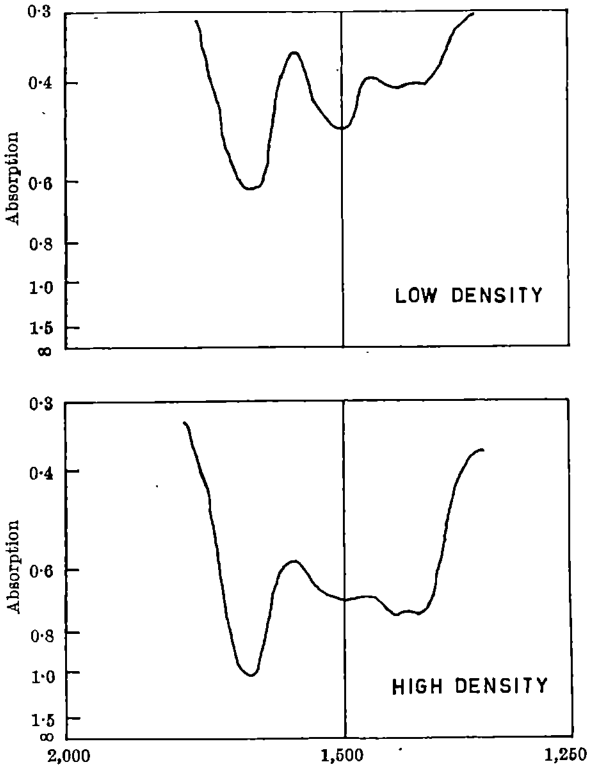

Fig. 1

Of the three regions of infra-red absorption by carbonate groups[3], two were satisfactorily demonstrated (860–875 cm^{-1} and 1,400–1,500 cm^{-1}) in both the low and the high specific gravity fractions. No absorption was observed at 710 cm^{-1}. Table 1 gives the location and intensity of all the absorption bands and Fig. 1 shows the spectra recorded between 1,300 and 1,700 cm^{-1}. In that region, three carbonate bands can be seen located at 1,506, 1,433, 1,401 cm^{-1} and 1,501, 1,433, 1,408 cm^{-1} respectively for the low and the high specific gravity fractions; but their relative intensity differs: in the low specific gravity fraction, the band located at 1,506 cm^{-1} is more intense than the bands found at 1,433 and 1,401 cm^{-1}, whereas the reverse is true in the high specific gravity pool. In both spectra, the band located at 1,639 cm^{-1} can be attributed to adsorbed water. The band located at 866 cm^{-1} was poorly resolved and showed no apparent difference from one spectrum to the other.

Two facts emerged from these results: (*a*) carbonate is present in both the early-calcifying and the fully mineralized parts of bone; (*b*) the change in the relative intensities of the three carbonate bands found in the 1,400–1,500 cm^{-1} region indicates that carbonate is present in more than one chemical form and/or environment, the proportion of which differs in the low and the high specific gravity fractions[4-6].

Table 1. FREQUENCIES OF THE ABSORPTION BANDS (CM^{-1})

Origin of the band	ν_3 $CO_3^{=}$	ν_2 $CO_3^{=}$	$PO_4^{\equiv}$	H_2O
Material				
Low density	1,506 m 1,433 w 1,401 w	868 w	ca 1,050 sb	1,634 s
High density	1,501 vw 1,433 w 1,408 w	868 w	ca 1,050 sb	1,639

Chemical analysis for carbon dioxide could not be carried out because only very small amounts of the low density material were available; this, however, appears to be critical before trying to propose any further interpretation of the infra-red data.

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Action of Rifamycin on Incorporation of Amino-acids into Protein in Cell-free Systems from *B. subtilis*

RIFAMYCINS are new antibiotics[1,2] having a marked inhibitory effect on the growth of many Gram-positive bacteria, including staphylococci[3,4], and have already been shown to affect bacterial respiration[5]. In this communication some results are reported concerning the effect of rifamycin *SV* and rifamycin M_{14} on the incorporation of amino-acids into protein by cell-free extracts of *B. subtilis* strain A.T.C.C. 6633.

Rifamycins have no effect on amino acyl-adenylate formation as studied by measuring the ATP-^{32}P pyrophosphate exchange or on amino acyl-RNA formation. Results of these experiments are shown in Tables 1 and 2. Fig. 1 shows that rifamycin does not affect pyruvate kinase activity as measured by the method reported by Bücher *et al.*[6].

For the examination of the incorporation of L-isoleucine labelled with carbon-14 into proteins by cell-free extracts, *B. subtilis* strain A.T.C.C. 6633 was cultivated in a medium containing 1·2 per cent casamino-acid, 0·3 per cent glucose, 0·1 per cent yeast extract, 0·45 per cent KH_2PO_4, 0·12 per cent $MgSO_4$, 0·81 per cent $NaHPO_4$. Cells were collected during logarithmic growth phase and disrupted by grinding with alumina Norton.

Results obtained using crude extracts prepared according to the method of Tissières[7], and by using washed ribosomes and 105,000*g* supernatant (*S*–105) prepared

Table 1. AMINO-ACID-DEPENDENT ATP-^{32}P-PYROPHOSPHATE EXCHANGE

	Additions	Percentage exchange
Complete mixture		55
,, ,,	− amino-acids	25·5
,, ,,	+ 0·0015 μmole/ml. rifamycin *SV*	56
,, ,,	+ 0·015 μmole/ml. rifamycin *SV*	55·4
,, ,,	+ 0·03 μmole/ml. rifamycin *SV*	50·4

The reaction mixture contained the following in μmole/ml.: 50 *tris*-HCl *p*H 7·4; 10 $MgCl_2$; 10 ATP; 10 each of 20 L-amino-acids; 10 ^{32}P-pyrophosphate (specific activity 14,000 c.p.m./μmole); 1·5 mg/ml. homogenate protein. ATP-^{32}P pyrophosphate exchange was measured as described by Demoss *et al.* (ref. 9). Radioactivity was determined by a windowless gas-flow counter.

Table 2. L-ISOLEUCYL-*s*RNA FORMATION

	Additions	c.p.m./mg *s*RNA
Complete mixture		194
,, ,,	+ 0·03 μmole/ml. rifamycin *SV*	188
,, ,,	+ 0·03 μmole/ml. rifamycin M_{14}	186

The reaction mixture contained the following in μmole/ml.: 50 *tris*-HCl *p*H 7·4; 30 MgCl$_2$; 10 ATP; 4·6 L-isoleucine ^{14}C (specific activity $2·68 \times 10^6$ c.p.m./μmole); 500 μg/ml. *s*RNA prepared by the method of Boman *et al.* (ref. 10); 1·5 mg/ml. homogenate protein. Amino acyl-RNA formation was measured as described by Berg *et al.* (ref. 11). Radioactivity was determined by a windowless gas-flow counter.

Table 3. ^{14}C-L-ISOLEUCINE INCORPORATION INTO PROTEIN BY CELL-FREE *B. subtilis* PREPARATIONS

Exp. No.		Additions	c.p.m./mg protein	Percentage inhibition
1	Complete mixture		880	
	,, ,,	+ 0·03 μmole/ml. rifamycin *SV*	467	59
	,, ,,	+ 0·15 μmole/ml. rifamycin *SV*	347	76
	,, ,,	+ 0·03 μmole/ml. rifamycin M_{14}	423	65
	,, ,,	Deproteinized at zero time	178	
2	Complete mixture		1,500	
	,, ,,	+ 0·03 μmole/ml. rifamycin *SV*	1,020	40
	,, ,,	+ 0·03 μmole/ml. rifamycin M_{14}	636	72
	,, ,,	deproteinized at zero time	300	

The reaction mixture contained the following in μmole/ml. In exp. 1: 10 *tris*-HCl *p*H 7·4; 10 magnesium acetate; 40 KCl; 0·34 ^{14}C-L-isoleucine (specific activity $2·68 \times 10^6$ c.p.m./μmole); 0·2 GTP; 1 ATP; 10 phosphoenolpyruvate; 84 μg/ml. pyruvate kinase; 120 μg/ml. DNA prepared by the method of Marmur (ref. 12); 2 mg/ml. homogenate protein prepared by the method of Tissières *et al.* (ref. 7). In exp. 2: 100 *tris*-HCl *p*H 7·4; 12 magnesium acetate; 50 KCl; 6 mercaptoethanol; 0·05 each of 20 L-amino-acids minus isoleucine; 0·17 ^{14}C L-isoleucine (specific activity $2·68 \times 10^6$ c.p.m./μmole); 0·03 GTP; 1 ATP; 5 phosphoenolpyruvate; 20 μg/ml. pyruvate kinase; 120 μg/ml. DNA (ref. 12); 1·2 and 0·6 mg *S*-105 and ribosomal protein prepared following the method of Matthaei and Nirenberg (ref. 8). In both cases the reaction was stopped with perchloric acid by the method of Ofengand *et al.* (ref. 13). Radioactivity was determined by a windowless gas-flow counter.

according to the method of Matthaei and Nirenberg[8], are given in Table 3.

These results indicate that rifamycin is an effective inhibitor of ^{14}C-L-isoleucine incorporation into proteins at molar concentrations much lower than those which produce the same inhibition by chloramphenicol or by other antibiotics active on protein synthesis[14-15].

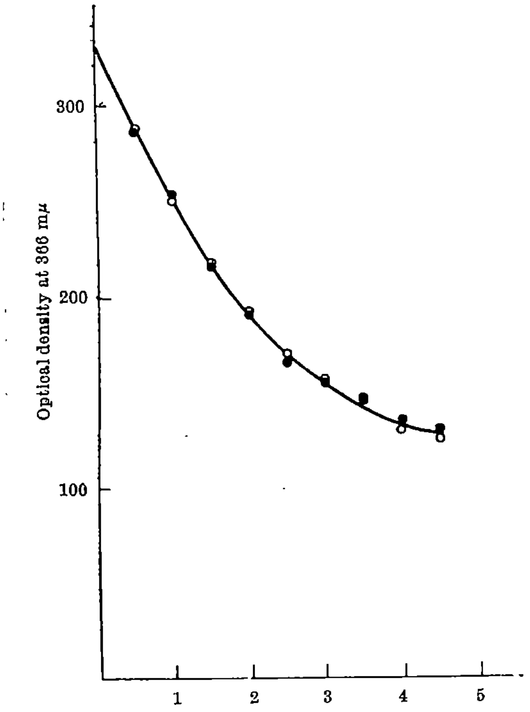

Fig. 1. Effect of rifamycin on pyruvate kinase activity, as measured by the method of Bücher *et al.* (ref. 6). ○, Without rifamycin; ●, in the presence of 0·075 mM rifamycin *SV*

Experiments are in progress to determine at which level the inhibition is effective.

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PHYSIOLOGY

Occurrence of Urinary Calculi in Rats exposed to High Concentrations of Oxygen with Added Carbon Dioxide

IN the course of an investigation[1] of the pulmonary responses of animals exposed to high concentrations of oxygen at atmospheric pressure (OAP) and to high concentrations of oxygen at atmospheric pressure to which 9–13 per cent carbon dioxide was added (OAP + CO$_2$), discrete, amorphous, non-crystalline masses were found in the urinary bladder at autopsy.

A total of 383 Sprague–Dawley male rats weighing 200–250 g were used in nine experiments. In each experiment 15–20 rats were exposed to OAP, another 15–20 to OAP + CO$_2$ and a third group of 8–12 were maintained in air. Each group had the same food and environmental temperature. Specially constructed identical chambers were used. The animals were observed frequently and autopsied after 40–60 h. Bladder masses, if present, were weighed (11·6 mg average) and their amino-acid composition was examined in a few cases.

The results of the observations of urinary calculi are presented in Table 1.

Table 1

	Air controls	OAP	OAP + CO$_2$
Total No. of rats	85·0	162·0	136·0
No. of rats in which masses were observed	4·0	32·0	61·0
% of total number showing masses	4·7	19·7	44·8

χ^2 comparison of OAP with air controls $P < 0·005$
χ^2 comparison of OAP with OAP + CO$_2$ $P < 0·001$

Two-dimensional chromatographic analysis of the calculi hydrolysed with 6 N hydrochloric acid indicated the presence of cysteine, histidine, arginine and/or lysine, aspartic acid, serine, glutamic acid, glycine, threonine, alanine, tyrosine, valine or methionine, proline, phenylalanine and leucine or isoleucine.

These results show a significant increase in the occurrence of urinary calculi after exposure to oxygen at atmospheric pressure for 2–3 days. The addition of carbon dioxide to the oxygen environment further significantly increased this tendency for calculus formation.

Signs of oxygen toxicity developed in both OAP and OAP + CO$_2$ rats during these experiments. At autopsy these animals showed hæmorrhagic and œdematous lungs, hydrothorax and a tendency for bleeding from the nose and mouth. These findings are typical of oxygen poisoning. In the air controls 2 of the 4 rats showing calculi also presented gross evidence of pulmonary lesions. It appears reasonable, therefore, to relate the formation of the calculi to hypoxia and hypercapnia which may exist in the late stages of oxygen poisoning. Since addition of carbon dioxide was associated with an increase in the incidence of calculi it is possible that hypercapnia and the attendant respiratory acidosis with consequent alteration of urinary *p*H predispose to the development of these urinary calculi. Anoxia as a cause cannot be ruled out since, at least in dogs exposed to OAP, severe anoxia develops immediately before death[2]. In these experiments autopsy of rats killed before the terminal stages of oxygen toxicity reveals bladder calculi both in OAP and in OAP + CO_2 animals. The significantly greater incidence of calculi in the latter case would argue in favour of hypercapnia as an aggravating condition since they are definitely hypercapnic but not necessarily hypoxic. In either case development of calculi is coincident with evidence of respiratory distress.

These findings are of interest in conjunction with the report by McGaughey[3] of excretion of uncrystallized urinary calculi in dystrophic mice. Muscular dystrophic animals frequently have compromised respiratory mechanics; therefore the development of calculi may be associated with the hypoventilation.

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Selective and Directional Transfer of 7*S* γ_2-Globulin across the Human Placenta

RABBIT γ_2-globulin can be hydrolysed by papain into three polypeptide fragments which are nearly equal in size: two of these fragments, I and II, each possess an active antibody site, whereas the third fragment, III, has no antibody activity[1]. Brambell *et al.* examined the maternofœtal transfer of such fragments in the pregnant rabbit, and reported that the amount of fragment III to be found in the fœtus after administration to the mother was 6–10 times the amount of fragments I or II and only a fourth the amount of the parent γ_2-globulin[2]. It was concluded that the fragments pass from mother to fœtus less readily than does the intact γ_2-globulin molecule, and that the transmissibility of γ_2-globulin from doe to fœtus is attributable to that part of the molecular structure represented by the non-antibody fragment. In the study reported here, this concept was reinvestigated as to its applicability to the metabolism of 7*S* γ_2-globulin in man.

Human 7*S* γ_2-globulin (Lot 330–2, Squibb) was hydrolysed with papain[1]. The *F* fragment, which is analogous to fraction III from rabbit γ_2-globulin, and the *S* fragments, or those components analogous to fractions I and II from rabbit γ_2-globulin, were isolated from the hydrolysate[3]. γ_u-Globulins having an average sedimentation coefficient of 1·5*S* were isolated from the pooled urine of normal adults[4]. The *S* and *F* components, the parent 7*S* γ_2-globulin and the 1·5*S* γ_u-globulins were iodinated with iodine-131 as described elsewhere[5].

Normal women in the last month of pregnancy were begun on 100 mg of potassium iodide orally per day, and then each received a single injection of less than 0·2 μc./kg body-weight of one of the radioiodinated proteins at a given time prior to delivery. The concentrations of radioactivity precipitable in 10 per cent trichloroacetic acid present in maternal sera at 10 min and again during delivery of the infant were determined, as were the concentrations in umbilical cord sera and in amniotic fluid. The concentrations of unlabelled 7*S* γ_2-globulin in maternal and fœtal sera and in amniotic fluid were determined immunochemically[5].

In Fig. 1, the concentration of labelled protein found in the serum of the infant at birth, C_f, relative to that found in maternal serum, C_m, at the same time, or C_f/C_m, is expressed as a function of time. It would appear that the transfer of *S* fragment, *F* fragment and γ_u-globulin from mother to fœtus exceeded the maternofœtal transfer of 7*S* γ_2-globulin and that *S* fragment as well as γ_u-globulin, which is related structurally to *S* fragment, traversed the maternofœtal barrier as readily as did the *F* fragment. These conclusions are quite different from those reached by Brambell *et al.*[2]; but it must be noted that the latter investigators related the amount of labelled protein found in the fœtus to the amount which was placed in the uterine lumen 24 h earlier without regard to possible differences in the turn-over rates of the various proteins: although two labelled proteins may cross the maternofœtal barrier at the same rate, a difference in their rates of catabolism will be reflected in differences in their concentrations in the maternal circulation over any period of time and this in turn will result in differences in their concentrations in the fœtus at any given moment. In the work recorded here, the half-lives for the different labelled proteins in the mother were found to be 20 days for 7*S* γ_2-globulin, 4 days for *F* fragment, 0·4 days for γ_u-globulin and 0·3 days for *S* fragment. By relating the concentration of labelled protein in the fœtus to that in the mother at the same time, C_f/C_m (Fig. 1), a correction was, in effect, introduced for the differences in the rates of disappearance of these proteins from the maternal circulation.

The concentration of 7*S* γ_2-globulin averaged approximately 1·7 g/100 ml. in both maternal and fœtal sera and 0·19 g/100 ml. in amniotic fluid. Amniotic fluid was obtained at delivery of five mothers given labelled 7*S* γ_2-globulin. Of four amniotic fluids obtained 4 h

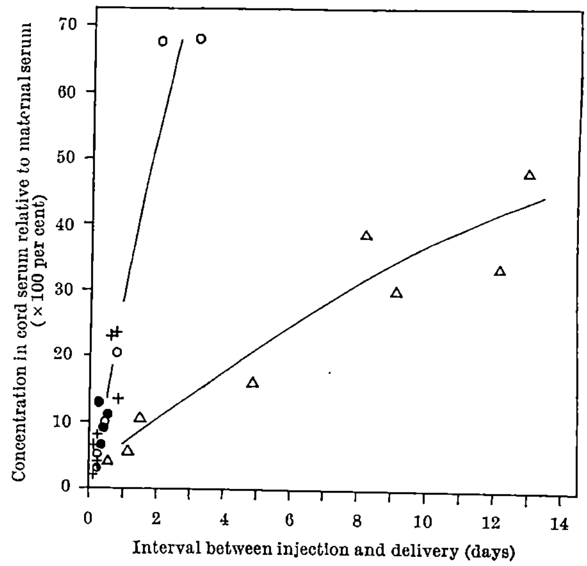

Fig. 1. Concentrations of labelled proteins in cord serum of the infant at birth relative to the concentrations of these proteins in the maternal serum at delivery: 7*S* γ_2-globulin (triangles), *S* fragment (solid circles), *F* fragment (hollow circles), and γ_u-globulin (crosses). The half-lives of *S* fragment and γ_u-globulin were 0·3 and 0·4 days respectively in the mother

after injection, no labelled protein could be detected in 2, while 2 had only 0·1 per cent of that in maternal serum; the corresponding cord sera contained 1·2–1·9 per cent of that in maternal serum. The remaining amniotic fluid, obtained approximately 23 days after injection, contained no detectable labelled protein, while the cord serum concentration was 125 per cent of that in the maternal serum. Thus, the specific activity, or counts per mg of γ_2-globulin, in amniotic fluid was lower than that in cord serum and the primary site of transfer of $7S$ γ_2-globulin to the fœtus was not via amniotic fluid. The placenta would appear to be the probable site of transfer, therefore, as has been demonstrated in the rhesus monkey[6].

The results also suggest that the permeability of the placenta to $7S$ γ_2-globulin in the direction of mother to fœtus is greater than that in the direction of fœtus to mother. The fœtus at term does not synthesize $7S$ γ_2-globulin[7]. Thus, the amount transferred from mother to fœtus, T_{mf}, must equal the amount degraded by the fœtus, A, plus the amount, T_{fm}, which passes from fœtus to mother, or $T_{mf} = A + T_{fm}$. The half-life of $7S$ γ_2-globulin in the newborn infant in this work was found to be relatively constant from birth and averaged 16 days; it would seem reasonable to assume that the fœtus at term just prior to delivery also catabolized $7S$ γ_2-globulin and that A is finite and significant. Therefore, T_{mf} would appear to be greater than T_{fm}. This directional difference in the permeability of the placenta to $7S$ γ_2-globulin would seem to explain why the fœtal serum concentration of this protein attains a level similar to that in the maternal serum despite fœtal catabolism of the protein and despite absence of synthesis of the protein by the fœtus. It also serves to explain why the serum concentration of $7S$ γ_2-globulin in the infant at birth may even be higher than that in the mother as is often found.

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Flow of Blood to the Pineal Body of the Rat

NUMEROUS cytological and histochemical observations indicate a secretory function for the pineal body of higher vertebrates[1,2]. Physiologically also, the pineal body of the rat satisfies most of the criteria for an endocrine organ[3-5]. Although anatomical evidence suggests that rat and mouse pineal vascularity is less than in some classical endocrine organs[6,7], in view of the relatively high blood flow rate to endocrine structures in general[8], it is reasonable to expect the pineal to be similarly well perfused. We have found that the minimum rate of rat pineal blood flow per g exceeds that of most endocrine organs, equals that of the neurohypophysis and is surpassed only by that of the kidney.

Pineal body blood flow was measured by a variation of Sapirstein's isotope indicator method which has been utilized successfully for other organs[9]. Briefly, the method depends on the fact that the quantity of radioactive rubidium or potassium indicator taken up by an organ is proportional to the blood flow through that organ, provided that the organ has the same extraction ratio toward the indicator as the body as a whole during the first minute after injection. This can be demonstrated if during this time uptake of the indicator by the organ remains unchanged, despite continuing recirculation; under such conditions, the ratio between organ uptake and body uptake of indicator is equal to the ratio between organ blood flow and cardiac output. If, on the other hand, the organ fails to retain its indicator after initial arterial delivery, as in the case of brain with respect to rubidium or potassium, then organ blood flow is underestimated. When a part of the body is non-homogeneous, such that the indicator is rejected by one part and taken up by another, there will be an initial peaking of the organ indicator content followed by a steady but lesser elevation[10,11]. Blood flow fractions can be estimated from the peak value of indicator content, but may underestimate true flow values considerably.

Fractions of the cardiac output perfusing the pineal body were independently measured as follows: In one laboratory (H. G.) rubidium-86 solution was rapidly injected through a chronically implanted femoral vein catheter in 185–225-g male and female Wistar rats anæsthetized with pentobarbital. The animals were decapitated 5 sec, 10 sec, 20 sec, or 60 sec later. In the other laboratory (R. W.) potassium-42 was rapidly injected through the tail veins of restrained, unanæsthetized, female Sprague–Dawley rats weighing about 200 g. These animals were decapitated 15 and 60 sec later. The pineal tissue was rapidly weighed and the organ fractional uptake of rubidium-86 or potassium-42 was measured in a γ-sensitive well counter.

The fractional uptake of rubidium-86 by the pineal body was very high at 5 sec after isotope delivery (Fig. 1). This indicates that no less than 0·0085 per cent of the cardiac output, or 8 per cent/g, perfused the organ. Rubidium-86 content then fell precipitously by a half to a relatively flat plateau 15–55 sec later. The 5-sec value differed significantly from those at 20 sec or 60 sec at the 0·001 level. There were no sex differences. A similar plateau (3·5 per cent/g) was found between 15 and 60 sec after injection of a potassium-42 indicator in another strain of rat at rest and without anæsthesia (Fig. 1).

On the basis of the rubidium-86 plateau figure and cardiac output values determined in a separate but comparable group of animals (about 250 ml./min/kg), the estimated minimum pineal body blood flow in the anæsthetized Wistar rat is 2 ml./min/g. A closer approximation to the true blood flow, based on the peak figure, is 4 ml./min/g; experience with similar peaks in nervous tissue leads us to believe that even this figure is below the true value.

The pineal body and whole brain[10,11] behave similarly toward alkali metal isotopes so far as the early time course of label is concerned. Later, the plateau value for whole brain is very low; that for the pineal body is about half the peak value. This suggests that the pineal is a two-compartment system. The first compartment loses its isotope content rapidly within 10 sec either by rejection, as in nervous tissue, or by shunting through arterio-venous anastomoses. The second compartment with a large and accessible reservoir captures its rubidium and retains it for a minute or longer, resembling non-nervous tissue such as thyroid or heart. Curve 1, an extrapolation of the peak figure, describes pineal isotope content as if it were composed entirely of nervous tissue. Curve 2, an extrapolation of the plateau, describes pineal rubidium content as if it were heart. A resultant curve closely resembling

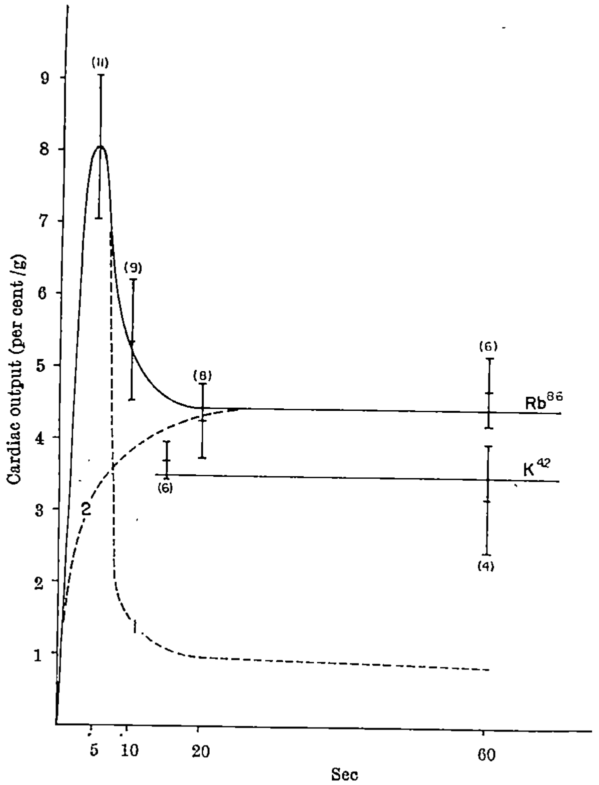

Fig. 1. Minimum pineal blood flow calculated from fractional uptake of ^{86}Rb or ^{42}K at different times after injection presented as per cent of cardiac output per g wet weight ± *S.E.* The numbers of animals in each group are in parentheses. Theoretical pineal behaviour towards ^{86}Rb based on all 'nervous' or 'non-nervous' tissue composition is presented in curves 1 and 2, respectively

our findings would be generated if the two tissue compartments were equally well perfused.

The structural correlates of these two physiological compartments are not yet clarified. An isotope peak, due to trapping in the blood vessels at the instant of death, indicates either rejection or *A–V* shunting. Since pineal body *A–V* shunts have not yet been described and a considerable mass of sympathetic nerve endings of extra-pineal origin terminates in the pineal perivascular spaces[12,13], the more reasonable hypothesis is that this sympathetic tissue constitutes at least part of a rubidium rejecting compartment.

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HISTOCHEMISTRY

Carbonic Anhydrase in the Mamillæ of the Hen's Egg Shell

THE shell of the hen's egg is formed in the oviduct of the hen within 19 h, and in this shell 5 layers can be morphologically differentiated. From the inner to the outer layer are, in consecutive order, the inner egg membrane, the outer egg membrane, the mamillæ, the matrix, and, finally, the cuticle. The egg which passes into the uterus is already covered by the inner and outer egg membranes; but whether the mamillæ are already formed in the isthmus or only later in the uterus has not been definitely established. The mamillæ and the egg shell matrix which are formed in the uterus are consequently incrusted with an inorganic substance which consists mainly of calcium carbonate. The porous cuticle finally surrounds the calcified egg shell on the outside. The CO_3^{--} ion as well as the calcium is of equal significance in the calcification process of the egg shell.

The observation that sulphonamides[1-3] and Cl^- ions[4] which inhibit the carbonic anhydrase *in vitro*, and which also influence the quality of the egg shell, has confirmed the supposition of Meldrum and Roughton[5] that the enzyme carbonic anhydrase plays a part in the calcification of the egg shell.

Carbonic anhydrase catalyses the reaction: $CO_2 + H_2O \rightleftharpoons H_2CO_3$ and was demonstrated in the uteri of laying hens[6]. We were able to show that the enzyme is predominantly situated at the apical pole of the epithelia of the tubular glands[7]. These glands release the Ca^{++} ions as well as the HCO_3^- ions into the lumen of the uterus.

Robinson and King[8] have reported on the histochemical demonstration of carbonic anhydrase in the region of the mamillæ in the egg shell. As the mamillæ which contain mucopolysaccharides are able to increase the concentration of calcium ions, the presence of carbonic anhydrase in these formations would be of extraordinary significance for the understanding of the calcification.

So far extracellular localized carbonic anhydrase was, of course, never demonstrated, and we know from our own experience that the histochemical demonstration of the enzyme is difficult and it must be very critically judged in respect to its specificity. Therefore it seemed justifiable to us to repeat the examinations of Robinson and King, and to submit the results to a test.

For the tests, partly freshly laid eggs of White Leghorns were used, and partly uncalcified egg shells which had been taken from the uterus of the same breed.

Decalcification proceeded according to Robinson and King[8] in disodium ethylenediamine tetraacetic acid with *p*H 8·5 and at 4° C. We followed the progress of the decalcification process by the use of a polarizing microscope.

The decalcified egg shells were torn up with pincers into pieces about 4 mm² large, a part of which was subjected to the periodic acid–Schiff reaction according to McManus[10]. The activity of the carbonic anhydrase was examined according to Häusler's method[11]. 'Diamox' (acetylamino-1,3,4-thiodiazole sulphonamide-5) or potassium cyanide was added to single incubating media:

Incubating media:
- (*a*) According to Häusler
  $CoSO_4$ (0·1 mol) 1 ml.
  H_2SO_4 (0·05 mol) 6 ml.
  $NaHCO_3$ 1 g
  Na_2SO_4 (0·1 mol) 50 ml.
- (*b*) Medium as above + 5 × 10^{-3} mol 'Diamox'.
- (*c*) Medium as above + 4 × 10^{-2} mol 'Diamox'.
- (*d*) Medium as above + 1 × 10^{-4} mol KCN.
- (*e*) Medium without $NaHCO_3$ (*p*H 7·5 was adjusted with *tris* buffer).

After an incubation period of 1 h at room temperature the cobalt carbonate was changed in the usual way, after thorough watering, into cobalt sulphide. The preparations were dehydrated and mounted with 'Entellan'.

After incubation for 60 min without the addition of inhibitors the preparations show a pattern of blackish brown, apparently enzymatic spots, distributed evenly across the outer egg membrane (Fig. 1). The pattern of distribution of these spots and their size correspond to the picture of the periodic acid–Schiff reaction. It is a question of the mamillæ which on account of their higher mucopolysaccharide content are to be seen as red zones in the periodic acid–Schiff reaction (Fig. 2).

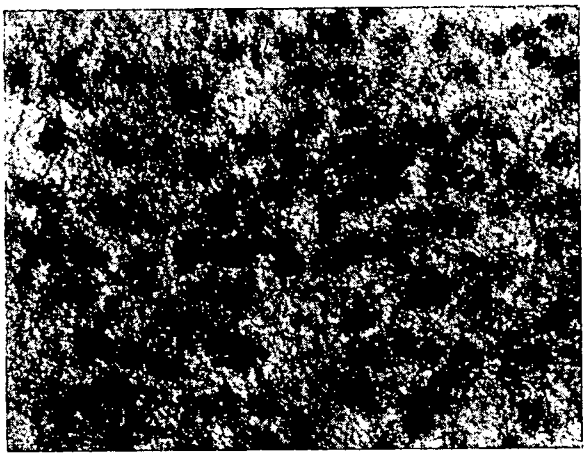

Fig. 1. Surface view of the decalcified egg shell. The mamillæ appear as black spots on the outer egg membrane. Carbonic anhydrase according to Häusler (×60)

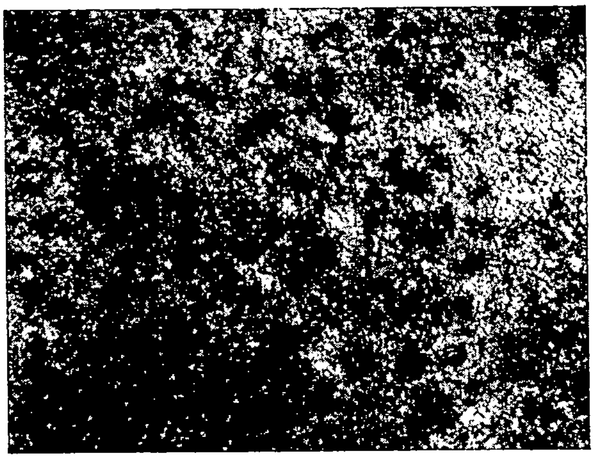

Fig. 2. Periodic acid–Schiff reaction. The mamillæ can be recognized as black spots by their higher mucopolysaccharide content (×60)

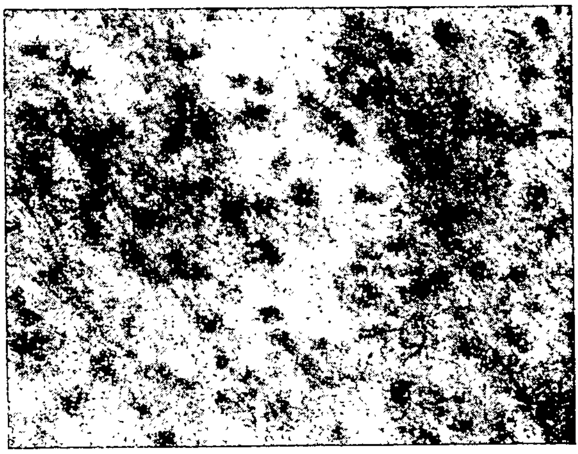

Fig. 3. Carbonic anhydrase according to Häusler. Addition of 4×10^{-2} mol 'Diamox' (×60)

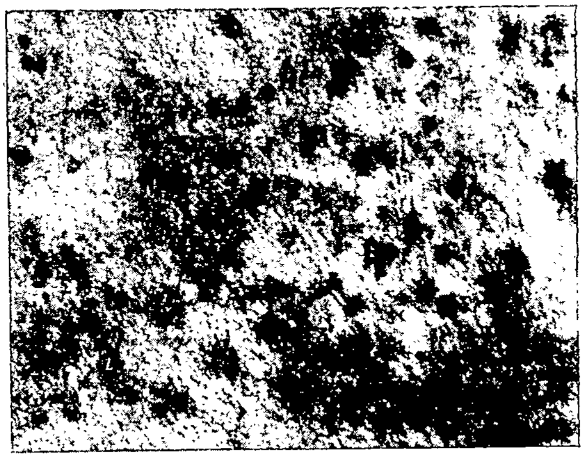

Fig. 4. Carbonic anhydrase according to Häusler. Incubation medium without sodium bicarbonate (×60)

However, in contrast to Robinson and King, we could neither inhibit the enzymatic reaction by the addition of 5×10^{-3} mol 'Diamox', which is sufficient to block completely the carbonic anhydrase in kidney sections and red corpuscles, nor suppress only partly the reaction by the addition of 4×10^{-2} mol 'Diamox' (Fig. 3). The same result followed the addition of potassium cyanide.

These observations demonstrate that it is here a question of an unspecific, non-enzymatic reaction of the mamillæ. For even with medium (*e*) the same reaction ensues in the mamillæ (Fig. 4). Consequently the reaction here also occurs in the absence of the substrate enzyme.

Evidently cobalt ions are bound to the mucopolysaccharides of the mamillæ. In order to verify this, the following model experiment was carried out.

1 ml. of a solution of 0·1 mol cobalt sulphate was mixed with 20 ml. of ethanol and added to 20 mg of chondroitin sulphuric acid in the form of its sodium salt. This mixture was put in a centrifuge and the residue was carefully washed out several times with altogether 100 ml. of ethanol. A sharp blackening effect which is due to the formation of cobalt sulphide could be distinguished in the residue after staining with an aqueous or even alcoholic ammonium sulphide solution.

The mamillæ of the egg shell contain mucopolysaccharides[9] which probably consist of up to 40 per cent chondroitin sulphuric acid[12]. These mucopolysaccharides can bind Co^{++} ions as well as other double valued positive ions. So, in the course of a histochemical carbonic anhydrase demonstration, enzymatic activity of the carbonic anhydrase is simulated by the Co^{++} ions which are bound to the mucopolysaccharides of the mamillæ.

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Fatty Metaplasia of the Filum Terminale of the Frog

THE following observations were made during a search for preparations which would permit assays of various enzymes in tissue samples containing glial cells only. Such an opportunity seemed to be provided by the filum terminale which is commonly considered a glial remnant of the fœtal sacral cord[1]. Tobias, Clark, and Gerard[2] measured the respiration of the filum terminale of the cat and concluded that glial tissue had a rather high rate of respiration, comparable to that of neurones. The filum terminale of *Rana catesbiena* was used in our work because of its unusual size. A smaller material of 8 specimens of *Rana pipiens* was also examined with the same results.

Assays of the frog filum terminale indicated very little, if any, succinic dehydrogenase activity but extremely variable DPN-diaphorase activity per wet weight or per mg of protein. Searching for histological differences which might reflect this variability, we found that the filum terminale of normal frogs undergoes various degrees of fatty metaplasia. Although we have not found previous reference to this in the literature, fatty metaplasia of the filum terminale of the frog seems to be a physiological phenomenon; we could not relate it to the state of health or nutrition of the frogs; it did not seem to represent a pathological fatty degeneration because the fatty changes were confined to the filum and no fatty changes were seen in the spinal cord. All our frogs were studied during summer and autumn. The extent of fatty metaplasia was usually the same throughout any given filum, but it varied greatly among animals. The following observations were made in histochemical preparations for lactic dehydrogenase and DPN-diaphorase which were counterstained with fat red; longitudinal and transverse sections of the filum were used.

'Normal' glial cells in the filum terminale of the frog showed intense lactic dehydrogenase and DPN-diaphorase activity throughout their cytoplasm and processes (Fig. 1). Compared with glial cells in mammalian brains, these cells could not be identified as being either astrocytes or oligodendroglia. A slight degree of fatty metaplasia was evidenced by a single fat droplet in the cell body. More advanced fatty metaplasia was indicated by various-sized large fat droplets, most of which by far exceeded the original volume of the cell (Fig. 2). The cytoplasm of such cells was stretched to a thin spherical shell and the perikaryon was flattened, as in adipose cells. The fat droplets had been removed from some cells during cutting, and these cells appeared as empty, spherical vesicles (Fig. 2). It appeared that the amount of enzyme activity was in a crude inverse proportion to the fatty metaplasia in the cells. At early phases of fatty metaplasia, the enzyme activity in the cells was not visibly weaker than normal, but very little enzyme activity was present in the cells of fila with extreme fatty metaplasia.

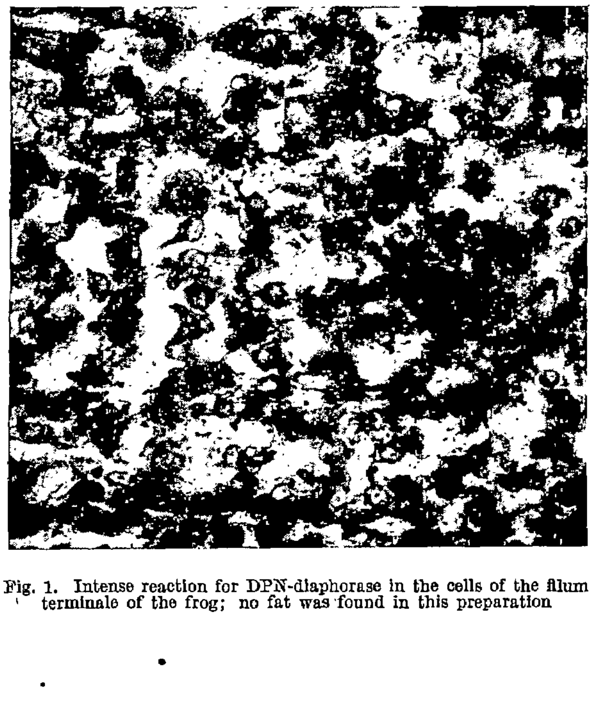
Fig. 1. Intense reaction for DPN-diaphorase in the cells of the filum terminale of the frog; no fat was found in this preparation

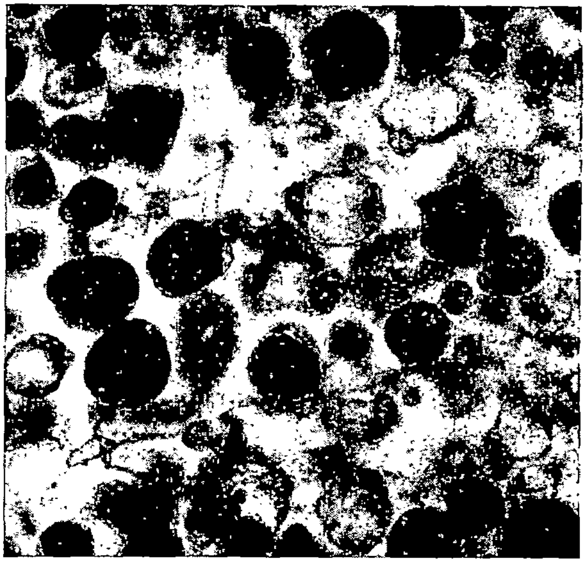
Fig. 2. Fatty metaplasia of filum terminale; DPN-diaphorase, counterstained with fat-red. The fat droplets had been removed from some of the cells, which show only an empty shell of cytoplasm, demonstrated by enzyme activity. In the original preparation, fat is bright-red and the enzyme reaction dark blue

These observations offer no clues as to the possible causes of this type of fatty metaplasia which seems to represent a physiological process in the filum terminale of the frog. The ability to store fat, much like adipose cells do, strikingly distinguishes the tissue of the filum of the frog from that of any other portion of the central nervous system. It seems quite clear that the filum terminale of the frog is not suitable for examination of glial metabolism.

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PATHOLOGY

Effect of Colchicine on Human Tumours transplanted in Cheek Pouch of Syrian Hamster

THE human tumour transplanted in the cheek pouch of the Syrian hamster has proved to be a useful tool for the examination of potential anti-tumour agents[1,2]. What appears to be an obvious advantage in using transplantable human tumours over transplantable animal tumours in chemotherapy investigations lies in the fact that a given compound can be assayed on cells of human origin growing in an *in vivo* system. However, at least two conditions would have to be fulfilled prior to making such a comparison: (1) transplanted human tumours should not

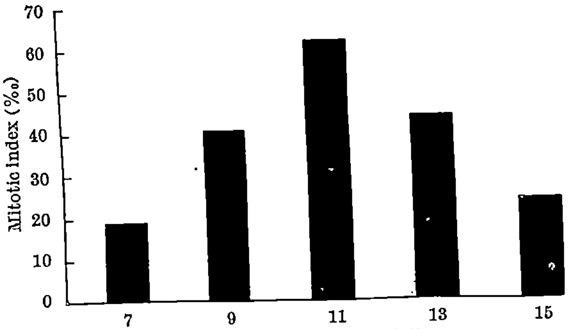

Fig. 1. Mitotic indices after colchicine (1·2 mg/kg) in *ME*-1 tumour growing in the cheek pouch of Syrian hamster

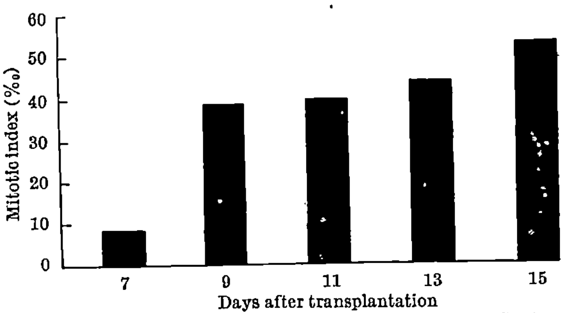

Fig. 2. Mitotic indices after colchicine (1·2 mg/kg) in *MFS*-1 tumour growing in the cheek pouch of Syrian hamster

undergo profound changes in their biological properties; (2) the host should not alter the effect of the tested agents on the human cells.

Heterologous transplantation studies of human tumours in hamsters, mice, and rats, by several investigators have revealed that very little alteration in the general histological appearance of human tumours occurs in the new host[2-5]. Furthermore, immunological studies conducted by Korngold[6,7] have demonstrated that human tumours transplanted in hamsters or rats retain, at least partially, their original antigenic constitution, even after undergoing repeated transplantations. It appears from these findings that no radical changes occur in transplanted human cells by serial passage in hamsters.

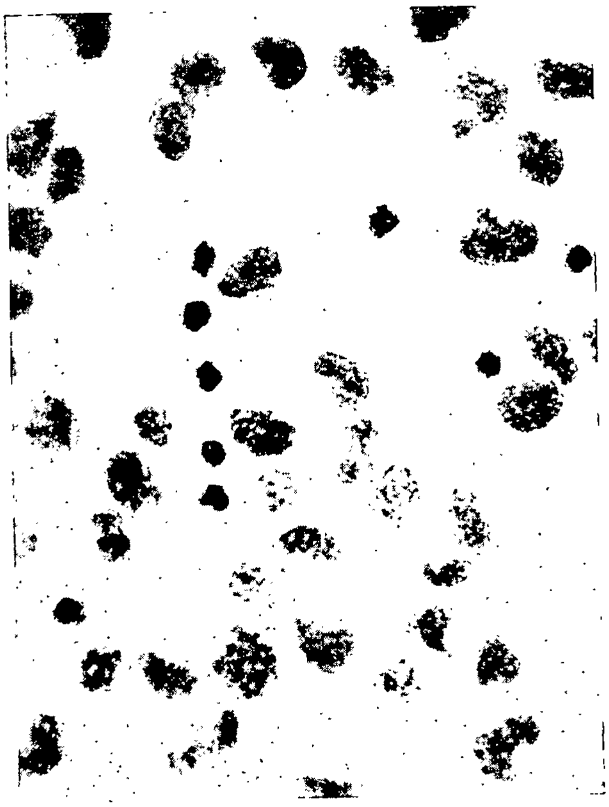

Fig. 3. Arrested metaphases, after colchicine treatment, in *ME*-1 tumour 11 days after transplantation. (Squash preparation, Feulgen; × 800)

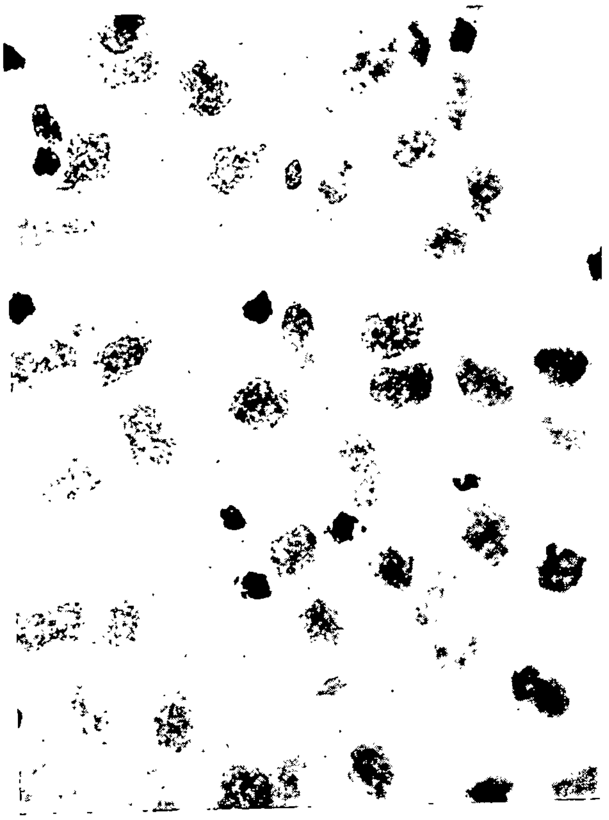

Fig. 4. Arrested metaphases, after colchicine treatment, in *MFS*-1 tumour 15 days after transplantation. (Squash preparation, Feulgen. × 800)

In evaluating the effect of a given compound on human cells transplanted in the hamster cheek pouch, consideration must be given to the influence of the new environment on the transplanted cells, and on the compound itself.

It has been known for some time that the Syrian hamster differs from other mammals in its sensitivity to the stathmokinetic effect of colchicine[8-11]. Orsini considered this animal to be completely resistant to the effect of this alkaloid[8]. In a recent investigation we have been able to demonstrate that the Syrian hamster is only partially resistant to the stathmokinetic effect of colchicine[11]. In fact, Syrian hamsters injected with a dose of 1·2 mg/kg colchicine, which is effective in causing metaphase arrest of dividing cells in other mammals, did not show any accumulation of arrested metaphases, while hamsters injected with 2·4 mg/kg or higher doses showed metaphase arrest in only some bone marrow dividing cells[11]. Midgley *et al.*[10] observed that colchicine induced metaphase arrest in human testicular tumours transplanted in the cheek pouch of Syrian hamsters, in spite of the resistance of the hamster to this alkaloid.

The purpose of the investigation reported here was to study the effect of colchicine on other human tumours transplantable in Syrian hamsters, and to investigate whether such tumours were sensitive to the effect of the alkaloid during all phases of growth.

Two human tumours, transplantable in the cheek pouch of the Syrian hamster, were used in these experiments: a myxofibrosarcoma (*MFS*-1) and an amelanotic melanoma (*ME*-1) (2). *MFS*-1 and *ME*-1 tumours were

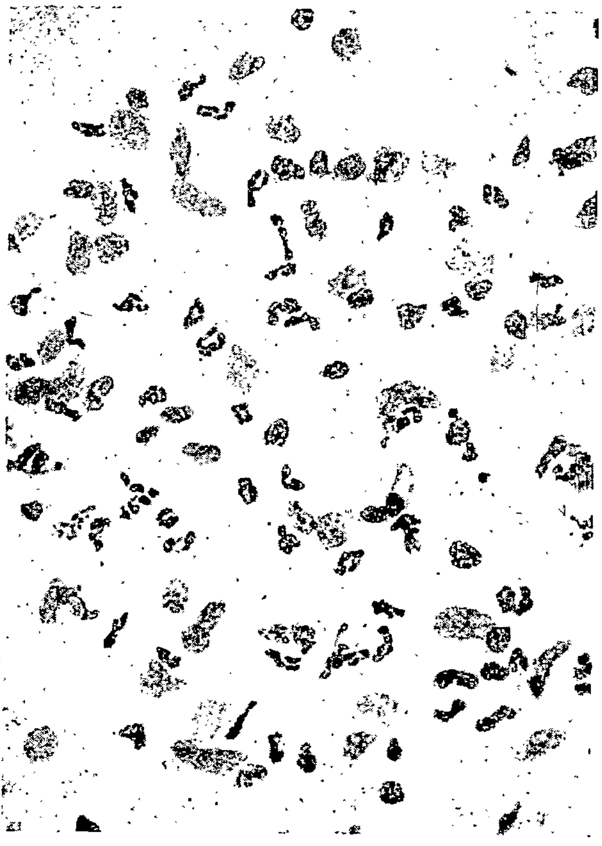

Fig. 5. Leucocytic infiltration in an *MFS*-1 tumour in regression. (Squash preparation, Feulgen; × 600)

transplanted into two groups of 40 hamsters each, 5–8 weeks of age, and of both sexes. The technique of transplanting tissues to hamster cheek pouches is described elsewhere[1,12]. On the fifth day following transplantation, hamsters bearing established tumour grafts were divided into groups of 5 animals each. At various time-intervals, starting from the seventh day after transplantation, a group of hamsters was killed 4 h after receiving, intraperitoneally, 1·2 mg/kg of colchicine. Immediately after death, the tumour was removed from the cheek pouch, minced with scissors, and fixed in 1 : 3 acetic/alcohol. Preparations for cytological study were made according to the Feulgen squash method. Mitotic indices were determined by counting at least 2,000 cells on each preparation.

In earlier experiments, it was observed that on the fifth day after transplantation, only a very few mitoses were present in the tumours; therefore, mitotic indices were determined at various time intervals from the seventh to the fifteenth day after transplant. In both *ME*-1 and *MFS*-1 tumours, arrested metaphases were observed on the seventh day after transplantation. The number of arrested metaphases increased progressively in the following days, reaching the maximum on the eleventh day in *ME*-1, and the fifteenth day on *MFS*-1 (Figs. 1, 2, 3, and 4). Post-metaphase figures were practically absent in almost all preparations. The progressive increase of the mitotic index paralleled pretty well the increase in size of the tumours. Some of the tumours which, after initial growth, showed a tendency to regress were also studied. These showed a large number of degenerating cells and extensive leucocytic infiltration. Mitotic figures in these tumours were always rare, or very low in number (Fig. 5).

Results obtained in this investigation indicate that human tumours growing in the cheek pouch of the hamster are fully sensitive to the stathmokinetic effect of colchicine during the entire period of growth.

Tumour sensitivity exists in spite of repeated serial transplantation in the hamster. The experimental evidence presented herein provides further strength for considering the hamster–human tumour system as a tool for assaying compounds as potential anti-tumour agents in man. Whether the hamster–human tumour system will indeed prove more suitable as a method for demonstrating human anti-tumour activity than the other systems currently in use will await clinical trial and evaluation.

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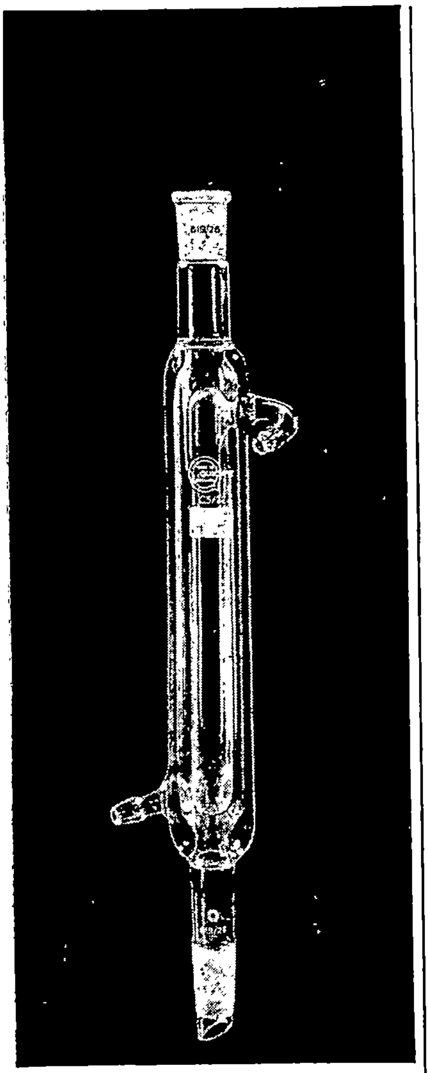
Proliferation and Maturation Defect in Acute Leukæmia Cells

PREVIOUS *in vitro* investigations have shown that thymidine incorporation in acute leukæmia cells is very low compared with that in hæmocytoblasts and myeloblasts of normal bone marrow or in chronic myeloid leukæmia[1–3]. This was taken to be a sign of reduced proliferative capacity in acute leukæmia cells, in accordance with stathmokinetic investigations of Astaldi and Mauri[4]. Lajtha and Craddock also noted a low degree of tritiated thymidine incorporation in acute leukæmia cells[5,6] and, by direct *in vivo* precursor administration Mauer and Fisher[7] and Killman *et al.*[8] were able to obtain direct evidence of the prolonged generation time of these cells.

Further *in vitro* and *in vivo* work has been undertaken to define more exactly the extent and significance of the phenomenon. For this purpose it was decided to: (1) establish whether or not the defect in proliferative capacity of acute leukæmia cells is related in some way to another defect long recognized as characteristic of these cells, namely, an absolute inability to mature; (2) define the significance of blast cell population heterogeneity, if any, in connexion with reduced proliferative capacity. To achieve the first objective we extended our investigations to intermediate clinical conditions, such as the terminal myeloblastic crisis of chronic myeloblastic leukæmia and the remission phases of acute leukæmia. For the second objective it was necessary to divide the cell population of each case into different classes according to cell diameter, so that proliferative capacity could be evaluated for each class.

In vitro investigations were conducted on 22 cases of acute leukæmia, 11 cases of chronic myeloid leukæmia, 7 cases of chronic myeloid leukæmia in various stages of terminal blastic crisis and 2 cases of acute leukæmia exhibiting a hæmatological remission after a complete course of 6-mercaptopurine therapy. The technique has



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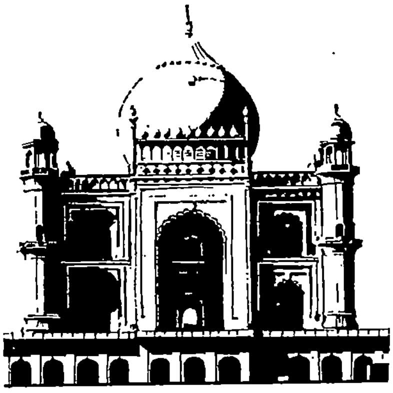

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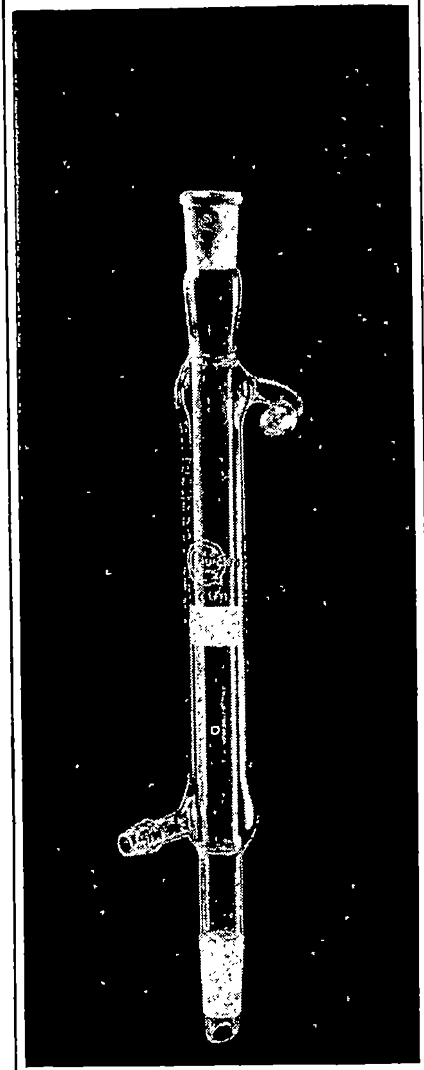

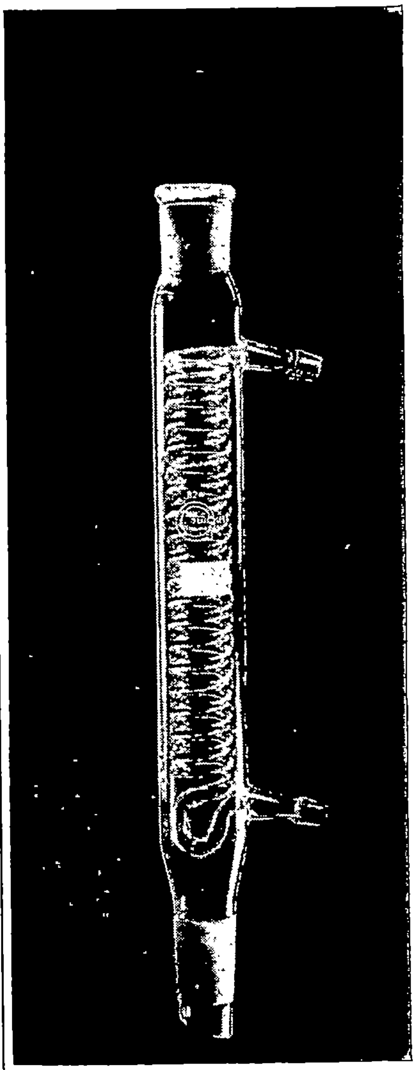

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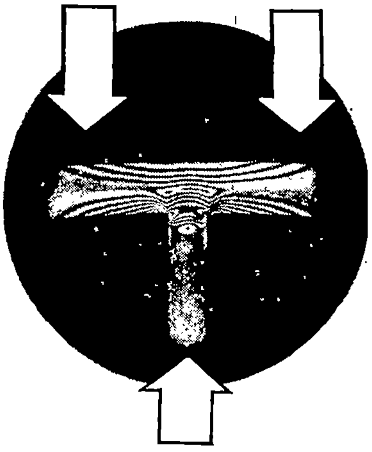

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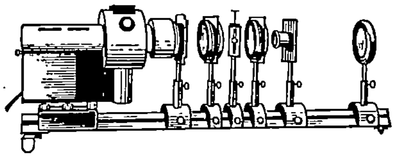

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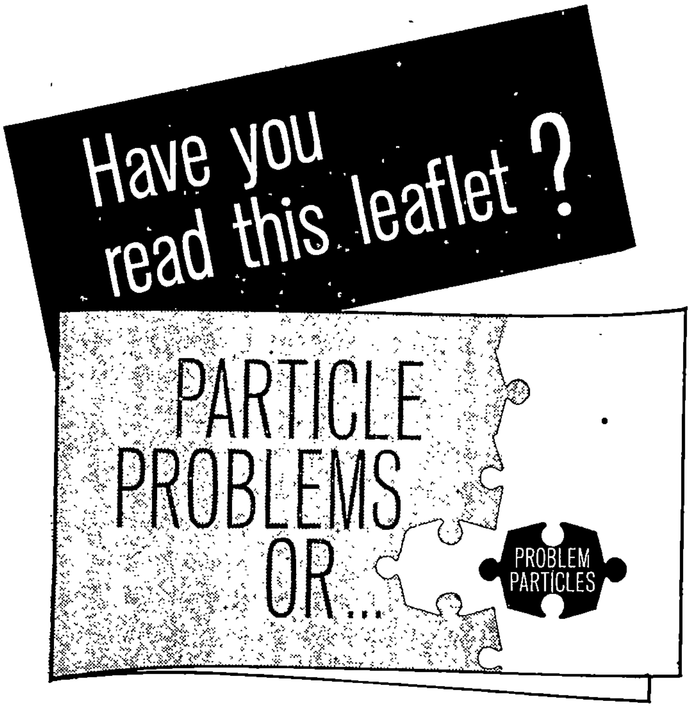
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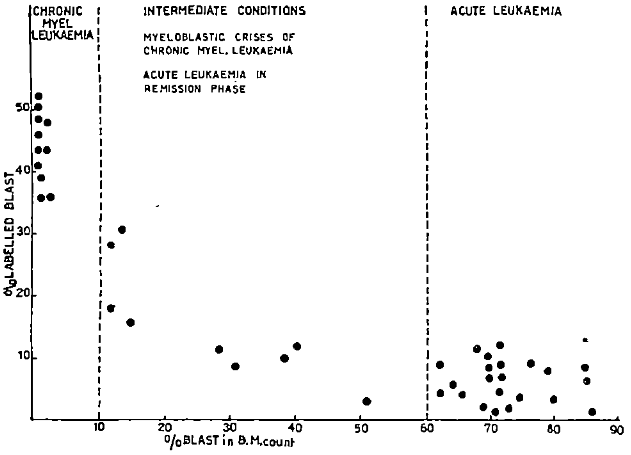

Fig. 1. Correlation between percentage of blasts and labelling index in the different leukæmia conditions

already been reported[3]. For the *in vivo* investigation, 0·15 mc./kg of tritiated thymidine (specific activity 1·4 c./m.mole) was injected intravenously into the acute leukæmia patients and peripheral blood samples drawn at various intervals for 4 days.

Fig. 1 shows the percentage of thymidine-labelled blast cells (or labelling index) of, respectively, chronic myeloid leukæmia, intermediate conditions and acute leukæmia. If the labelling index is correlated with the percentage of blast cells in bone marrow smears it is clear that the higher the percentage of blasts the lower their proliferative capacity. This is clearly seen in the individual cases of chronic myeloblastic leukæmia in which the course of the disease could be followed closely up to the terminal myeloblastic crisis. The phenomenon was also observed during hæmatological remission in a case of acute leukæmia.

In 4 cases of acute leukæmia and in 1 case of blastic crisis studied *in vitro* and in the 2 cases of acute leukæmia examined *in vivo* the blast cell population was divided into different classes according to cell diameter. Heterogeneity was evident in all cases, with larger blast cells containing a great number of nucleoli at one extreme and much smaller cells with few nucleoli (and often a lesser degree of cytoplasmic basophilia) at the other. The percentage degree of tritiated thymidine incorporation was determined for each class. Incorporation was highly heterogeneous between the different classes, those with greater cell diameter giving values of normal or chronic myeloid leukæmia cells (Table 1). It should be borne in mind that a higher labelling index in larger cells may be due simply to the fact that cell diameter increases during the *S*-period[9]. An examination of blast cells of normal bone marrow (a homogeneous cell population) gave a mean diameter ratio of labelled to non-labelled blasts of 1·1. Since the differences in incorporation between the various classes of leukæmia cells are of the order of 20- to 30-fold we may dismiss this factor of increasing cell diameter as having no appreciable effect on the findings of our investigation.

Hale and Cooper recently reported an increased rate of DNA synthesis in larger blasts[10].

Table 1. Labelling Index in the Different Blast Cell Classes

Patients:	B.C. b.m.	B.C. p.b.	G.C. b.m.	G.C. p.b.	T.L. b.m.	F.M. p.b.	C.B.* p.b.	F.A.† b.m.
Cell diameter (μ)					39·0			
21					21·4			48·0
19			50·0		29·3	47·3	76·5	46·2
17	60·0		38·0	21·7	22·8	25·2	46·4	37·7
15	46·0	52·0	30·2	20·5	15·3	17·0	13·3	23·9
13	28·0	32·0	15·2	5·8	6·1	5·6	3·2	10·4
11	7·4	8·4	5·7	1·6	3·8	0·8	1·3	6·2
9	2·6	0·7	1·8	1·3	1·9		1·8	3·7
7	0·4	0·5	0·4	0·4				

b.m., bone marrow; p.b., peripheral blood.
* *In vivo* experiment. † Blastic crisis of chronic myeloid leukæmia.

In two untreated cases of acute leukæmia, tritiated thymidine was injected *in vivo*. Intravenous tritiated thymidine administration is known to correspond to a pulse-labelling process[11]: for about 20 min the precursor is incorporated in all the cells which are in the phase of DNA synthesis. Thereafter, up to the ninety-sixth hour, the labelling index was evaluated on peripheral blood in each of the cell-diameter classes. In both cases the behaviour of the labelling index differed markedly according to the different classes. Fig. 2 illustrates the situation in one case (F. S.); that in the second case (C. B.) is very similar. In the larger cell-diameter classes the labelling index (initially very high) fell rapidly during the first 24 h; in the smaller cell-diameter classes the labelling index rose gradually from very low values (1–3 per cent) to high values (10–20 per cent).

Comparative studies on cases of acute leukæmia, intermediate forms of the disease and chronic myeloid leukæmia point decisively to a link between the gradual increase in blast cell population and the fall in average proliferative capacity. However, a gradual percentage increase in the blast cell population also implies the appearance and progressive intensification of a maturation defect. Consequently, it is reasonable to assume that the existence of a maturation defect is connected with that of a proliferation defect.

Is there a causal link between the proliferation defect and the maturation defect, both of which characterize and define every picture of acute leukæmia ? An affirmative answer to this question is suggested by the findings of our second series of investigations, which provided evidence of different cell classes with strikingly different percentage degrees of thymidine incorporation both in acute leukæmia cell populations and in those of leukæmia in blastic crisis. Labelling indices ranged from near-normal or chronic leukæmia values to extremely low values. *In vivo* investigations undertaken to examine the fate of the various classes of thymidine-labelled cells have shown clearly that in a single population the larger cells are generally the less aged and that over subsequent divisions (altogether ineffective as regards maturation) these cells gradually become smaller in diameter, thereby swelling the ranks of the smaller cell-diameter classes. In both cases the significance of the finding was beyond question. Clearly, then, the reduction in proliferative capacity observed in leukæmia cell populations is not a homogeneous phenomenon: the values obtained from the population as a whole are simply the mean values between the younger blast cells still in possession of a high proliferative capacity and the older blast cells which have

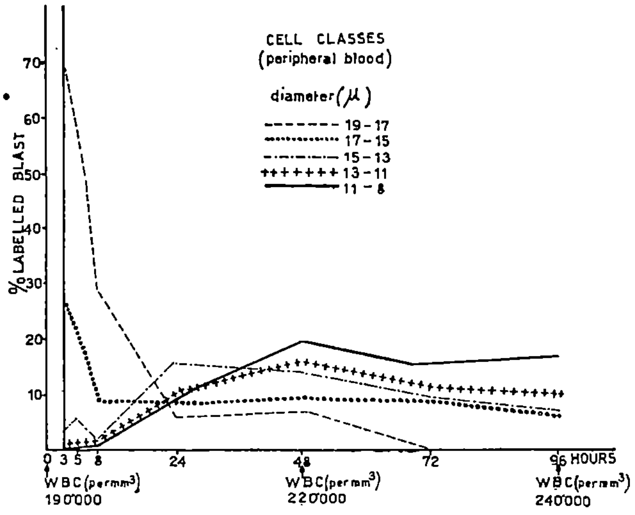

Fig. 2. (Patient F. S.). Tritiated thymidine labelling index in the different blast cell classes of peripheral blood after the intravenous administration of 0·15 mc/kg precursor

gradually lost their proliferative capacity. In other words, the ageing of a population of blast cells lacking the capacity of differentiation involves the progressive loss of their proliferative capacity. Young blast cells are found in greater numbers in bone marrow than in peripheral blood. This explains the differences in the mean proliferative capacity revealed by simultaneous analysis (Table 1)[12].

In conclusion, the foregoing findings provide a basis for the postulation of a unitary theory on acute leukæmia. In this disease there is only one major functional cell defect, namely, an absolute incapacity for differentiation and maturation. As the cells grow older without maturing they gradually lose their proliferative capacity. The existence of this defect in acute leukæmia cells or its development during the blastic crisis in chronic cases probably indicates the existence or development of new lines of blast cells which have lost nuclear control over differentiation, so that the genetic information governing the synthesis of specific proteins is lost, inhibited or suppressed. As pointed out in a previous communication[1], this concept of acute leukæmia suggests the need for developing new lines of approach to the rational treatment of the disease.

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BIOLOGY

A Haploid Tissue Culture from the Female Gametophyte of *Ginkgo biloba* L.

Haploid tissue cultures of the female gametophyte of *Ginkgo biloba* L. have been obtained successfully for the first time. The tissues are green, show a considerable degree of differentiation, and grow well on a chemically defined medium.

The female gametophyte tissue is located in the seeds, and it develops in a definite pattern over a period of four months[1]. It goes through a free nuclear stage, segmentation, growth, archegonia formation, and finally, after fertilization, it matures as storage parenchyma. In its mature form it serves as food for the germinating embryo[2].

After investigating the female gametophyte for several years using a variety of media, it was found that the stage of development best suited for growth *in vitro* was that occurring at 11–13 weeks after pollination, that is, during growth and the formation of archegonia, but before fertilization and maturation took place. At this stage, the gametophyte tissue consisted of a green, egg-shaped structure about 10–15 mm in size surrounded by the hard and fleshy seed coats. The intact seeds were surface sterilized in a 1 per cent hypochlorite solution, washed in sterile water and then dissected on sterile paper towelling. The seed was bisected at right angles to th median longitudinal axis; this divided the female gamet phyte into equal halves: an apical (archegonial) half an a basal (non-archegonial) half. The seed coats an nucellus were removed, and the apical and basal portion of the gametophyte grown in separate test tubes. Sinc it was known that archegonia sometimes occurred dis placed from the apical region[1], the two halves wer cultured separately to reduce the possibility of archegoni tissue being included in the basal end. An examination the neck canal cells of the archegonia in the apical end showed these cells to be turgid. This was evidence tha fertilization had not occurred. Moreover, fertilization wa very unlikely to have occurred, since it normally too place 17–19 weeks after pollination, whereas excision wa carried out at 11–13 weeks.

The apical and basal halves of the female gametophyt were initially cultured on White's medium[3] supplemente with 6 p.p.m. 2,4-dichlorophenoxyacetic acid (2,4-D) an 18 per cent coconut water (v/v). On this medium th gametophyte proliferated at the junction of the tissue an medium; the apical tissue also formed tissue outgrowth in the area surrounding the archegonia. After severa sub-cultures on this medium, the tissue masses wer removed and transferred to a medium with the followin conposition (mg/l.): KCl, 900; KNO_3, 80; $MgSO_4 \cdot 7H_2O$ 760; $NaNO_3$, 1,800; $Ca(NO_3)_2 \cdot 4H_2O$, 280; $NaH_2PO_4 \cdot H_2O$ 300; Na_2SO_4, 200; KI, 0·5; H_3BO_3, 0·2; $MnSO_4 \cdot H_2O$, 0·8 $ZnSO_4 \cdot 7H_2O$, 0·5; $CuSO_4 \cdot H_2O$, 0·02; MoO_3, 0·01; $CoCl_2$ $6H_2O$, 0·01; ferric citrate, 5·0; calcium pantothenate, 1·0 pyridoxine·HCl, 0·5; thiamine·HCl, 0·5; nicotinic acid, 1·0 sorbitol, 100; adenine, 10·0; kinetin, 0·5; naphthalene acetic acid. 1·0; sucrose, 20,000; agar, 10,000; glutamine 200; *m*-inositol, 100; cytidylic acid, 100; guanylic acid 100. The last four components were filter-sterilized an the *p*H of the medium was adjusted to 5·5. This mediu was based in part on one used by Wood and Braun fo *Vinca* tissue cultures[4], but was modified with respect t the trace elements, vitamins, sorbitol, and adenine. Not all the constituents of this medium are known to b required for growth, hence this is not considered a minimal medium. The tissue has been maintained on this mediu by transferring at monthly intervals for more than a year.

Later experiments indicate that the synthetic medium is adequate for starting the tissue cultures, but that the medium containing coconut water and 2,4-D is better. Once good growth is initiated (after 2–3 months) the synthetic medium is better for continued proliferation o the tissue. The tissue is friable when young but becomes harder and more compact as it ages. Microscopic examination shows a fairly abundant development of tracheid-like cells in the interior of tissue masses; chloroplasts are conspicuous in the younger cells. Chromosome counts show 12 chromosomes and confirm the haploid origin of the tissue.

Newcomer[5] reported the parthenocarpic development of *Ginkgo* seeds and Favre-Duchartre[1] reported on the abnormalities and the *in vitro* culture of the female gametophyte; but neither paper mentioned the development of a tissue or organs. Favre-Duchartre did observe some cellular growth and divisions in his cultures, but no tissue formation, continued growth, or organ development. In the cycads, however, apospory was described by Duchartre[6]; he noted the spontaneous formation of roots from the female gametophyte of *Cycas thourarsii.* LaRue[7] noted the development of both roots and shoots on gametophytes of *Zamia floridana* and *Cycas revoluta*; he grew gametophytes for more than a year on culture media of sterile sand or coconut water and obtained whole plants. However, LaRue reported no tissue cultures from these plants.

Tissue cultures have been obtained from the male gametophyte (pollen) of *Ginkgo biloba* L.[8]. These tissues are haploid and are made up of white, undifferentiated parenchyma cells which are derived from meristematic centres and may be cultured continuously on a defined medium[9]. Thus, it is believed that this is the first case where tissue cultures of both the male and the female gametophytes of a higher plant have been grown *in vitro*.

As a diœcious species, *Ginkgo* shows no phenotypic sex differences except in the production of male or female strobili. Hence, it is presumed that the genomes of the two haploid tissue cultures derived from the gametophytes of the male and female trees are identical in all respects except for sex-linked characters. The gross differences seen *in vitro* are the white, undifferentiated character of the male tissue and the green, differentiated character of the female tissue. Recent chromosome counts of both tissues show a basic haploid number of twelve, but nothing is known of the karyotype of the tissues. Previous reports[10,11] indicated the presence of a sex-linked satellite, but more recent investigations[12] did not confirm these findings.

It is suggested that the two haploid gametophyte tissues of *Ginkgo* provide an experimental system which may be useful for work on experimentally controlled regeneration, for investigations on the genetic and biochemical basis of sexual differences in higher plants, and perhaps for examination of conditions which affect chloroplast formation. In addition, the female gametophyte of *Ginkgo* is known to have growth-promoting properties similar to those found in coconut water, corn endosperm and *Aesculus* endosperm[13]; the tissue culture from the gametophyte may be useful in studying the nature of these substances. It has also been shown by Yih and Clark[14] that the male gametophyte tissue of *Ginkgo* has a boron requirement; it will be of interest to see whether the analogous green tissue of the female gametophyte of *Ginkgo* also has this requirement, in which case both tissues may be useful in examining the role of boron in cellular metabolism.

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Cytoplasmic Inheritance in Amœbæ: Nuclear Transfer Investigations

INVESTIGATIONS into the molecular basis of cytoplasmic inheritance in the large, uninucleate amœbæ, *Amoeba proteus* and *Amoeba discoides*, have shown that certain self-replicating cytoplasmically inherited determinants can be transferred from one strain to the other, by the micro-injection of relatively small quantities of non-homologous cytoplasm[1,2].

In addition to characters determined by nuclear factors, *A. proteus*, ${}_{T1}P$ differs from *A. discoides*, ${}_{T1}D$ in several cytoplasmically influenced characters. Two characters selected as experimental markers were mean nuclear diameter[3] and streptomycin sensitivity[4]; ${}_{T1}P$ having a higher mean nuclear diameter and greater streptomycin sensitivity.

After the injection of relatively small quantities of ${}_{T1}D$ cytoplasm into whole ${}_{T1}P$ amœbæ, 25 per cent of the receptor cells survived and formed flourishing mass cultures. Of these clones, approximately 20 per cent show decreased mean nuclear diameter and decreased streptomycin sensitivity. In normal amœbæ mean nuclear diameter and level of streptomycin sensitivity are not associated. Some indication of the degree of assimilation, and presumably replication, of the foreign cytoplasm in these clones could be obtained by transferring nuclei from 'parent' clones into injected cell lines and vice versa.

In addition, these clones of 'injected' amœbæ offered another means of investigating the incompatibility that exists between nucleus and cytoplasm of different strains, which is similar to that met with in transplantation experiments with other organisms, for example, *Stentor*[5] or *Amphibia*[6]. Thus when ${}_{T1}D$ nuclei are transferred into the enucleate cytoplasm of ${}_{T1}P$, the cytoplasm may be re-activated and divide several times, but a viable clone is very rarely obtained. Homotransfers, transfers between nuclei and cytoplasms of the same strain, that is, ${}_{T1}P_n \longrightarrow {}_{T1}P_c$, are at least 90 per cent successful and give flourishing mass cultures.

The viability of nuclei and the state of the cytoplasm of one of the 'injected' clones, namely, one obtained from ${}_{T1}P$ injected with ${}_{T1}D$ cytoplasm which was apparently assimilated, as judged by decrease in mean nuclear diameter and decreased streptomycin sensitivity, were investigated. Using the technique of nuclear transfer[7], experiments were carried out in order to ascertain:

(*a*) the viability of ${}_{T1}D$ nuclei in the cytoplasm of the clone obtained from the injected cell, $({}_{T1}D_c \dashrightarrow {}_{T1}P)_c$; (*b*) the viability of ${}_{T1}P$ nuclei in the cytoplasm of the clone obtained from the injected cell $({}_{T1}D_c \dashrightarrow {}_{T1}P)_c$; (*c*) the viability of nuclei from the 'injected' clone $({}_{T1}D_c \dashrightarrow {}_{T1}P)_n$ in ${}_{T1}P$ cytoplasm; (*d*) the viability of nuclei from the 'injected' clone $({}_{T1}D_c \dashrightarrow {}_{T1}P)_n$ in ${}_{T1}D$ cytoplasm.

These experiments were carried out on amœbæ separated by at least 50 cell divisions from the original injected cell, and the results obtained are given in Table 1.

Table 1

Type of transfer	Transfer	Expected percentage of clones	Actual percentage of clones obtained
Homotransfer	${}_{T1}P_n \longrightarrow {}_{T1}P_c$	100	90
Homotransfer	${}_{T1}D_n \longrightarrow {}_{T1}D_c$	100	90
Heterotransfer	${}_{T1}P_n \longrightarrow {}_{T1}D_c$	< 5	< 1
Heterotransfer	${}_{T1}D_n \longrightarrow {}_{T1}P_c$	< 5	< 1
Transfer into 'injected' clone	${}_{T1}D_n \longrightarrow ({}_{T1}D_c \dashrightarrow {}_{T1}P)_c$	< 5	48
Transfer into 'injected' clone	${}_{T1}P_n \longrightarrow ({}_{T1}D_c \dashrightarrow {}_{T1}P_c$	100	0
'Injected' clone nuclei	$({}_{T1}D_c \dashrightarrow {}_{T1}P)_n \longrightarrow {}_{T1}P_c$	100	0
'Injected' clone nuclei	$({}_{T1}D_c \dashrightarrow {}_{T1}P)_n \longrightarrow {}_{T1}D_c$	< 5	0*

* 30 per cent transfers divided to give more than 30 cells before death.
$\rightarrow$ Nuclear transfer. $\dashrightarrow$ Injection of cytoplasm.

Results of transfer experiments using a clone of *A. proteus* which had received and assimilated an injection of *A. discoides* cytoplasm, compared with results obtained from homotransfer and heterotransfer experiments.

From these results it was apparent that ${}_{T1}P$ nuclei were no longer viable in the cytoplasm of the 'injected' cells (that is, *A. proteus* which had received and presumably assimilated an injection of *A. discoides* cytoplasm), while the considerably increased percentage of viable clones obtained from the transfer of ${}_{T1}D$ nuclei into these cells indicated the extent of the change in these formerly 'proteus' amœbæ. The nuclei of the 'injected' clone were no longer viable in ${}_{T1}P$ cytoplasm, neither were they viable in ${}_{T1}D$ cytoplasm, although in the latter experiments many transfers divided to give 30 or more cells before death. The behaviour of 'injected' amœbæ in these nuclear transfer experiments is a further indication of the successful assimilation of non-homologous cytoplasm.

Nuclear transfers between more widely separated species, for example, *A. dubia* and *A. proteus*, have proved unsuccessful so far[8]. Prior injection of non-homologous cytoplasm and its successful assimilation, even in a very low percentage of cells, would be a means of obtaining clones of a mixed type able to accept foreign nuclei. Experiments of this type are in progress.

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Moulting Frequency of a Deep-sea Crustacean, *Euphausia pacifica*

Most of the observations on moulting in crustaceans have been confined to littoral animals kept in aquaria[1]. Because planktonic, deep-sea crustaceans are difficult to maintain in the laboratory there has been virtually no information available on moulting frequency in these forms. In connexion with experiments designed to determine filtering rates and the quantitative aspects of carbon metabolism in the deep-sea planktonic euphausiid shrimp, *Euphausia pacifica*[2], observations were made on the moults and moulting frequency of this animal.

E. pacifica is an important and ubiquitous component of the deep-sea plankton in a broad oceanic region ranging from the north of Japan across the Bering Sea to Baja, California, Mexico[3]. In the laboratory *E. pacifica* has been kept alive for more than 7 weeks on a diet of the green flagellate *Dunaliella primolecta* and/or *Platymonas subcordiformis*.

Each animal, ranging in dry weight from 1·2 to 4·8 mg, was kept in 1 l. of sea water and the water changed every three days. One drop of algal suspension was added daily, but no attempt was made to determine how much food was actually ingested by the euphausiids. All containers were examined daily for cast moults. Recovered moults were rinsed in distilled water, dried at 80° C on a microscope slide and weighed with a Cahn electrobalance to ± 0·002 mg. Temperature was not rigidly regulated but normally held within narrow limits. Only those animals which had moulted more than once are included in this work.

The results summarized in Table 1 show that within the range of experimental temperatures (9°–14° C) the moulting frequency was 5 days with a standard deviation of o day (range 4–7 days). Each cast moult averaged 10 cent of the animal's dry weight. There is no appare correlation of moulting frequency with temperature or a of the animal, although it is quite likely that furcilia a calyptosis stages have more frequent moults as do mo developing crustaceans[1]. Temperature may have had small effect, but because observations were made daily a not hourly these may have been masked.

Table 1. Moulting Frequency of *Euphausia pacifica*
(*S.D.*, standard deviation)

Euphausiid	Temperature range	Final animal dry weight (mg)	No. of moults produced	Average weight moult	Time (days) alive in laboratory	Average moulting frequency (days)	Moults = percentage of animal dry weight
1	9·5–12·5	2·333	2	0·247	10	5	10·6
2	9·8–11·2	2·143	2	0·182	14	7	8·5
3	9·8–11·2	4·810	2	0·479	8	4	10·0
4	9·8–13·7	4·800	3	0·416	18	6	8·7
5	9·8–13·7	—	3	—	15	5	—
6	10·8–14·5	1·541	2	0·198	10	5	12·9
7	10·8–14·5	1·975	3	0·167	12	4	8·4
8	10·8–14·5	—	2	0·187	14	7	—
9	10·8–14·5	—	2	0·109	12	6	—
10	10·8–14·5	1·244	2	0·157	9	4	12·7
11	12·3–13·2	—	3	0·249	13	4	—
12	12·3–13·2	—	2	0·263	12	6	—
13	12·5–13·2	4·798	2	0·327	9	5	6·8
14	12·5–13·2	—	2	0·161	7	4	—
15	12·6–14·3	1·300	11	0·128	50	5	9·8
16	12·8–14·2	3·476	3	0·380	12	4	10·9
17	15·4–17·5	—	2	0·146	8	4	—
18	15·4–18·9	1·305	10	0·211	36	4	16·1
19	15·4–18·9	1·326	8	0·121	40	5	9·1
						Av. 5 ± 1 (*S.D.*)	Av. 10·4 ± 2·4 (*S.D.*)

One animal lived 50 days in the laboratory and h passed through 11 moults, while another of similar si (1·3 mg) went through 10 moults in 36 days. Ash weigh averaged 54·4 per cent of the moult dry weight (range f four determinations 52·9–56·9 per cent) and the organ components 45·6 per cent by difference. Nitrogen w 1·5–2·5 per cent of the moult dry weight or between 3 an 5 per cent of the ash-free moult dry weight.

There are large populations of euphausiid shrim throughout the deep oceans. If moulting occurs in th sea as frequently as it has been observed in the laborator the moults represent a tremendous amount of organ material added daily to the rain of materials known a detritus in the sea. For example, each euphausiid con tributes its full body-weight in cast moults every 50 day The importance of organic detritus in the sea was discusse by Parsons and Strickland[4]. The results presented her show that the moults of planktonic crustaceans coul contribute a substantial portion of this material.

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Body Temperature of Yellowfin and Skipjack Tunas in Relation to Sea Surface Temperature

Tunas are frequently cited as the exception to the generality that fishes are poikilothermic. These references to temperatures higher in tunas than in their environment are mainly based on measurements made with mercury thermometers on isolated specimens at one or two sea temperatures[1], circumstances which result in less-than-accurate temperature measurements, and which fail to describe adequately the body temperature–sea temperature relation over the thermal range of the tunas. The single report[2] of tuna body temperatures over a wide range of sea temperatures is based on measurements made with the mercury thermometer.

In the course of other investigations, we had the opportunity of measuring, with thermoelectric techniques, the

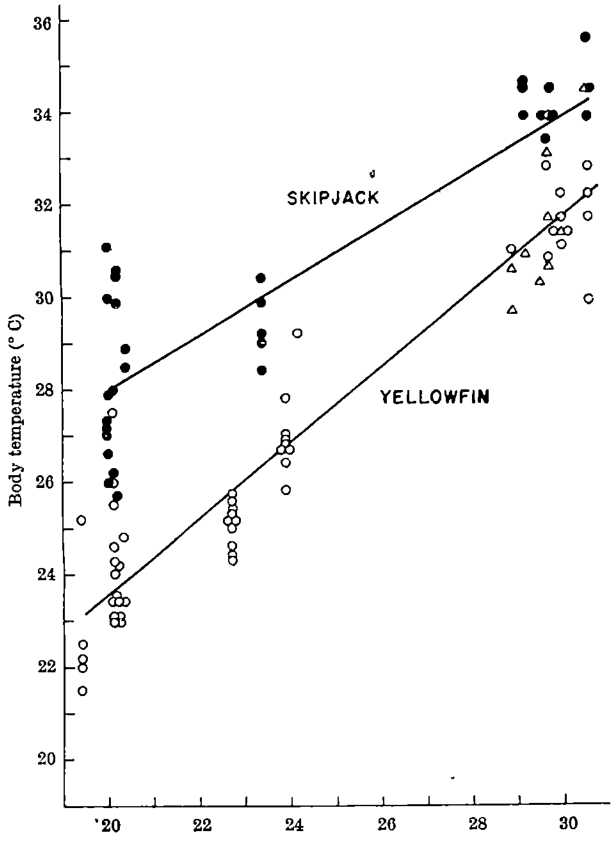

Fig. 1. Relation of body temperature to sea surface temperature. ●, Skipjack; ○, yellowfin; △, yellowfin in sea

deep-muscle temperatures of 62 live yellowfin tuna (*Thunnus albacares*) (length-range, 530–882 mm) and 31 live skipjack tuna (*Katsuwonus pelamis*) (length-range, 467–555 mm) captured in the eastern tropical Pacific Ocean, at sea temperatures of 19·4°–30·6° C. Body temperatures were measured with either the *YSI* model 47 telethermometer or the Electrotemp remote reading thermometer, both calibrated against standard mercury thermometers. The thermistor probes were inserted deeply into the lateral muscles, midway between the origins of the dorsal and pectoral fins. Most readings were taken on live fish within 1 min after being brought on deck, after capture by live bait methods[3], by purse-seine, or by trolling; the remainder were made on hooked yellowfin swimming in the sea at the end of a line.

The plot of these results (Fig. 1) shows the linear least squares regression of body temperature of each species on the temperature of the environment at the time of capture. The equations are:

$$\begin{aligned}&\text{yellowfin body temperature °C} = 0{\cdot}81 \text{ water temperature °C} + 7{\cdot}47\\ \text{and } &\text{skipjack body temperature °C} = 0{\cdot}58 \text{ water temperature °C} + 16{\cdot}39\end{aligned}$$

The regression lines are significantly different in both slope and level. The correlation coefficients are 0·95 and 0·87 for the yellowfin and skipjack, respectively.

Over the size range of the fish tested, length was of minor importance in the body temperature–sea temperature relation for the yellowfin (body temperature °C = 6·49 + 0·86 water temperature °C–0·0002 body length mm, for the 53 fish for which length measurements were available). In the skipjack, however, the small fish tended to have lower body temperatures than did the large (body temperature °C = 8·63 + 0·59 water temperature °C + 0·015 body length mm).

Although the effects of stimulation, handling, heat gradients within the fish, and fish size must be considered in assessing these results, the similarity of the temperatures of yellowfin in the sea (Fig. 1, triangles) to those of yellowfin on deck immediately after capture (Fig. 1, open circles) suggests that the line for the yellowfin is valid. No such confirmatory results are available for the skipjack, but there is little reason for suspecting the validity of the line.

Examination of blood hæmoglobin-levels[4], muscle glycogen content[5], blood lactate accumulation[6], and mortalities after handling[6] and tagging[7] in both the yellowfin and skipjack indicates that these two species live at a higher physiological rate than do other fishes which have been examined, the skipjack more so than the yellowfin[5–7]. The temperature results given here offer confirmatory evidence of this.

We thank the masters and crews of the motor vessels *San Juan*, *Julia B.* and *Red Rooster* for their help in this investigation. Mr. Ami Dolev, University of California at Berkeley, helped with the measurements aboard the M.V. *San Juan*. One of us (I. B.) was supported in part by funds from contract 14–17–001–844 between the U.S. Bureau of Commercial Fisheries and the Institute of Marine Resources, University of California.

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Another Giant Enteropneust from the Atlantic

THE largest known hemichordate is *Balanoglossus gigas* Fr. Müller. First discovered on the Atlantic coast of South America[1], this member of the family Ptychoderidae was found afterwards in considerable numbers at São Sebastião, São Paulo, Brazil[2]. Spengel[3] estimated its maximum length as 2·5 m, but specimens collected recently[4], although presumably complete, have not exceeded 2·3 m. The majority of the larger enteropneusts belong to this family.

Recently, March 1962, and again on the Atlantic coast of South America, at Praia do Araça, São Sebastião, Brazil, yet another giant enteropneust has been discovered. Unfortunately, the extreme fragility of the post-branchial region, especially when gorged with sand, has made it impossible to remove a complete specimen from its burrow. The largest and almost complete specimen obtained was 1·6 m long. A fully grown and fully extended specimen may well attain a length of 2 m, or even more.

Preliminary investigations show that this is a new species and a member of the family Spengelidae. By comparison the spengelids are only moderately large animals, although *Glandiceps talaboti* (Marion), also found in the Atlantic, may exceed half a metre in length[5]. It is remarkable that the two largest known enteropneusts have been found at Praia do Araça. This shore is also sparsely populated by *Balanoglossus clavigerus* Delle Chiaje. All three populations overlap one another and are confined to a region of the shore where the substratum remains saturated throughout the ebb-tide—a requirement common to many enteropneusts[6,7].

This is the first record of a spengelid from the South American coast, although *Tornaria* larvæ comparable to *Tornaria dubia* Spengel have been recorded off the coast of Brazil[8]. According to Stiasny-Wynhoff and Stiasny[9] *T. dubia* are the larvæ of *Glandiceps talaboti*, and in consequence Björnberg[8] suggests that this spengelid may turn up on the Brazilian coast. It also seems probable that these *dubia*-like *Tornaria* may prove to be the larvæ of the new spengelid species. Of the four specimens collected during March to August, one was a ripe female which released several hundred ova in an aquarium some hours after collection.

It is also another record of a spengelid inhabiting the same shore with more than one species of ptychoderid[10,11]. The remainder of the associated fauna included a large population of the lamellibranch *Anomalocardium brasiliana* Gmelin, the gastropod *Olivella verreauxi* Ducros, some burrowing decapod crustacea, a variety of small errant polychætes primarily *Ammotrypane aulogaster* Rathke, *Arenicola* sp., and numbers of very large *Eunice rousseaui* Quatrefages, commonly more than 1 m in length. This giant polychæte and the new spengelid lived at a slightly higher level on the shore than the *Balanoglossus gigas*.

The presence of a deep median dorsal sulcus on the proboscis, a small rudiment of a vermiform appendix on the buccal diverticulum and of peribuccal spaces, coupled with the absence of hepatic sacculations, lateral trunk septa and of synapticulæ, justify the identification of this spengelid as a new species of the little-known genus *Willeyia* Punnett 1906. This genus was first established by Punnett[12], who described *Willeyia bisulcata* from fragments of two specimens collected at Zanzibar, where it is said to be abundant. His description was not very accurate or as extensive as it might have been, and so doubt existed concerning the validity of the genus, until Horst[5] described *W. delagoensis* from a fragment of a single specimen collected at Inyack Island. This new discovery of several specimens in Brazil now makes it possible for us to corroborate Horst's observations and establish beyond doubt the validity of the genus *Willeyia*.

Little is known about the burrowing habits of the Spengelidae. The related genera *Glandiceps* and *Spengelia* are thought not to make permanent burrows. The new *Willeyia* species formed coiled casts on the surface, which were easily confused with those voided by the *Arenicola* sp. common in the same area. The burrows descended vertically from the cast to a depth of 30–35 cm before turning and following a long sinuous course at 50–70 cm below the surface. One burrow penetrated to a depth of 80 cm. They appeared to be devoid of any well-defined lining. No entrance holes were discovered.

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Significance of Clutch-size in Swift and Grouse

WYNNE-EDWARDS[1] has so misunderstood the problem of clutch-size in the swift *Apus apus* at Oxford reported by Perrins[2] that further clarification seems desirable. First, he confused clutch-size (number of eggs laid) with brood-size (number of young at hatching). This is important because we often manipulated brood-size in the tower, as Perrins stated for broods of four, while most broods of one were due either to our manipulation or to hatching failures. Wynne-Edwards was, therefore, wrong in stating that clutches of four have been commoner in recent years, and that clutches of one, two and three have been in the proportion of 24 : 61 : 15. The swifts at Oxford have normally laid either two or three eggs, and natural clutches of one and four have been extremely rare.

The most critical question is what restricts the clutch to three, and Perrins's experiments, far too lightly dismissed by Wynne-Edwards, show that pairs with broods of four raised, on the average, fewer young than those with broods of three. In these circumstances, any tendency to lay four eggs will be strongly discouraged by natural selection, and this is sufficient to explain the extreme rarity of clutches of four.

Since, on the average, each brood of three gave rise to more young than each brood of two, a second and more complex question is why any swifts should lay clutches of two. Wynne-Edwards omitted to discuss the three reasons which I advanced earlier[3,4] and which I still hold. First, in cold, wet summers such as 1948, 1954 and 1956, swifts found it difficult to bring enough food to raise even two young successfully. Hence, in some years, birds with clutches of two have probably been at a selective advantage over those with clutches of three, and therefore a hereditary tendency to lay two eggs may persist in the population. Secondly, clutches of two are unusual at the start of the breeding season, but become commoner later, and nearly all late clutches consist of two. This regular seasonal decline could have been evolved through natural selection if, on the average, a late brood of two gives rise to more young than a late brood of three. The latter point has not been tested by experiment, and as no late swifts lay three eggs, observations alone are inadequate. However, in late summer as compared with mid-summer, the hours of daylight available for feeding are shorter, and the weather tends to be wetter, when air-borne insects are sparser, so it seems likely that late broods are more difficult to raise.

Thirdly, if cold weather occurs during the main laying period in May, those pairs which have not yet started are restrained, while it has been shown that those which have just started tend to lay their successive eggs three instead of two days apart, and often lay only two in all instead of three. This variation, unlike the others, is obviously not connected with the future raising of the brood. Instead, it is surely a consequence of the physiological strain of egg-production on the female when food is sparse, under which conditions it will presumably be advantageous to lay two eggs rather than three, even though this eventuates in a rather smaller brood.

Summing up, the normal clutch of the swift at Oxford can be satisfactorily explained through the operation of natural selection on hereditary variations, combined with two or three phenotypic variations, most of these variations being adaptations to the capacity of the parents to find food for their brood, and one to the physiological condition of the female at the time of laying. Several points in this explanation have not been proved, but all seem probable. Wynne-Edwards wrote that "on my alternative hypothesis that inter-group selection has fixed the fecundity range around the optimum level for recruitment to the population as a whole, the facts . . . seem reasonably intelligible". But he has provided no positive evidence for this statement, and in particular he has given no supporting figures for correlated variations in fecundity recruitment or size of population. Hence there is no means of knowing whether recruitment is at the optimum level'.

Turning more briefly to the red grouse *Lagopus lagopus scoticus*, this bird does not bring food to its young in the nest, so it would be extremely unlikely that natural selection would restrict brood-size for the same reason as in the swift. Instead, I would suggest that a factor of minor influence in the swift may be much more important in the grouse, namely, the physiological condition of the female at the time of laying. Jenkins *et al.*[5] showed that the heather on which red grouse basically depend for their food was in good condition in the spring in 1957, 1960 and 1961, in which years the average clutch was between 7·8 and 8·1 eggs, but that the heather was in bad condition in the spring in 1958 and 1959, when the average clutch was only 6·9 and 6·1 eggs, respectively. It is reasonable to attribute this variation to the food available to the females prior to laying. Further, this agrees with the earlier work of Siivonen[6] on other gallinaceous birds in Finland, where the partridge *Perdix perdix*, capercaillie *Tetrao urogallus* and Blackgame *Lyrurus tetrix* likewise have larger clutches in those seasons when food is more abundant prior to laying. The physiological condition of the female prior to laying may not be the sole factor determining clutch-size in gallinaceous birds; for one thing, in all species so far studied, the average clutch is smaller later in the season. But the food for the female is clearly important, and there is, once again, no evidence for Wynne-Edwards's alternative view. Indeed, he has not yet produced any positive or quantitative evidence for it in any species of birds.

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DR. LACK's communication clarifies some important points, among them the influence of weather at the time of laying in the swift, and the earliness or lateness of the laying date, on clutch-size. But, notwithstanding the errors in the figures I extracted on clutch-size frequencies, he does not challenge my statement that the mean clutch-size in the common swift both at Oxford and in Switzerland is well below the 'most efficient' clutch-size, this being the size that on average has yielded the largest number of surviving young. Lack outlines three suggestions as to how this situation could be accounted for on the basis of natural selection acting at the individual level.

The first concerns the cold, wet summers, when broods of two (derived from clutches of two or more) can turn out as well as or even a little better at Oxford than broods of three. In fact, such summers are in the minority, and the margin is at most rather small, so that any transient selective effect would be outweighed by the more substantial advantage to be gained in average and good years by starting off with a clutch of three. The breeding life of common swifts averages something like 4–5 years[4]; those that consistently lay three will, therefore, always tend to leave more offspring than those that lay two. In the alpine swift (*Apus melba*), studied at Solothurn, Switzerland[3], a good many parallels were found to the common swift in breeding biology, including a distribution of clutch-sizes between 1 and 4 eggs; but the extensive data in this case do not reveal any parallel switch of advantage between good and bad years.

The second, that there is a selective advantage in reducing clutch-size as the season advances, is speculative with regard to the swifts at Oxford, not being supported by adequate evidence. In the alpine swift again, where there is a corresponding fall in the frequency of 3-egg layings as the season advances, the threes nevertheless appear to remain more productive than the twos, even after the latter have come to outnumber them[7].

The third suggestion concerns the relationship established in the swifts at Oxford between the weather just prior to laying and the number of eggs laid. As Dr. Lack says, this appears to be a short-term physiological effect or phenotypic variation, modifying the innate fecundity-level. Natural selection could promote this kind of adaptability if it were hereditary and advantageous, but would be unlikely in so doing to have any effect on the basic fecundity level. Even in years when cold weather before laying depresses the average clutch-size, the threes that are laid can still be expected to do better than the twos.

I have emphasized elsewhere the fact that fertility in animals is widely influenced by contemporary events, particularly by changes in population density and economic conditions. In view of this, experiments of the kind reported by Perrins[2] seem to me to assume far too simple a conception of what is involved in determining breeding success. Environmental stress can condition such responses as the number of eggs laid, the tenacity of the parents in incubation and food-getting, the eviction or desertion of eggs or young, and so on; and where a pair of birds have become physiologically conditioned to making a reproductive effort of a certain magnitude it is hardly to be expected that they will automatically adjust themselves to a larger one, if the experimenter presents them with an extra chick. If they then fall down on the job, what valid conclusion can be drawn ?

Finality on the difficult problems discussed here is not likely to be attained by a further exchange of correspondence in *Nature*, however stimulating. It can reasonably be concluded that there are unresolved difficulties about establishing Dr. Perrins's objections[2] to the concept of intergroup selection[9], which is where the correspondence started. Whether the concept itself has suffered at all in the discussion it is for others to judge.

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A Hermit Crab New to Britain

IT is of interest to record the occurrence on the shores of the Isles of Scilly of a hermit crab new to Britain. During the course of a collecting trip with my students to Porth Hellick, on the south-east corner of St. Mary's, in September of 1962 my attention was attracted by a small hermit crab with brilliant violet antennæ. Being unable to determine it to my satisfaction, I sent it to Dr. I. Gordon at the British Museum (Natural History), who identified it as a male *Catapaguroides timidus* (Roux), a species which she knew well in the Mediterranean. I have now taken a second male of the species, confirmed by Dr. R. W. Ingle, of the British Museum, this time from a bay near Gulf Rock at the south-west tip of the island of Bryher (Isles of Scilly). The two records, from widely separated localities among the islands and at an interval of two years, coupled with the fact that no systematic search has been undertaken in the interim, suggest that the species is established there.

The hitherto known localities are summarized by Bouvier[1] as the Atlantic at Roscoff and the Canaries, perhaps as far as Angola, and the eastern Mediterranean as far as the Adriatic, between shallow littoral and 1,200 m. These two records, both from littoral waters between L.W.N. and L.W.S., extend the known range of this southern species by about 1° northwards.

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MICROBIOLOGY

Identification of the Bacteriostatic Component of 'Scotch' Brand of Cellulose Adhesive Tape

WE have recently shown that the 'Scotch' brand of cellulose adhesive tape of the Minnesota Mining and Manufacturing (Australia) Pty., Ltd., contains a volatile component the antibacterial activity of which is virtually restricted to coagulase-positive strains of *Staphylococcus aureus*[1]. In view of this unusual specificity it seemed important to identify the active agent.

The anti-staphylococcal component was extracted from the tape by a number of organic solvents, but not by water. For chromatography, approximately 30 g of tape was extracted with 100 ml. of boiling alcohol and the extract concentrated to a final volume of about 10 ml. About 0·2 ml. of extract was applied to strips of Whatman 3 *MM* paper 4 cm wide and developed with a variety of solvents. After drying, the position of the active component was detected by cutting the strips into short lengths which were then placed in the lids of nutrient agar plates inoculated with *Staphylococcus aureus* *E*169[1]; the absence of growth on an incubated plate indicated the presence of the active component in that section of the paper strip. With all solvents used, the active component either remained at the origin or moved with the solvent front.

Because of the apparent insolubility of the active component in water and its behaviour on conventional paper chromatograms, separation was attempted by reversed phase chromatography, using Whatman 3 *MM* paper impregnated with paraffin oil, and methanol/acetone/paraffin oil (3 : 1 : 1) as the mobile phase[2]. Under these conditions the active component was confined within R_F values of 0·4 and 0·5. Examination of strips under ultra-violet light revealed a fluorescent area extending for some distance before and behind the area within which the active component was confined. In order to remove this interfering fluorescent material, descending chromatograms were developed overnight with acetic acid/butanol/water (the aqueous phase of a 1 : 4 : 5 mixture) in which the active component was immobile. After drying, the strips were impregnated with paraffin oil and then developed with the methanol–acetone–paraffin oil solvent. Under ultra-violet light these strips showed only a slight fluorescence along the length of the paper, with an absorbing spot containing all the anti-staphylococcal activity having an R_F value of 0·46. The active spot gave no reaction with a number of detecting reagents. However, reducing activity was shown with both alkaline silver nitrate and a mixture of ferric chloride and potassium ferricyanide; these reagents showed brown and blue colorations, respectively, both streaking towards the front. In addition, strong oxidizing activity was shown with alcoholic leuco-methylene blue. These results suggested that the active component could exist in both a reduced and an oxidized state, forming a readily reversible oxidation-reduction system.

Examination of patents[3] covering the manufacture of 'Scotch' brand cellulose adhesive tape revealed that the adhesive layer of the tape contains, as an antioxidant, either 2,2′-methylene-*bis* (4-methyl-6-*tert*-butylphenol) or 2,5-di-*tert*-amylhydroquinone. When tested for anti-staphylococcal activity by direct addition to the lids of inoculated plates, only the latter substance was active.

Reversed phase chromatography of 2,5-di-*tert*-amylhydroquinone and 2,5-di-*tert*-amyl-*p*-benzoquinone (prepared by oxidation of the hydroquinone with silver oxide) gave major spots with R_F values of 0·9 and 0·5 respectively; both substances showed minor spots of the corresponding oxidized and reduced forms. These results suggested that the anti-staphylococcal activity of the tape was due to 2,5-di-*tert*-amyl-*p*-benzoquinone. This was confirmed by tests in which variants of *Staphylococcus aureus* *E*169 selected for resistance to either the tape or to 2,5-di-*tert*-amyl-*p*-benzoquinone were found to be completely cross-resistant.

The lowest inhibitory concentration of 2,5-di-*tert*-amyl-*p*-benzoquinone for *Staphylococcus aureus* *E*169 was discovered by preparing serial dilutions of the inhibitor in nutrient agar; as little as 3 μg/ml. was sufficient to reduce the colony count to less than 0·01 per cent of that on control plates. In comparison, under similar conditions a concentration of 300 μg/ml. of *p*-benzoquinone was required to produce the same reduction in colony count.

Oxford and Raistrick[4] investigated the activity of several *p*-benzoquinones against a limited range of bacterial species; they found that strains of *Staphylococcus aureus*, *Bacillus anthracis*, and *Vibrio cholerae* were particularly susceptible. In view of the role of naturally-occurring quinones in biological electron transport[5] and the inhibitory effect of *p*-benzoquinones on this system[6], it would be of interest to determine more precisely the 'spectrum' of activity of *p*-benzoquinones in relation to the distribution of ubiquinone and menaquinone in bacteria.

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CYTOLOGY

Chromosome Duplication and the Cell Cycle in Lens Epithelium

PREVIOUS experiments have shown that a penetrating needle injury to the central area of rabbit lens epithelium *in vivo* produces a response in which a large number of cells synthesize DNA and divide[1]. This response begins characteristically at the site of injury and progresses farther and farther away from the wound with time. Experiments have indicated that DNA synthesis is initiated in the cells closest to the wound at about 12–14 h after injury[2]. Mitosis is first seen at about 24 h after injury. The rate at which the wave of DNA synthesis travels was calculated to be about 17 μ/h. The typical picture obtained at 48 h after injury shows a band of cells which are synthesizing DNA and completely encircle the wound. Another band of mitotic figures is seen encircling the wound and lying between the DNA synthesis band and the injury but close to the DNA synthesis band. Frequently, when the injuries are larger (these can be obtained by using a larger needle) a 'second wave' of DNA synthesis and mitosis can be seen.

The classical experiments of Taylor *et al.*[3], which demonrated in plant material that the chromosomes duplicated ι a semi-conservative fashion, indicated that examination f chromosomes with labelled precursors of DNA synthesis an be of great value.

The present experiments have yielded information egarding the mode of chromosome duplication *in vivo*, nd the nature of the cell cycle of the centrally located ells after injury.

Young American Dutch rabbits (1·5–2 lb.) were anæshetized with sodium pentobarbital and the eyes injured vith a fine steel insect needle (0 gauge) by inserting the eedle through the cornea and into the lens at right angles o the corneal surface. Tritium-labelled thymidine (spec. ct. 3 c./mM), 5 μc./ml. in Eagle's basal medium, without erum or glutamine, was injected into the anterior chamber f the eye about 18 h after injury. Colchicine (1 μg/ml. in asal medium) was injected into the anterior chamber at pproximately 46 h after injury and the eyes enucleated at 8 h. Chromosome spreads from lens epithelium were repared for autoradiography according to the technique f Srinivasan and Harding[4].

Non-radioactive preparations in which well-spread chromosomes were observed showed that the rabbit lens epithelial complement consists of 44 chromosomes. Examination of many spreads did not reveal the presence of heteroploidy. When tritium-labelled thymidine was injected at 18 h after injury and the cells fixed at 28 h, radioactive chromosomes were observed[4]. A very high percentage of chromosome spreads were labelled and the label was present along the lengths of both chromatids of the chromosome.

When tritiated thymidine was injected 18 h after injury and the eye fixed at 48 h, radioactive chromosomes were seen. Examination of the location of the label on some of the larger chromosomes revealed that the label was primarily localized on only one of the two chromatids of the chromosome. Taylor *et al.*[3] exposed plant cells to tritiated thymidine and found that the chromosomes of the first division were labelled on both chromatids. If the cell was allowed to divide once more in the absence of tritiated thymidine, the chromosomes of the second division showed label primarily on only one of the two chromatids.

Within the same preparation chromosome spreads varied in the intensity of label seen on them. This would be expected since the tritiated thymidine would have been incorporated by cells in different stages of DNA synthesis before the first division. There also seems to be asynchrony of DNA synthesis among the chromosomes *in vivo* since some of the chromosomes are not labelled at all.

The fact that radioactive chromosomes have been demonstrated after a 30-h time-interval between the introduction of the labelled precursor and fixation may not necessarily be an indication that these cells are dividing for a second time. Indeed, in other systems $S + G2$ times of a much longer duration have been reported[5]. However, the segregation of label, 48 h after injury, on the chromosomes of some cells is similar to that obtained by Taylor *et al.*[3] at the second division in cells of *Vicia faba* and also in human cells in culture[6]. This could be taken as strong evidence in favour of some lens epithelial cells dividing twice after injury. Earlier experiments by Harding *et al.*[2] showed that the cells around the injury were the first to incorporate labelled thymidine about 12–14 h after injury. The first mitotic figures appeared about 24 h after injury. Harding and Srinivasan[7], using tritiated thymidine, found that the time between the onset of DNA synthesis and cell division was about 10–12 h. Preliminary experiments indicate that the $G2$ period of these cells is 4 h or less. Therefore, the maximum time for DNA synthesis in these cells is about 8 h. When tritiated thymidine is injected into the anterior chamber at 18 h after injury, it would remain in the anterior chamber for about 2 h, since Maenza and Harding[8] showed that the concentration of tritium in the aqueous humour after 2 h is negligible. Therefore, the tritiated thymidine would remain available to the cells until about 20 h after injury. During this period of time, cells which are in all stages of DNA synthesis would incorporate the labelled nucleoside. The range of time during which one would expect to see radioactive mitotic figures of the first division is between 24 and 32 h after injury. Assuming that the $S + G2$ time remains the same for the second division as well, the generation time for the cells which divide for a second time would lie within the range of 16–24 h.

These figures are in general agreement with the timings reported by Thomson *et al.*[9], who examined the cell cycle of germinative zone cells in lens epithelium from Dutch rabbits. They found that $G2$ was between 1 and 4 h. $S + G2$ was between 9 and 13 h. These results indicate that the germinative zone cells and the cells of the central area of rabbit lens epithelium after injury have approximately the same $S + G2$ time. Similar findings have been reported for lens epithelium from very young rats[10].

This work shows that there can be a redivision of cells near the site of injury. Such a redivision occurs within 16–24 h after the first division and therefore corresponds to the timing of the second wave of stimulation of DNA synthesis.

This work was supported in part by U.S. Atomic Energy Commission contract *AT* (30–1) 2456 and U.S. National Institutes of Health grant 5*T*1 *GM* 690–03. I thank Prof. C. V. Harding for his advice.

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Tetrad Analysis by Autoradiography

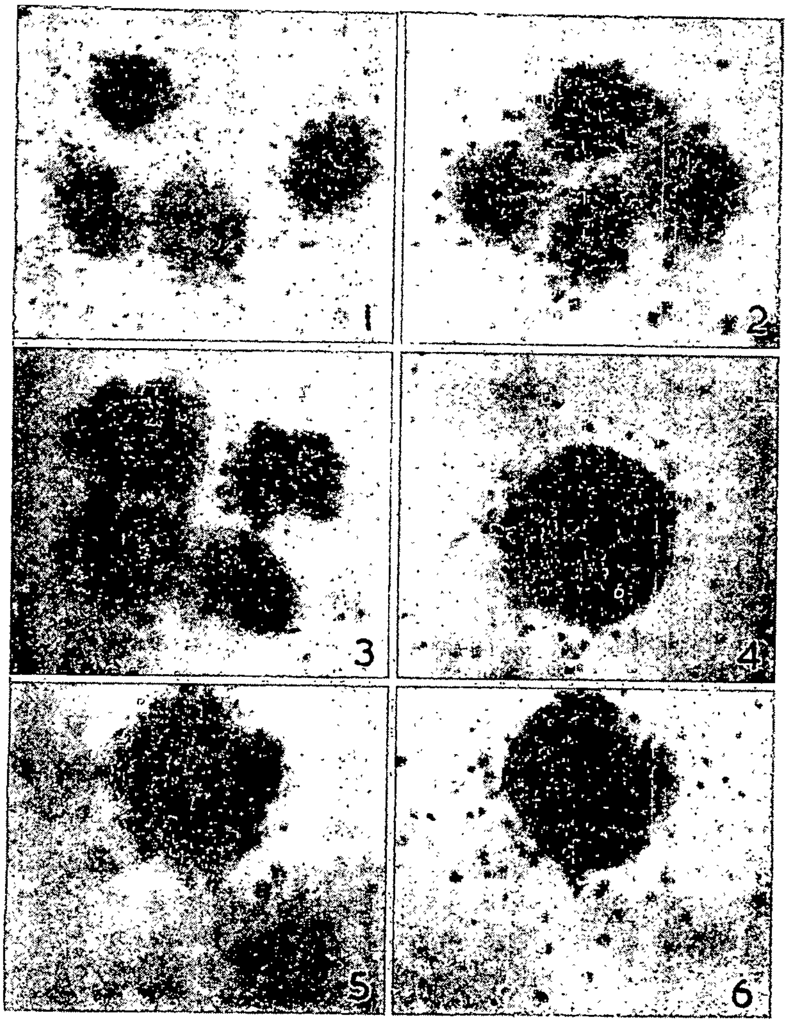
THE phenomenon known as 'gene conversion'[1], which results in the production of tetrads in which a pair of alleles segregates in a 3 : 1 ratio, instead of the usual 2 : 2 ratio, has been explained on the basis of a copy-choice theory[2]. Such an explanation is unacceptable unless the conservative replication of chromatids, at least in part, is also hypothesized[3]. It is generally accepted that both the DNA double helix[4] and chromatids replicate semiconservatively[5]. However, there exists evidence which suggests that the DNA double helix may replicate conservatively[6].

Evidence for the mode of chromatid replication has been derived from mitotically dividing chromosomes[6] only: it is possible that chromatids may divide conservatively at meiosis[3], but evidence on this point is lacking. We wish now to report the development of a simple system capable of providing information on chromatid replication at meiosis.

Use is made of the flowering plant *Haplopappus gracilis* ($2n = 4$). Tritiated thymidine is fed to the buds of the intact plant at the time sporogenous tissue is in the premeiotic interphase. The method of feeding the radioactive isotope is based on the use of a cotton wick as described elsewhere[7]. Following this treatment slides are made of cells in the tetrad stage; the incorporation and distribution of the label are followed by examining autoradiographs of these preparations. The low chromosome number of the plant makes it possible to predict that semi-conservative chromatid replication at meiosis will always result in equally labelled nuclei within a tetrad; whereas the presence of tetrads with unequally labelled nuclei would suggest conservative replication of chromatids.

Figs. 1–6 are photographs of autoradiographs of some of the tetrads studied. These results indicate: (1) That it is possible to label tetrads by this method. The success of the method may partly be due to the short length of time the cells take to go from premeiotic prophase to the tetrad stage in *Haplopappus gracilis*. (2) That chromosome replication at meiosis may be conservative. Figs. 2, 3, 4, 5 suggest unequal labelling of the four nuclei within a tetrad; Fig. 6 is the tetrad shown in Fig. 5, with the nuclei in focus, showing no other labelling than that in Fig. 5. Fig. 4 is of particular interest as it clearly shows that the two nuclei nearest the emulsion have little or no labelling, whereas the two nuclei farthest away from the emulsion (out of focus) are heavily labelled. Thus Fig. 4 suggests against the possibility of differential labelling of nuclei reflecting the distance of nuclei from the emulsion. Fig. 1 shows a tetrad with equally labelled nuclei.

We believe that with further refinements of our technique we should be able to reach a conclusion as to whether chromatid replication at meiosis is conservative or semi-conservative.



Figs. 1–6. Fig. 1 (× c. 1,260); Figs. 2–6, photographed under oil immersion (× c. 1,700)

This work was supported by grant A1386 from the National Research Council of Canada.

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GENETICS

Balanced Combinations of Polygenes

Thoday and Gibson[1] have criticized some points in my discussion[2] of their results[3] in relation to Mather's ideas[4–6] concerning the action of stabilizing selection on the generation of "balanced combinations" of polygenes.

First I will follow up their points:

(1) Of course, it is possible to find some places where Mather mentions the possibility of pleiotropy of polygenes. But this is always followed by rejection of the importance of pleiotropy in quantitative genetics (for example, "Pleiotropy in the classical sense is therefore almost useless as a concept for application in biometrical genetics"[7]). Especially there was no room for pleiotropy in Mather's theory of the origin of balanced combinations of bristle polygenes which was a main aspect of my communication.

(2) The question whether Thoday's methods for the localization of polygenes are biometrical or classical genetics is only an amusing side-track leading into the terminology of polygenes. The interested reader is referred to a paper where Thoday himself[8] contrasts his "more or less classical" genetical analysis with the biometrical approach represented by quotations from Sheppard and Kempthorne, who emphasize the impossibility of recognizing individual polygenes. Thoday does not quote Mather here, from whom the following statement could have been used: ". . . the methods of analysis, so successful in the Mendelian genetics of discontinuous variation, cannot be applied where variation is continuous, because . . . the single factor cannot be separated from its fellows and followed as an individual . . ."[9].

(3) I do not think that Gibson and Thoday[3] have proved pleiotropy of the genes affecting bristle number with regard to their viability interactions. Proving pleiotropy is clearly difficult in organisms with a limited number of offspring when the developmental effects of the genes concerned are not known. But in view of the large number of generations in Gibson and Thoday's experiments and the large number of flies handled, apart from pleiotropy only very tight linkage could be responsible for the continued association of bristle and viability effects. Pleiotropy, then, seems the simplest explanation, only to be abandoned when further evidence makes it necessary.

It seems relevant to mention that Gibson *et al.*[10] claim that at least part of the effect on bristle number of the very same chromosomes under discussion here works via body size.

Reasons for thinking that pleiotropy will be the rule rather than the exception in polygenic inheritance can be found in Mayr's recent book[11].

(4) When discussing my statement that differences in bristle number are neutral or almost so in relation to natural selection Thoday and Gibson clearly missed my eference to Waddington's book[12], where the experiments and conclusions of Clayton *et al.*[13] are discussed. In addiion they can be referred to Falconer[14], where a lucid ummary of the arguments concerning the minor direct elective value of chaeta number can be found. There the results of the "extensive experiments" (there are several replicates, a feature not found in other selection experiments on chaeta number) of Clayton *et al.* are one of the main arguments.

Now we come to the main point of my communication, which was the interpretation of Gibson and Thoday's results in relation to Mather's theory of the role of stabilizing selection in the origin of balanced combinations of polygenes.

Thoday and Gibson avoid going into this. They do not dispute my statement that Mather's theory involves stabilizing selection on chaeta number itself. According to this theory natural selection would favour animals with intermediate bristle numbers because deviation from the optimal mean value is disadvantageous. This would cause an association of plus and minus factors in balanced combinations.

In Gibson and Thoday's experiments the association of plus and minus genes is not caused by natural selection on bristle number itself but by lethality, not related to bristle number, of certain combinations of their 'factors'. This seems to me an essential difference.

Therefore the statement that their experiments "demonstrate and locate in a wild stock just such a repulsion linkage balance as Mather[5] has argued should occur. . . ." is not justified.

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In the comment by Thoday and Gibson[1] on a communication by Scharloo[2], they quote a paper[3], of which I was an author, in a way which seriously misrepresents its conclusions on a point which is of importance in the theory of evolution.

Scharloo had said "extensive experiments have shown that differences in this character (chaeta number) do not have much selective value". In their reply, Thoday and Gibson say the "extensive experiments" are those of Clayton, Morris and Robertson, who only say that "it has been suggested that there is little direct connection between bristle number and fitness", quoting an earlier theoretical discussion. "They do not seem to have thought their evidence firm enough to justify a statement as strong as that required to support Scharloo's thesis".

They have chosen for quotation a sentence from a section on the theoretical aspects of the dominance relationships of the genes affecting bristle characters in *Drosophila*. Contrary to their final sentence, there are in our paper strong statements about our experiments which would justify Scharloo. On presentation of the results of relaxing artificial selection, we say, "It seems from these results that many of the genes controlling bristle numbers must have little or no connection with fitness". Again in the discussion, on the same page as the sentence quoted by Thoday and Gibson, we deal with the selective forces maintaining this genetic variation in our population in the following terms: "The results of relaxation of selection in lines selected for five generations, which showed that the line means had returned only about one-third of the way back to the original mean after nineteen generations of relaxation, suggest that these forces are not very strong and that many of the genes controlling the character must be effectively neutral in their effects on reproductive fitness. We have sufficient evidence on the effects of relaxation to feel justified in relying on the results".

Our last sentence would seem strong enough to support Scharloo's thesis on this important point.

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Our criticism of Scharloo's publication has done its work in eliciting replies and demonstrating that whether, and if so when and where, polygenic balance arises through selection operating on effects of genes on bristle number, or on pleiotropic effects of those genes, through linkage of those genes to others with more drastic effects on fitness or through random effects, is a more open problem than Scharloo suggests. Prof. Mather points out to us that it has also shown the dangers of taking statements out of their context, for his comment, quoted by Scharloo, that "Pleiotropy in the classical sense is therefore almost useless as a concept for application in biometrical genetics" followed a discussion of the difficulty, general with a biometrical methodology as distinct from classical, of analysing into their ultimate units groups of linked genes (or effective factors as Mather called them) which might indeed be expected to show apparently pleiotropic action (just as they can show apparent over-dominance) even where the ultimate genes themselves did not. The effective factor is the finest unit whose properties biometrical genetics will normally reveal; but its pleiotropy cannot be held to imply pleiotropy of the genes which compose it, and indeed in cases which Mather has analysed demonstrably does not do so.

We only wish to add two points. First, we had not believed Dr. Robertson attached so firm a conclusion to the results he refers to, because the conclusion can only follow on the assumption of a large number of genes none of large effect. That some of the relevant polygenes (or effective factors) are of relatively large effect is one thing we do claim to have demonstrated. Such genes might readily be fixed in some lines in short periods of selection and these lines could not return to the starting point under relaxed selection. Second, the two "genes" we described[1], and which Scharloo chose to discuss, clearly have extraordinary properties. They giv [illegible] interaction, show an apparent 20 map unit posit [illegible] 'loci' are polymorphic in populati [illegible] sive discussion of general [illegible] in relation to what may [illegible] unwise. These two a [illegible] which have been clo [illegible] have different [illegible] and studi [illegible] importa [illegible]

Depar [illegible]
Univers [illegible]

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[1] Gibson, J. [illegible]
[2] Gibson, J. [illegible]
[3] Wolstenh [illegible]
[4] Thoday, [illegible]

Base Ratios in DNA in Male and Female *Pseudococcus citri*

The mealy bug, *P. citri*, affords a unique opportunity for analysis of biochemical changes underlying chromosome behaviour. In the male embryos, the entire paternal set, that is, one half the total number of chromosomes, remains condensed in the non-dividing nuclei; this facultative behaviour is similar to that of typical heterochromatic elements such as sex chromosomes[1,2]. Experiments with tritiated thymidine show that, like heterochromatic elements in other organisms[3,4], the paternal set in the males reproduces asynchronously from the maternal, or euchromatic, set[5]. As measured by dominant lethality[2] and mutant genes[6], the paternal set is genetically inert in the males, but some 'residual' influences can be demonstrated[7,8].

Except for a few tissues, the paternal set maintains its heterochromaticity throughout the life of the male; at spermatogenesis it is segregated from the maternal, or euchromatic, set, and is not included in the sperm. In certain related forms, however, the heterochromatic state reversed during spermatogenesis and the paternal romosomes are segregated, apparently at random, to sperm, to be transmitted to offspring of both sexes[9]. seems likely, therefore, that the chromosomal changes ponsible for facultative heterochromatization are ones ich block rather than alter or destroy the genetic inmation. As a beginning we have isolated DNA from le and female insects and determined the base ratios m melting temperature and buoyant density measurents.

Mealy bugs were grown on potatoes, *Solanum tuberosum* .; the large, gravid females containing both male and emale embryos provided abundant material for establishment of experimental procedures; the second instar, prior to the extreme sexual dimorphism of later states, was used for the comparisons of males and females. No attempt was made to separate a yeast-like symbiont, present to about the same extent in males and females of the second instar; the DNA isolated includes symbiont nucleic acid also. DNA was isolated from 1 g of mealy bugs by the method of Marmur[10]. Phosphate was determined by the method of Fiske and SubbaRow[11], scaled down for the analysis of 10 µg of DNA. The best preparation of DNA had an $E(P)$ of 7,240. Chromatographic analysis was done on 1 mg of DNA from gravid females, hydrolysed 2 h in 70 per cent formic acid at 175° C. No methylcytosine was detected by Wyatt's procedure[12]. The average results from six analyses for base content are given in Table 1.

Table 1. Base Content of DNA from Gravid, Female Mealy Bugs

Base	Mole (%)
Adenine	34·2 ± 0·7
Thymine	31·9 ± 1·2
Guanine	16·2 ± 0·7
Cytosine	17·8 ± 1·3
Mole (%) guanine-cytosine	34·0 ± 1·1

Table 2. Melting Temperatures of DNA from Mealy Bugs

Source	Tm	Mole (%) guanine-cytosine
Gravid females	84·4 ± 0·35	37·5 ± 1·0
Second in	84·9 ± 0·20	38·7 ± 0·6
[illegible]	4·9 ± 0·28	38·7 ± 0·8

[illegible] were determined on a *DU* [illegible] a heated cell compart- [illegible] M sodium chloride– [illegible] ng temperatures of [illegible] female second [illegible] mole per [illegible] ublished

[illegible] males is [illegible] males is [illegible] f 1·39. [illegible] omatin

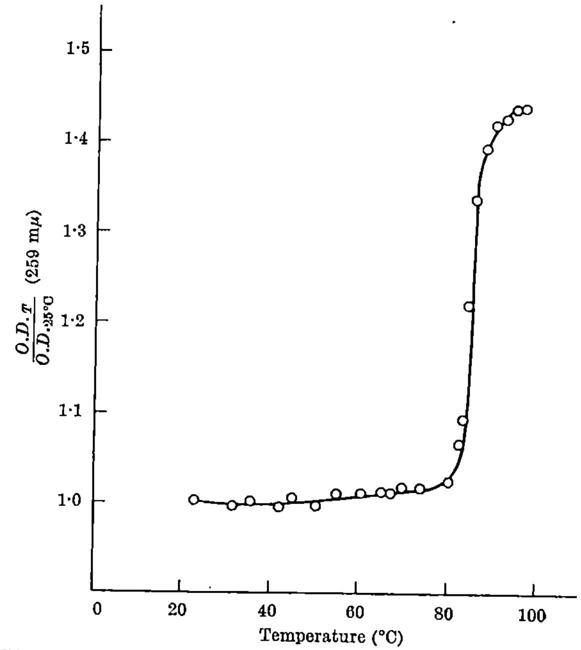

Fig. 1. Melting temperature profile of DNA from male mealy bug. Solvent used was 0·15 M sodium chloride–0·015 M sodium citrate. (Optical densities uncorrected for volume expansion)

The buoyant densities of DNA from both male and female second instar insects was 1·695 ± 0·001, corresponding to 35·8 ± 1·0 mole (per cent) guanine-cytosine[14].

The difference in base ratios by these three methods is not outside the variation reported in the literature[14]. It may be concluded that there is no evidence of a difference in base ratios greater than 1 per cent between DNA from male and female mealy bugs. Although genetic information no doubt could be destroyed without producing grossly detectable changes in base ratios, the fact that such cannot be demonstrated here is certainly in conformity with, rather than opposed to, the independent conclusion, from reversals of facultative heterochromatization, that the genetic information is masked rather than disrupted. Work is in progress to determine what differences may be found in other chromosomal components.

Buoyant densities in $Cs^{14}Cl$ were determined by Dr. John Hearst, of the Department of Chemistry, University of California, at Berkeley. The λ phage DNA used as a density standard was a gift of Dr. Monica Reilly, of the Department of Microbiology, University of California, at Davis.

This investigation was supported in part by a research grant (*GB*413–Brown) from the U.S. National Science Foundation and (*G*–4236–McLaren) from the National Institutes of Health, U.S. Public Health Service.

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FORTHCOMING EVENTS

Monday, July 6

Royal Society of Medicine (at 1 Wimpole Street, London, W.1), at [illegible] p.m.—Prof. E. B. Chain, F.R.S.: "Some Contributions from Chemical Microbiology to Medicine" (Jephcott Lecture).

Tuesday, July 7

Royal Society of Medicine (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Annual Meeting.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer in Geography—The Assistant Secretary, London School of Economics and Political Science, Houghton Street, London, W.C.2 (July 8).

Assistant Lecturer (with a good honours degree) in Geography—The Registrar, King's College (University of London), Strand, London, W.C.2 (July 8).

Assistant Cataloguer (graduate with good academic qualifications and experience or training in librarianship, and preferably with professional diplomas or experience) in the University Medical Library—The Registrar, The University, Manchester, 13 (July 10).

Lecturer in the Department of Medicine at the Royal Infirmary, Edinburgh—The Secretary, The University, Edinburgh (July 10).

Lecturer or Assistant Lecturer (with a special interest in inorganic *chemistry and preferably some teaching and research experience*) in the Department of Chemistry—The Secretary (F), Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (July 10).

Soil Chemist (with an honours degree or equivalent qualification), for duties which will include advisory work in soils and the supervision of routine soil analysis—The Secretary, School of Agriculture, West Mains Road, Edinburgh, 9 (July 11).

Assistant Lecturer (*with a degree in mathematics or the physical* sciences and preferably some postgraduate experience) in Computing—The Registrar, University College of North Wales, Bangor, North Wales (July 13).

Demonstrator (with an honours degree in chemistry or biochemistry and prepared to engage in research in biochemistry with a view to obtaining a higher degree in this subject) in the Department of Biochemistry—The Dean, St. Thomas's Hospital Medical School, London, S.E.1 (July 13).

Sub-Dean (preferably with experience as a university lecturer in science and also some administrative experience) in the Faculty of Science—The Registrar, The University, Liverpool, 3 (July 13).

Research Assistant (with a degree in pharmacology, physiology or a related subject), to join a team concerned with the identification of a urinary polypeptide which inhibits gastric secretion—Prof. H. O. Schild, Department of Pharmacology, University College, Gower Street, London, W.C.1 (July 15).

Graduate Research Assistant (with a degree in science or engineering, and competent to set up experimental apparatus and equipment involving vacuum production and measurement, power supplies up to 100 kV, electrical circuits and *controls, oscillographs and electronic measuring devices,* and preferably some knowledge of optics or spectroscopy) in the Research School of Physical Sciences, to take part in the general field of the physics of ionized gases—The Registrar, Institute of Advanced Studies, Australian National University, Box 4, G.P.O., Canberra, Australia (July 17).

Assistant Lecturer in Geology at Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (July 18).

Special Research Assistant to Dr. J. W. Smith in the Department of Chemistry, for work on the nature of complexes formed between acids and amines in aprotic solvents—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (July 18).

Assistant Veterinary Investigation Officers (men or women aged 23 or over, M.R.C.V.S., and preferably with postgraduate research experience) for at least 10 posts (mostly in provincial towns in the southern part of England)—Civil Service Commission, Savile Row, London, W.1, quoting Ref. 266/64 (July 20).

Demonstrator (with, or taking, an honours degree in botany, and either *have studied or have an interest in bacteriology or microbiology*) in the Department of Botany—The Registrar, The University, Nottingham (July 20).

Lecturer or Assistant Lecturer (medically qualified or non-medically qualified) in the Department of Bacteriology—The Registrar, The University, Leeds, 2 (July 20).

Demonstrator/Senior Demonstrator (with experience in the fields of *geophysics, solid state physics, or optical spectroscopy*) in the Department of Physics—The Registrar, The University, Newcastle upon Tyne, 2 (July 22).

Assistant Lecturer or Lecturer (veterinary graduate) in Veterinary Anatomy—The Registrar, The University, Liverpool, quoting Ref. No. CV/204/N (July 24).

W. R. Grace Chair of Chemistry at the University of the West Indies, St. Augustine, Trinidad—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (July 28).

Lecturer or Assistant Lecturer in the Department of Inorganic and Structural Chemistry—The Registrar, The University, Leeds, 2 (July 30).

Lecturer (preferably with research experience on polymerization reactions) in the Chemistry of High Polymers in the Department of Textile Industries—The Registrar, The University, Leeds, 2 (July 30).

Post-doctoral Fellow for research into the chemistry of hydrocarbon metal nitrosyl complexes—The Professor of Chemistry, The Royal College of Science and Technology, George Street, Glasgow, C.1 (July 31).

Research Fellow (medical or non-medical graduate) in Cancer in the Cell Metabolism Unit—Dr. R. A. Holman, Department of Bacteriology, The Welsh National School of Medicine, 34 Newport Road, Cardiff (July 31).

Reader (with a Ph.D. or higher degree in microbiology and have made a substantial contribution to research in some field of microbiology) in Microbiology at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 7).

Curator (with specialized knowledge of early scientific instruments and their history) of the Museum of the History of Science—The Registrar, University Registry, Oxford (August 31).

Lecturer (graduate in any subject with an interest in any branch of *educational studies*) in the Department of Education, University of Adelaide, Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, August 31).

Assistant Director of Research in Mechanics of Solids in the Department of Applied Mathematics and Theoretical Physics—The Secretary of the Appointments Committee, Faculty of Mathematics, The University, Arts School, Bene't Street, Cambridge.

Assistant Lecturers and Lecturers (with a good honours degree or an honours diploma in technology in electrical engineering and some industrial experience) in Electrical Engineering with opportunity to specialize in any branch—Head of the Department of Electrical and Electronic Engineering, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

Botanist (Plant Pathologist) (*national of the United Kingdom or the* Republic of Ireland, with an honours degree in botany or agricultural botany and at least one year's postgraduate training in plant pathology) in Uganda *to diagnose the cause of crop diseases and to recommend control measures,* to control trials on the comparison of methods for the control of crop diseases and to study the epidemiology of specific crop diseases and to relate any findings to field practices—Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/09.

Departmental Research Assistant (Physics/Engineering), to design, assemble and test *electromagnetic flowmeter heads for measuring blood* flow—Dr. D. G. Wyatt, Nuffield Institute for Medical Research, 43 Woodstock Road, Oxford.

Experimental Officer or Senior Experimental Officer (physicist or engineer with graduate or equivalent qualifications) in Electronics, to set up a group responsible for the maintenance and design of electronic equipment used for teaching and research in the Physics Laboratory—The Professor of Experimental Physics, Physics Laboratory, University of Sussex, Falmer, Sussex.

Head of the Department of Electrical Engineering—Clerk to the *Governing Body, Borough Polytechnic,* Borough Road, London, S.E.1.

Junior Hospital Medical Officer (Psychiatry)—The Physician Superintendent, Oakwood Hospital, Maidstone, Kent.

Laboratory Instructor (graduate with suitable experience) in the Department of Inorganic and Structural Chemistry, to assist in teaching modern inorganic and analytical chemistry at all levels up to Final Honours Degree standard—Prof. H. Irving, Department of Inorganic and Structural Chemistry, The University, Leeds, 2.

Lecturer (national of the United Kingdom or the Republic of Ireland, training and experience in agronomy (arable crops), practical experience of soil conservation practices in arid, tropical or semi-tropical areas, and preferably experience of practical demonstration and lecturing in these subjects) at Karaj Centre, Iran, to train students on soil conservation practices in arid, tropical and semi-tropical areas—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/86/01.

Mathematician—The Director of Science Studies, Brock University, St. *Catharines, Ontario, Canada.*

Mathematician (well qualified) to teach mathematics to all levels—The High Master, The Manchester Grammar School, Manchester, 13.

Physicist or Physical Chemist (degree standard, preferably with some research experience in rheology) to be concerned with *a fundamental* research programme in polymer melt rheology, for which a Weissenberg Rheogoniometer is available—The Secretary, Rubber and Plastic Research Association of Great Britain, Shawbury, Shrewsbury, Shropshire.

Post-Doctoral Fellow (pure or applied scientist with, or about to obtain, the degree of Ph.D.) in the Department of Chemical Engineering, to investigate dimensional changes occurring in polyurethane foams consequent upon the sorption of water vapour—*The Registrar, University College* of Swansea, Singleton Park, Swansea.

Post-Doctoral Fellow (spectroscopist, physicist or physical chemist) in the Department of Chemistry, to co-operate with Dr. A. J. Hyde on the determination of molecular space-time correlation functions in liquids using scattering from laser beams—Dr. Hyde, Department of Chemistry, The Royal College of Science and Technology, Glasgow, C.1.

Research Assistant or Research Technician (A.I.M.L.T. or equivalent or degree, and preferably experience of tissue culture, bacteriological or serological techniques), to take part in an investigation of the synthesis of antigens by cells growing *in tissue culture* system, and immunological and biochemical studies on cellular antigens—Department of Pathology, The University, Cambridge.

Research Assistant (physicist or electrical engineer with a good honours degree and preferably some research experience) in the Department of Building Science, to take part in a study of the thermal response of buildings to climatic variations and its electrical simulation—The Registrar, The University of Liverpool, Liverpool 3, quoting Ref. No. CV/211.

Research Assistants and Students for research in the following fields: properties of gaseous ions; physico-chemical aspects of cumbustion; inter*molecular forces in solutions*; inorganic complexes; structure and reactivity of organosilicon compounds; selected topics in natural product chemistry; and the kinetics of polymerization processes—Head of the Department of Chemistry, College of Advanced Technology, Gosta Green, Birmingham, 4.

Research Assistants (eligible to register as *full-time* students for a higher degree of the University of London) in the Department of Botany and Zoology, one in Botany (cytochemistry or chromosome studies), two in *Zoology (freshwater ecology and histology of marine mammals)*—Head of the Department of Biology, Sir John Cass College, Jewry Street, London, E.C.3.

Research Fellow or Senior Research Fellow (social scientist with research experience) to take a leading part in a *project concerned with some* of the sociological aspects of the construction of a new university—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST 64/69.

Research Students in Biochemistry—The Registrar, The University, Hull.

Research Students in Physics of the Solid State—Prof. G. W. Hutchinson, The University, Southampton.

Scientific Information Officers (Abstractors) (with a degree in agriculture or natural sciences, together with (a) ability to abstract from Russian or (b) ability to abstract from Japanese or Chinese)—*The Director,* Commonwealth Bureau of Pastures and Field Crops, Hurley, near Maidenhead, Berks.

Soil Chemist (with either an honours degree in agricultural chemistry (soils and plants), *or a degree in chemistry* with postgraduate experience in agricultural analysis (soils and plants), and preferably experience in soil and plant analysis) with the East African Common Services Organization, to carry out and supervise soil and plant analyses and to help develop and investigate new methods—The Recruiting Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/214/04.

Technician or Senior Technician (*preferably* with experience in the handling of radioactive materials) in Chemistry—The Director, Scottish Research Reactor Centre, East Kilbride, near Glasgow.

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AIMS AND OBJECTIVES IN EXPANDING HIGHER EDUCATION

IN a talk "Educating Half Ourselves", broadcast on the B.B.C. Third Programme and published in *The Listener* for March 19, Dr. G. H. Bantock suggested that neither the authors of the Robbins Report nor Dr. A. E. Sloman in his Reith Lectures, "A University in the Making"*, had appreciated the extent to which the crisis in education to-day arose out of a crisis in the level and in the range of consciousness. Accordingly, both the Robbins Report and the Reith Lectures were too much concerned with organization to the exclusion of cultural needs. Little thought was given to the cultural needs of the many students to whom the way to further education was now opened and whose home-life and surroundings did not equip them to explore it fully.

Properly conceived, Dr. Bantock pointed out, a culture embraced the ways in which we structured and communicated our common world, intellectually and emotionally, and on it depended our ability to understand the world in its various guises; from it there stemmed the spiritual strength or weakness of a generation. It formed, he rightly maintained, an essential part of the human predicament in an advanced industrial society, and the dangers involved intensified as automation, for example, extended. Yet, on all these dilemmas and cultural needs, the Robbins Report and the Reith Lectures had little or nothing to say. Their conception of the service which the university should render to the community was that of community service, the supply of special skills, rather than of the development of human personality and the equipping of men and women to meet the demands of the age in which they live.

Dr. Sloman did indeed suggest that a university should stimulate the curiosity of its students with subjects relevant to the social, cultural and scientific realities of to-day and to their own personal lives, and provide both a specialized training in depth and a truly liberal education. While the University of Essex is to be restricted initially to the physical sciences, to social studies and to comparative studies, there is to be a centre for creative arts, though its nature is not made clear, and, in discussing what he called the "fulfilment of lives", Dr. Sloman was concerned with the physical conditions of residence rather than the psychological or cultural needs stressed by Dr. Bantock. No more than the Robbins Report did he desiderate an education that would meet deeper requirements of individuals living in a civilization faced with a crisis of abundance, implying not only much more leisure in the near future but increased social mobility—in an age which from the cosmic to the personal level can fairly be characterized as an age of anxiety.

In that context the failure of the Robbins Committee to consider at any length the purposes which the vast extension of higher education is to serve appears as an unmistakable weakness, and, for all his admission at the outset that a new university must decide at the start what kind of a university it is going to be, Dr. Sloman equally failed to ask the fundamental questions about education and society. Much indeed that he said was sound and unexceptionable: on the relations between research and teaching; on self-government; and on the relations between the university and the community, including industry. Nevertheless, his approach was too pragmatic and seemed to lead to a subservience to professional or industrial needs that overrides those of the individual or even the deeper needs of society itself, which the rising debate on automation and leisure is now tending to bring to the forefront.

Dr. Bantock himself did not indeed ignore the requirements of technological change which a system of education must meet and for which proposals for expansion may reasonably be expected to provide. What he argued was that even those technical needs cannot be properly met without a clear conception of the purpose of education—of university education in particular, and of what constitutes an educated man or woman. If higher education is indeed to be expanded essentially to increase productivity, then we are in grave danger of becoming enslaved by the immediate necessities of our time, and unable to find the detachment which will permit a true perspective and the service of the deeper and enduring needs of society. Conceived and pursued in that way the expansion of higher education could indeed betray society, and the universities would fail to fulfil their most important function.

Prof. P. M. S. Blackett did not fall into this error in his Granada Lecture, "The Universities and the Nation's Crisis", which he delivered in October 1963, just before the Robbins Report was published and later published in *Advancement of Science* (20, 379; 1964). Prof. Blackett saw the greatest act of communication in the modern world in the passing on, to each new generation, the knowledge, wisdoms, skills and visions of the older generation. Much of this act of communication takes place in the universities, and it was for the complete communication that Dr. Bantock pleaded. While, however, Prof. Blackett did not assume that a university education was always a good thing for all young people and actually urged expansion on a somewhat larger scale than, in the event, the Robbins Report recommended, he was more alive to the human aspect and he urged as one reason for the expansion of the universities the contribution it could make to the progress and welfare of the newly independent nations.

This would be rendered in three ways. First, by providing academic and other professional staff to work in these countries, at their request, on secondment from professional posts in Britain. Secondly, by providing still wider training facilities in Britain's universities, mainly for postgraduate and post-doctoral work. Thirdly, by increasing Britain's financial aid to those emergent countries, within the limits set by the health of the British economy as a whole and its foreign exchange position in particular.

In so far as Prof. Blackett sees university expansion as directed to the proper training and use of all the brains which Britain possesses, his approach could equally be described as pragmatic, but the distinction lies in the interpretation of 'proper' and the attention given to other elements than the acquisition of technical skills. Even at the pragmatic level the omission of the Reith Lectures or the Robbins Report to deal adequately with the purpose of university education is unfortunate. It leads to a tendency to disregard some of the changes in the character of the university student now that increasing numbers are drawn from homes with little or no tradition of learning or culture. Some of these changes were discussed by Mr. D. Johnson in a talk broadcast on the B.B.C. Home Service and later published in *The Listener* (71, 185; January 30, 1964), and if serious attention is given to the purpose of a university education, such weaknesses as immaturity, irrelevance, or decreasing independence cannot be disregarded.

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* *A University in the Making*. By Dr. Albert E. Sloman. (The Reith Lectures 1963.) Pp. 90. (London: British Broadcasting Corporation, 1964.) 12*s*. 6*d*.

Over the past ten years, Mr. Johnson finds students becoming less independent and characterized by a great personal immaturity, surrounding an acquired and perhaps shallow intellectual maturity. As he rightly observes, education should at least affect the character and outlook of the student and promote a link between a student's approach to the past and his or her approach to the present. Something of Mr. Johnson's mind would seem to be influencing the plans which Dr. Sloman outlined for the new University of Essex, for those plans should encourage self-confidence among students, though they are not free, as already suggested, from some danger of subservience to other interests. Only, however, such an understanding and sympathetic approach to the human problems of the student such as Mr. Johnson displays in this talk, however, will suffice to meet the fundamental need, but that found comparatively slight expression when the Conference of the Universities of the United Kingdom discussed the Robbins Report in December 1963.

This is not to suggest that the Conference was unmindful of such issues or obsessed with organization. There was no complacency, and Lord Robbins himself insisted that there was too much slack in Britain's present organization—in the defective use of man-power, of obsolete teaching methods, etc.—and pleaded for a new and impartial outlook on many subjects, such as that of the teacher training college. Nevertheless, neither he nor other speakers raised the issue of the purpose of university education. While the reference by Dr. B. V. Bowden (as he was then) to the way in which the American universities and technological institutions recognized a fundamental obligation to try in every way possible to help industry was not unreasonably pragmatic, that could not be said of Mr. D. R. O. Thomas's endorsement of the paragraphs in the Robbins Report on education as an investment. These paragraphs formulate a view of education as a coldly calculated investment to promote national productivity such as to justify the hardest comments of Dr. Bantock. They completely overshadow the earlier reference in the Report to the transmission of a common culture and common standards of citizenship as among the aims of higher education—aims which Mr. Thomas described as magnificent in concept and clarity.

For all the characterization of this particular objective in the Robbins Report as 'fundamental' and the observation that universities and colleges have an important part to play in the general cultural life of the communities in which they are situated, there is unfortunately too much support for Dr. Bantock's belief that, in the expansion of higher education, there is too much conc
with organization and that insufficient thought is b
given either to its purpose or to the needs of the
dividual student. Nor is concern on these points remo
by the Godkin Lectures on the uses of the univer
delivered at Harvard University in April 1963 by
Clark Kerr†, president of the University of Califor
For, while Dr. Kerr attempts to describe and to eval
some of the significant new developments in Ameri
higher education, particularly the rise of what he te
the 'multiversity', it is rather the problems of organiza
and administration arising out of the sheer size of a uni
sity of 50,000 or more students, the interrelations of
universities with one another, the Federal Governm
and the community, with which he is concerned. E
in discussing the future of the "City of Intellect"
does not discuss either the purpose of a university
its contribution to safeguarding the cultural and spiri
linkage of society.

The admission that undergraduate tuition must disco
how to treat the individual student as a unique hu
being in the mass of students, giving individual atten
at that stage and not simply after graduation, is as
as Dr. Kerr gets to recognizing the range of problems emp
sized by Dr. Bantock. He does not pretend that s
parts of higher education are not poorly done, though
maintains that the general test is how much is d
superbly, as a good deal is, to the nation's great bene
He quotes A. N. Whitehead's words across almost
a century: "In the conditions of modern life, the ru
absolute: the race which does not value trained int
gence is doomed", and he proclaims the ends of the
versity the preservation of the eternal truths, the creat
of new knowledge and the improvement of ser
wherever truth and knowledge of high order may se
the needs of man. The means are ever to be impro
in a competitive dynamic environment, and it co
be argued that his third end would embrace much
if not all, the human needs for which Dr. Bantock plea

Nevertheless, this lucid but concise account of
versity development in the United States, of the relati
between universities and the Federal Government
of the forces which within the past two decades or
have ranged those universities into three outstand
clusters or concentrations of talent is essentially a st
of structure or administration. It is concerned with
corridors of power and, while the idea of science to
community is in the background, it is only in scale t
the picture differs from that portrayed in the Re
Lectures or the Robbins Report. The same mena
are there, so also the same *tempo*. While the implicati
of the factors may not always be the same and the patt
unfolded by Dr. Kerr may be suggestive in the Brit
context, and sometimes warn us of dangers we would
wise to forestall, rather than indicate measures we mi
adopt, the general impression is still that left by
Robbins Report—of the university as a prime instrum
of national purpose.

That is, above all, what needs to be challenged
re-examined, rather than accepted uncritically.
balance between the advancement of knowledge,
fessional education, general education and the dema
of the student has always been changing and will conti
to change as society itself changes. To expand hig
education in general, or university education in particu

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† *The Uses of the University*. By Clark Kerr. Pp. vi+140. (
bridge, Mass.: Harvard University Press, 1963.) 2.95 dollars.

with no clear conception of the real purposes of university education is a sure way to frustration and to waste national resources and talent, rather than to achieve the return on investment urged by the Robbins Committee. Even though expansion is already beginning, we would do well to look at our real objectives and think deeply about the human needs of society, including the potential students, before committing irrevocably limited resources on a scale and in ways that are ill-judged to serve effectively the long-term needs of individuals or society, even of industry itself.

EXPERIMENTAL PSYCHOLOGY

Traité de Psychologie Expérimentale

Sous la direction de Paul Fraisse et Jean Piaget. Fascicule 1: Histoire et Méthode. Par Jean Piaget, Paul Fraisse et Maurice Reuchlin. Pp. 191. 12 francs. Fascicule 2: Sensation et Motricité. Par Henri Piéron, René Chocholle et Jacques Leplat. Pp. 161. 12 francs. Fascicule 5: Motivation, Émotion et Personnalité. Par Joseph Nuttin, Paul Fraisse et Richard Meili. Pp. iv+226. 15 francs. Fascicule 6: La Perception. Par Jean Piaget, Paul Fraisse, Éliane Vurpillot et Robert Francès. Pp. 231. 15 francs. (Paris: Presses Universitaires de France, 1963.)

TRAITÉ de Psychologie Expérimentale is the first treatise of its kind since the Harvard *Handbook of Experimental Psychology*, edited by S. S. Stevens and published in 1951. In a way, it is a little odd that it should come from France, for although there has been a distinguished French psychological tradition, experimental psychology has never held a central place in it. Nor has the output of the French psychological laboratories been large or, for that matter, particularly important. It might seem, however, that interest in experimental psychology is rapidly growing in the French-speaking countries and this treatise will undoubtedly do much to shape its development.

The complete work is to comprise nine volumes, covering the experimental psychology of sensation and perception, learning and memory, the psychophysiology of behaviour, intelligence, communication and decision processes, motivation and personality, and experimental social psychology. Inevitably, the choice of topics reflects both contemporary fashion and the special interests of the Editors. It also reflects in some degree the availability of contributors, all of whom appear to have been drawn from France, Belgium and Switzerland. Whether this geographical limitation was altogether wise is debatable: in cultural as in economic matters national pride can be a potent source of inflation.

The first volume, *Histoire et Méthode*, starts with an account of the evolution of experimental psychology by Prof. Paul Fraisse. Although perfectly adequate, it leans heavily on secondary sources (particularly Boring's *History of Experimental Psychology*) and the judgments expressed are for the most part conventional. It is followed by chapters on experimental method by the same author and on psychological measurement by Dr. M. Reuchlin. These are quite well done, if unexceptionable. More exciting, however, is Prof. Piaget's discussion of explanation in psychology and the problem of psychophysical parallelism. This is both lucid and original and it is interesting to note that the author expresses hope in the possibilities of a sophisticated isomorphism. This chapter will interest philosophers of science as well as experimental psychologists, and should be widely read.

The second volume, entitled *Sensation et Motricité*, opens with a long and detailed account of psychophysics by Prof. Henri Piéron, whose grasp has clearly been in no way impaired by age. Indeed, the author passes from classical Fechnerian psychophysics to modern decision theory with masterly ease and detachment. Dr. R. Chocholle writes on reaction times, showing himself to be well acquainted with the ways in which information theory has been applied to their measurement. Dr. J. Leplat considers human sensori-motor performance; recent British work is particularly well represented in his survey. This whole volume is altogether of much interest to British experimental psychologists and can be warmly recommended to their attention.

The fifth volume, *Motivation, Émotion et Personnalité*, is inevitably less satisfactory. As is well known, the topics with which it deals are fraught with snags, conceptual, linguistic and methodological, and have on the whole proved resistant to experimental analysis. However, Dr. J. Nuttin surveys experimental investigations of drives and incentives with commendable balance, covering both animal and human enquiries. His knowledge of recent American work is particularly impressive. Prof. Fraisse writes on emotion; if he has rather little to say, this is due largely to the paucity of convincing experimental data. Finally, Dr. R. Meili outlines investigations of personality structure, mainly the large-scale, statistical variety which Prof. Eysenck and others have made their special province. (But he does have a kind word or two for psychoanalysis.) Although little positive emerges, the author concludes that thirty years' work on personality has at all events led to a clearer conception of the problems and of the methods which might lead to their solution. Possibly.

The sixth volume is concerned with perception. As might be anticipated, Prof. Piaget leads off with an account of the genetic development of perception, much of the material being derived from the work of his own laboratory. Prof. Fraisse writes on time perception, a topic to which he has himself devoted much study. Dr. E. Vurpillot writes on space perception; this chapter is impressive and might well justify translation as a monograph. Finally, Dr. R. Francès discusses form and object perception. This is a bit limited, no consideration being given to recent investigations of form perception in animals, which have given new impetus to the whole field of enquiry. Oddly, too, auditory perception is little discussed and tactual perception scarcely at all. but perhaps these omissions will be remedied in later volumes. Nor do neurophysiological aspects of perception receive their due.

This treatise is comprehensive, scholarly and well balanced. Although drawing heavily on American (and to a lesser extent British) sources, it certainly cannot be regarded merely as an attempt to market Anglo-American psychology in a form appropriate for Continental consumption. It does distil a flavour of its own, critical, at times ironic, and above all lucid. It will have both impact and influence, and the Editors deserve congratulation on their enterprise. Psychologists everywhere will await the forthcoming five volumes with lively anticipation.

O. L. ZANGWILL

SOCIAL MISBEHAVIOUR

Crime and the Social Structure

By Dr. John Barron Mays. (Society Today and Tomorrow.) Pp. 256. (London: Faber and Faber, Ltd., 1963.) 30*s*. net.

DR. J. B. MAYS has in recent years made a significant contribution towards our understanding of some of the intractable problems of juvenile delinquency, notably in his investigation, published in 1959, appropriately called *On the Threshold of Delinquency*. It was, therefore, with keen anticipation that I looked forward to his latest book *Crime and the Social Structure*.

The central thesis of this investigation is that "society itself is in some respects criminogenic and to a considerable extent precipitates its own delinquency". There is nothing really novel in this argument, but what is unusual is the extent to which Dr. Mays tries to prove that "criminal behaviour arises naturally enough out of the ordinary daily affairs in which all of us are engaged". But, does he provide the proof to validate his thesis? In my view he does not, despite the wide range of social factors associated with crime which he has examined.

There is, of course, a real difficulty in making any attempt to put forward a particular thesis about the cause of crime, and that is the difficulty of finding, by scientific methods, supporting evidence. Dr. Mays has had to rely in the main on his own experiences as a youth club leader, and on the research he carried out among the boys of the youth club with which he was connected, and of course on the results of research carried out by others in Britain and the United States. Unfortunately, one cannot satisfactorily translate to Britain the results of research carried out in the United States, for as Dr. Mays admits "British society is in some important respects very unlike American". Furthermore, much of the research carried out in limited areas of Britain has not been repeated elsewhere and therefore he has to rely all too often on 'localized' evidence, as, for example, when he argues that the offences of the youth club members he knew in Liverpool were caused essentially by the environment in which they lived. The interesting question is, would these same boys have committed offences had they been born and brought up in any other part of Britain? Unlike the natural scientist the social scientist cannot easily repeat his experiment, and therefore that interesting question cannot readily be answered.

For anyone who is unfamiliar with theories of the causation of crime and such facts as are known about crime in Britain this book provides a useful introduction. Dr. Mays, as always, writes extremely well and, unlike many writers on criminology, he avoids jargon. This is a very readable book, it stimulates thought about criminal behaviour and draws a useful distinction between 'normal' and 'abnormal' crime which helps the reader to see in perspective the alleged increase in criminal behaviour. With his plea that "we should take a hard long look at the nature of our society to see whether or not there are social and environmental forces which foster delinquent attitudes" one must agree whole-heartedly, but has he himself shown us what these forces are? I'm afraid the answer must be no, and having finished reading this book I was left with the uneasy feeling that the claim made on the dust-jacket that Dr. Mays "reveals the relationship between long-established institutions and criminal attitudes and behaviour" is not substantiated.

D. C. MARSH

THE WAYS OF SCIENCE

The Nature of the Natural Sciences

By Dr. Leonard K. Nash. Pp. xix+406. (Boston and Toronto: Little, Brown and Company, 1963.) n.p.

Philosophy of Science

The Delaware Seminar. Vol. 1, 1961–1962. Edited by Bernard Baumrin. Pp. xvi+370. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1963.) 75s.

THE author of *The Nature of the Natural Sciences* is a professor of chemistry at Harvard University, and as a member of Conant's Harvard General Education Programme for the teaching of science to non-scientists he has always been particularly conscious of the need to give an answer to that most difficult of all questions, "What is science?". In this book he sets out to sketch such an answer, with refreshing simplicity of approach and copious illustration from actual cases of our scientific past and present. His objective, he explains, is not to present the reader with some abstract 'ideal science' which may be found in logicians' text-books, but to attend closely to its dynamic aspects though all the time keeping attention directed towards the history, the philosophy, the internal organization of science, its social ties, and so forth. This is a formidable programme, and one will not, for that reason, expect a very searching enquiry at all levels; but, as an introductory exploration of a many-faceted field, the book has its value. Its examples are chosen so as to be comprehensible to the scientific amateur, but it should be noted that they turn out to differ not so very much from the stock-in-trade of the defamed logician: Boyle's Law, Archimedes's Principle, the Kinetic Theory of Gases, Newton's theory, Ptolemy and Copernicus spatter the pages, even though supplemented by such 'modern' representatives as Moseley's law and Mendel. Again, for someone who sets out to offer us a fresh appraisal of an old subject from a working scientist's point of view, the book is curiously cluttered up with quotations from the pens of a vast number of philosophers, chosen quite at random. This gives the enterprise a somewhat eclectic appearance, and a teacher should be warned to press the work into students' hands only on condition that he is prepared to supervise their reading himself.

This said, I must nevertheless register my approval of the author's attempt to present science neither as a straightforward enterprise of collecting facts followed by generalization and explanation, nor as a purely deductive and theoretical enterprise with only an occasional exploration into the laboratory. On the contrary, the two sides of science, intelligible explanation and theoretical approach on one hand, and the whole apparatus of instrumental control on the other, are presented with illuminating care. The element of 'colligation by laws' in the construction of scientific concepts is emphasized, as is the weight of certain 'methodological principles', such as those of parsimony, penury, clarity and analogy all acting as often unconscious devices in the day-to-day operations of the scientist, particularly when faced with the task of the choice between hypotheses.

Prof. Nash is always concerned with exposing the many-sidedness of the scientist's thinking. The almost dialectical way in which the demand for 'intelligibility' of explanation—misunderstood by the early scientists and philosophers as the request for 'self-evidence'—is checked by certain constraints, for example, eschewal of a total explanation of everything, the need for experimental control coupled with a rigidly deductive stratification. Other methodological principles discussed are those of continuity, determinism, corrigibility. There are the usual chapters on theories and models, sympathetic discussions of the claim (ultimately rejected) that there is "*a* scientific method", and considerable attention is directed towards psychological and social factors inherent in scientific research experience. For good measure, at the end we get also a discussion of questions that have exercised academic philosophers since the seventeenth century, such as the debate of 'realism' versus 'phenomenalism', the author arguing for a particularly trivialized version of optimistic realism.

Philosophy of Science is far headier stuff, with contributions by some of the most eminent of our contemporary philosophers, mathematicians, biologists and physicists to the Delaware Seminar, a running colloquium intended to be indicative of work in progress in the philosophy of science. This is the first of a series. Here, issues are argued with greater care and attention to detail, though, of course, with less relevance for the everyday concerns of the working scientist. This is, however, a price that has to be paid by every branch of intellectual endeavour as soon as it comes of age: in this case, progressive depth in its searchings and the establishment of an agreed forum of discussable 'technical' problems, however much these may originally have arisen out of the corpus of scientific activity itself, soon take on the aspect of a separate academic discipline; nor is it easy to see how an honest interest in truth could prevent such a splintering process from coming to pass. Philosophy of science, in this respect, is in the condition in which dynamics, geology, psychology, and most other scientific disciplines have found themselves at their own respective periods of gestation. The complication, however, in the present case is that philosophy of science is (and always has been) a tremendously composite hotchpotch of interests and influences; ranging from the extreme of the foundations of logic and mathematics—not to mention the metaphysical enterprises of the great classical philosophers, many of whom saw themselves primarily as writers on the foundations of science—to the investigations of the statisticians and probability theorists into the theory of games.

The Delaware volume is evidence of a similar width of interests. Its six main parts include sections on "Scientific Explanation and Prediction"; "Philosophical Aspects of the Foundations of Mathematics"; "Philosophical Aspects of Biology"; "Philosophical Aspects of the Social Sciences"; and finally "Philosophical Aspects of Physics". The section on the foundations of mathematics argues the virtues (and vices) of Platonism and Nominalism, through an explanation of the notion of 'existence', of sets, for example. Another article is concerned with the notion of mathematical truth. A. Landé, in "Causality and Dualism on Trial", reiterates his thesis, already made in an earlier book of his, seeking an interpretation of the formalism of quantum mechanics primarily in terms of a particle model with an inbuilt statistical element from the start. Probabilistic theory is then considered a fundamental and irreducible element in all physical situations.

Naturally, as always in a co-operative effort, the value, thoroughness and relevance of the individual contributions vary tremendously; but as an insight into the workshop of contemporary discussions on the logic of the sciences and mathematics this book can be strongly recommended. GERD BUCHDAHL

SCIENCE IN THE HISTORY OF CIVILIZATION

Ancient and Medieval Science from Prehistory to A.D. 1450

Edited and with a General Preface by René Taton. Translated by A. J. Pomerans. (A General History of the Sciences.) Pp. xvi + 552 + 48 plates. (London: Thames and Hudson, Ltd., 1963.) 126*s*.

THIS is the first volume of a series, published in 1957 in French, which aims to bridge the gap between specialized papers in the history of science and general history written for the educated man. The apparatus of historical scholarship and critical discussion have been kept to the minimum, and extensive bibliographies have been included to enable readers to pursue particular interests. Each major section has been written by a specialist. This volume covers the period from prehistory to A.D. 1450, and covers not only the ancient civilizations which provide the first chapters of every general history of science—Egypt, Mesopotamia and Greece—but also China, India, ancient and medieval Jewish science, Byzantium and pre-Columbian America. Geographical unity is maintained not only by description of transcultural influences, but also by some comparative discussion of method and scientific attitude. This is especially valuable in the case of Chinese science, which started so promisingly but scarcely came to theoretical fruition, and is admirably done in the space of two short chapters by A. Haudricourt and J. Needham.

The further subdivision of each cultural unit is generally into the various special sciences, with more extensive attention to medicine than is usual in such works. In the case of the Greeks, the unity is rightly seen as one of philosophical school rather than of subject-matter, and a sustained exposition in four chapters of Greek science and mathematics from Thales to Aristotle by P.-H. Michel provides a firm centre-piece for the book, for subsequent specialized developments took their origin from this period. Indeed, fundamental views of the nature of scientific knowledge, and theories of the structure of matter and the cosmos, continued until the seventeenth century to be directly indebted to the conflicting philosophies of Democritus, Plato and Aristotle. It is good to see in a volume of this kind a proper place given to philosophical influences, and particularly to the Platonic tradition, which has often been denigrated by historians of science. But since the monumental researches of Alexandre Koyré and other students of Renaissance and seventeenth-century science, no historian can ignore the vast influence of the Platonic mathematization of Nature, which can be regarded as one of the major sources of the science of Copernicus, Galileo, Kepler and Descartes. Within the book's brief compass, full value is given to the continuity discerned by recent historians between ancient and modern science.

How successful is the volume in its aim of introducing history of science into the general historical perspective of the educated man? In spite of many good things, one must still confess to some doubts as to whether the method of approach is the most suitable for this purpose. There is a serious attempt in many of the sections to relate the details of scientific development to their cultural background, as well as to face squarely the question how far this was 'science' in any sense we would now recognize. A fairly coherent view emerges among the various authors as to what we do in fact recognize as the essentials of science. This is an empiricism somewhat modified by theoretical systematization: "The true scientist joins accurate knowledge of the facts to the demands of reason" (p. 258), and examples of mere observation or technique without rational system are condemned as pre-scientific. But there are still too many phrases which echo an earlier and less satisfactory style in the historiography of science: "In Galen's physiology we must distinguish between far-fetched biological theory and brilliant individual discoveries" (p. 359) is one example among many. The implication is that theories ought not to be far-fetched, and that discoveries are independent of theories. But what could have been more far-fetched in their historical context than Newton's action-at-a-distance, or Einstein's curved space-time? And what discoveries would have been made without the aid of these conceptions? The historian's besetting temptation is to condemn earlier science because it turned out to be 'false', instead of judging it according to the evidence available and the criteria recognized in its day. The brevity of the general discussion of historical background which is dictated by the scope and form of this book makes it difficult for many of the authors to guard against such distortions. It is flattering to our self-esteem to look to the distant past and to think we recognize faint premonitions of our own science among much wrong-headedness in the best intelligences we find there, but a deeper historical insight will often reveal that they shared few of our interests, criteria or values, and to a modest historical view they are not necessarily the worse for that. All of which leads one to wonder whether the cause of history of science is not better served by works of general history devoted to more limited periods and cultures, which show science, philosophy and technique as integral parts of a context developed in terms of its own values in more detail than is possible here.

If the project does not quite succeed in giving historical significance to the science of the distant past, it is nevertheless a unique mine of information, beautifully illustrated, and exceptionally well translated.

MARY HESSE

TECHNIQUES IN ENDOCRINE RESEARCH

Techniques in Endocrine Research

Edited by Peter Eckstein and Francis Knowles. (Proceedings of a NATO Advanced Study Institute held at Stratford-upon-Avon, England, under the auspices of the University of Birmingham.) Pp. xvi+319. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1963.) 63*s*.

IN September 1962 a meeting on the techniques used in endocrine research was held at Stratford-upon-Avon under the auspices of the North Atlantic Treaty Organization. Most of the papers which were read are now published in book form.

The volume consists of communications dealing with the following topics: X-ray crystallography (D. C. Hodgkin); hormone-containing sub-cellular particles (U. S. von Euler); antibody staining techniques (F. A. Pepe); neurosecretion (F. Knowles); ultrastructure of the mammalian pituitary (R. L. Holmes); autoradiography (A. Ficq and J. Flament-Durand); chromatography (E. Lederer); hormones in blood (H. N. Antoniades); immunochemical methods (R. G. White); interpretation of biochemical techniques (R. B. Pennell); the experimental animal (W. Lane-Petter); the pituitary complex (J. M. Dodd); hormones and gene action (P. Karlson); transplantation (P. L. Krohn); preservation and storage of endocrine tissue (A. S. Parkes); purified protein hormones in human endocrinology (C. Gemzell); endocrinology of reproduction (M. C. Shelesnyak); ovum implantation (G. Mayor); hormones in development (A. Raynaud); trends in methodology (M. Klein).

It is unfortunate that not all the participants were able to present written accounts of their papers for publication. This omission is, however, not so important to the reader as the failure to include the discussion which followed each communication. In the form in which it is published, *Techniques in Endocrine Research* thus presents a series of discrete papers rather than an integrated account of the subject as a whole. The inclusion of the discussions might well have overcome this defect, as well as providing further insight into the problems associated with the methods discussed. A less-important factor, but one which detracts from the value of the book as a whole, is the inadequacy of the subject index. Thus, to give one example, in her paper Dr. Hodgkin discusses the X-ray crystallography of insulin in some detail, but there is no mention of this in the index. This means that although the book can be used as a reference work, it will be of greater value to peruse the relevant chapters in detail.

The value of the book lies in the fact that it consists of the description of a wide range of techniques at present used in endocrine research. In the introduction Sir Francis Knowles reminds us that "developments in physics and chemistry during the past thirty years have revolutionised biological research". Approximately half the papers included in the volume are devoted to illustrating this point. The relevant communications generally consist of a brief historical introduction followed by a more detailed discussion of the recent applications of a particular technique to endocrinology. Most of the remaining chapters deal with the interrelationship or application of a variety of procedures to a given problem. This is well illustrated in the chapter by Dr. Shelesnyak, which describes the way in which biological and chemi techniques have been used to investigate the mechani of nidation.

Laboratory animals have been used extensively experimental work throughout this century. It is, ho ever, only relatively recently that the well-being of t animal has been considered. Because of this fact, t inclusion of a paper by Dr. Lane-Petter on the expe mental animal in research is very welcome.

A large number of different experimental techniques a described and it would, therefore, be inappropriate discuss any particular method in detail. In view of t many specialized technical journals in which such metho are published, the value of the book to the chemist and the biologist is considerable, in that it provides descriptio of both recent and classical methods in endocrinolog This volume may, therefore, be recommended with co fidence for perusal by anyone interested in endocrinolog

E. TREVOR BELL

RADIATIVE TRANSFER IN THE U.S.S.R. AND THE WEST

A Treatise on Radiative Transfer

By V. V. Sobolev. Translated by Dr. S. I. Gaposchk Pp. xi+319. (Princeton, N.J.: D. Van Nostrand Co pany, Inc.; London: D. Van Nostrand Company, Lt 1963.) 76*s*.

THE development of the theory of radiative trans during the years 1940–60 proceeded in the U.S.S. and in the West along roughly parallel lines, but until t past few years of the period contacts were few. Weste astrophysicists were strongly influenced by the work S. Chandrasekhar, while Russian astrophysicists f lowed V. A. Ambartsumian and V. V. Sobolev.

It is sobering to read this translation of Sobole treatise, first published in 1956, and to realize th methods which were only then being developed in Weste journals had been in use in the U.S.S.R. for some yea The comparison and appreciation of results and metho were made more difficult by the use of different notatio for standard functions. Thus, Chandrasekhar a Western writers used, and still use, $H(\mu)$, $X(\mu)$, $Y($ where Sobolev and the Russians used $\varphi(\eta)$, $\varphi(\eta)$, $\psi(\eta)$, notation which has been adhered to in the translation.

For an experienced astrophysicist, *A Treatise on Rad tive Transfer* is an exciting book, but unfortunately it w be of little value to a beginner until a corrected version available. Many misprints have come from the origir Russian version, while others have been introduced by t translator or the printer. On p. 65, for example, the on formulæ without some sort of omission are (46) and (4 The translation itself is sometimes misleading. Thus, p. 110, in the sentence "Let us consider the transmissi coefficient only for the case of the spherical scatteri indicatrix", 'only' should be 'also' and 'spherical' should 'non-spherical'.

When the difficulties caused by misprints have be overcome, the reader will find much to interest hi Sobolev first develops most of the known methods dealing with transfer problems. Special mention mu be made of the so-called probabilistic method, d originally to Sobolev, which is based on the probabili that a quantum absorbed at depth τ below the surfa will leave the medium in a certain direction. The methods are applied to various physical problems, su as the scattering of polarized light in planetary atm spheres and in the electron atmosphere of a star; t luminescence of a medium bounded by a reflecting surfa light scattering when there is a redistribution in frequen and the consequent effect on the contours of absorpti lines; and non-stationary radiation fields.

It is in the detailed applications of general results that Sobolev's book will most delight the experienced reader. Besides applications to planetary atmospheres and nebulæ, one finds a discussion of the brightness of an overcast sky at sea allowing for reflexion of light by the sea, and the brightness of the sea itself allowing for both reflexion from the surface of the sea and scattering of light in the sea. In the final chapter, Sobolev uses transfer theory to explain why a flat fish, in order to be nearly invisible, is white below and dark above!

Much perplexity has been caused in recent years by the non-uniqueness of the solutions of some of the integral equations of transfer theory. These problems have now been cleared up, mainly through the recent work of T. W. Mullikin in the United States. Sobolev has nothing much to say about existence and uniqueness of solutions, but happily he has selected the best function whenever a family of solutions exists. This makes his book valuable as a basis for future work.

Dr. S. I. Gaposchkin is to be thanked for making Sobolev's book available to Western astrophysicists. He has himself added to the book some of Sobolev's latest articles, adapting them slightly so that they fit in with the book. Thus the version in English is an enlargement of that in Russian.

I. W. BUSBRIDGE

LAND AND WATER USE IN THE UNITED STATES

Land and Water Use

Edited by Wynne Thorne. (A Symposium presented at the Denver Meeting of the American Association for the Advancement of Science, 27–29 December, 1961.) (Publication No. 73.) Pp. x + 364. (Washington, D.C.: American Association for the Advancement of Science; London: Bailey Bros. and Swinfen, Ltd., 1963.) 80*s*.

LIMITED land and water resources in relation to present and future population is a problem which confronts not only Britain but increasingly other parts of the world. Not least of these areas is the United States, and although the problems in this case are on quite a different scale from those confronting Britain much can be learnt from the study of somewhat similar situations in other localities. This new publication of the American Association for the Advancement of Science is therefore especially welcome for it summarizes a symposium which took place at the Denver, Colorado, meeting which had as its objective a consideration of land and water as related resources with special reference to the mountain and plains region of the United States—wherein we find concentrated many of the United States' most critical problems of land and water use.

Whereas most of the plains region is now in private ownership, more than half the inter-mountain area remains in Government hands. Competition for the region's resources brings about pressures on both the private and public sectors for courses of action which are often conflicting. The symposium was therefore largely concerned with a consideration of many of these conflicting claims and an attempt is made to evaluate the long-term consequences of the application of selected policies.

Contributors to the symposium were drawn from many disciplines and organizations and were carefully selected as individuals who could talk authoritatively concerning these basic natural resources. The scene is set by an introductory chapter on "Land and Water Use" by Wynne Thorne, who edits the collection of papers. This is followed by a group of articles under the collective title of "The Resource Setting", the topics included ranging from population demands on land and water, potential uses, livestock interests, public grazing, evaluations of dryland environments at differing technical levels, recreation as a competitive segment of multiple use, management and vegetation of watershed areas, hydroclimatology, to methods of securing additional water supplies.

Part 3 deals with "Criteria and Policies", with chapters on economic priorities for water use in arid regions, management of wildlands, multiple use in the management of land and water, and criteria and planning for optimum use. Part 4 covers the role of Government in seeking solutions to the problems which lie ahead, with contributions on wilderness problems, public and private investment in development, and legal aspects of a national water programme and a concluding chapter on "Government Responsibility for Land and Water—Guardian or Developer?".

It will be clear that the symposium is wide-ranging and comprehensive and that it touches on many points. Throughout, however, there is a significant repetition of references to the concepts of multiple-use and optimum-use. Specialists in the field of resource management argue that the concept of multiple-use provides the most practical approach in seeking maximum benefits from land resources. Management for multiple-use is based on human value systems and fulfils human needs by increasing total useful production. It is argued that such policies should be flexible enough, however, to serve the future welfare of the nation. Recreational uses are vital but difficult to evaluate financially.

Others have argued in favour of the concept of optimum-use since the concept of multiple-use has limited value as a basis for determining public and private policies in planning and management. But a determination of optimum-use requires extensive research on resources and better criteria for estimating exactly what would be the optimum-use in particular cases. A complete inventory of all land resources would in fact be necessary before foolproof decision-making on optimum-use becomes possible.

It is the responsibility of Government to assure the maximum benefit to the country of the available resources with particular reference to the 'public good'. But it is important to remember that financial statistics cannot always be used to evaluate competing interests. A recreational national park cannot be judged by its turnover of "hot dogs, fishing tackle and gasoline sales"; its true worth cannot really be measured in any financial terms.

Among the major recommendations which emerge from these papers are: (1) the need for a detailed inventory of water and land resources evaluating specific characteristics; (2) the need to place æsthetic enjoyment and leisure time use in balance with economic demands; (3) the need for a clearer definition of governmental responsibilities and functions in relation to land and water resources; (4) the need for policy criteria to guide future governmental actions.

It will be clear that most of the principles discussed in this important symposium are immediately relevant to the problems which Britain faces in the proper use of land and water resources. The concepts of multiple-use and conservation are slowly gaining ground together with a need for a detailed inventory of water and land resources. Government, however, moves but slowly and Britain has not yet reached the stage where land utilization survey has become a regular and official activity for the whole country. Meanwhile we have to rely on the private enthusiasm of dedicated geographers. Adequate data on water resources should, however, be forthcoming once the Water Resources Act, 1963, has been brought into full force. As the pressure of population on both land and water amounts with increasing numbers and increasing leisure the community will be forced into undertaking more detailed inventories of both water and land resources, of resolving the problems of multiple-use as against optimum-use, and of accepting the need for more Government action. This symposium from the United States makes valuable background reading for all those concerned with finding the solution to the problems that are approaching all too rapidly.

W. G. V. BALCHIN

Annual Review of Nuclear Science

Volume 13. Edited by Emilio Segre. Pp. vi+590. (Palo Alto: Annual Reviews, Inc., 1963.) 8.50 dollars.

THE mixture as before comes from Annual Reviews; one dozen assorted articles on various aspects of nuclear science. No one will want to read them all, but each will be interesting and useful to many. This kind of review tends to remain a standard reference for several years.

In the field of nuclear reactions, Glendenning discusses stripping at considerable length, while Stelson and McGowan survey Coulomb excitation more briefly. In high-energy particle physics, Puppi and Dalitz deal with the pionic and strange-particle resonances, respectively. As usually happens with such reviews, several new particles have appeared while they were in the press, but Dalitz has partly insured against this risk by discussing the predicted Ω^-. The surprise particle of two years ago is covered by Feinberg and Lederman, in a paper on the muon and the muon neutrino.

There are two correlated papers on the slowing-down of charged particles in matter; Fano deals with protons, α-particles and mesons, while Northcliffe takes the rather different case of heavy ions. Cumming contributes a review of data and techniques used for monitoring high-energy proton beams. The automatic analysis of bubble chamber data is a new technology; Rosenfeld and Humphrey tell the inside story, full of evocative code-names—*Franckenstein* is a machine, *Quest* and *Fake* are computer programmes.

Finally, there are three shortish surveys representing some other sciences in the nuclear field. Sayre summarizes the methods and uses of activation analysis, Björnerstedt and Edvarson describe some questions needing further research in the physics, chemistry and meteorology of fall-out, and Hutchinson writes about the effects of radiation on biological macromolecules.

On the whole, these reviews are meant for serious students and research workers in the subjects concerned, but several of the shorter ones will interest the casual onlooker who simply wants to widen his horizons. This volume is a worthy continuation of a useful series.

R. J. N. PHILLIPS

Atomic Structure and Chemical Bonding

A Non-Mathematical Introduction. By Fritz Seel. Translated from the Fourth German edition and revised by N. N. Greenwood and H. P. Stadler. (Methuen's Monographs on Chemical Subjects.) Pp. vii+112. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1963.) 15*s*.

THE translation of Prof. Seel's *Atombau und Chemische Bindung* by Prof. Greenwood and Dr. Stadler makes readily available to English students a monograph which has proved very popular in Germany. It provides an introductory treatment in non-mathematical terms of the theory of chemical bonding, being concerned with the development of principles underlying the whole of chemistry without arbitrary sub-division. Undoubtedly students will appreciate the clear synoptic view of the subject which it develops clearly and concisely. Not only are the more conventional aspects of bonding theory introduced descriptively but these ideas are also very widely applied. Having developed the theory underlying the formation of molecules and crystals the author provides a discussion, supported by pictorial models, of material ranging from stereochemistry and chemical reactivity, over intermetallic compounds and electrical conduction to the strength of materials and the elasticity of rubber. This coverage in a small book is achieved only as the result of a very concise style of writing. In places the material is condensed to the point of terseness, and a student will probably gain little here, if this is his first encounter with the ideas, although he might well find it useful in providing perspective in the area after making a more detailed investigation.

Some parts of the book can be criticized for over-simplification. The directions of chemical processes are discussed without reference to entropy changes and in one place relative rates of two reactions are accounted for by writing seven versus six resonance structures for the transition state. Ideas are frequently introduced rather loosely: "The electron in each cell traces out a particular pattern or *orbital* . . ." serves to define an orbital; transition state is introduced by referring to "the energy level of the *transition state*".

More seriously, it seems a pity that some feeling for the relative strengths of chemical bonds is not developed. The book is weak generally on energetics; there is little on lattice energies, almost nothing on solvation energies and bond energies are not mentioned.

The great merit of the book is the way in which it relates bonding theory to chemistry at a simple descriptive level. It can confidently be recommended as a stimulating introduction to the subject.

D. J. MILLEN

Introduction to the Constructive Theory of Functions

By Prof. John Todd. (International Series of Numerical Mathematics, Vol. 1.) Pp. 127. (Basle and Stuttgart: Birkhäuser Verlag, 1963.) 27.50 Sw. francs.

THE constructive theory of functions deals with approximations to a given function in terms of simple elements, such as polynomial approximation to a continuous function in a basic interval, or approximation to a periodic continuous function by trigonometric polynomials. It is, therefore, a well-defined field of mathematical analysis, which may serve as a theoretical basis to much recent work on numerical methods.

Weierstrass's fundamental theorem, that a polynomial can be found which will not differ from a given continuous function over a finite interval by more than a prescribed ε, leads us at once to ask what may be meant by a 'best' polynomial approximation, and how, when defined, it may be found. Here the Chebyshev polynomials which, of all polynomials in $(-1, 1)$ with leading coefficient unity, deviate least from zero become relevant, and this book gives a very concise but reasonably comprehensive table of the main formal properties of these and of other orthogonal polynomials arising from a suitable weighting of the mean square error. The Bernoulli polynomials are discussed so far as is needed for dealing with the Euler–Maclaurin summation formula. A brief chapter on interpolation stresses, by means of exercises, the simplification, due to A. C. Aitken, which reduces n-point interpolation to successive linear interpolations. The concept of 'best' approximation is generalized by the use of function spaces, enabling a discussion of existence and uniqueness to be given in economical form. Finally, numerical integration is briefly developed on a Gaussian basis.

The text, apart from the solutions to the exercises, takes up only ninety pages, so the large amount of ground covered necessitates a terse exposition. The author demands full co-operation from the reader, but shows remarkable skill in emphasizing the key-points of an argument, so that the conscientious reader should never have any difficulty in filling in details. There are about 100 exercises, with solutions; some develop more fully matters which are briefly sketched in the main text, others arise fairly from the text but seldom degenerate into points of routine drill. Obviously much care has gone into the construction of these problems, and a young student could scarcely fail to find his curiosity strongly stimulated. The book reads as if the author had thoroughly enjoyed writing it, which should encourage many students to enjoy reading it. The new series of texts on numerical mathematics is off to a flying start.

T. A. A. BROADBENT

FOUNDATIONS OF QUANTUM MECHANICS

By Prof. P. A. M. DIRAC, F.R.S.
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In view of the profound difficulties met with in quantum field theory the foundations of quantum mechanics, which were established more than thirty years ago, are under suspicion. Let us re-examine these foundations and see if they can be amended.

Quantum mechanics, as introduced by Heisenberg in 1925, involved dynamical variables u which do not obey the commutative axiom of multiplication and which satisfy the Heisenberg equation of motion:

$$i\hbar\, du/dt = uH - Hu \tag{1}$$

where H is the Hamiltonian. These dynamical variables were first represented by matrices. Later it was found that they could be considered as operators operating on the vectors of a Hilbert space. It is convenient to denote the Hilbert vectors by symbols such as $|A)$ and the result of applying a dynamical variable u to $|A)$ by $u|A)$.

Shortly after Heisenberg's discovery, another quantum theory was discovered independently by Schrödinger, based on different ideas. Here the emphasis was on physical states, represented by wave functions. The wave functions may be considered also as vectors in Hilbert space, so they may also be denoted by symbols $|A)$. They differ from Heisenberg's Hilbert vectors in that they vary with the time, in accordance with Schrödinger's wave equation:

$$i\hbar\, d|A)/dt = H|A) \tag{2}$$

while Heisenberg's were constant.

For a while physicists had two quantum theories to work with; but it was soon proved that there was a simple connexion between them, resulting in their being equivalent. The Hamiltonian H is the same operator in both theories. The dynamical variables and the Hilbert vectors in the two theories are connected by a unitary transformation:

$$u_S = e^{-iHt/\hbar}\, u_H e^{iHt/\hbar}, \quad |A_S) = e^{-iHt/\hbar}|A_H) \tag{3}$$

where the suffixes S and H refer to Schrödinger and Heisenberg respectively. Thus there was just one quantum mechanics with two ways of looking at it, Heisenberg's picture and Schrödinger's picture.

This quantum mechanics had enormous success when applied to the simpler problems of the atomic world, but ran into serious trouble when one tried to set up an accurate relativistic theory involving fields. The trouble was most acute for electrodynamics, because the other atomic fields were not very well known and one could ascribe the difficulties for them to our imperfect knowledge of the forces involved. But electrodynamics was so well understood classically that it was most puzzling to find that quantum theory fails for it.

To focus our attention on the trouble, let us put it in its simplest form. Let us confine our attention to electrons and positrons interacting with the electromagnetic field. Then it is not difficult to obtain the Hamiltonian H. One can be confident that this H is essentially correct because, when one uses it in the Heisenberg equations of motion (1), one gets sensible field equations in agreement with what one would expect from classical electrodynamics. However, when one inserts this H into Schrödinger's wave equation (2), one cannot find any solutions, not even approximate ones. Presumably solutions do not exist. Physically, one would expect there to be a trivial solution corresponding to the vacuum state, but there is not even this trivial solution.

We have here the dilemma which has hindered the development of theoretical physics for three decades. There is a simple way out of it. Evidently the Schrödinger picture will not work at all, but the Heisenberg picture is not so bad. We may consider that: *The Heisenberg picture is a good picture. The Schrödinger picture is a bad picture. The two pictures are not equivalent.*

The reader will immediately say, "What about the proof that the two pictures are equivalent ?". Let us examine this proof and see if it is sound. It involves a unitary transformation to connect the two pictures, in accordance with (3). The unitary transformation exists, of course, only if the operators $e^{iHt/\hbar}$, $e^{-iHt/\hbar}$ exist. Now the H with which we are dealing is quite a complicated operator, and it is not immediately evident that exponentials of it should exist. We may check whether $e^{-iHt/\hbar}$ exists from first principles, taking a definite Hilbert vector $|B)$, say, and seeing whether $e^{-iHt/\hbar}|B)$ then exists. If it did exist, we see at once that it would be a solution of the Schrödinger equation (2). But such solutions do not exist. Hence the operator $e^{-iHt/\hbar}$ does not exist and the proof of the equivalence of the two pictures fails.

Let us make a closer examination of the two pictures to see the advantages of Heisenberg's. In electrodynamics one may treat the interaction between the particles and the electromagnetic field as a small perturbation, and can then seek a solution of the equations in powers of $e^2/\hbar c$.

If one tries to solve the Schrödinger equation on these lines one gets an expansion, each term of which corresponds to a Feynman diagram. All the terms except the first few involve divergent integrals. Among the terms there are some that correspond to v–v (vacuum to vacuum) transitions with no external lines in the Feynman diagrams. These terms are the worst ones. They prevent one from getting a solution to represent the vacuum state. The divergent integrals in the remaining terms can be dealt with by the technique of re-normalization.

One may try to solve the Heisenberg equations of motion by a similar perturbation method. One then gets an expansion, the terms of which again correspond to Feynman diagrams, with similar divergent integrals. But there are now no v–v terms. The reason for the difference is that, if one starts with zero in the Heisenberg picture, it remains zero, while if one starts with the vacuum in the Schrödinger picture, it does not remain the vacuum. In this way the Heisenberg picture avoids the worst difficulties of the Schrödinger picture. Those that are left, the divergent integrals in the non-v–v terms, can be tackled by limiting processes corresponding to re-normalization.

In the Schrödinger picture the perturbation is so violent that, if one starts with a particular Hilbert vector to represent the initial state, *it gets knocked right out of Hilbert space* in any time-interval, however short. The v–v terms are responsible for this. With such a violent perturbation the Schrödinger picture is unworkable.

In the Heisenberg picture there are no such violent reactions. However, the Heisenberg dynamical variables can no longer be taken to be operators in Hilbert space. They must be something more general, the precise nature of which is at present unknown. We may call them q-numbers. All that is known about them is that they are non-commuting quantities satisfying definite commutation relations. Calculations in the Heisenberg picture consist of algebraic deductions from the commutation relations, which one performs without knowing the mathematical nature of the quantities with which one is working.

The foregoing discussion shows that one should establish quantum field theory entirely in the Heisenberg picture, with all references to the Schrödinger picture cut out. We now have the big problem of setting up a method for the physical interpretation of the results of calculations in the Heisenberg picture. The usual interpretation of quantum mechanics involves the Schrödinger picture, the square of the modulus of the wave function being interpreted as a probability, and so cannot be used. I propose the following new interpretation.

Each physical state throughout all time corresponds to a solution of Heisenberg's equations of motion. This is a q-number, K_t, say, a function of the Heisenberg dynamical variables at time t containing t also explicitly, which is a constant of the motion, so that:

$$\frac{dK_t}{dt} = \frac{\partial K_t}{\partial t} + [K_t,H] = 0$$

For each value of t, K_t will be a function of the emission operators η_t of various particles and the corresponding absorption operators $\bar{\eta}_t$ and also of t explicitly. We suppose the q-numbers can operate on vectors $|A)$ of a more general kind than Hilbert vectors. For each time t we set up a vacuum vector $|V_t)$, satisfying $\bar{\eta}_t|V_t) = 0$ for all absorption operators $\bar{\eta}_t$. Then we can interpret $K_t|V_t)$ like the wave function at time t in the old theory. We may express K_t as a sum of terms each having all the η's to the left of all the $\bar{\eta}$'s. Those terms that contain $\bar{\eta}$'s will give zero when multiplied into $|V_t)$. The coefficients of the remaining terms will then be physically significant, the squares of their moduli giving the probabilities of various particles being present at the time t.

It will be seen that, so far as concerns conditions at one instant of time, the new interpretation corresponds exactly to the old one, with $|V_t)$ representing the vacuum state and $K_t|V_t)$ a general state. Under variations of t the new theory is quite different, since $|V_t)$ is not a solution of the Schrödinger equation, nor is $K_t|V_t)$. The most striking departure from the old theory lies in the fact that the quantity $K_t|V_t)$, playing the part of the wave function at time t, does not fix the quantity $K_{t'}|V_{t'})$, playing the part of the wave function at time t', since one may have $K_t|V_t) = 0$ for a particular t without having $K_t = 0$, and thus without having $K_{t'}|V_{t'}) = 0$. There is no longer determinism for the wave function, as in the Schrödinger theory, but only determinism for the q-number K_t.

The usual formulation of quantum electrodynamics is a set of working rules and is not a logical deduction from the general principles of quantum mechanics. It has had great success in giving correctly the Lamb shift and the anomalous magnetic moment of the electron.

We may now formulate quantum electrodynamics as a logical deduction from Heisenberg's quantum mechanics with the foregoing physical interpretation. This leads to the same results for elementary problems of radiative transitions, the calculations being essentially the same as before, except that the v–v terms of the interaction automatically drop out and no longer have to be arbitrarily discarded. I have checked that the new theory leads to the correct Lamb shift and anomalous magnetic moment for the electron, to the order of $e^2/\hbar c$. Thus the successes of quantum electrodynamics can all be maintained with the increased standard of logic that the new formulation provides.

In conclusion, I would like to say that the development of quantum theory proposed here should not be considered as detracting from the value of Schrödinger's work. The Schrödinger picture is a very good one for all those problems in which only a finite number of degrees of freedom are effective, such as problems in which the electromagnetic interaction can be represented by Coulomb forces, and it will continue to be used extensively as it is then simpler and more convenient than Heisenberg's. Only when one goes to an infinite number of degrees of freedom does one find that the Schrödinger picture is inadequate and that the Heisenberg picture has more fundamental validity.

GREY SEALS AND FISHERIES

By Dr. E. B. WORTHINGTON

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In July 1955 the Nature Conservancy was asked by the Ministry of Agriculture, Fisheries and Food to advise on the problem of damage caused to fisheries by grey seals (*Halichoerus grypus*). The complaints were mainly from south-eastern Scotland near the mouth of the River Tweed, whereas the seals alleged to be troublesome were assumed to come from the Farne Islands, south of the border.

At that time there was not much scientific knowledge about the grey seal or its relation to fisheries. Several biologists had investigated aspects of its natural history, behaviour, breeding biology and population structure in Cornwall, Pembrokeshire and the Hebrides[1-6]. In 1951 Hickling[7] had begun marking young seals on the Farne Islands to trace their movements in the sea, but accurate counts of the breeding population there were not started until 1956. However, the challenge was accepted, and two years later Lockie[8] had made a preliminary investigation of the damage caused on the Northumberland and Berwickshire coast. With this rather scanty background, the Nature Conservancy advised that, in addition to initiating a programme to discover means of protecting nets against seals, the Ministry should, as an experimental and interim measure, limit further increase of the Farne Islands colony by conducting an annual cull of calves.

Although population control on any significant scale was not in fact undertaken at that time, research on many aspects of the situation was augmented. In addition to continuation of work by Hickling on the Farnes, and by Lockley in Pembrokeshire, annual visits to North Rona were started by Boyd[9] and his collaborators. In 1959 a Consultative Committee on Grey Seals and Fisheries was set up by the Development Commission and the Nature Conservancy. E. A. Smith was appointed as seal research officer and has already presented results on a number of aspects of the problem, in the Committee's Report[10]. He has also investigated grey seals in other parts of their distribution range, during visits to north-east Canada, Norway and the Baltic. One of the few remaining areas where little is yet known about the status of the species, around the Irish Republic, will be studied in the next year or so.

On the much-debated question of the influence of grey seals on fisheries, Rae[11] described the kinds of damage caused. Seals are frequently seen near salmon nets and have been observed killing and eating salmon. Regular recordings of the presence of seals at fixed-net fishing stations have been made over the years 1959–63; these results have been analysed by Rae and Shearer, of the Scottish Fisheries Department, who will soon publish their report. The records of a representative sample of salmon packing stations indicate that at least 6 per cent of the total catch of salmon bear seal claw or tooth marks. Fish, evidently mutilated by seals, are frequently found in nets or washed ashore near by. Almost all fixed-net stations on the Scottish coast suffer damage to nets; during the period 1959–61, the average number of nets damaged by seals was 10 per cent of the total fishing effort.

The fishery for cod and other white fish also suffers damage from seals; but it is less easy to assess this quantitatively than in the fishery for salmon and sea-trout. However, the organization now exists for collecting data on seal damage in a form which is susceptible to statistical analysis.

As a result of these investigations, many of which are still in progress, it can be claimed that there is a much better scientific background for attempting to solve the seals/fisheries problem to-day than there was nine years ago when it first came into prominence. Some of the salient results can be summarized as follows:

The world population of grey seals is now estimated at about 46,000 all but 10,000 of which live around the British Isles. Recent censuses during the breeding season have shown some 10,000 to be based on Orkney and 3,500 on the Farne Islands. In the early years of the century, before the Grey Seal Protection Acts of 1914 and 1932, the species was thought to be so rare as to be in danger of extermination, although valid evidence for that opinion is now difficult to find.

The rate of increase of population is best illustrated by the Farne Islands, where there are several early estimates and since 1956 detailed counts of calf production[12]. These show an overall annual rate of increase since 1956 of more than 7 per cent, which implies doubling of the population in less than ten years. Some of the Farnes breeding beaches are densely crowded, with calf mortality sometimes as high as 20 per cent; but there is no evidence as yet of a flattening of the population curve. In recent years small breeding groups have become established on the Scroby Sands and Isle of May. It may be significant that Scroby, where the colony would appear to be an overflow from the Farne Islands, is the only place where grey seals are known to breed on a sand bank.

The grey seal population of North Rona seems also to have undergone a rapid increase. It is fairly certain that none bred there until the human population was evacuated in 1844. In the 1880's, 100 or so were killed each year. In 1939 Fraser Darling estimated that 1,500 pups were born there, which indicates an adult population of rather more than 5,000. Counts by Boyd and his collaborators put it at more than 9,000 to-day. No other colonies have records adequate to estimate increase in population, but Orkney in 1960, the first year of census, had far more seals than had previously been thought. The only major colony where it would seem that the population has not increased markedly is around Shetland, where a tradition of seal hunting has persisted throughout the years and the shore habitats are for the most part unsuitable.

Longevity has been established through reading the age of seals from tooth sections. The oldest bull so far examined from British waters was 23 years and the oldest cow 35 years. Females are capable of conception from the age of 5 years and sexual maturity is attained in males at 6 years, although the successful maintenance of breeding territories appears to be restricted to bulls between the ages of 10 and 20 years. Facts such as these, observations and counts at breeding grounds, the study of post-mortem material, and comparisons with other species of pinnipeds the biology of which is better known have enabled Hewer[13] to prepare a provisional life-table of the species.

Exact information about the movements of grey seals in the sea is so far limited mainly to their first year, because no tag has yet been devised which will remain attached to the animal for much longer than that. In all some 3,300 young seals have been tagged on the breeding beaches of the Farne Islands, 1,700 on Orkney, 1,100 on North Rona and 1,200 in Pembrokeshire, with smaller numbers in the Hebrides and Shetland. Occasional recoveries have come from as far away as north-west Ireland, Norway, Iceland, Holland and Spain, but the general pattern of returns shows a rather uniform dispersal from the points of origin with no obvious drifts or bias in particular directions. In the region where damage to fisheries is reported as most intense, from Durham northwards to Caithness, a large proportion of young seals come from Orkney or the Farnes. It seems probable that the older seals distribute themselves in a similar pattern to the young ones; definite information about them should become available before long, since a substantial number of seals from North Rona and Orkney are now carrying brand marks which last much longer than tags.

The food of grey seals is known at present only from about 200 stomach examinations. Animals eaten range widely, with salmon and sea-trout, cod, plaice, sand eels, squid and crab among the more prominent. It is clear that seals, like fishermen, are opportunists, so when fisheries are operating at a maximum, seal activity is also greatest in the same area. Hence the apparent anomaly that complaints of damage are apt to be greatest when the fisheries appear to be most prosperous.

The incidence of cod-worm (*Porrocaecum decipiens*) is a matter of concern to the white fish industry. Rae[14] has examined the incidence of the larvæ, of which the grey seal and to a lesser extent the common seal are definitive hosts, and as such necessary for the worms' full development. He presents much evidence that a big increase in the proportion of infested cod in some areas is associated with the increase in the number of grey seals.

The Consultative Committee on Grey Seals and Fisheries[10], having considered all this and other evidence, and taking all interests into account—the fishing industry, the naturalists and the seals themselves—reached the conclusion that some control of the seal population was necessary. The Committee recommended, however, that such control should be limited to the two colonies which, from the marking experiments, were suspected of doing a large proportion of the damage to the vulnerable fisheries of the east coast of Scotland and northern England. It would not be sufficient to take from these two colonies an annual crop equivalent to the estimated annual gain, but some inroads should be made into the future breeding potential, provided such could be achieved without endangering the continuation of these colonies in flourishing condition.

It was decided that the aim should be to reduce the breeding potential of both these colonies by 25 per cent in the course of five years. Assuming that the Orkney colony has been increasing at approximately the same rate as that on the Farnes, such a reduction would bring the numbers of each colony down to what they were in 1959. It was appreciated, of course, that such a reduction would not meet the demands of the fishermen; but the Committee thought that a modest reduction in the number of seals, coupled with continued efforts to scare seals away from the fishing centres and the use of more resistant nets, was likely to reduce the damage to tolerable limits in years to come.

This general recommendation by the Consultative Committee, which is supplemented by detailed proposals made from year to year in the light of experience and of continuing research, was endorsed by the Nature Conservancy and accepted by the Ministry of Agriculture, Fisheries and Food and the Secretary of State for Scotland. After the appropriate orders had been laid before Parliament, the proposed culling operation was carried out by the Ministry of Agriculture, Fisheries and Food on the Farnes during the seal breeding season of 1963, and the operation in Orkney was arranged by the Department of Agriculture and Fisheries for Scotland in 1962 and 1963.

It is important to note how this unpopular work was carried out, because in the process evidence was collected which will help to ensure the sensible management of grey seal populations in future years. In the Farnes the culling was done during three days in December 1963 by agents of the Ministry in a well-planned operation. The number needed to achieve the object was 85 adult cows with their calves, or, failing that, 360 female calves, or a combination of the two. In the circumstances prevailing, with a good many onlookers, it was difficult to take adults cleanly and safely, so the cull was limited almost entirely to calves, the bodies of which were removed immediately for disposal of the products. An important result was that the operation was performed without any hint of cruelty and the survivors took remarkably little notice, as described by Hickling[15]. This, together with comparable experience from Orkney, provides a reassurance that management of these seal populations can be undertaken without the risk of upsetting the breeding biology through disturbance.

In Orkney, licences were issued by the Department to a number of local men. Since under such arrangements it would be impossible to limit killing to females, substantially larger numbers would be needed to achieve the object. The calculations showed that approximately 1,800 calves would be required annually, four calves being the equivalent in this context to one adult. However, since there were unknown factors in this operation, the Committee thought it wise to err on the safe side and recommended that the numbers killed in each of the two seasons 1962 and 1963 be limited to 750. In 1962 the returns did not exceed this figure, but in 1963 the number had reached 974 before licences could be withdrawn.

Following a wide ventilation of the pros and cons of controlling the numbers of this species, in the newspapers, on television, and in sections of the scientific Press, we now move forward to the third year of the proposed operation on Orkney and the second year on the Farne Islands.

The philosophy of controlling populations of wild animals, even those living in nature reserves, has been well described by Fraser Darling[16]. There are numerous examples where this has proved necessary in the interest of the species concerned as well as of other species, including man; deer and wapiti in North American national parks, red deer on the island of Rhum, hippopotami in the Queen Elizabeth National Park, elephants in the Murchison Falls and Tsavo National Parks. The effects of population pressure are somewhat different in large herbivores, such as these, from a predator such as the grey seal; but the implications are similar. If we protect a species from its normal hazards, in the case of grey seals by providing sanctuaries and an Act of Parliament, we must expect an increase in population. Indeed, this is the purpose of protection. The consequences may become severe in time, either to the species itself or to others, and in such cases a re-introduction of the hazards to survival may be desirable, especially if they can be kept under scientific control. Such a case is that of the grey seal in the north-east parts of Britain.

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SECOND PARLIAMENTARY AND SCIENTIFIC CONFERENCE

By SIR WILLIS JACKSON, F.R.S.

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IN March 1961 a Parliamentary and Scientific Conference was held in London under the joint auspices of the Council of Europe and the Organization for Economic Co-operation and Development (O.E.C.D.), and afforded an opportunity for parliamentarians and scientists from several countries to discuss matters of common interest and concern. Since this Conference the subject of scientific policy has been receiving increasing attention, and no doubt its meaning and scope have become more clarified. An important contribution towards clarification was made by a report entitled "Science and the Policy of Governments" prepared in 1963 by an international committee set up by the Secretary-General of O.E.C.D., the publication of which was followed by a meeting of Ministers of the member countries of the Organization in Paris in October of that year. This meeting revealed a growing recognition that a scientific policy has become an essential component of national and international policy, but also the widely differing steps which the various countries have taken to cater for its formulation and implementation. An interim committee set up by the Ministers is now preparing material for a further meeting to be held probably during next year.

In varying degrees the scientist, technologist, economist, sociologist, employer, Trade Union official and parliamentarian all find themselves involved increasingly in the consideration of, if not in the taking of decisions on, such questions as the place which scientific research and technological development must occupy in national affairs, the resources which should be devoted to them, how these resources should be allocated and balanced in the light of overall national policy, and how to deal with their sociological consequences. The role of the parliamentarian in these matters is, or should be, a particularly important one. Yet all too few of them possess a sufficient knowledge and understanding of science to permit their effective participation in the discussion of many matters of major national and international importance. Correspondingly, however, many scientists are not good at communicating with the non-scientists, nor at appreciating the complexity of the issues, involving science, which fall to the parliamentarian for ultimate resolution.

The decision of the Council of Europe and of O.E.C.D. to organize a second Parliamentary and Scientific Conference, held in Vienna during May 23–27, was, therefore, a welcome one. Its objectives were stated to be to enable

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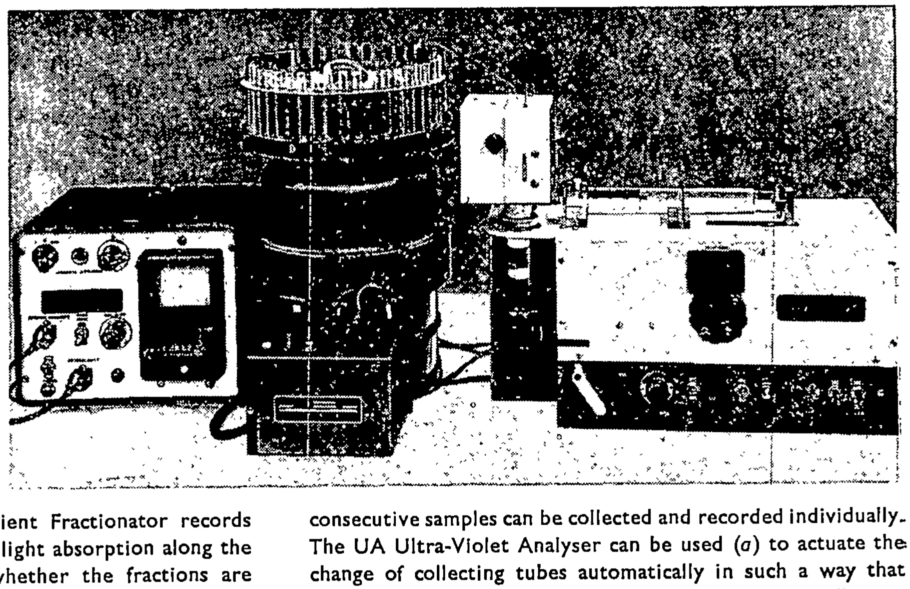

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legislators to gain a better understanding of the problems, the needs and the perspective of science; to estimate better its effect on the national economy and on the development of the State; and to enable scientists to partake more fully in considerations which may give rise to political decisions.

The formal papers prepared for presentation at the Conference were a review of the aforementioned O.E.C.D. report and of the scientific situation in European countries and the United States by Mr. Kenneth Lindsay, a past Member of Parliament for the English Universities; and addresses by Prof. Gerhard Hess, President of the German Research Association; Mr. Quintin Hogg, Secretary of State for Education and Science in the United Kingdom; Mons. G. Palewski, French Minister of State for Scientific and Atomic and Space Research; and Mons. Karl Czernetz, Member of the Austrian Parliament.

Prof. Hess surveyed the history and philosophy of European science; the main theme of Mons. Palewski's address was that a national policy for scientific research had become essential to the independence of a country in the broadest sense, namely to retaining the possibility of a voice in world affairs and of expressing its own national genius; while the essence of Mons. Czernetz's contribution was concentrated in his statement that parliamentary democracy is in great danger if parliament is not asked to take part in the formulating of scientific policy, and where such policy is devised only by scientists and the government. The substance of Mr. Quintin Hogg's address follows.

It would be an over-statement to claim that the Conference made much progress towards its defined objectives, notwithstanding the many distinguished participants from twenty countries, due mainly to the generalized and diffuse character of much of the discussion, and to the way in which it alternated somewhat uncontrollably between the two themes, which Mr. Hogg tried at an intermediate stage to separate, of 'science for policy' and 'policy for science'. It is to be hoped that in similar future conferences an effort will be made to direct attention, and to sectionalize preliminary discussion, on a limited number of specific topics falling within these two aspects of scientific policy.

The communiqué which was approved at the conclusion of the Conference drew particular attention to the value of joint Parliamentary and Scientific Committees within individual countries; to the supplementary possibility of regular committees for scientific policy as part of parliamentary machinery; to the desirability of directing international co-operation in science to those areas in which international effort offers advantages as compared with national effort; and to the fact that the growth of scientific policy needs the support of an informed public opinion, involving much increased attention to scientific matters in the Press and other media of mass communication, and closer collaboration between journalists and scientists.

SPECIFIC PRESENT AND FORESEEABLE IMPACTS OF SCIENCE ON POLITICAL LIFE

By The Right Hon. QUINTIN HOGG, Q.C., M.P.

Lord President of the Council and Secretary of State for Education and Science

The impact of science on politics, and even the field of international affairs, has grown out of all recognition during the past seven years in which I have been responsible for the conduct of civil scientific research and development in the United Kingdom.

I propose to avoid, partly because it will be dealt with by others, and partly because I do not wish to traverse again ground which I myself covered last October, the specific questions of scientific organization and policy with which Parliaments and Ministers are increasingly concerned in their day-to-day life; not even the fascinating and increasingly important field of international, and specifically European, co-operation in the scientific field, exemplified among other things by the development of C.E.R.N., E.L.D.O., and E.S.R.O. in the field of space research, and *Dragon* in the field of applied nuclear science.

The Scientific Revolution

My task is to survey the specific present and foreseeable impact of science on society and political life. By 'science' I mean nothing less than thought systematically applied to the results of systematic measurement and observation. This is the specifically new factor in our modern civilization. Many ancient civilizations developed highly sophisticated and complex systems of thought which flowered as religions or philosophies. Some also indulged in a limited field in systematic observation and measurement with results which have left traces in modern astronomy and mathematics. But over a wider range and in the field of applied science, in the sense of the application of mechanical power to production and exchange, the achievements of the ancient world were, by modern standards, rudimentary. Genuine experimentation as distinct from the collection and classification of material was rare, and, so long as the individuals remained isolated, systematic enquiry in the modern sense (which is distinctively collective in character) was scarcely possible. The true scientific revolution began when scientists developed a corporate sense and when the era of collection and classification gave way over an extended field to data acquired by systematic experimentation.

My task, therefore, is to assess the impact of this revolutionary development in human thinking on society. Of the extent of this impact there can scarcely be any question. It is trite that we live in an era of change, rapid, cumulative, and accelerating. It is beyond question that these changes affect the size and viability of political organisms, the relations between classes and nations, the whole organization of society for production, the standards and distribution of education, property, wealth, hygiene and comfort, religious and moral beliefs, methods of waging war, the structure of the family, and the expectation of life. Moreover, it is beyond question, too, that the predisposing cause of all this change has been precisely the scientific revolution of which I speak. I would go so far as to claim that, in the wider sense in which I am accepting the subject set for me, the impact of science on society and political life is precisely the one question which, above all others, scientists and parliamentarians must set themselves to answer.

Expectation of Life

Perhaps the most fundamental change which science has brought about has been in the physical life and environment of the human species itself. I place these two factors above even the application of mechanical power to production, and communications. With the advent of surplus farming and modern hygiene in the nineteenth century, followed by the conquest of infectious disease and by modern surgery in the twentieth, the expectation of life has rocketed. Populations starting with those of the developed countries have exploded in size, an increase which shows every sign of intensifying as longer expectation of life extends, as it is already doing, to the less-developed parts of the world.

The productivity of agriculture and industry has in general kept pace with the rise in the expectation of life, but has not risen at such a pace as wholly to discredit the neo-Malthusians, who continue to predict food shortages on an immense scale by the end of the century and claim that the limits of agricultural productivity are visibly at hand. That this is a serious problem no one would doubt; indeed, it is something of a surprise to me both that it is so little discussed and that its effects are so little noticed. It is foreseeable, predictable and specific. Its impact on the society and politics of the latter half of the twentieth century may well be the major theme of the future political histories of our time. Nevertheless, I doubt the pessimistic views which are sometimes expressed —particularly in the West (but less in the East, since Marxism is traditionally anti-Malthusian)—as to the limits of possible increases in production. I would myself expect that we can immeasurably develop the food resources of Asia, Africa and South America. I suspect that we may soon be able to desalinate sea-water on a commercial scale and so irrigate the deserts. I would not put it beyond the bounds of possibility that we may discover means before long of actively cultivating the hitherto trawled but still unharvested sea.

I do not propose at this stage to raise the *ignis fatuus*, usually discussed *ad nauseam* on these occasions, of the perfect contraceptive as the complete answer to the problems of over-population in the developing countries. For aught I know this magic potion is just around the corner; on the other hand, its existence is not yet proved and even though it were found to exist its use in the conditions of Indian, African or Amer-Indian village life is problematical. What is certain to my mind is that the high birth-rate in the developing countries is less the product of the absence of such methods (since cruder methods of control have existed since the dawn of history) as of the simple desire for children in a society not yet used to low infant mortality rates and accustomed to regard a quiverful of offspring as a poor man's old age pension, a personal bodyguard or the answer to the servant problem. Social insurance, monogamy, later marriage, improved living and housing conditions, and a higher degree of personal security will, in my judgment, do more in the long term to lower the birth-rate than all the contraceptive pills in the world. These conditions will, I believe, follow on the development of agriculture and industry and an intelligent social policy.

Confrontation of Races

Perhaps the most spectacular and dramatic of the effects of modern science has been the confrontation between races, nations and classes. The scientists tell us that man has been on this planet for a few hundreds of thousands of years—even more if some interpretations of recent discoveries be accepted. By far the greatest part of this time we have spent our lives in isolated groups, removed from one another by countless generations of separation, behind insurmountable geographical barriers or untraversable distances, and such groups have remained for the most part innocent of elaborate political or social organization.

But modern communications make light of oceans, mountains, forests and deserts. Races, previously almost complete strangers, are now near neighbours. It is perhaps not surprising that in this new reunion of the human family, there have been heightened tensions and unedifying incidents. Modern methods of production have altered no less long-established relationships within communities, and as a result of developments in military science the nature of the relationships between established sovereign states is also in a state of near flux.

The process of confrontation will continue to occupy the front of the stage as the Earth becomes surrounded by an increasing number of orbiting satellites, continents conjoined by an ever larger number of radio networks and airlines, and individual countries laced by growing numbers of motorways. Served by large numbers of mechanically propelled vehicles, with towns sprawling ever wider, and expanding into vast densely populated conurbations utilizing ever-increasing quantities of water (the one natural product universally necessary for which there is no effective substitute), man is becoming more than ever a social creature and is becoming conscious more and more of a common destiny.

Environmental Problems

So much, for the moment, for the species. Now let us look at our physical environment. Not the least of our political problems arises from its increasingly artificial nature. This constantly presents new problems to the town planner, the traffic engineer and the social scientist. Complaints are made by all the developed countries of the depopulation of the countryside. But the centres of large towns are empyting, too. An American airline pilot is reported in a recent book as saying that as he flew across the American countryside now at night there were large black spaces which, not many years ago, were coloured with the lights of farms, but that the formerly discrete patches of light which showed the towns has broadened into great bands of lighted streets and suburban villas. This is happening in Britain, too. The population of the countryside is converging. But the population of the centre of cities is moving out. The population of Central London is less than it was fifty years ago in my boyhood. In the south-east of Great Britain great urban regions are developing into a regionalized subtopia. But the subtopia itself is not one of suburban villas and Manhattan skyscrapers. In the modern city high buildings are no longer allowed to form the artificial canyons of New York streets, but are spaced to reconcile the requirements of space and light with density. A new type of urban, or rather suburban, civilization based on the high building and the internal combustion engine is clearly on the way.

Our Natural Surroundings

Our natural environment is changing, too. Man has always, within the limitations of his technology, been more or less destructive of his natural surroundings. But his powers in this regard have changed in scale, and, therefore, in kind as well as in degree. Environmental problems are not confined to the growth of the cities and are not merely due to the lack of understanding of rural ecology shown by those nurtured in towns. New sporting —or unsporting—weapons, new methods of agriculture, and new industrial products present an increasing hazard to wild life and our natural biological environment. The large scale of modern scientific and military experiment also presents hazards to the wider physical surroundings in which we are placed. The rainbow bomb may have permanently altered the radiation belts in our immediate surroundings; bipole curtains outside the atmosphere could blanket out radiation from outer space. Military operations with nuclear weapons—or even renewed atmospheric tests if persisted in—would affect beyond human tolerance the actual physical environment in which we live. All these have a clear social and political implication calling for active effort on our part.

The Motor-car and Society

Scientific methods of transport and building stand among the major environmental influences at work. The automobile itself is a product of scientific achievement. Increasing ownership and use are a result of rising prosperity and increased leisure. Science has created a new problem here—how to obtain the benefits of the motor-car without losing the amenities of a modern urban society. To solve this problem we must use the resources of a whole range of scientific disciplines. Computer techniques enable us to analyse and apply vast quantities of information in the study of urban problems, and there is a role for mathematicians, statisticians and engineers in developing the use of new techniques. But, in the last resort, this is a human problem—there is vast scope for workers in the social sciences, as well as for planners and architects. This field is a good example of the need for bringing together the skills of many different disciplines to ensure that the benefits from scientific achievement are applied to the true advantage of society.

Construction Industries

A result of what has been happening has been an immense outburst of building and construction. But the ability of the construction industries in almost every country to carry the load required for the physical reconstruction of our urban and industrial environment is beyond its present means, man-power or methods. For the construction industry has been one of the last even to be industrialized, let alone automated. But this is clearly moving fast ahead. Modular construction, standardized and factory-built components, and new building materials are rapidly becoming standard practice, and new on-site methods and layouts are rapidly appearing. The architects' profession, once regarded as one of the fine arts, seems to be developing rapidly into a branch of civil engineering; the management and constructional design of buildings, once split up between the surveyor, the architect, and the builder, is rapidly becoming the task of an integrated team of professionals; while various methods of handling materials to and on the site are slowly taking the place of traditional habits.

Recreation and Leisure

One of the main results of the scientific revolution has been to bring adequate leisure and facilities for recreation within the reach of the masses. Catering for recreation and leisure is becoming a vast industry, but the emphasis is less exclusively on the traditional games. Sea, rivers, lakes, mountains, canals are becoming, and even further afield, the desert and the ice-cap may become, the playgrounds of the masses where formerly they provided the fields of exploration or of sports for the wealthy. Indoor entertainment and sport reach new elaboration and expense. It is also a mistake to think that the scientific age is contemptuous of the traditional arts and culture. Whatever we may think of popular standards of taste, enjoyment of literature, music and painting has never been more widely spread. The use of leisure in all its forms is part of the scientific and social revolution of our times. Yet the picture is not all bright. To win the constructive interest—and thus engage some of the leisure time—of the less talented and imaginative in worthwhile pursuits or social service is a common problem whether we are dealing with the 'stilyage' of the U.S.S.R., the hoodlums of New York or the mods and rockers of London's East End.

Energy Requirements

Cheap power is one of the main spearheads of the scientific revolution. The relative economics of nuclear and fossil fuels appear to vary from year to year, but it is still expected that nuclear stations will, at base load, provide the most economical means of generating electricity by the 1970's. By the end of the century we can surely expect that nuclear generation will be far cheaper than any of the older methods and that means will be found of consuming the plutonium which is a by-product of uranium-fuelled stations. Soon after it is possible that nuclear fusion processes will be contributing to energy supplies. But it is also, I would have said, certain that other new methods of generation, the fuel cell and perhaps heat convectors using cæsium vapour, will have been developed.

Advent of Automation

I now turn from the broad prospect of man and his environment to the more specific issue of methods of industrial production. We are already beginning to enter the age of automation and to feel the political and social effects of control engineering. As the century progresses this series of technological changes will produce important social and political consequences. The industrial methods of production of the nineteenth century created the urban working class, the miner, the factory worker, the railwayman, the clerk in the city office. It is possible that the control engineering of the twentieth century may end by destroying this class and substituting others more highly trained, better educated, with different economic functions. The computer and automatic processes of production, inspection, packaging and despatch will take the place of much unskilled labour. The demand is for new skills with different applications. I have some doubt whether this change need cause the structural unemployment which is said to have occurred in the United States. I cannot help feeling that a little more foresight in diversifying industry, a more careful phasing of redundancies and perhaps a more vigorous attitude to re-training, might at least have been worth trying. In the long run, however, the age of automation will produce its own demand for labour. Maintenance and other services can absorb the skill and labours of a population whose rising wages and improving educational standards and higher degrees of discrimination constantly increase levels of effective demand.

New Skills

The advent of automation, however, brings new problems to management, labour and educationists. While the nineteenth century created a network of highly specialized craft skills superimposed on an unskilled proletariat, and the early twentieth brought into being a huge army of semi-skilled factory operatives, the automative age calls for skills of a higher, more flexible, and less-specialized type. Where automation brings unemployment, it strikes the under-educated and unskilled earlier and several times more severely than the skilled. This means that the age of automation demands a higher general level of education and can do with far fewer specialized craftsmen trained by the traditional methods of apprenticeship to a single trade. It also throws up a special problem of adult training and re-training in fresh skills or in a higher degree of accuracy and perfection in old skills.

Management

It is, however, in the field of management and of decision-making that automation may effect the greatest social change. It is not simply that the computer eliminates the black-coated clerk from the processes of record keeping and calculation. It is that much that previously depended on hunch and flair in the realm of decision-making has proved to be capable of precise calculation by feeding a sufficient number of carefully programmed variables into an 'electronic brain'. This does not, of course, mean that there is less scope for scientific management, but far more. With such a flexible and sophisticated tool at his command, the modern business manager needs greater understanding exactly in proportion as he can pursue his predictions into ever greater accuracy.

Transport

It is quite certain that new methods of transportation will link countries even more together and lead to even more intimate economic interdependence. Not merely water, oil, and gas, but solids, possibly encapsulated, or in suspension, will be transported by pipeline. It is probable, though not certain, that new methods of land transport will have evolved. No one knows at present the future of the monorail, the hovercraft, or the hydrofoil. Ships will have automated engine rooms, and will probably be designed for automatic loading and unloading at the port. It is uncertain whether they will be served by nuclear reactors. Aircraft will be supersonic with perhaps vertical take-off and landing and fully automatic landing for passenger aircraft. Radio and telephone communications will almost certainly be increasingly by satellite.

Agriculture

The movement towards subtopianism already indicated will be intensified by modern agricultural methods. The agricultural industries of Europe will continue to produce more food with fewer employees. This process continues in Britain, but in Germany and France it will have even more drastic effects. Fertilizers, pesticides, and intensive methods will continue to boost output. It is probable that new pesticides will continue to create ecological problems needing prohibition and control. It may also be that the introduction of biological controls and more selective chemical pesticides will diminish the hazards which at present exist.

The primitive methods of agriculture in other parts of the world will gradually become mechanized. As the chemical industries contribute increasingly to the production of new materials, more land should become available for food production. One hopes that tobacco culture will gradually become a thing of the past.

The food-producing industry will make further advances in freeze-drying, packing and processing. It is possible that irradiation techniques will be widely used to prevent or delay spoilage from bacteria.

Medicine

Recent medical advances will clearly accentuate the problems attendant on old age. The doctors can prevent us dying, but there is no sign yet that they have learned how to keep us young. Further virus diseases may well be conquered by vaccines or new chemical inoculations. A surgery may possibly be developed to overcome the difficulty of engrafting spare organs and for utilizing electronically controlled artificial limbs. With the increasing numbers in society who will live on to a green old age, it seems that medicine will concern itself increasingly with the conditions and diseases attendant on old age and with mental health. Meanwhile social and preventive medicine, sanitation and human genetics are already coming to the fore as fields of study.

In the hospital world, the impact of new scientific advances, and especially of automation, is requiring much consideration in the context of the designing of new and improved hospital facilities. Equipment is becoming even more complex and varied and this has its impact on the staffing of our hospitals.

Education

The varying effects of the strange changes through which we have passed have already brought the question of the education of the young very much to the fore. A generation ago it would have been almost unthinkable even for a society modern by the standards of that time to afford a secondary education for all and a university education for all who can make use of it and desire to do so. This, and nothing less, is the proclaimed object and almost the accomplished achievement, of a number of European States, Great Britain among them. In the meantime the growing *tempo* of technical change makes the refresher course for adults in the more technical professions, medicine, management, engineering, teaching, and soon, perhaps even law, virtually a necessity, while the proportion of students going on to do postgraduate work and research, or attending schools of advanced study, is steadily increasing. If the age of automation makes this economically possible, it is also a technical requirement of the age of automation, and probably a condition of its success. So far from being the century of the common man, the twentieth may well become the age in which the qualified man holds the reins of political and economic power and adequate specialized qualifications may become within the reach of all. The uneducated tycoon of plutocracy, and the anonymous mass of the uneducated, may well be on the way out.

The 'Sixty-four Thousand Dollar' Question

Having said all this, I am conscious of having said almost nothing at all in the sense of answering the questions we all ask and to which we must all find answers if we are to survive. Most of the conditions of our future existence are all discernible in the present, and, though there may, and almost certainly will, be new scientific discoveries which are not discernible in present trends, I know of no method short of the necromancer's crystal ball to enable me to predict them.

The real issues, however, are altogether different. What has all this to say about peace or war, about life and death, freedom or enslavement, the abolition of poverty, the possibility of civilized amenities for the vast under-developed areas in Asia, Africa and South America, the relationship between 'East' and 'West', or the organization of our society?

These are not questions to which science as such can provide an answer. Yet it is precisely the development of the scientific revolution which I have been attempting to describe which presses the necessity for an answer upon us.

I do not think that politics is likely to become a purely scientific occupation. Let anyone who thinks the contrary consider the number of parameters to be identified and the extent to which human purposes are incapable of being settled by agreement. Yet can we be satisfied with a negative answer? In one specific direction the politician must become a scientist—a social scientist—or he will become a demagogue and a charlatan.

It is a commonplace, which nevertheless has some truth in it, that society has not grown up as fast as technology. We have not yet learnt how to live on tolerable terms with many of the new tools it has put in our hands. For Governments this situation presents the particular difficulty that, alongside all the quasi-certainties which the physical sciences present to them, there is a large area of uncertainty and ignorance about human nature and human behaviour, both individual and in society. It is largely this uncertainty which makes political decisions so hazardous and difficult. A Minister must work with, through and for the benefit of human beings. I think this means at least that far greater attention should be directed to the social sciences, and the necessity of Parliamentarians knowing something about them and the arts of decision-making as well as of the traditional humanities and physical sciences.

My visit to the U.S.S.R. last summer convinced me that, contrary to the official doctrine of both sides, the effect of technical and scientific change will be to drive the economic and social systems of developed nations more closely together. The environmental problems of developed industrial society are driving the free-enterprise nations into heavier and heavier investments of social capital despite their continued emphasis on private-consumer choice. The rising standards within the U.S.S.R. are driving the Soviet Government to produce a wider range of consumer goods in the shops. It is doubtful how far the former can be indefinitely accommodated within the previously accepted assumptions of capitalist society. But I also feel certain that a wide range of consumer choice available to the individual is quite inconsistent with the hitherto accepted norms of socialist planning. Indeed, it is not without significance that the Soviet doctrine is that Communism, that is, the phase at which Socialism with the State itself according to orthodox Marxism withers away, is coming within the next twenty years. Should it come, I fancy that it may not turn out to be so different from the American way of life as the inhabitants of the Kremlin or the White House might expect.

Moreover, under the impact of increasing wealth, the level of education is rising everywhere. On the whole this must favour freedom of speculation in the East and in the West. University graduates, whether in the sciences or the arts, are kittle cattle. But at least it is difficult to keep them to a rigid orthodoxy of any kind, or to prevent them from asking themselves, or one another, the questions, perhaps insoluble, which have haunted the human race wherever their standard of living has risen above the level of poverty. A scientific friend of mine was being shown round a scientific institute library by his Soviet host, when they passed the political section filled with the orthodox literature of Marxism–Leninism. "This is our theological section," said the Russian to my friend, and while the remark undoubtedly illustrates the absence in both of orthodox religious belief it is a scarcely less-convincing argument for the lack of any real conviction about the official political theory. A Soviet scientist visiting Britain was asked in a university common room what changes he had seen in Britain since he was last in this country more than thirty years ago. His astonishing reply was that Britain had travelled further on the road to Socialism than the Soviet Union. I do not profess to understand, far less agree, with this judgment; but at least it goes to show that the preconceived categories of opinion among the Soviet intelligentsia are less rigid than might be supposed.

With the capitalist and the socialist worlds converging towards each other from opposite directions and each competing for the favours of the politically developing but economically undeveloped nations, what in the end can we foresee as the impact of the scientific revolution for mankind?

Need for a Common Culture

We see, I believe, the urgent need for a common culture, a generally accepted standard of social and political values, a commonly enforced and recognized ethical and political code between men, between nations, between races, between classes. We see also the necessity for an international mechanism more effective than that provided simply by diplomatic contacts, functional organizations, military alliances, or even the United Nations.

I am well aware of the dangers of advocating, in any era of ideological struggle, anything which even savours of the co-existence of ideas as distinct from the co-existence of people. But if we are to co-exist as people, and if our mutually incompatible ideas are to lock in the dialectic of history, it is above all things necessary that races, classes and states—yes, and individuals—should work out, understand, and observe some objective rules of behaviour mutually acceptable and universally understood if only for the conduct of the struggle. For this purpose there must be communication, there must be established dialogue, and those who seek to explore, each with loyalty to his own people, the minds of the other side must not be condemned as revisionists or fellow travellers. The logic of history demands that human beings, as well as being the masters of their environment, should also learn to control and mitigate the violence of the very forces which make for conflict. I myself was proud last summer to play, with others, a part in the negotiation of the limited test ban agreement later signed in Moscow. It caused me real pain to see some of those who had taken part with me in the negotiation, which seemed to me a logical political corollary of the scientific revolution, condemned by doctrinaire and sectarian extremists of their own camp as traitors to their own side.

To my mind, the necessary corollary of the scientific revolution, the urgent necessity of our time, is to break down cultural, political and racial barriers and especially to break down the barriers between East and West, between the developed and the developing, and all the other remaining psychological barriers which, after all, are the relics of an age when we could afford to ignore races and nations who were not our immediate neighbours, and could afford without ultimate disaster to go to war with those who were. These days the scientific revolution has brought to an end. I do not mean that, in the world of the future, differences of language, culture, personality, colour should cease to exist. Freedom under the law does not extinguish—perhaps it even enhances—a variety of individual and national expressions. That at least has been our experience of it in Europe, where we have been, for upwards of four thousand years, at once the guardians and the heirs of the slowly evolving and dynamic tradition of freedom under the law, self-disciplined freedom under a law carrying moral as well as positive authority. But the need for such a system of law and morality acceptable to the different races and creeds of the re-united human family is perhaps the most specific question which the scientific revolution compels us to face. Unless we face it, not all the increased control of our physical environment will save us from disaster. If we do, there is, perhaps, ahead of us an age of magnificence and splendour equalling the rosiest dream of poets, prophets and philosophers.

OBITUARIES

Prof. Ø. Winge, For.Mem.R.S.

Prof. Øjvind Winge, director of the Physiological Department of the Carlsberg Laboratory, Copenhagen, died on April 5, after a long illness at the age of seventy-seven.

Some years ago, at a ceremony in memory of the founder of the Carlsberg Laboratory, the brewer J. C. Jacobsen, Prof. Aksel Tovborg Jensen expressed that he felt he had a right to say that the men of the Carlsberg Laboratory have always administered the founder's rich endowments well ; and he continued: "Emil Chr. Hansen's and Joh. Kjeldahl's successors – Johs. Schmidt, S. P. L. Sørensen, Kaj Linderstrøm-Lang and Øjvind Winge – stand as leading lights of their sciences in Denmark and in the wide world as the equals of the greatest. They have done credit to their country and to J. C. Jacobsen, Denmark's great son."

A finer monument can scarcely be imagined, and with this as a background we shall remember Prof. Winge's achievements in genetics and brewing science.

Øjvind Winge was born in Århus in 1886; he started his career as an assistant to Prof. Johs. Schmidt at the Carlsberg Laboratory, and in 1921 he was appointed professor in genetics in the Royal Veterinary and Agricultural High School in Copenhagen. During this period he wrote a most outstanding text-book on genetics.

After the death of Johs. Schmidt in 1933, Øjvind Winge succeeded him as director of the Physiological Department of the Carlsberg Laboratory. He immediately took up research on yeast and here introduced the breeding of new hybrids by crossing and by the combination method, and contributed largely to our knowledge of the sex-life of yeast, its meiosis and its fertilization mechanism. The micromanipulation technique, elaborated by Winge and Laustsen as a necessary new microscope technique, is now commonly used in heredity investigations and yeast breeding.

Theoretical investigations of yeast first of all comprise the heredity conditions of the fermentation enzymes, and they have contributed to a knowledge of the nature of the gene and its mode of operation. Inheritance of the morphological properties has also been thoroughly investigated, and of heterosis, even to a pronounced degree, in certain crossing combinations of yeast, has been demonstrated.

Degeneration phenomena of normal brewer's bottom yeast, poor formation of spores, and low fertility in the spores have resulted in the fact that the combination breeding could not be utilized in the brewing industry. With the production of baker's yeast, on the other hand, both breeding and heterosis effect have been utilized.

In the science of brewing, Prof. Winge will be specially remembered from his breeding work with barley and hops. The plant-growing part of the work was carried out at 'Nordgården', the experimental farm belonging to the Carlsberg Breweries, where Prof. Winge was the scientific leader during 1938–62. With regard to barley, the variety Carlsberg II, on the market in 1953, attained a wide distribution as a high-yielding and acknowledged malting barley. Its outstanding qualities have left their marks on the European barley-breeding, often being used as crossing material in many European countries.

Winge's work on breeding of hops is especially familiar to specialists, but here it is highly esteemed. We are grateful to Prof. Winge for his review last year in *Comptes Rendus des travaux du Laboratoire Carlsberg* of his experiences: "Fifty Years of Hop Breeding at the Carlsberg Laboratory and Nordgården".

Prof. Winge received many scientific honours, among those many honorary doctorates, and he was elected a member of numerous scientific academies all over the world. For several years he was chairman of the group of natural scientists in the Royal Danish Academy of Science and Letters. He was an inspiring teacher, and his researches have been in front ranks both in connexion with yeast and with barley and hop breeding.

BIRGER TROLLE

Prof. R. McKay

ROBERT MCKAY, who died on May 4 at the age of seventy-five, was emeritus professor of plant pathology in University College, Dublin. Born in County Antrim, he received his early education there. He entered the Royal College of Science for Ireland in 1912, but his studies were interrupted from 1915 until 1919, during which time he served with the Royal Army Medical Corps in France. In 1920 he became an Associate of the Royal College of Science, and after a short period of service with the Seed Propagation Division of the Department of Agriculture, he joined the Plant Disease Division as Assistant to the late Prof. Paul A. Murphy. Following the death of Prof. Murphy in 1938, McKay became head of the Plant Pathology Department, which had been transferred to University College, Dublin, in 1926. In 1940 he was appointed to the post of lecturer and in the same year was awarded a D.Sc. degree of the National University of Ireland. He was appointed professor of plant pathology in 1945.

Prof. McKay's earliest work, in collaboration with Prof. Murphy, was concerned mainly with investigations on potato virus diseases. The technique worked out by these investigators of testing nucleus stocks of potatoes in the glasshouse and propagating such stocks under conditions of good isolation and rigid inspection has been largely responsible for the high repute in which Irish seed potatoes are held.

Onion mildew was probably the first of the fungal diseases worked on by Prof. McKay. With Prof. Murphy, he demonstrated the presence of hibernating mycelium in bulbs from diseased plants and that such mycelium could be killed by heat treatment. This is one of the first references to control of a fungus disease by heat treatment. Prof. McKay was the first to observe the germination of the oospores of the onion mildew fungus, and he showed that such spores could remain viable in the soil for up to twenty years. His work on diseases of cereals, particularly on *Septoria* leaf spot of barley, *Helminthosporium* leaf spot of oats and diseases of wheat caused by *Gibberella* has added much to our knowledge concerning these maladies. During the Second World War, flax growing was an important industry in Ireland, and Prof. McKay made a study of the diseases occurring on that crop. With his co-workers, he was the first to demonstrate clearly that the fungus causing the pasmo disease of flax was seed-borne. He carried out spraying trials on the control of apple scab over many years and he pointed out the importance of bud scale infection as a source of the apple scab fungus under Irish conditions. He studied beet diseases, and it was mainly due to his advice to the Irish Sugar Company that the practice of growing stecklings and sugar-beet crops in isolation was adopted many years ago. This practice has resulted in considerable reduction in such diseases as virus yellows and downy mildew in these crops. Among other diseases which he investigated were blight and scab of potatoes.

As adviser in plant pathology to the Department of Agriculture, he came in contact with most of the diseases occurring on commercial crops in Ireland. The knowledge thus gained he committed to writing in a series of monographs on diseases of individual crops. These monographs, so well and so profusely illustrated, are of immense value to growers, advisers and students of plant pathology.

His monographs on diseases were subsidized by the Irish Sugar Co. (sugar beet diseases), the Irish Potato Marketing Co. (potato diseases), Messrs. Guinness (cereal diseases), and the Flax Development Board (flax diseases). He was honoured by the Royal Horticultural Society of Ireland by the award of the Medal of Honour in 1948 and by the Royal Dublin Society by the award of the Boyle Medal in 1957.

Prof. McKay's work covered a wide field of research, but it was always carried out with thoroughness and perseverance. Whether fundamental or applied, all of it is sound and reliable.

As a teacher, Prof. McKay is remembered with affection and respect by hundreds of graduates in agriculture. As a lecturer he was painstaking in keeping up to date with his subject and in delivery he was clear and lucid. His lectures were often interspersed with witticisms, for he had a lively sense of humour. Under the auspices of the Royal Dublin Society he lectured on plant diseases to growers in almost every county in Ireland. His practical approach to the subject, and his awareness of their needs, earned him the appreciation of growers throughout the country.

J. B. LOUGHNANE

Miss E. H. Ekins, O.B.E.

MISS E. H. EKINS died at Hitchin, Hertfordshire, on June 4. She was eighty-four.

Emily Helen Ekins, daughter of A. E. Ekins, public health analyst for Hertfordshire, was educated at St. Albans High School, the University of Birmingham and Studley College, Warwickshire. She joined the staff of Studley College as a lecturer in horticulture in 1911, became acting warden in 1922 and principal in 1924, which position she held until August 1946. She was awarded the O.B.E. in recognition of her services to agricultural education in 1934.

Miss Ekins will long be remembered as one of the pioneers of agricultural and horticultural education for women. During the twenty-two years she held office as principal of Studley College she put her whole-hearted energy into consolidation of the administrative and educational sides. Building developments began in 1925; two appeals were launched, one to purchase the freehold of the property from the Warwick Estate and the second for the building of science laboratories and study bedrooms, and despite difficulties and frustrations, all-round progress in the history of the College was evident, brought about by her integrity and single-mindedness. She will also be remembered by hundreds of students who recognize and appreciate the vast amount of service which she so generously gave; she was the chief link in binding together each generation of students throughout her long association with the College, and many of them will remember with gratitude the help and encouragement she gave during their training and early days of their career.

NEWS and VIEWS

International Meteorological Organization Prize for 1964: Dr. F. W. Reichelderfer

Dr. F. W. Reichelderfer, formerly chief of the National Meteorological Service, U.S. Weather Bureau, has been awarded the International Meteorological Organization Prize for outstanding work in meteorology and international collaboration.

Dr. Reichelderfer was born on August 6, 1895, in Harlan, Indiana. He obtained a B.A. at the Northwestern University and joined the U.S. Naval Reserve Flying Corps in May 1918. After meteorological studies at the Harvard Meteorological Observatory he was appointed chief of Naval Aerological Service from 1922 and continued in that capacity until 1928. From 1928 until 1938 he served on U.S.S s *Oklahoma* and *Lexington*, and as executive officer on board U.S.S. *Utah* and in the naval air station at Lakehurst, New Jersey, for lighter-than-air craft. In this latter capacity he participated in a flight of the German zeppelin *Hindenburg* from Lakehurst to Rio de Janeiro and Frankfurt and back in 1936. He was appointed chief, United States Weather Bureau, in 1938, and retired from office in 1963. Dr. Reichelderfer actively participated in international meteorological work both in the International Meteorological Organization (1938–51) and the World Meteorological Organization (1951–64). He participated in preparing the International Convention adopted in Washington in 1947 which created the World Meteorological Organization. He served as the first president of the World Meteorological Organization, after the Convention entered into force, from 1951 until 1955. He was a member of the Organization's Executive Committee from 1951 until his retirement in 1963. He also participated in the international scientific activities in meteorology undertaken by the International Union of Geodesy and Geophysics. Dr. Reichelderfer is still active in a large number of scientific bodies in the United States, such as the National Academy of Sciences, the American Association for the Advancement of Science, the Institute of Aeronautical Sciences, the American Geophysical Union, the American Meteorological Society, over which he presided during 1945–46, and was a member of the National Advisory Committee for Aeronautics for many years.

Physical Chemistry in the University of Exeter: Prof. M. L. McGlashan

Dr. M. L. McGlashan, reader in chemistry in the University of Reading, has been appointed to the newly established chair of physical chemistry in the University of Exeter as from October 1. Dr. McGlashan was born in New Zealand. He graduated with a first in chemistry from the University College (now University) of Canterbury, Christchurch, and joined the teaching staff there. In 1949 he entered the University of Reading with the first Sims Empire Scholarship and obtained a Ph.D. degree for his work on theoretical chemistry, which he carried out with Prof. E. A. Guggenheim. He returned to Christchurch and was made senior lecturer; but in 1954 he joined the staff of the University of Reading. At Reading, Dr. McGlashan has won a reputation for his theoretical and experimental research in the field of intermolecular forces. He collaborated with Prof. Guggenheim on a variety of problems in statistical mechanics, and he built up an important research group making measurements of the thermodynamic properties of liquid mixtures and of gases. He was awarded a D.Sc. degree of the University of Reading in 1962 and in that year was given the title of reader. He is an enthusiastic mountaineer and is keenly interested in the theatre. His three Shakespearean productions for the University Drama Society are well remembered.

Department of Pure and Applied Chemistry: University of Strathclyde

The granting of a charter to the University of Strathclyde, formerly the Royal College of Science and Technology, Glasgow (*Nature*, 199, 1137; 1963), has approximately coincided with the completion of a new building to accommodate the University's chemical activities. The opportunity has been taken to reorganize these activities within a new framework by the formation of a Department of Pure and Applied Chemistry from the former separate Departments of Chemistry and of Chemical Technology. The new Department will be headed by a committee of professors, initially consisting of Profs. P. D. Ritchie (chemical technology), P. L. Pauson (organic chemistry) and M. Gordon (physical chemistry). Prof. Ritchie will be chairman of this committee for the academic year 1964–65. The Organic Chemistry, Physical Chemistry and Chemical Technology Sections of the Department have now moved into the new building on Cathedral Street, which provides greatly increased facilities for both teaching and research. Part of the old George Street building is being retained for the Inorganic Chemistry Section, and for the use of first-year classes. The staff has been augmented by the appointment to lectureships of Drs. S. R. Logan (physical chemistry), A. McAuley and G. A. Melson (inorganic chemistry). Dr. D. Pugh has been appointed to an assistant lectureship in theoretical chemistry, and Dr. W. E. Watts to a temporary lectureship in organic chemistry; and Dr. S. Affrossman has been promoted from assistant lecturer to lecturer in physical chemistry.

The Morecambe Bay Barrage

In reply to a question in the House of Commons on June 11, the Parliamentary Secretary to the Board of Trade, Mr. D. Price, said that Mr. E. Heath had asked the North-West Study Group to make a thorough assessment of the material already available about the proposals for a Morecambe Bay barrage. Under the scheme, a road and rail link would be incorporated enclosing the estuaries of the Leven and Kent and the waters would be used for public supply and for cooling locally sited power stations. The assessment was to be made in order that the Minister might determine whether a survey was needed and, if so, on what lines it should be undertaken. He would willingly receive any further information on the proposals, and proposals for such projects as a barrage and road links across the Solway Firth would also be readily received by the Study Group. The Ministry conceived it as part of the Group's duties to take these into its general reviews.

Aid to Developing Countries

In reply to a question in the House of Lords on June 18, the Chancellor of the Duchy of Lancaster, Viscount Blakenham, said that aid to developing countries from British public funds in 1963 was £158 million, compared with £150 million in 1962. For the financial year 1963–64, expenditure was £175 million compared with £148 million in 1962–63 and £160 million in 1961–62. These figures did not include the substantial flow of private investment, nor the £24 million of military aid provided in 1963 or the £30 million of military aid made available in 1963–64.

The Minister of State for Commonwealth Relations and for the Colonies, the Marquess of Lansdowne, said that, according to a joint statement issued by the British and Southern Rhodesian Governments on June 4, the latter Government had now agreed to assume responsibility for maintaining and developing the University of Rhodesia and Nyasaland as an independent institution open to all races. The British Government agreed to contribute £750,000 towards recurrent expenses up to 1967; a capital grant of £800,000 for building the teaching hospital; and some £300,000 towards general capital expenditure.

Commonwealth Bursaries Scheme

THE tenth annual report of the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme covers the year ended December 31, 1963, in which 24 bursaries were awarded, five for visits from the United Kingdom (two to Australia and one each to Canada, the Falkland Islands and Uganda), 15 for visits to the United Kingdom (four from Australia, five from India, and one each from Canada, Jamaica, New Zealand, Nigeria, Sierra Leone and Singapore), and four for visits between countries of the Commonwealth other than the United Kingdom (Pp. 8. London: The Royal Society and the Nuffield Foundation, 1964). Of the 56 applications considered, 43 were for visits to and 8 for visits from the United Kingdom. During 1963, besides the £5,000 from the Nuffield Foundation and the Parliamentary grant-in-aid to the Royal Society of £4,000, funds available included £1,200 from Canada, £796 16*s*. 3*d*. from Australia, £561 10*s*. 5*d*. from Pakistan, £500 from India, £400 from New Zealand, £150 from Sierra Leone and £100 from Ceylon.

Southern Hemisphere Telescope

FOUR British astronomers, led by the Astronomer Royal, Sir Richard Woolley, have just left for Australia for exploratory discussions with Australian astronomers of a proposal to build a 150-in. optical telescope there. These discussions may then enable the Royal Society and the Australian Academy of Science to put proposals to the United Kingdom and Australian Governments for the support of a full design examination for the telescope. At present there are 74-in. telescopes at the Radcliffe Observatory, Pretoria, South Africa, and at Mount Stromlo, Australia, but a larger telescope would improve ability to explore the southern sky. Such a telescope could examine such interesting objects as the Magellanic clouds and the centre of the Milky Way, which is not easily observed by existing telescopes in the Northern Hemisphere. It would also supplement the superb facilities for radio-astronomy already established in Australia. Funds for this exploratory visit are being provided by the Department of Scientific and Industrial Research. The other members of the party are: Prof. R. O. Redman (University of Cambridge) and Dr. A. Hunter and Mr. J. D. Pope, both of the Royal Greenwich Observatory.

Expenditure on Research and Development in France

ESTIMATES of French expenditure on research and development given in a pamphlet issued by the French Embassy, Service of Press and Information, indicate that the French Government expended some 4,000 million francs on research and development in 1962, or about 4 per cent of the budget (*France's Expenditure on Research and Development*. Pp. 17. London: Ambassade de France, Service de Presse et d'Information, 1964). Of this expenditure, 1,973 million francs went on civil and military atomic research and investigations and 1,519 million francs on military research, designing and prototypes. Of the total, 1,327 million francs were distributed through the Minister of State for Scientific Research and Atomic and Space Questions and 784 million francs through the Ministry of Education. Research expenditure by business firms in 1962 amounted to 2,300 million francs, or some 3·5 per cent of the turnover. This represented an increase of about 24 per cent on the previous year, and of it 40 per cent was in the engineering sciences (excluding electronics and electro-techniques), 28 per cent in electronics and electro-techniques, 15 per cent in chemistry and 10 per cent in physics other than electronics. Two-thirds of the industrial expenditure on research was borne by some fifty firms.

Railway Services in France

A FURTHER note issued by the Press and Information Service of the French Embassy describes the Société Nationale des Chemins de Fer and the French Railways, including its general organization, the magnitude of the traffic and the changes made since the Society was established in 1938 (Pp. 16. London: Ambassade de France, Service de Presse et d'Information, 1964). While the staff was reduced from 515,000 in 1938 to 348,500 in 1962, the productivity of staff increased by 160 per cent, the productivity of engine units by 360 per cent and that of passenger-carrying vehicles by 240 per cent, together with a relative drop of some 70 per cent in the consumption of energy compared with that prior to the Second World War. There are now 7,780 km of electrified track compared with 3,340 in 1938, and some 1,100 Diesel rail car units are in operation together with about 900 slip coaches, providing about 40 per cent of passenger train runs.

Society for Developmental Biology

THE inaugural meeting of the new Society for Developmental Biology was held on June 20 in Oxford. The programme consisted of scientific papers given by biochemists, botanists and zoologists. Dr. J. Gurdon (Department of Zoology, Oxford) discussed the significance of differences in the ribosomal RNA of haploid and diploid embryos of *Xenopus laevis* and described some changes resulting from nuclear transfer experiments. Prof. C. W. Wardlaw (Department of Botany, Manchester) then gave a general review of recent work on plant embryos, and Dr. E. Huehns (Department of Biochemistry, University College, London) concluded the morning session with a summary of his work on the structure of human embryonic hæmoglobins. In the afternoon, Dr. P. Burch (Department of Medical Physics, Leeds), in a paper entitled "The Molecular Basis of Self-recognition and Mitotic Control", speculated that the same genes may encode for both these types of control mechanism. His ideas provoked some discussion, which was continued after Prof. W. S. Bullough (Department of Zoology, Birkbeck College, London) had described the effects of adrenaline on mammalian epidermis, where a constant cell population appears to be maintained by parallel changes in mitotic rate and rate of cell death. The next two papers, given by Dr. K. R. Ashby (Department of Zoology, Durham) and Dr. J. M. Ioannou (Department of Anatomy, Birmingham), were concerned with the development of gonads and germ cells. Dr. R. M. Gaze (Department of Physiology, Edinburgh) discussed the development of neuronal connexions after combinations of two temporal or two nasal halves of the eye in *Xenopus laevis*. After tea, Dr. P. R. Bell (Department of Botany, University College, London) described histochemical and autoradiographic investigations of the distribution of DNA in fern eggs, then Dr. Webster (Department of Zoology, King's College, London) discussed enzyme activities in normal and regenerating *Hydra*. At a business meeting immediately after the scientific proceedings, the Society's constitution was adopted and its officers and committee elected, and it was planned to arrange two or three meetings each year in the future.

British Food Manufacturing Industries Research Association

THE annual report for 1963 of the British Food Manufacturing Industries Research Association has recently been published (Pp. 68. Leatherhead: The British Food Manufacturing Industries Research Association, 1964).

The topics in the research programme covered work on a wide range of materials, including cocoa and chocolate, sugar confectionery, bakers' prepared materials, jam, pickles and sauces, meat and fish products, oils and fats. During the year, the Association published and distributed to members six research reports, two scientific and technical surveys, one review of research and twenty-eight technical circulars. Members of the staff also contributed to fourteen other scientific and technical publications during the year. Both the Analytical and the Bacteriological Services report an increased number of samples examined for Association members. The Information and Library Services report that the number of abstracts published during 1963 rose to 2,306 (1,844 in 1962) and their value to members is shown by the fact that the Association received requests for a sight of the original article for one-third of this number (excluding patents). The Director reports that the Association is now prepared to accept 'sponsored' work, provided that the results are likely to be of interest to a substantial number of members. Special projects already in progress at the beginning of 1963 included two "Public Law 480" contracts for the U.S. Department of Agriculture on the biochemistry of bacon curing and the glyceride composition of cottonseed oil. This should assist the Association in many ways, not least in aiding in the procurement of some of the more expensive items of equipment that are becoming increasingly necessary in food research to-day.

Immunochemistry

It is a sign of the deep interest that is now being shown throughout the scientific world in the chemical aspects of immunology that a distinguished group of scientists should have launched a new scientific publication—a journal of immunochemistry—under the title *Immunochemistry* (1, No. 1; April 1964. Annual subscription rates: (*A*) for libraries, government establishments, research laboratories, etc., £15 (40 dollars); (*B*) for individuals who place their orders directly with the publisher and certify that the journal is for their personal use, £5 (15 dollars). London and New York: Pergamon Press, 1964). The editor-in-chief is the eminent American immunochemist, Prof. Dan Campbell, of the California Institute of Technology, who is supported by three regional editors and a distinguished honorary editorial board of international character. The remarkable increase in the number of scientific publications falling within the field of immunochemistry has obviously stimulated the production of this new publication by the editors and the Pergamon Press. With the passage of time, no doubt the size of the volumes issued will increase so as to justify, on physical grounds alone, the segregation of immunochemistry from the broader subjects of immunology and biochemistry, of which it must remain a part and which, at the present time, are already covered by numerous national journals completely or largely devoted to the subject. Past experience has shown repeatedly that a new specialist journal, when once launched by a competent editorial board and an established publishing house, is assured of success because the appropriate scientists cannot afford to be without the new journal, and university libraries and research institutes must see to it that it is available for consultation. It is probable that the many international journals of biochemistry and/or immunology could easily contain the present output of papers on immunochemistry for some years to come. However, if in future papers are to be published in full, one could hazard a guess that the number of papers in this exciting field will increase considerably within the next few years. In order that they should not be buried in the great mass of biochemical and immunological publications, the editors have shown real foresight in starting a new journal at this phase in the development of immunochemistry.

An Engineering Buyers' Guide

The Engineer Buyers Guide, 1964, gives particulars of nearly 2,000 firms which advertise in *The Engineer* or in the *Guide*, and covers chemical plant, electrical equipment, engines, machine tools and accessories, sheet metal machinery and a wide range of general products (Pp. 1016. London: *The Engineer*, 1964. 10*s*.). Firms are listed under the headings of the products and, in addition, an address section gives the various addresses, telephone numbers and codes for each firm. Details of trade and technical associations, national undertakings and exhibitions are also given. There is also a list of United Kingdom agents for foreign firms. This *Guide* will be a useful addition to the reference books in any workshop or laboratory.

Seismology at Eskdalemuir Observatory

In July 1910, Prince Galitzin himself supervised the installation of Galitzin seismographs at Eskdalemuir observatory, Dumfriesshire, the Observatory having been established two years before, primarily so that geomagnetic recording could be obtained free from the artificial disturbances, mainly from electric trams and trains, increasingly affecting Kew Observatory. When geomagnetic recording finally ceased at Kew, in 1925, the Galitzins were transferred from Eskdalemuir to the basement of the main building at Kew from whence, to eliminate disturbance due to wind shaking the building, they were transferred, in 1937, to a new vault in the grounds. In recent years, owing partly to an increased interest in the science of seismology and partly to the need for distinguishing between man-made nuclear explosions and natural earthquakes, more sensitive seismographs have been devised and thus vaults in quiet localities with solid rock foundations are required. The Eskdalemuir area proved to be one of the best in the British Isles and was adopted by the U.K. Atomic Energy Authority as the site of their seismic array which is centred 4 km north-east of the Observatory. Following a recommendation of the International Seismological Survey that all countries work towards the ideal of a world network, having stations at intervals of about 1,000 km, and after a recommendation by the Council of the Royal Society, the Meteorological Office decided that Eskdalemuir Observatory should be developed as its main seismological recording base, with research facilities, in the United Kingdom. A start has now been made by the building of a new vault in the hill-side near the observatory and the installation therein of the standard seismograph system being presented by the U.S. Coast and Geodetic Survey to some 125 stations over the world. The equipment was installed in time to record the large Alaskan earthquake of 28 March 1964. It is appropriate that Eskdalemuir, the field station, is near to Edinburgh where there is the Royal Observatory Seismic Research Group and where there is also to be the International Seismological Research Centre and the International Seismological Summary Unit. There is space in the new vault for research workers generally to install seismic apparatus under test, and there will be available a shielded cable from the vault to a planned new surface laboratory where data recording and processing equipment can be located. It is expected that routine seismological recording with the older instruments will cease at Kew at the end of 1964, and that the *Kew Seismological Bulletin* will then be replaced by an *Eskdalemuir Bulletin*.

Memorial Lectures of the Institute of Physics and the Physical Society

The Institute of Physics and the Physical Society has announced the following arrangements for its memorial lectures during 1964: 1963 *Guthrie Lecture*, the postponed 1963 Guthrie Lecture entitled "Magnetic Processes in

Weak and Moderate Fields" will be delivered by Prof. L. F. Bates (University of Nottingham), in the Physics Department of the University of Nottingham on September 9; 1964 *Rutherford Memorial Lecture*, Dr. P. H. Fowler (University of Bristol) will give the 1963 Rutherford Memorial Lecture entitled "Pi-mesons versus Cancer ?" in the School of Physical Sciences, the University of Sussex, on September 9; 1964 *Guthrie Lecture*, the 1964 Guthrie Lecture entitled "Radio Telescopes" will be delivered by Prof. Martin Ryle (University of Cambridge) in the Physics Department of the University of Keele, on October 1. All three lectures will be held in conjunction with conferences, and for those not attending the conferences admission will be by ticket, obtainable from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

Senior Fellowships for Short-term Visits to Australia

The Australian Academy of Science, from its J. Ellerton Becker Fund, is to provide substantial assistance to senior scientists to make short-term visits to Australia. Great flexibility is intended in the administration of the fellowship scheme; no restrictions are imposed with respect to nationality, occupation of the men to be appointed, or subject (within the natural sciences). The Academy welcomes enquiries from scientists of professorial standing or the equivalent who may wish to visit Australia in connexion with their own scientific researches or to confer with Australian scientists. Applicants are expected to have had prior correspondence with the relevant laboratories in Australia to ensure that their visit would be of advantage to scientists in Australia, as well as to the applicant. Fellows would normally be expected to participate in colloquia, and to deliver a limited number of lectures in one or more of the major cities of the Commonwealth. They would, however, largely determine their own programmes. A brief report is expected at the end of the visit. Maximum support will be on the basis of economy class return air fare and a subsistence allowance at the rate of £A5 per day. A minimum period of six weeks is desirable for any visit, and visits of longer duration would be welcomed. In special circumstances a minimum period of four weeks might be considered. Application forms for fellowships can be obtained from the Executive Secretary, Australian Academy of Science, Gordon Street, Canberra City, A.C.T., Australia. Applications will be considered and awards made quarterly; applications closing on January 31, April 30, July 31 and October 31 each year.

University News : London

At his request, Prof. P. M. S. Blackett will stand down as Pro-Rector of the Imperial College of Science and Technology at the end of the present academic year. Prof. O. A. Saunders has been appointed in his place as from October 1. Prof. Blackett will continue to hold a chair of physics. Promotions to senior lectureships at the Imperial College of Science and Technology are as follows: Mr. L. R. Barrett (chemical engineering and chemical technology); Mr. C. J. Carpenter (electrical engineering); Mr. R. G. Davies (zoology and applied entomology). Appointments to lectureships in the College are as follows: Dr. D. W. G. Ballentyne, Mr. R. S. Sidorowicz and Mr. R. W. Wilde (electrical engineering); Dr. D. E. Newland (mechanical engineering); Dr. J. M. Charap and Dr. S. Doniach (physics).

Newcastle upon Tyne

Mr. J. W. House, who is at present directing research schemes on "Rural Problems in North East England" for the Development Commission and "Migration and Mobility of Labour in North East England" for the Ministry of Labour, has been appointed to the second chair of geography. Appointments to lectureships are as follows: Dr. A. J. McComas (physiology); Dr. R. H. Stobbart (zoology); Dr. P. Gray (theoretical physics in the Department of Physics).

Southampton

Prof. J. E. G. Raymont, who has held the chair of zoology at Southampton since 1946, has been appointed to the chair of biological oceanography. Prof. Raymont will be head of the new Department of Zoology and Oceanography. Appointments to lectureships are as follows: A. F. E. Zagni (civil engineering); G. J. Rogers (electrical engineering); H. A. Kemhadjian and Dr. R. C. Smith (electronics); D. Harding, R. C. King and Dr. G. Lederer (mathematics); Miss P. Rothwell (physics); Dr. J. D. Burton (zoology and oceanography).

Announcements

Dr. Henri-Paul Koenig, at present a member of the staff of the National Research Council of Canada, has been appointed Scientific Counsellor at the Canadian Embassy in Paris. Dr. Koenig was previously professor of physics at Laval University and a past vice-president of the Canadian Association of Physicists. Dr. Koenig succeeds Dr. J. Gordon Malloch, who is returning to Canada and will retire shortly.

The Department of Scientific and Industrial Research has awarded a grant of £142,000 to the University of Southampton towards the cost of research on sound and vibration under the direction of Prof. E. J. Richards, professor of aeronautics and astronautics and director of the Institute of Sound and Vibration Research, for the four years ending July 31, 1967.

The summer congress of the Universities Federation for Animal Welfare will be held at Redland College, Bristol, during September 4–11. Further information can be obtained from the Universities Federation for Animal Welfare (Summer Congress), 7*a* Lamb's Conduit Passage, London, W.C.1.

A symposium on "High Temperature Inorganic Chemistry", arranged by the Inorganic Chemistry Division of the Chemical Institute of Canada, will be held at Carleton University, Ottawa, during September 2–4. Further information can be obtained from the Chemical Institute of Canada, 48 Rideau Street, Ottawa 2, Ontario.

An international summer course on "Some Physical Aspects of Oceanology in Shallow Seas", arranged by the Netherlands Universities Foundation for International Co-operation and sponsored by the North Atlantic Treaty Organization, will be held at Lunteren during September 1–18. Further information can be obtained from the Registrar, Netherlands Universities Foundation for International Co-operation, 27 Molenstraat, The Hague.

An international seminar for research and education in physics, sponsored by the Swedish Agency for International Assistance, the International Atomic Energy Agency and Unesco, will be held at the Institute of Physics, University of Uppsala, for one year commencing September 1. Forms of application and further information can be obtained from International Seminar, Institute of Physics, University of Uppsala, Uppsala.

A summer school in "Organic Spectroscopy", organized by the Royal Institute of Chemistry in collaboration with the Society for Analytical Chemistry, will be held at the School of Pharmacy, University of London, during September 6–12. The school will provide an organized and concise presentation of infra-red and nuclear magnetic resonance spectroscopy in terms of organic structures. Emphasis will be placed on fundamental principles and basic techniques. Further information can be obtained from the Royal Institute of Chemistry, 30 Russell Square, London, W.C.1.

GAS CHROMATOGRAPHY

An informal symposium organized by the Gas Chromatography Discussion Group of the Institute of Petroleum was held on April 7 at the Shell Centre, Waterloo, London, with Mr. C. S. G. Phillips in the chair. The symposium was preceded by the annual general meeting of the group followed by an introduction, on behalf of Shell, Ltd., by Lord Rothschild.

The first paper was given by Mr. J. C. Hawkes on "The Use of the Gas Density Meter for Process Monitoring and Control" in which he described the use of this detector for following the progress of vapour phase oxidation processes such as the production of maleic anhydride from benzene. Mr. Hawkes first listed the factors affecting the design of an instrument for applications of this sort. These were: (1) accuracy; (2) ease of calibration; (3) stability; (4) ease of maintenance; (5) facility for sample handling; (6) reliability; (7) provision of an output suitable for the control of the process. Passing on to a consideration of the detector itself, he said that the diameter of the orifice leading to the anemometer had an effect on the linearity of the detector. Enlargement of this orifice over a limited range increased both the linear range and the sensitivity. Increase of the carrier gas flow-rate through the cell, on the other hand, gave greater linear range without affecting the sensitivity, and by adjustment of these two parameters a detector having suitable characteristics could be obtained. A plot of gas flow-rate against thermocouple output rose sharply at first and then levelled out and, from a stability point of view, it was best to work in the level region where small changes in flow-rate would not affect the detector response. Other factors controlling the output of the detector were the nature and pressure of the carrier gas and its moisture content.

Mr. Hawkes next described a complete plant control system in which two density balances were utilized. The first of these was used alone as an on-stream detector to monitor continuously the input benzene concentration. In oxidation plants of this type the hydrocarbon reactant is carried through the system in a stream of air and this could be passed through the sample cell of the density balance to give a signal proportional to the benzene concentration. Process air from the same source, but containing no hydrocarbon, was used as reference gas, thus eliminating any response due to water in the carrier. The second detector was used with a column and sample loop to measure input hydrocarbon, output product and impurity concentrations. Use of a single chromatograph, with suitable valve system, for all these purposes avoided difficulties due to differences in sensitivity which might arise with more than one detector. Sample lines from the plant to the chromatograph were steam heated right up to the column oven. This was electrically heated with forced convection in order to achieve control to within 0·1° C with no temperature gradient. Pneumatically controlled solenoid valves were used for gas stream switching and these were normally automatically cam-operated by a programme unit. Results could be displayed on a recorder either as normal chromatograms or in compressed 'bar-graph' form. Concentrations were measured by peak height and, in order to obtain an accuracy within 0·1 per cent, it was necessary to maintain variables to within the following limits: gas flow-rate 0·3 c.c./min; oven temperature 0·1° C; gas pressure 0·4 mm; bridge current 0·002 amp. In order that sample switching and result presentation could be automatic it was also important that retention times of components did not vary by more than 1 sec. This required control of carrier flow-rate to within 1 c.c./min, column temperature within 0·3° C, and column pressure within 7 mm. Mr. Hawkes concluded by saying that he had had a number of instruments using density meters working for some time and had found this detector very satisfactory from the point of view of ease of calibration and stability. Other detectors, such as flame ionization, would, of course, give greater response, but for most of their applications the sensitivity of the density balance was quite adequate.

The second paper was given by Dr. C. S. F. Pine on the subject of "On-line p.p.m. Analysis by Gas Chromatography". He commenced by defining his field as that of direct measurement of substances present in vapour or liquid process streams at concentrations usually less than 100 p.p.m. Preconcentration techniques were generally unsuitable for on-line applications, but their use had become largely unnecessary due to the advent of ionization detectors.

On the use of in-line instruments by Imperial Chemical Industries, Ltd., Dr. Pine said that the first one had been installed in 1955 and used a katharometer detector for the determination of percentage amounts of mixed gaseous hydrocarbons. Their first p.p.m. instrument, installed in 1959, monitored hydrocarbons in oxygen over the range 0–3 p.p.m., sounding an alarm if the concentration rose above 2 p.p.m. This instrument used an argon ionization detector. Since this time instruments had been installed at the rate of about two per year, the earlier ones being home-made, but more recently some commercially produced instruments had also been used. The greatest advance in the development of in-line instruments during this time was the use of reliable automatic sampling valves for injection of liquids and gases. The type now widely employed was the linear movement sliding face valve developed by Imperial Chemical Industries, Ltd. Reasons for the spread of in-line instrumentation included its speed of analysis which was ideal for process control. Prevention of the formation of off-specification products had economic benefits in reducing the amount of re-purification necessary. In the intermediate stages of production, on-line analysis detected impurities which might be undesirable or dangerous in later stages.

Discussing the ways in which on-line chromatographs differ from their laboratory counterparts, Dr. Pine listed the following characteristics of control instruments: (*a*) fixed volume samples are used so that the recorder trace can be calibrated in terms of peak height; (*b*) multiple columns with switching and by-pass valves are used to reduce analysis time to a minimum; (*c*) column materials must be stable over a period of at least 12 months; (*d*) electrical circuits are arranged to show only signals from components of interest; (*e*) attenuators are used to adjust the scale range for each component and the recorder is usually started and stopped to give compact 'bar-graph' presentation; (*f*) programming units perform all the repetitive operations, thus necessitating stability of peak elution times; (*g*) lay-out and housing of the units are designed for easy sample handling and to comply with factory safety regulations; (*h*) on-line chromatographs must be designed to operate for long periods with a minimum of maintenance.

On-line p.p.m. analysis could be applied to most organic substances boiling below 300° C, but analysis of permanent gases was not practicable at the moment due to lack of a satisfactory detector. Concerning the problem of sample injection, Dr. Pine said that satisfactory sliding valves were now available from W. G. Pye, Ltd. With samples in the 1–20 µl. range the valve had sufficient heat capacity to act as an efficient flash vaporizer. Realizable peak height reproducibility was within about 1·5 per cent although the actual volumetric reproducibility of the valve was known to be better than 0·1 per cent in 5 µl. Failure of the sample to evaporate as a compact plug, among other factors, was thought to be responsible for this discrepancy. It would be preferable to work always with vapour samples, but if these were condensable complications arose with accurate pressure and temperature control. The sliding valves were also used, connected in a different manner, for other functions such as back-flushing and column switching. Difficulties often arose in the choice of a suitable stationary phase and in some cases multiple columns using different stationary phases were necessary. The greatest trouble usually arose from the large tailing peak due to the major constituent. If this was the last substance to be eluted time could often be saved by back-flushing it from the column after all the impurities had gone through. When some impurities followed the major constituent and were contaminated by its tail the position was more difficult. The best solution found was to collect the eluent over the period when it contained the impurities and to re-chromatograph this material on another column. The impurity peaks were then often completely resolved from that due to the now much reduced amount of major constituent. Preparation of columns for on-line p.p.m. work required that great care be taken to ensure as complete a removal as possible of volatile impurities and residual solvent. All the columns in present use were of the packed type as capillary columns had been found to lack sufficient long-term stability. In setting up these analysers all carrier gas pipework required to be thoroughly degreased and then baked out *in situ*, sometimes for some weeks, until contamination was reduced to a very low level.

Surveying the present position, Dr. Pine put forward a plea to manufacturers to provide more equipment for this field at a reasonable cost. Application engineering, normally supplied by the manufacturer, might well be left for at least the larger users to provide for themselves with consequent reduction in purchase price. The position was, he said, improving at least so far as American and Continental manufacturers were concerned. He concluded his paper by suggesting that future development might be aimed at increasing speeds of analysis, improving liquid handling techniques, collecting and publishing adequate retention data and developing linear and stable detectors which would extend the applicability of this field of gas chromatography.

Owing to the absence of Mr. J. Mawson, the paper on "Some General Aspects of Process Control with G.L.C. Analysers" could not be given, and it was left for Dr. H. J. Noebels of Beckman Instruments to put forward the view of the manufacturers in a contribution entitled "Applications of Industrial Gas Chromatographs to Process Monitoring and Control". He said that they had considerable experience in this field and that analysis of their instruments sold over the past year showed that 1 in 4 were equipped for continuous control of plant. The first chromatographs of this type were made in 1957, and since then about 2,000 instruments of all manufacturers had moved into the process field. Some six months ago Beckman produced an instrument which incorporated some new and important features. A major difference between process and laboratory instruments was that the latter were controlled by a competent operator who could take into account a number of factors such as varying base-lines. Control instruments, on the other hand, had to work completely alone practically continuously with perhaps, at the most, 2-h maintenance per month. The instrument must therefore be exact, automatic, dependable and capable of providing as short an analysis time as possible.

The last point became even more important when, as sometimes occurred, 5 min was taken for the sample to travel from its point of extraction from the plant stream to the analyser. A process instrument, Dr. Noebels said, must handle complicated streams and often was required to produce results expressed in a number of different ways. It might, for example, be required to estimate C_3, C_4 and C_5 compounds separately but to give a combined figure for C_6–C_{10} compounds. For this reason a considerable amount of stream switching was necessary. The valves utilized for this purpose were similar in design to those described by Dr. Pine and, when used for sample injection, had a lower limit of 1 µl. 'Teflon' was used in their construction for applications below 225° C, and they could be used at pressures of up to 350 lb./in.^{2}. Two types of detector were normally used: (1) four-element katharometer having a sensitivity such that full-scale deflexion was obtained with 50 p.p.m. of acetylene in ethylene; (2) flame ionization detector giving full-scale deflexion for 1 p.p.m. of acetylene in ethylene. Difficulty had been experienced in introducing flame detectors for plant control particularly in refineries, but their high dynamic range made them of great value for process monitoring applications. Dr. Noebels said that it was desirable to keep the number of controls on the front of the instrument as small as possible. Those which required occasional adjustment were mounted inside the case and pre-set, while repetitive operations such as gas flow switching, attenuation adjustment, etc., were carried out by a programme unit. This was normally of the type using magnetic tape but could be replaced by a cam-operated unit for those operators who desired a visual indication of the progress of the control cycle. Columns were heated by circulation of preheated air and required to have a life of at least six months with only a very slow change in retention times.

Eight basic column configurations were used: (1) single column; (2) single column with back-flush; (3) single column with back-flush and stripping: this elutes light compounds individually and the heavier ones as a group; (4) dual column: low boiling materials go through to a second column while high boiling compounds are detected after the first; (5) dual column with back-flush; (6) dual column with back-flush and stripping; (7) heart cut column: this was similar to the system described by Dr. Pine for chopping out a portion of the tail of a major peak; (8) 'flip-flop' column. This system comprised three similar sections designated the pre-splitter, storage and regrouping columns which were connected in series in the order given. It could be used for recombining a number of peaks when it was desired to estimate these together. Thus a sample containing substances eluting normally in the order A, B, C, D, etc., could be put on the column and allowed to run for a calculated time such that it was known that substance B had just entered the regrouping section. At this time fractions C, D and E could be at various points in the centre storage section, and if the flow through this part of the column were now reversed they would pass from it to the regrouping section in the reverse order (E, D, C). Fractions A and B would therefore emerge normally as separate peaks, but C, D and E, having the same distance to travel to the end of the column as they had each already travelled from the start, would recombine and would emerge as a single peak.

On presentation of results, Dr. Noebels said that 'bar-graph' readings were often insufficient and that a steady-state signal was useful for conversion to a pneumatic output for control of the plant. Such a signal could be obtained by amplification of the detector output and storage in capacitors, one for each component. Of actual analysis, 90 per cent was accomplished by peak height measurements, but integration was sometimes necessary. Features which made the gas chromatograph an ideal instrument for plant applications were its great speed (three components could be estimated in less than 1 min) and its high sensitivity. Applications had included such things as: (1) estimation of 0–50 p.p.m. of acetylene in benzene; (2) control of phthalic anhydride production; (3) determination of hydrochloric acid, chlorine and phosgene from 'Fluolube' on 'Teflon'; (4) determination of hydrogen in water in the range 0–5 p.p.m.; this estimation could be carried out in 5 min on a 50-µl. sample; (5) heart cut analysis of ethylene; (6) cascade control of distillation columns. He concluded by saying that generally a pay-off time of one year could be considered a good application of instrumentation.

C. D'Oyly-Watkins

PLACE OF BIOCHEMISTRY IN THE NEW UNIVERSITIES

At the Middlesex Hospital Medical School on April 16, the Biochemical Society held an evening discussion on "The Place of Biochemistry in the New Universities", a topic which aroused considerable interest and not a little feeling. In framing the discussion it had been agreed not to limit it strictly to the new universities but to include all universities in which biochemistry had only recently been introduced or was about to be taught.

Sir Rudolph Peters took the chair and, in the course of his opening remarks, pointed out that nowadays many aspects of biochemistry masqueraded under other titles. The new term 'molecular biology' had been introduced by Astbury and was, perhaps, valuable for getting money, but the claim that biochemistry only dealt with small molecules was just not true—we had always dealt with substances like proteins. With the present ramifications of biochemistry it was especially pertinent to discuss its role in the new universities.

Prof. R. A. Morton (Liverpool), under the title of "Biochemistry—Past and Present", traced the development of biochemistry with special reference to universities where the subject is now firmly established. The first department of biochemistry in Great Britain was founded at Liverpool in 1906, the inspiration of the department and of biochemistry in general at that time being derived from work on vitamins and nutrition. This and other departments were first set up in faculties of medicine, for it was from this direction that the need for teaching and research first arose. Very few honours courses in science faculties were started before 1939, but since the War the number of such courses had multiplied rapidly so that to-day most recruits to the subject have been trained as biochemists.

Over the years, the courses for medical, dental and veterinary students have changed and continue to do so. Although in the teaching, applications are not forgotten, they are subordinated to the need to expound the basic principles of biochemistry so that, for example, the clinical aspects of the subject come later from specialists.

Prof. Morton stressed that the biochemist should be competent in chemistry but must also have biological training and insight. Much had been achieved in the past, and no doubt would be in the future, by those who had detected, isolated and determined the structure of metabolites. Theories, for example, of photosynthesis or oxidative phosphorylation could be elegant even with an incomplete roll-call, but truer theories follow a full muster of the molecular species engaged—whether in biosynthesis or enzymatic mechanisms.

Biochemistry is now a central part of many curricula in biology. The sharp boundaries between sciences have gone; trespassing invokes only a shrug and territories are 'invaded' without apology. Chemistry, biochemistry and microbiology interpenetrate, and terms like genetics, biochemical pharmacology, neurobiochemistry and biochemical systematics all point to unification within diversity. This calls for psychological readjustment and reconstruction of teaching, organization and administration, on a scale easier to realize in a new university than an established one. There must be flexibility, perhaps, especially in M.Sc. courses. Prof. Morton emphasized that there is, however, a hard core of biochemistry which cannot safely be watered down.

In a period of expansion the penury of the past was easily forgotten, but it may be more difficult to sustain the peculiar excellence which raises university studies above the trivial.

In the subsequent discussion, Prof. J. N. Davidson (Glasgow) pointed out that biochemistry has tended always to suffer from being a hybrid subject. It grew up under the wing of physiology in most universities (occasionally under pathology) and only within recent years has begun to lead an adult independent life as a scientific discipline in its own right, rather than merely as a subdivision of some other subject. However, there are now disturbing trends. The chemists, who used to despise it, are now beginning to realize its importance and are tending to say that biochemistry should certainly be developed in all universities, but only as part of the chemistry department.

The biologists are belatedly taking an interest in biochemistry and are tending to follow the same unfortunate course as the chemists. By showing less interest in taxonomy and ecology and a greater interest in cell biology, they have realized that they cannot go very far in any biological discipline without biochemical help and they are therefore tending to say biochemistry must certainly be developed, but only as a part of a biological department.

Prof. Davidson went on to say that he was all in favour of breaking down barriers between sciences and of having the utmost collaboration between representatives of different disciplines, but was utterly opposed to the trend that biochemists may develop into mere laboratory assistants helping 'big brother biologist' to solve his problems.

Prof. E. A. Dawes (Hull) then presented the results of a survey he had made on present and future developments in Britain with particular reference to the place of biochemistry in the development of biological sciences in the new universities. There are seven new universities in England of which five have either planned or already initiated biological studies, and a sixth has it under active consideration. In Scotland, at the new University of Strathclyde, existing biology departments of the Glasgow Royal College of Science and Technology are to become part of a School of Biological Sciences, housed together in a separate new building and offering honours degrees in biochemistry, food science, biology, microbiology and general biological sciences.

The development of biological sciences in the new English universities has, without exception, followed the suggestion of the *ad hoc* Committee of the Royal Society (*Ann. Rep. Advisory Council on Scientific Policy, 1961–62*, H.M.S.O., 1963) that multi-professorial departments be created and under the one roof a variety of different biological disciplines be represented. East Anglia, at present, is the only school to have started teaching along these lines; Sussex and York both commence operations in October 1965, while Kent expects to do so in 1966.

At Sussex advanced teaching and research are being arranged round a number of research groups each headed by a senior member of staff; the units at present envisaged include animal physiology, biochemistry, microbiology and plant physiology, and the research activities of most of them will be strongly biochemical in outlook. The three-year honours course in biology will precede a fourth-year course of advanced study leading to an M.Sc. degree. At York fairly close co-operation between the departments of biology and chemistry is planned and the three-year honours course in biology will include specialization in the third year; biochemistry may be selected as one of the topics for this. Clearly, courses in the new departments are in the process of evolution and present information is of a provisional nature. It was evident, however, that belief in the necessity for a fourth-year M.Sc. course was universal in the new universities offering degrees in biology. To conclude, Prof. Dawes mentioned the newly created professorial departments in universities which do not have a medical school—Leicester, Southampton and Hull—and gave a brief description of the honours course in his own Department which includes a one-year course in cell biology, two years of chemistry and biochemistry in all three years.

In discussion, Prof. J. F. Sutcliffe amplified the plans for Sussex and emphasized the importance of the biochemistry unit to their scheme. Other speakers expressed misgivings on the brevity of the basic chemistry courses in many of the biological sciences syllabuses and pointed to the flimsy foundations on which the biochemical content of the course would have to be built. The chairman stressed that all biochemists should be taught to work with small amounts of substances.

Prof. H. L. Kornberg (Leicester) outlined the development of biochemistry within the framework of a three-year course leading to a degree in cell biology, and discussed the reasons for this approach rather than the more conventional honours course in biochemistry. At Leicester it was felt that, at the undergraduate level, biochemistry might be most effective as the central theme of an inter-departmental degree course jointly planned and executed chiefly, if not exclusively, by specialists in biochemistry, genetics and physiology, but that chemistry to an advanced level, and other physical and biological specialities to less-advanced levels, would also form indispensable parts of the degree structure. Such a course would provide graduates suitable for teaching at the secondary modern school level, Civil Service experimental officer grade, or the equivalent level in hospital or industry. More able students would take one-year M.Sc. courses in the particular speciality that has aroused their interests and this would be a prelude to the orthodox three-year period leading to a Ph.D. The flexibility which this proposed course, planned for 1967, would confer, by virtue of the wide biological interests of the academic staff concerned, was emphasized.

Prof. Kornberg then described plans for the new School of Biology at Leicester, which, with more than 90,000 sq. ft. of usable space, will house not only all the existing biological science departments but possibly also biological specialities as yet unborn. A department of genetics has recently been established and similar developments in general physiology are to occur, while the appointment of Dr. E. R. Redfearn to a second chair of biochemistry at Leicester had just been made.

The development of biological chemistry in the University of Manchester was described by Prof. A. J. Birch. An honours school of biological chemistry was established in 1961 in the Faculty of Science with studies extending over three years and with considerable emphasis on chemistry and biology. At present, this is achieved by exclusion of biochemistry from the first-year courses, and the intermediate examination at the end of the first session includes chemistry, physiology and either botany or zoology. The first two terms of the second year are divided equally between chemistry and biochemistry, both of which are included in the Part I examination held at the end of the Easter vacation of the second session. Biochemistry occupies the whole of the students' time during the summer term of the second year (this being a full teaching term) and throughout the third year, with the exception that during the third year students attend short ancillary courses chosen from a wide range.

A full tutorial system is operated during the summer term of the second year and throughout the third year, and less frequently in the earlier parts of the course. It is hoped in future to extend the full tutorial system to all three years. Further developments planned for the future include an introductory biochemical course in the first year and it is hoped that a single, fully integrated course in biology may be established.

In planning the courses for the new honours degree, the aim has been to give students a thorough understanding of the chemical and biological bases of the subject and a good appreciation of general biochemistry without emphasizing any one specialized aspect. Dr. G. R. Barker elaborated on some aspects of the course.

The final speaker was Prof. T. A. Bennet-Clark (Norwich), who discussed the role of biochemistry within a school of biology and described developments at East Anglia, where a special feature is being made of the biological sciences. Biochemistry is fundamental and must be incorporated in the courses, but this could only be achieved by elimination of dead wood from the more classical aspects of the biological parts of the curriculum. A critical problem is that of recruitment, for the ideal aspirant should have a good background of mathematics, physics and chemistry in addition to his biology; this poses almost insuperable difficulties in the schools and forces the university school of biology to teach in the first year appropriate aspects of mathematics and physics.

A three-year undergraduate course is operated at Norwich which provides during the first two years, in addition to chemistry, mathematics and physics, a background having a general physiological and genetical basis. The third year permits specialization in which three options are selected from a present list of eight, of which (1) biochemistry and (2) biophysics and biophysical chemistry are two. A fourth-year M.Sc. course permits further specialization: a course in biophysics is run by Prof. J. Dainty and, in the future, a course in food science will be available. Prof. Bennet-Clark made the point that a three-year first degree course is by no means an adequate training for any career and hence the desirability of having a fourth-year M.Sc. course.

Sir Rudolph Peters then declared the meeting open for general discussion and some lively comments ensued. Concern was expressed that the plans for the new universities did not include strong biochemistry departments in which the teaching was securely based on chemistry. Latent fears for the emergence of a breed of 'tame biochemists' were in evidence.

Dr. G. R. Barker aroused much interest and support for his proposal that a new advanced-level subject 'Physics with Mathematics' should be instituted by the examining boards. Such a paper would go a long way towards solving the problem of university entrance requirements for the biological sciences. The meeting passed a resolution that the Committee of the Biochemical Society consider whether the Society might approach the examining boards in this connexion. Prof. Morton said how important it was that faith should be kept with the school teachers so that they knew precisely what the university biologists wanted of their pupils.

The meeting was universally adjudged to have been a timely and instructive one, and the large attendance reflected the widespread interest in developments in the new universities.

E. A. DAWES

AGRICULTURAL SOIL SCIENCE

TEN years have elapsed since the popular Easter School in Agricultural Science was inaugurated at the School of Agriculture in the University of Nottingham. The eleventh meeting, which was held during March 23–26, was marked by a symposium and colloquium on "Experimental Pedology". Some 130 scientists, experts in a wide range of disciplines and assembled from the British Isles, Europe, Israel, the British Commonwealth and the United States, testified to the general interest in soil science and to the particular concern for that aspect of the subject dealing with the genesis, properties and classification of the soil profile.

After a welcome to the University from Mr. B. L. Hallward, the vice-chancellor, the delegates settled down to a programme developing logically from organic geochemistry and the chemistry of rock surfaces through the organic and inorganic agencies of soil profile development studied *in situ*, in the laboratory and by model simulating processes, to the final analysis of experimental pedology in relation to soil classification.

The assessment of release and redistribution of major and trace elements in weathering rocks, in the soil profile and of their loss in drainage water has always been at the basis of soil research. The construction of a rough balance sheet from such data can be instructive and informative, but its value is greatly enhanced when the agencies responsible for the changes can be identified qualitatively and quantitatively.

The symposium not only illustrated the enormous wealth of information that exists on the results of soil changes but also the dearth of precise knowledge of the processes and their dynamics. Too little is known of the movement of either simple ions or of more complex substances, nor do we yet know enough about the natural processes of clay formation or of the precise roles of living organisms or their products in the development of soils. Discussion of the kind provided by this School serves an excellent purpose by directing attention to these needs and should serve in turn to kindle the fire of fresh ideas and extend the mind to further effort.

This stimulating and provocative symposium was noteworthy for its unobtrusive yet skilful organization, tribute for which must be paid to Prof. Hallsworth and his many helpers. His forthcoming departure to the Commonwealth Scientific and Industrial Research Organization (*Nature*, 199, 1237; 1964) carries both our good wishes and thanks for his inspiration of the Schools.

R. I. DAVIES

BRITISH COAL UTILIZATION RESEARCH ASSOCIATION

THE annual report for 1963 of the British Coal Utilization Research Association*, which includes a list of publications and of staff, records a total staff at the end of the year of 67 scientists, 109 research officers and research assistants, 47 in clerical and administrative grades, and 90 in industrial grades. An extensive survey was completed of the behaviour of a variety of types of coals with a wide range of ash-fusion characteristics, in an attempt to relate coal type and ash composition with the formation of clinker and slag in shell boilers. There was very little clinker formation with any of the coals but sufficient slag on the ignition arch to attempt corelations. The opportunity was taken to investigate the formation of deposits on end-tube plates which, with some coals, can be very troublesome.

In domestic heating, the first of four main programmes was to try to improve the amenities and performance of the conventional central heating boiler fired with smokeless fuel. The second programme is to try to devise a means of burning bituminous coals smokelessly in a central heating boiler; the third, to design a reliable and cheap heat meter; and the fourth, to obtain reliable information on the amount of smoke emitted from domestic appliances for a range of fuels. Investigations of mineral deposits and corrosion in water-tube boilers are increasingly directed to problems which may arise in future in the large power-station boilers operating at very high steam temperatures and pressures. Four proprietary alloy steels of different composition have been tested above 600° C in the biggest of the three laboratory combustors when using a range of coals including the normal test fuel of high chlorine content, and further extensive trials have been carried out on the town-gas-fire combustor.

Much work on slagging gasification during the year has been concerned with filling gaps in the knowledge of slag formation and behaviour and other facets of the operation of the slagging fixed-bed process. Confirmatory laboratory tests in the high-temperature viscometer have shown that, where refractory mineral matter is being dissolved in a slag, the apparent viscosity may attain a value several times that of the viscosity of the homogeneous slag that is finally formed. Investigations have been made into the changes of the chemical composition of slags under particular operating conditions, and particularly of the volatilization of silica and of the loss of iron. Systematic investigation of the slagging properties of the ashes of British coals was completed during the year, and some attention was also directed to the crystallization properties of the slags.

Basic investigations, during the year, of combustion and gasification were directed at exploring the phenomena of pre-ignition heating and the devolatilization of pulverized fuel particles in the laminar flow furnace. Measurement of the thermal conductivities and diffusivities of coals of temperatures up to 900° C has been completed. In observing the structure and reactivity of reactor graphites, comparisons have been made of the porous structure parameters measured at high and at room temperatures, and the structure of graphites has been further examined before and after their oxidation. More fundamental investigations of the chemical structure of coals have thrown further light on the distribution of groups containing hydrogen, and particularly the differences in fractions of aromatic hydrogen and of hydrogen in methylene groups in coals of different ranks. A two-pronged attack on the mechanism of decomposition in the coking temperature range has been further advanced and, from now on, basic investigations of the physical and chemical structure of coal will be directed more to understanding the reactions that occur in various combustion systems.

Fundamental investigations of simple breakages in commercial mills have been greatly advanced during the year. The examination of the stress distribution in thin particles loaded in a model of a ring-mill using photoelastic techniques shows that the primary failure occurs along lines of maximum tensile stress. The distribution of mineral matter in samples of pulverized fuel has also been examined.

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* The British Coal Utilization Research Association. Annual Report, 1963. Pp. 92. (Leatherhead: The British Coal Utilization Research Association, 1964.)

THE ARTHUR D. LITTLE RESEARCH INSTITUTE

IN the annual report for 1963 of the Arthur D. Little Research Institute, Dr. F. N. Woodward, the director, reviews some of the outstanding developments in the first seven years operation of the Institute *. These include the work on interaction between certain metals and water at very high temperature, hydrogen embrittlement investigations, which appear to be revealing at least one pickling method which could safely be applied to very strong steels after heat treatment, and tempering without causing hydrogen embrittlement.

Fundamental investigations of the release of corrosive vapours from wood under tropical conditions have given a technique which appears to be capable of reducing significantly the corrosiveness of kiln-dried woods. Observations on the chemical modification of cotton have given a technique for increasing crease-resistance, and work on the chemistry of ethylene sulphide has also yielded patentable techniques and products. Two basic investigations of potential long-term significance are concerned with the crystallization and properties of high polymers and with gaining an understanding of photoconductivity in organic compounds generally and polymers in particular.

In the Biochemistry and Biophysics Division, wheat proteins and anti-tubercular factors in milk whey received considerable attention, while the work on peat isolates of industrial value was expanded and preliminary work on the fractionation of inorganic polyphosphates carried out. In carbohydrate chemistry, several promising new polymers were prepared from glucose and its derivatives and the principle of co-polymerizing small amounts of carbohydrate with cheap, readily-available monomers. to give reactive co-polymers, has been demonstrated, These afford an easy route for introducing reactive groups to improve the dyeing properties and hydrophilic groups to improve the water-adsorptive power of conventional man-made fibres. The investigation of the emission of corrosive vapours from wood continued for a third year and methods for the analysis, by gas liquid chromatography, of the volatile acids and neutral compounds of wood have been worked out and applied to the assay vapours arising from the exposure of wood-shavings to high humidity and tropical temperatures.

In sulphur chemistry, white crystalline polymers have been prepared from ethylene sulphide; mercaptans have been found to react with ethylene sulphide to give products which are also mercaptans and which further react with ethylene sulphide. In physical-organic chemistry, an extensive search has been made for high-temperature-resistant materials in organic, organo-metallic and inorganic chemistry, while the physical chemistry section has been mainly concerned with the crystallization and properties of polymeric materials.

Recently, facilities have been developed for investigating the electrical properties of materials. In metallurgy, the reaction between metals and water vapour up to 1,500° C and above has been further investigated for aluminium and aluminium–uranium alloys and zircaloy-2. The investigation of the adhesion between molten glass and metals has been continued in order to improve understanding of the fundamental principles governing sticking between hot glasses and a metal mould while the glass is being blown to shape during manufacture of glassware.

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* Arthur D. Little Research Institute. Annual Report for 1963. Pp. 48. (Inveresk, Midlothian: Arthur D. Little Research Institute, 1964.)

LUMINESCENCE OF ENSTATITE ACHONDRITE METEORITES

By C. J. DERHAM*, Dr. J. E. GEAKE and G. WALKER

Department of Physics, Manchester College of Science and Technology

WE recently reported the red luminescence of three meteorites of the enstatite achondrite type under proton excitation[1], and are now able to give some further information, including revised estimates of the radiant efficiency.

Our published spectrum for each meteorite shows a weak blue peak at about 4000 Å and a strong red peak at about 6700 Å (for example, Fig. 1), but with different red/blue peak intensity ratios for the three meteorites; we afterwards found that the differences were just as great between different samples of the same meteorite as between the three different ones. This suggested that two separate constituents of an inhomogeneous mixture were giving the red and blue luminescence, respectively. We then concentrated mainly on the Bustee meteorite, and a crushed sample was separated, by visual appearance under a microscope, into its main constituents. These were identified as follows: white enstatite ($MgSiO_3$), the main constituent; diopside ($CaMgSi_2O_6$), present as grey grains showing exsolution lamellæ of enstatite; brown crystalline oldhamite (CaS); and finally osbornite (TiN), present as a few minute golden octahedral crystals †. The latter two minerals are only known in meteorites, and do not occur in terrestrial rocks. These observed minerals agree with the known constituents of this meteorite[2] We then investigated the luminescence behaviour of each constituent separately, under proton excitation. The enstatite part was found to give a spectrum like that already found for samples of the mixture. The diopside luminesced weakly, if at all—it was difficult to free it from enstatite, and the resulting spectrum was a weak version of the enstatite one, with no additional features. It was, therefore, probably just the spectrum of the enstatite contamination. The oldhamite constituent, which was only present in small quantities, also showed the spectrum of residual enstatite, but with an additional yellow peak at about 5800 Å. This peak is not usually evident in the spectrum of the mixture, probably because of the small amount of this substance present. Also, this constituent was found to damage very quickly, and would not contribute for long to the spectrum of the mixture. A laboratory sample of CaS was found to show the same yellow peak, and also to damage rapidly. There was insufficient osbornite to obtain a spectrum, but when a few crystals of it were observed visually under proton excitation no luminescence was seen.

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* Now at Barnet College of Further Education.

† We also found osbornite crystals in a sample of the Bishopville meteorite; this mineral has not, so far as we know, previously been reported anywhere except in the Bustee meteorite and its presence further emphasizes the great similarity between these two meteorites.

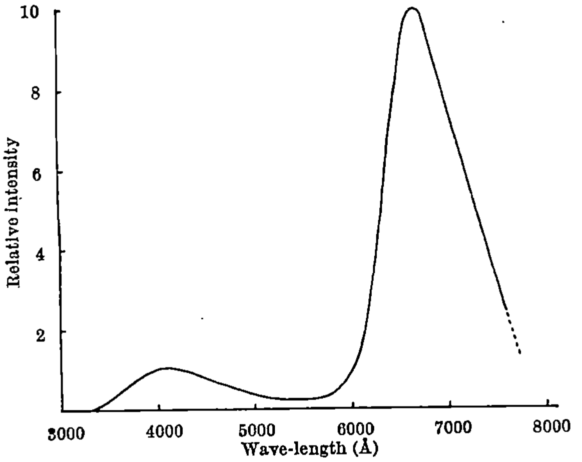

Fig. 1. The luminescence emission spectrum of a dust sample from the Bustee meteorite under 40-keV proton excitation

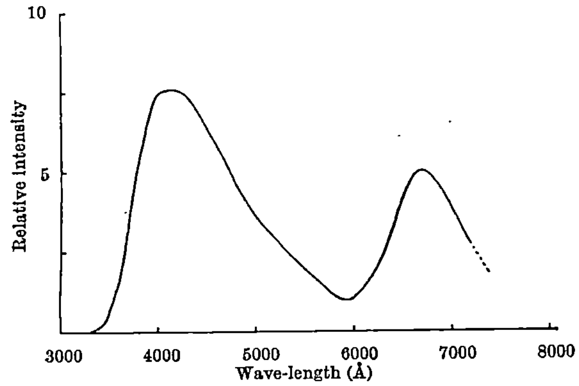

Fig. 2. The spectrum of the 'blue' constituent of Bustee

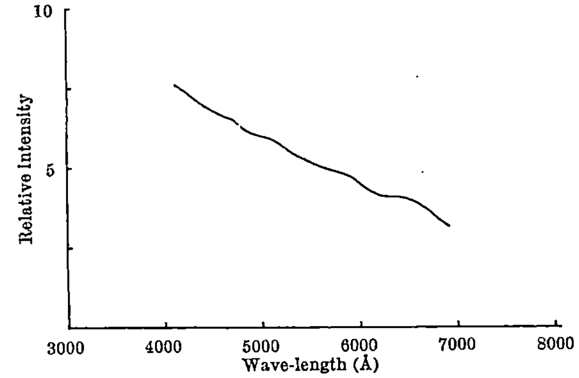

Fig. 3. The luminescence emission spectrum of Bustee under ultra-violet excitation

We conclude that the enstatite is probably responsible for all the observed luminescence. It is interesting to note that Garlick[3] has suggested, from a study of the published luminescence spectra of synthetic phosphors, that the red peak of the luminescence we observed was probably due to $MgSiO_3$ with manganese present as an impurity. The blue peak has not yet been explained.

However, it appeared that the enstatite was producing both the red and the blue peaks, whereas the variability of the red/blue peak intensity ratio suggested that two separate constituents were responsible. In an attempt to resolve this we then observed visually a mixed sample of the crushed meteorite, under proton excitation. It was seen that there were indeed some grains luminescing red and a smaller number luminescing blue (and some with a mixture of these colours). By noting their positions, the most distinctly 'red' and 'blue' grains were then separated out; the luminescence spectra of a crushed 'red' grain and a crushed 'blue' grain were then obtained separately. The 'red' grain gave a spectrum which was much the same as that for an average sample of the meteorite as a whole, with a slightly weaker blue peak; the spectrum of the 'blue' grain is shown in Fig. 2. The two spectra thus show red and blue main peaks, respectively, but each shows a certain amount of the other colour. The efficiency of the 'blue' constituent is about the same as that of the 'red' one, but its contribution to the total emission is small because it is only present as a small proportion of the mixture. X-ray powder analysis showed both grains to be orthoenstatite.

Later these observations were reinforced by obtaining a thin section of the Bustee meteorite, so that it could be observed visually under proton excitation with its constituents in their original configuration. It was found to luminesce red over most of its area, but with one small zone bright blue. When the section was transferred to a microscope, this 'blue' area appeared to be part of an enstatite region, but with no consistent differences in appearance as compared with its 'red' surroundings.

We conclude that while the enstatite constituent of this meteorite is responsible for its luminescence, it may luminesce either predominantly red or predominantly blue, depending on some factor as yet unknown. Investigation of this continues. We find, however, that samples of the meteorite as a whole always luminesce predominantly red, because the blue part is only present as a small proportion.

In our earlier communication[1] we gave a rough estimate of 20 per cent for the radiant efficiency of the meteoritic material, by comparing it with a readily available phosphor—ZnCdS–Ag, in the form of 'Sirius' screen. We have since been able to test this by comparison with other known phosphors, kindly supplied by Prof. G. F. J. Garlick. These include ZnS–Ag which emits in the blue, Zn_2SiO–Mn a green emitter, and $MgSiO_3$–Mn, the red emitter mentioned above. Efficiency figures for these phosphors under proton excitation do not seem to be available, but Bril and Klasens[4] give efficiencies under excitation by 20-keV electrons as follows: ZnS–Ag, 21 per cent; Zn_2SiO_4–Mn, 8·5 per cent; $MgSiO_3$–Mn, 6 per cent. We have now compared these phosphors with each other and with the meteoritic material under 40-keV proton excitation, with the same beam conditions. The relative emission efficiencies have been calculated from the relative intensities and integrated areas of the corrected spectra; we find them to be related as follows: ZnS–Ag : Zn_2SiO_4–Mn : $MgSiO_3$–Mn $= 2 : 1 : \frac{1}{4}$. The first two of these are relatively in rough agreement with the electron figures, whereas the $MgSiO_3$–Mn is low by a factor of about 3 compared with the other two. This may be because the relative efficiencies of these phosphors really are different under proton as compared with electron excitation, or because of sample variability.

The efficiency of the meteoritic material was found to be about one-sixth of that for $MgSiO_3$–Mn, which gives an efficiency ranging from just under 0·5 to about 1 per cent, depending on which phosphor one compares it with.

Of the meteoritic material we now find samples of Khor Temiki to be generally more efficient than Bustee or Bishopville, although variation by factors 2 or 3 from sample to sample is common. The brightness of the emission is also seen to vary from place to place on the dust samples, and the brightest parts may well have an efficiency consistent with its identification as $MgSiO_3$–Mn[3], which is itself one of the most efficient of the known red-emitting phosphors. The meteoritic material differed from this phosphor in showing no visible afterglow, presumably due to impurities.

We now conclude that although our original comparison with ZnCdS–Ag was instrumentally correct, the figure we quoted for the efficiency of this phosphor was too high.

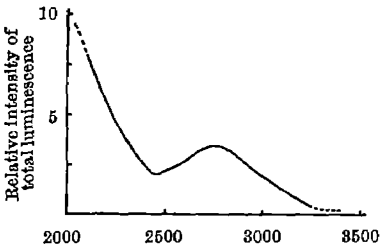

Fig. 4

The discrepancy seems to be due to the rapidity with which this material damages under proton bombardment. We find other phosphors to damage slowly, and we allow for this; however, the efficiency of ZnCdS–Ag is found to fall by a factor of 10 in the first 90 sec, for a typical beam energy and current (40 keV, 0·2 μamp). This was only revealed when we completed a sample-holding turret which enables four samples to be loaded at once and irradiated alternately without disturbing the vacuum or the proton beam. The intention was to permit more reliable comparisons of sample efficiencies; but it also means that the beam can be set up on one target, and the luminescence emission of the others can then be recorded right from the instant of first exposure. Any rapid initial damage is then easily seen. We conclude that previously our ZnCdS–Ag samples had always been appreciably damaged while the beam was being positioned and adjusted, and that the efficiency by the time we came to make measurements was usually less than 1 per cent. One of the other comparison phosphors, ZnS–Ag, was also found to damage rapidly, and it has already been mentioned that the CaS constituent of the meteoritic material damaged rapidly. It is interesting that all three of these materials are sulphides. In contrast to this very rapid damage, we find that the main material of the Bustee meteorite takes about 4 h, under the same beam conditions, for its efficiency to drop by a factor of about 10. We also find that it is possible to produce almost complete recovery of Bustee samples by heating. Investigation of this recovery process continues, as it is clearly of interest in considering the possibility of phosphors continuing to work on the lunar surface.

In considering the relevance of this meteoritic luminescence to that of the Moon, it is of interest to know the effect of different types and energies of excitation on the luminescence emission. Samples of Bustee dust were therefore excited in several different ways.

In our apparatus we can irradiate samples with protons of energies from 5 keV to more than 100 keV. We find the spectral profile of a sample of Bustee dust to be the same over this range of energies (Fig. 1), with an overall intensity roughly proportional both to accelerator voltage and to beam current. However, under ultra-violet excitation (mainly 2537 Å) the luminescence is quite different; it is almost white in colour, with the spectral profile shown in Fig. 3, and a very low efficiency—about 1/100th of that for proton excitation. The relative effectiveness of different ultra-violet wave-lengths in producing luminescence is shown in Fig. 4; this was found by recording, with a photomultiplier, the total luminescence emission from a sample under excitation by the output of a calibrated monochromator which was scanned in wave-length. The source was a deuterium lamp, and a Wratten *2B* filter in front of the photomultiplier absorbed any ultra-violet reflected by the sample. The peak of effectiveness appears to lie below 2000 Å.

We have also been able to observe visually the luminescence produced by several other sources of excitation; in each case it was red, and we estimated that both the colour and the relative efficiency appeared to be about the same as that seen under excitation by our low-energy proton beam. The excitation sources tried were: (1) protons at 2 MeV and 4 MeV from a Van der Graaff generator; (2) electrons from 5 keV to 40 keV; (3) X-rays, from a tube with a copper anode, run at 40 kV, giving a peak at about 1·5 Å and a continuum extending to a few Å. There appears to be an energy threshold in the region we are unable to investigate, between 2000 Å and a few Å, at which the low-efficiency white luminescence changes to high-efficiency red luminescence.

We thank Prof. W. S. MacKenzie for his geological help and advice and for carrying out the X-ray powder analysis. We also thank the British Museum (Natural History) for the loan of the meteorite samples; also Dr. J. M. Calvert for the use of the Van der Graaff generator, Mr. J. B. Shepherd for help with the electron excitation, Mr. W. Hughes for help with the X-ray excitation, and Mr. J. McConnell for help in constructing the apparatus. This work was sponsored by the U.S. Air Force Cambridge Research Laboratories, under contract *AF* 61(052)–379 through the European Office of Aerospace Research, U.S. Air Force.

[1] Derham, C. J., and Geake, J. E., *Nature*, **201**, 62 (1964).

[2] Prior, G. T., and Hey, M. H., *Catalogue of Meteorites* (1953).

[3] Garlick, G. F. J., *Nature*, **202**, 171 (1964).

[4] Bril, A., and Klasens, H. A., *Philips Res. Rep.*, **7**, 401 (1952).

AN ELECTRICALLY CHARGED TORQUE PENDULUM

By DR. ERWIN J. SAXL
Pin Hill, Harvard, Mass.

USING precisely timed parts of each period of an oscillating torque pendulum, a determination of individual short-time events was made in sequence.

The disk of the torque pendulum with which the subsequent observations were made is suspended from an isoelastic wire, the modulus of which is constant to 0·5 parts in 10^6/deg. C. Within the multiple enclosures, the temperature was maintained to ± 0·5 deg. C. The elastic restoring force of the wire remains, therefore, constant; only the difference is shown that is produced due to the slight winding-up and winding-down of the rotating pendulum. The pendulum oscillates in a Faraday cage which can be charged electrically together with the pendulum.

The disk is set automatically for each swing by attracting it electro-magnetically against a stop and holding it there by means of a time-delay for a pre-set length of time prior to release. This rest period frees it from the influence of ambients such as parastic oscillations of mechanical nature, eliminates the influence of the Earth's precession and restores the potential mechanical energy of the pendulum quantitatively for each swing. A mirror is attached to the mounting of the disk in a manner similar to a galvanometer.

Part of each period was timed by photoelectrically gating the output of a crystal controlled oscillator (which is precise to 3·5 parts in 10^{-8} sec) after the release from the stop and while the disk rotates at a position about midway between release and return where the speed of the disk approaches maximum. The time is measured during a part of the total cycle and includes both clockwise and counter-clockwise rotation. The beam of light starts the electronic counter on its clockwise motion and stops it on its counter-clockwise return. Direct digital printing is obtained without analog-to-digital conversion since the readout constitutes elapsed time. This simplifies rapid and accurate recording and facilitates input into data-handling systems.

Unexpected phenomena were noted as follows:

(1) When the pendulum was charged electrically with different, carefully controlled electro-static voltages (together with its equipotential shields), it was observed that positive and negative charges caused different delays (Fig. 1). A positive charge caused the pendulum to rotate slower, as a rule, than when the pendulum was charged negatively. The grounded pendulum swung fastest. (There are exceptions to this rule at times.)

(2) The time of swing follows in first approximation a square law (Fig. 2) with a small, negative bias. In view of this asymmetry there must exist, therefore, effects in addition to the usual eddy current damping since the latter would cause a symmetrical time delay.

(3) There exist differences between the influence of voltage on the pendulum at different times. While consistent within themselves, there may be one time-delay in spring and another one in mid-summer. Diurnal as well as seasonal variations were found.

(4) Unusual variations of the pendulum period were noted particularly during solar and lunar eclipses (Fig. 3).

In the foregoing precise determinations the influence of seisms, microseisms and changes in the Earth's magnetic flux was eliminated as was the influence of temperature, barometric pressure (and thus weight of the mass of air), humidity and other ambients. Possible variations in the electric supply voltage are limited to less than one part in 10,000.

The signal-to-noise ratio of the instrumentation (as shown in Fig. 1) is but a small fraction of the observed significant values.

The phenomena described here seem to go beyond what could be legitimately calculated from an extrapolation of present theory.

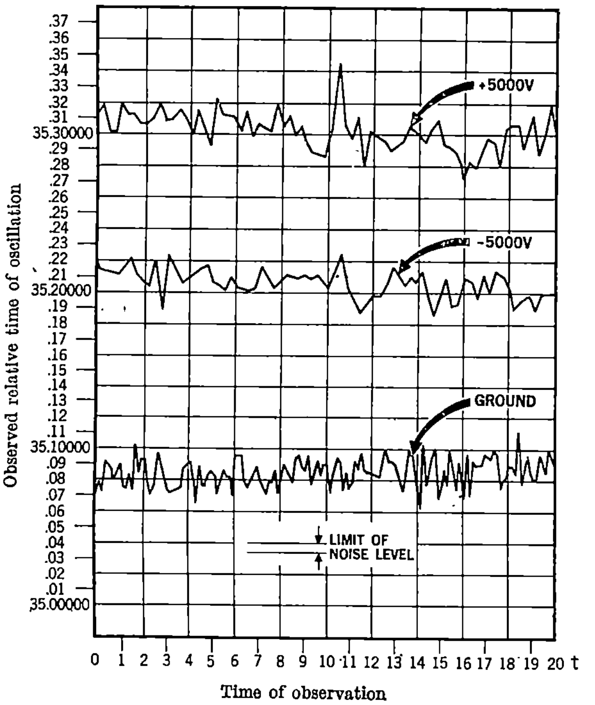

Fig. 1. Time delay of a pendulum when grounded and when charged positively and negatively with 5,000 V d.c.

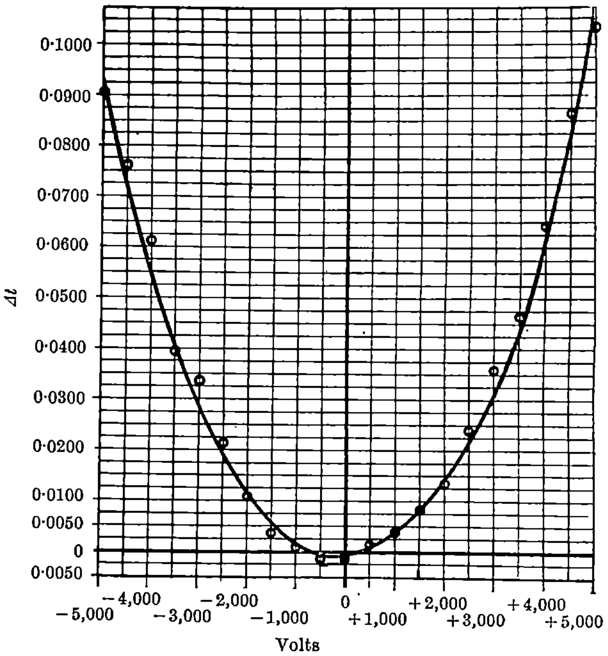

Fig. 2. Voltage versus period

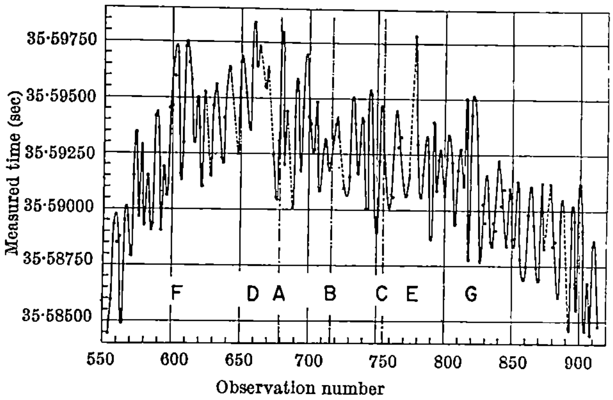

Fig. 3. Record of timing of the grounded torque pendulum during lunar eclipse of March 13, 1960

Referring to Fig. 1, it should be noted that the automatic cycle of observations, taken at the different voltages and ground, took place according to the following schedule: positive, ground, negative, ground, positive, ground and so forth in rotation. Each observation constitutes an individual determination. It is separated from the next one by the period of dwell (about 20 sec) on the resetting location, during which time all the cycling and voltage-changing operations occur automatically.

It is significant, therefore, that the deviations shown on the recordings, as made with the +, ground and − potentials, show a high degree of synchronicity despite the fact that one measurement is separated from the next one by more than 1 min. Whatever factors influenced the delay in the pendulum's period, they repeated themselves independently on consecutive measurements. 'Noise' would not do so.

Each point of the voltage-versus-period curve (Fig. 2) was determined at least 50 times and least squares taken for each point shown. The accuracy of the readings taken for each point therefore exceeds changes of the time of the pendulum oscillation due to its electro-static charge.

Consideration should also be given to such facts as, for example, those shown in the recordings taken during the lunar eclipse (Fig. 3). Herein the points of registration lie repeatedly on a harmonious curve with reference to their preceding ones as well as the subsequent ones thereafter. This exceeds the probability of a random occurrence.

The physicist hesitates to form a working hypothesis for such observations. Having accounted carefully, however, for all other influences, he is driven reluctantly to the conclusion that there may exist variations in *g* even if such cannot be noted with grounded, quasi-stationary instruments. These new effects may become noticeable if geo-gravitic flux-lines are cut dynamically and electrically charged apparatus is used.

When working as a post-doctoral student with Einstein, we discussed the possibility that there were interrelations between electricity, inertial mass and gravitation. These experimental results make me wonder whether they may properly be so interpreted.

Thousands of careful observations made under reproducible conditions, and records taken for more than ten years, indicate the possibility of significant short-time variations when measured dynamically with a conductive object rotating at an angle of 90° to its suspension which latter coincides with the geo-gravitic vertical.

These deviations were found with a torque pendulum but not with conventional pendulums of Kater's type and the usual gravity meters operating under equivalent conditions.

Apart from the foregoing phenomena, the precise torque pendulum has many other applications. One of them is a seismograph of ultra-long period. When charged electrically, it constitutes also a means for the recording of other phenomena the electric parameters of which have scarcely been investigated so far.

Among others, the electric pendulum is a powerful analytical tool for the indication of electrical events that are co-variant with major geophysical phenomena. Preliminary recordings seem to indicate that an electrical disturbance can be noted not only during but also preceding earthquakes, volcanic eruptions and hurricanes. It is submitted as a working hypothesis that changes of geo-electric currents may precede the major mechanical stress-relief that appears afterwards in the form of an earthquake. The electric pendulum under the proper conditions may be capable of sensing such changes in the electronmagnetic conditions ahead of time. Data found here occasionally offer a fascinating possibility although they are not conclusive at this time since such work can be done only by around-the-clock observations and requires also other refinements.

The electrical phenomena apparently associated with major perturbations are of such a nature that further work seems to be indicated. This includes also man-made events such as nuclear detonations which are known to cause electric disturbances. These, together with other sensors, offer the fascinating possibility of an additional sensing means of high sensitivity, specificity and resolving power together with an increased signal-to-noise ratio.

I thank the Rev. Fr. Daniel Linehan, S.J., director of Weston Observatory, who, after inspection of my instruments and recordings, co-ordinated their comparison with his records for the elimination of seismic and magnetic disturbances from the observations shown.

I also thank Prof. Edwin B. Wilson for his advice and Prof. Hans Thirring, who put on record the original form of these findings before the Austrian Academy of Sciences.

REACTION BETWEEN PORTLAND CEMENT CLINKER AND WATER

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IT has been recently suggested that the reaction between Portland cement clinker minerals and water (hydraulic action of cement) may be an 'acceptor'-type semiconductor surface reaction[1]. To verify this suggestion one has to find if individual clinker minerals are semiconductors and, if so, their type of semiconductivity. Further, and, this is more important, it is necessary to see if the electrical conductivity of individual clinker mineral is related with its hydraulic action. This is to be expected, if the foregoing suggestion is correct, as the electrons responsible for electrical conduction are also the electrons which take part in this type of reaction.

As individual minerals in the clinker (di- and tri-calcium silicate, tri-calcium aluminate, tetracalcium alumino ferrite) suitable for semiconductor studies were not available, experiments were carried out with ordinary Portland cement clinker, which consists of a mixture of the aforementioned minerals and of some lime (removed later) and MgO.

The clinker was powdered and the fraction passing B.S. 300 mesh sieve was made free of lime by repeated washing with absolute alcohol, and the material thus obtained was used in these experiments. Within the limitation imposed by the material, the results indicate that the amount of hydration of Portland cement clinker in a given time and its electrical conductivity change in opposite direction (anti-parallel). This means that the hydraulic action of Portland cement clinker is a semiconductor surface reaction. Since the type of semiconductivity of clinker is unknown, it is not possible at this stage to specify the 'type' of the reaction ('acceptor' or 'donor').

To examine the semiconducting property of the clinker, variation of electrical conductivity with temperature was the only measurement which could be undertaken. To correlate electrical conductivity of clinker with hydration, it is necessary to change the electrical conductivity of the clinker, and this was effected (1) by heating the clinker at 1,000° C for 4 h and (2) by heating the clinker in a similar manner after thoroughly mixing it with 1 per cent (by weight of clinker) of each of these oxides individually: (*a*) Cr_2O_3, (*b*) ZnO, (*c*) MoO (possibly so, the material was actually heated with ammonium molybdate), (*d*) MnO_2, (*e*) CoO and (*f*) NiO. As no sintering was observed, the surface area was taken to have remained unchanged. Both fired and unfired materials were made into tablets under a total pressure of 3,500 lb. as done previously[1]. Electrical resistance of the tablets was measured at room temperature, without a conducting layer. The variation of electrical conductivity of the tablets with temperature (rising by steps of 25° C up to 950° C) was also measured in the manner described earlier[1].

For hydration, fresh tablets, made from both fired and unfired materials, after measuring their resistances at ordinary temperature and their weights, were put for hydration at ordinary temperature over water in a moist atmosphere (vapour hydration) in a closed container for 3 days. The tablets were then dried at 110° C for 24 h and weighed and their increase in weight found. These were then allowed to hydrate for a further period of 7 days by immersing them in de-ionized distilled water. Three tablets, of the same treatment, were placed in 50 c.c. water at ordinary temperature. These were dried,

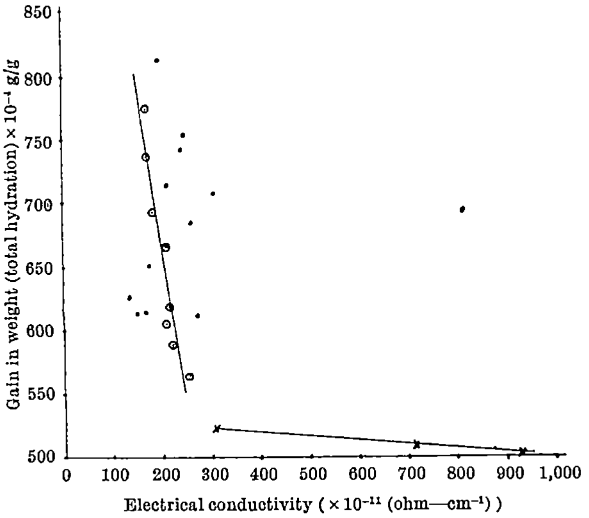

Fig. 1. Gain in weight due to total hydration and electrical conductivity of clinker

Table 1. Electrical Conductivity and Hydration of Portland Cement Clinker

Serial No.	Treatment	Electrical conductivity ($\times 10^{-9}$ ohm-cm^{-1})	Gain in weight, vapour hydration (g/g)	Gain in weight, total hydration (g/g)	Corrected gain in weight, vapour hydration (g/g)	Corrected gain in weight, total hydration (g/g)
1	Heated alone	1·36	0·0387	0·1121	0·0217	0·0628
2	Heated with CoO	1·52	0·0414	0·1098	0·0232	0·0615
3	,, ,, Cr_2O_3	1·70	0·0510	0·1099	0·0286	0·0615
4	,, ,, CoO	1·73	0·0402	0·1316	0·0225	0·0737
5	,, ,, ZnO	1·73	0·0576	0·1388	0·0323	0·0777
6	Heated alone	1·77	0·0327	0·1163	0·0183	0·0651
7	Heated with NiO	1·88	0·0489	0·1236	0·0274	0·0692
8	,, ,, Cr_2O_3	1·94	0·0479	0·1452	0·0268	0·0813
9	Clinker untreated	2·08	0·0397	0·1079	0·0222	0·0604
10	,, ,,	2·08	0·0386	0·1193	0·0216	0·0668
11	Heated with MnO_2	2·11	0·0377	0·1279	0·0211	0·0716
12	Clinker untreated	2·20	0·0396	0·1052	0·0222	0·0589
13	Heated alone	2·20	0·0421	0·1104	0·0236	0·0618
14	Heated with ZnO	2·42	0·0551	0·1329	0·0309	0·0744
15	,, ,, ZnO	2·46	0·0563	0·1349	0·0315	0·0755
16	,, ,, NiO	2·51	0·0492	0·1202	0·0276	0·0673
17	,, ,, Cr_2O_3	2·52	0·0476	0·1006	0·0267	0·0563
18	,, ,, CoO	2·73	0·0413	0·1096	0·0231	0·0614
19	,, ,, NiO	3·05	0·0457	0·1264	0·0256	0·0708
20	,, ,, MnO_2	3·08	0·0367	0·0933	0·0205	0·0522
21	,, ,, MoO	7·12	0·0413	0·0909	0·0231	0·0509
22	,, ,, MoO	8·13	0·0417	0·1245	0·0234	0·0697
23	,, ,, MoO	9·31	0·0402	0·0900	0·0225	0·0504

and gains in weight noted. Gain in weight per gramme of clinker was calculated for vapour hydration and for both vapour and water hydration combined (total hydration). The gains in weight were corrected for the weight of calcium hydroxide formed during the hydration of dicalcium and tricalcium silicates on the basis of the equations given by Hansen[2]. This correction covers about 75 per cent of the clinker. Correction for reaction products other than the cementitious hydrates for the rest of the material was neglected. Loss of lime during water hydration was also neglected.

When logarithms of electrical conductivities of the treated and untreated clinker at different temperatures are plotted against $1/T$ (T in deg. K), the conductivity is seen to fall up to 200° C and then rises continuously except for those heated without any addition and heated with ZnO and MoO. In these, a slight rise in conductivity up to about 50° C is observed, which then falls to a minimum at about 200° C and then rises continuously with temperature. Variation of electrical conductivity with temperature indicates that the material may be behaving as a semiconductor, and, since these measurements do not yield any information regarding the type of semiconductivity of these materials, the curves are not produced.

Electrical conductivity at ordinary temperature of the treated and untreated cement clinker and the gain in weight on hydration through vapour hydration and total hydration, corrected and uncorrected, are given in Table 1. Results of hydration of 23 tablets are given; one, with MnO_2 added, was broken during experiment. There is no relationship between the (corrected) gain in weight by vapour hydration and electrical conductivity. This is apparently due to all minerals not taking part in hydration during this part of the hydration while conductivity takes into consideration the entire mass. However, when the corrected gain in weight after total hydration is plotted against electrical conductivity some of the points fall along one straight line and a few along another with a different slope (Fig. 1). Such behaviour is quite possible. There is considerable dispersion of points also. This is expected when one takes into consideration the state of inhomogeneity of the material together with the fact that the change in the electrical conductivity produced in individual minerals on treatment cannot be uniform.

The nature of the curves indicates that the amount of hydration of Portland cement clinker, in a given time, is related to its electrical conductivity in an anti-parallel way. This means that the reaction between Portland cement clinker and water (hydraulic action of cement) is a semiconductor surface reaction. It may either be '*n*' type reaction on '*p*' type semiconductor or '*p*' type reaction on '*n*' type semiconductor. Taking into consideration the arguments given earlier[1], and also the similarity in the hydration of lime and cement, and, as the reaction between lime and water may be an '*n*' type reaction[3], it appears that the hydraulic action of Portland cement clinker is '*n*' type reaction on '*p*' type semiconductor, that is, the clinker material is behaving as a '*p*' type semiconductor.

[1] Chatterji, A. K., and Phatak, T. C., *Nature*, **197**, 656 (1963).

[2] Hansen, W. C., *Materials Research and Standards*, **2**, 490 (1962).

[3] Chatterji, A. K., Phatak, T. C., and Dhariyal, K. D., *Nature*, **203**, 61 (1964).

ALPHA-RADIOACTIVITY OF PLANKTON

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In recent years considerable attention has been directed to the problem of radioactive contamination of the marine environment, and several measurements of artificially produced radioactive isotopes in plankton have been reported (for example, Osterberg *et al.*[1]). By comparison, the naturally occurring radioactivity of plankton has received scant attention. The activity due to the mixed β–γ emitter potassium-40 has been the subject of some discussion, and Rama *et al.*[4] have made brief mention of lead-210. The α-activity appears to have been almost totally neglected since the early work of Evans *et al.*[2] and Føyn *et al.*[3] on the radium contents. A recent report by Marsden[5], which indicated high α-activities and interesting problems associated with possible disequilibria in the radioactive series, has revived interest in this problem, and as a result of this report a programme for investigating the α-radioactivity of plankton has been started here.

So far, only 'total' α-counting plus thorium-series 'pairs' measurements have been made. The experimental set-up is based on that of Turner *et al.*[6]. This consists of a sealed phosphor-sample system of high sensitivity and simplicity. This type of system has been used with success in several investigations[7] of samples from the natural radiation environment, and it provides: (*a*) a measurement of the total α-activity; (*b*) an estimate of the percentage of this activity which is due to the thorium series. The particular set-up used in these experiments utilized Levy–West *G* 86 ZnS(Ag) as phosphor, E.M.I. 9530 *B* photomultiplier tubes and low cathode-to-first dynode resistor values[8]. This combination resulted in the low background value of less than 1 count/h for a 4½-in. diameter phosphor. It is perhaps worth mentioning that, on such a phosphor, one sample count/h above background will correspond to less than 0·1 pc./g α-activity in the sample, or to a thorium content of less than 0·1 p.p.m. in a hypothetical sample containing only the thorium series in equilibrium.

Sample preparation is very simple. The plankton is dried at 105° C, finely ground and then packed on the Scotch tape–'Perspex' ring–ZnS phosphor system as described by Turner *et al.*[6]. The sample is sealed on top with additional tape. No emanation problems arise provided that the sample is left for 2–3 weeks after preparation in order to allow radon to regain equilibrium. Ashing of the sample was avoided in order to prevent loss of the volatile element polonium; in any event, the extra concentration provided by ashing was not required.

Results have been calculated using the usual 'thick-source' α-counting formula (for example, Finney and Evans[9], Turner *et al.*[6], Cherry[10]). This requires a knowledge of the range of the α-particle in the sample material, and the formula which assumes proportionality to $Z^{2/3}$ has been used in the present work. The range problem has been discussed previously[11] by me; here it will suffice to mention that lack of consistent experimental knowledge concerning α-particle ranges in solids results in an inherent uncertainty in the absolute values of results obtained by thick-source α-counting techniques. Should some recent α-particle range data (for example, those of Kamke and Kramer[12]) be substantiated, then all the activity values in the present article might have to be lowered by about 25 per cent. $Z^{2/3}$ has, of course, to be calculated on the usual 'atomic fraction' basis. For dry zooplankton a composition of major elements with an atomic ratio of 212 : 176 : 106 : 16 : 1 for oxygen : hydrogen : carbon : nitrogen : phosphorus has been assumed. These values give $Z^{2/3} = 2{\cdot}81$. The phytoplankton samples measured are mostly diatomaceous, and atomic ratios of 191 : 158 : 95 : 38 : 15 : 4 : 2 : 1 for oxygen : hydrogen : carbon : silicon : nitrogen : calcium : iron : phosphorus have been used to calculate $Z^{2/3} = 3{\cdot}11$. The compositions used are based on data given by Sverdrup *et al.*[13], and might be subject to some error. Fortunately, however, $Z^{2/3}$ is a slowly varying function, and the resulting error in $Z^{2/3}$ is likely to be unimportant at this stage; the 10 per cent difference between the zoo- and phyto-plankton values of $Z^{2/3}$ is probably indicative of the order of magnitude of the most serious discrepancy which is likely to arise from this source.

The five sections of Table 1 represent different groups of samples. Section 1 comprises samples consisting predominantly of zooplankton which were collected at various dates from 1899 until early in 1962. With the exception of the last sample listed (No. 6) all these specimens were obtained from the collections of the South African Museum, Cape Town, and were supplied by Mr. J. G. Grindley of the Museum. Sample No. 6 and those in Sections II and III were collected by the University of Cape Town research vessel *John D. Gilchrist*. The Section II samples are also zooplankton; they are being collected at approximately monthly intervals from the 'Slangkop Station'. Section III comprises phytoplankton data; these samples are collected at the same time as the zooplankton specimens of Section II, but with a different net. Finally, Section IV gives data for phytoplankton collected either during the 1958 International Geophysical Year cruises of the S.A.S. *Natal* or during the 1962 International Indian Ocean Expedition cruises of the same vessel. The *John D. Gilchrist* and the S.A.S. *Natal* samples were provided by Messrs. J. Stone, F. J. R. Taylor and P. Zoutendyk of the Council of Scientific and Industrial Research Oceanographic Research Unit at the University of Cape Town. The solitary sample in Section V represents the only pre-war phytoplankton specimen so far obtainable. It came from the same source as the samples of Section I.

Results are also given in Table 1. Total α-activities are quoted in picocuries per gram (that is, μμc./g). Relative standard deviations due to counting statistics are in all cases 5 per cent or less, and are generally of the order of 3 per cent. The most serious errors will probably arise from the α-range problem already discussed; as previously indicated, it is possible that this could result in all the values quoted being too high by as much as 25 per cent. Percentage contributions to the total α-activity by the activity due to the thorium series alone are also quoted. These are obtained by counting the 'pairs'[6,10] due to the successive α-decays of the isotopes emanation-220 and polonium-216. Since these 'pairs' rates are, typically, lower by a factor of about 40 or 50 than the total count-rates, the errors due to counting statistics are appreciably larger than for the total α-activities. A standard deviation of about 20 per cent would be average. A further uncertainty is caused by the fact that it is not known for certain whether the thorium series activity is in complete equilibrium from the isotope thorium-232 downwards. This has been assumed to be the case in calculating the percentage quoted; should either radium-228 or thorium-228 prove to be the true parent of the 'pairs' activity, the quoted values would have to be lowered by a factor of about 0·9.

The data in Table 1 require to be supplemented by some comments about the time-dependence of the counting rates of samples. All the data quoted in the table were obtained from counts during the period December 1963–March 1964. Samples in Sections II and III were, if possible, counted about one month after collection (and about three weeks after drying and mounting); in some cases, however, the interval between collection and counting was as great as ten weeks. In addition to the results quoted, additional counts, separated in time by varying intervals, have been made on several of the samples listed. The project has not been under way for a sufficiently long time for definite conclusions to be drawn, but the results so far obtained can be summarized thus:

(1) None of the phytoplankton samples has exhibited any time-dependence in the α-activity within the limits of statistical error.

(2) Several of the zooplankton samples in Section II, which are all recent samples, have shown slight decreases (of up to 25 per cent) when re-counted two to three months after the original count.

(3) With the exception of sample No. 6, which will be discussed separately, none of the zooplankton samples in Section I has exhibited any time-dependence over a period of about six months.

(4) None of the samples has exhibited a rise in α-activity with time, apart, of course, from the small rise sometimes observed due to radon build-up if the samples are counted too soon (within two or three weeks of drying).

(5) The pairs rates statistics are at present too poor to permit of any serious conclusions on variations with time. It is, nevertheless, worth mentioning that, within the wide

limits of statistical error, no time-dependence in the pairs rate has been observed for any of the samples.

(6) Sample 6 (zooplankton) is worthy of special mention since its α-activity has been followed intermittently over a period of two years. A month after preparation its α-activity was as high as 19 pc./g. This declined steadily, until eighteen months later (in September 1963) the level was down to 2·1 pc./g. In January 1964, the value was as given in Table 1 (2·1 pc./g) indicating no significant change during the intervening four-month period. The rate of fall-off between March 1962 and September 1963 as measured by a set of six readings fitted an exponential decay with a half-life of about 140 days. It is difficult to see how this can be ascribed to anything other than the presence of the well-known α-emitter polonium-210, which has a half-life of 138 days. The experimental facts support the hypothesis that the sample initially contained about 17 pc./g of unsupported polonium-210 plus a 'long-lived' (half-life $\geqslant$ 2 years) α-activity of about 2 pc./g. No statistically significant variation in the pairs rate was observed between March 1962 and January 1964; the percentage contribution of the thorium-series activity has thus risen from about 2 per cent to the present value of 22 per cent.

Total α-activity of plankton. The results presented indicate that fresh plankton contains in general (if the samples tested are typical) upwards of 4 pc. α-activity/g dried sample. The means for the 'routine' samples of Sections II and III are in fact 6·7 pc./g for zooplankton and 5·1 pc./g for phytoplankton. It is clear, moreover, that for the zooplankton some of the initial activity is often of 'short-lived' ($\leqslant$ 2 years) duration, and that this short-lived activity can, as in the case of sample 6 and possibly *GIL* 526 as well, contribute an activity considerably in excess of the 'basic' 4 pc./g. No such short-lived activity has been observed in phytoplankton samples; but this may merely be a reflexion on the relatively small number of samples thus far tested. Some phytoplankton samples, on the other hand, do show almost sensationally high values of α-activity, and this high level seems to persist for a time of five years or more—see the International Geophysical Year samples in Section IV.

It is of interest to consider the foregoing comments in relation to the radiation dosage received by the living planktonic organism. The range of a typical α-particle in living plankton will be about 10–15μ. If we consider a plankton specimen with dimensions very much larger than this figure (true for most zooplankton), then to a good approximation all the α-particles emitted by the specimen will be absorbed in the specimen itself. This will, of course, not be true if the α-particles are distributed on the surface alone, but even in this event only a small correction factor of the order of 2 or 3 will be involved. Suppose that the plankton contains 4 pc. α-activity/g dried material. The concentration factor between 'wet' and dry plankton will be about 16 (ref. 13), thus giving us a concentration of about 0·25 pc. α-activity/g living plankton. If we next assume that the average α-particle energy is 5 MeV, then we find that the radiation dosage to the organism from its own inherent α-activity is about (2·6) (10^{-3}) millirads per hour or about 23 millirads per year. Furthermore, α-radiation is generally considered to have a relatively high biological efficiency ; if we use the usually quoted relative biological efficiency (R.B.E.) of 10 for naturally occurring alphas, then we end up with a dosage of about 230 millirem per year. Folsom and Harley[14] quote radiation doses in millirads per year in "eleven radiological domains". They estimate the radiation dose to a micro-organism near the sea-surface to be about 39 millirads per year from external sources ; since most of this is due to minimum ionization radiation, this can be considered to be equivalent to about 39 millirem per year. It is thus apparent that the radiation dose to our plankton specimens will be derived very largely (80 per cent or more) from internal α-radioactivity. Contrast this with the situation in human body tissue: Eisenbud[15] quotes tissue dose rates of between 120 and 140 millirem per year to various body tissues, and the contribution of internal α-activity to these figures varies between 4 and 15 per cent. Furthermore, it should be emphasized that the 'basic' figure of 4 pc./g is definitely at the lower limits of the α-activity concentration in fresh plankton so far as the present samples are concerned. Consider the phytoplankton sample *NGY* 16, with about 96 pc. α-activity/g dried plankton. This sample has been identified by Mr. F. J. R. Taylor as a mixed bloom with the relatively large diatom *Rhizosolenia hyalina* Ostenfeld predominating. The diatoms in this sample have typical dimensions of about 300μ long and 35μ diam., and with typical α-ranges of about 10–15μ it is reasonable to assume that something like half the α-energy is internally absorbed. A simple calculation then shows that the α-radiation will deliver a dose of about 2·8 rems per year to the diatom. This is exceptionally high, and bears comparison with the large (external) radiation doses received by the human population living on the thorium-bearing sands in Kerala, India[15]. Most phytoplankton are, of course, smaller and will thus tend to 'lose' more of their α-particle energy to the surrounding sea. What has been said will nevertheless suffice to indicate that at least some plankton must carry an abnormally high internal radiation burden of natural origin. It is perhaps relevant to compare the dried plankton figure of 4 pc./g with the reported α-activities of foodstuffs. Thus Turner *et al.*[22] quote values of up to 0·005 pc./g for meats. 0·04 pc./g for fish and 0·6 pc./g for cereals. Only in exceptional cases (for example, Brazil nuts, 17 pc./g) do the activities rise above the dried plankton figure. Another interesting 'high' α-activity is that reported by Hill[16] in lamb's kidney, namely, about 2 pc./g wet tissue.

α-Emitting elements in plankton. The results presented here involve no chemistry or α-spectroscopy, and do not therefore permit definite identification of the nuclei responsible for the α-particle emission. However, the use of the 'pairs' technique, the measurements of time-dependence of the α-activity and the fact that a few measurements have been made on relatively old samples do allow of a few reasonable deductions. We shall consider the figure of 4 pc./g dried material to be 'basic' for fresh plankton, as in the previous section. It must, however, be borne in mind that this figure is probably on the low side, and is certainly subject to very wide variation. We shall discuss elemental abundances as follows:

(1) *Polonium.* As already suggested here, there is strong evidence that the 138-day isotope polonium-210 can in some cases be responsible for a substantial portion of the initial activity of plankton specimens. This is not surprising: polonium-210 is, after all, a 'natural fall-out' product, and Hill[16,17] and others have measured this nuclide in grass, various tissues and other environmental samples. It is interesting that the measurements on sample 6 and on some of those in Section II seem to indicate that the polonium is, in part at least, unsupported by its parent lead-210. Lead-210 has a 22-year half-life, and is generally considered to be an important contributor to natural fall-out. Since the residence time of lead-210 in the atmosphere is estimated to be of the order of a few weeks[18,23] it is unlikely that polonium-210 will accompany fall-out lead-210 in amounts approaching equilibrium activity. In fact, Burton and Stewart[18] found an average lead-210 to polonium-210 activity ratio of 10 in rain-water samples. After fall-out lead-210 will, of course, decay to polonium-210, and one would therefore expect an exponential increase of α-activity with the 138-day polonium-210 time constant if the plankton samples at the time of collection contained appreciable amounts of fresh, unsupported, lead-210 fall-out nuclei. No such increase has been observed in any of our samples, although increases in activity with time have been reported by

Marsden[5]. Factors such as the residence times of polonium and lead in the upper layers of the ocean must also be taken into account; but it is possible that the results quoted here might be construed as indicating preferential intake of polonium-210 by planktonic organisms. Obviously many more results are required before the situation can be clarified. It is perhaps worth directing attention to the fact that polonium-210 radioactivity appears to be assuming increasing biological importance; thus Hill[17] states that "in bone and many of the soft tissues of normal unexposed human subjects, polonium-210 appears to be responsible for a large part of the total natural activity". He quotes polonium-210 concentrations in various human tissues which are typically of the order of 4×10^{-3} pc./g of wet tissue, and in another paper[16] finds that the α-activity of 2 pc./g in lamb's kidney previously quoted is due almost entirely to polonium-210. If we assume (see Sections I and II, Table 1) that about half the 'basic' 4 pc./g in dry zooplankton is due to polonium-210, then a typical value would appear to be of the order of 125×10^{-3} pc./g 'wet' plankton—considerably larger than for human tissue. The 'concentration factor' for polonium-210 from sea-water to zooplankton is also of interest. Rama *et al.*[4] quote a lead-210 concentration in surface waters of about 0·1 disintegrations/min/l., or about 5×10^{-5} pc./g. Picciotto[19] quotes a radium-226 concentration in sea-water of about 9×10^{-5} pc./g. Both these nuclei are parents of polonium-210, and it seems reasonable to assume that the polonium-210 content of sea-water will not be more than 10^{-4} pc./g. Comparing this with our figure of 250×10^{-3} pc./g in wet plankton implies a concentration factor of more than 2,500; even this figure is likely to be a conservative estimate.

(2) *Thorium series isotopes.* The thorium series comes into equilibrium rapidly after the 1·9 year nuclide thorium-228, and it is plain that the thorium series activity in the plankton must be supported by either thorium-228 or its precursors, 6·7 year radium-228 and/or $1 \cdot 4 \times 10^{10}$ year thorium-232. The fact that the 1899 and 1904 samples still show considerable thorium-series activity indicates that the long-lived thorium-232 is probably at least partially responsible. For the purposes of the present discussion it seems safe to assume that the thorium series will contribute, typically, upwards of 0·4 pc./g of dried plankton to the α-activity. This is equivalent to about 0·025 pc/g of wet plankton. Calculations based on Picciotto's[19] results (which give $1 \cdot 2 \times 10^{-7}$ d.p.s./ml. for thorium-228 in sea-water) give a thorium series contribution to the sea-water α-activity of about 2×10^{-5} pc./g. This gives a concentration factor of about 1,250, and again this is likely to be a conservative estimate.

Rama *et al.*[4] have suggested in their paper on lead-210 in the ocean that "the marine biosphere is responsible for the conveyance of lead from surface to deeper waters". The results just discussed indicate that the same may be true for the nuclides at the head of the thorium series, although, of course, the ferric hydroxide precipitation mechanism suggested by Pettersson[20] to account for the deficiency in sea-water of ionium (that is, thorium-230, a uranium series nuclide) might also operate.

(3) *Uranium and radium.* After allowing for short-lived activity and for the thorium series contributions of (1) and (2) we are left with upwards of 1–2 pc./g of α-activity unaccounted for. This must presumably be due to the uranium-series, and is probably supported by 1,620-year radium-226 and/or one or both of its uranium parents. 1·6 pc./g dry plankton would give about 0·1 pc./g wet plankton; Picciotto's sea-water data give us a concentration of just under 0·002 pc./g sea-water for the uranium series contributions, implying a concentration factor of the order of 50. It is interesting to note that the pre-war measurements of Evans *et al.*[2] on a single zooplankton sample gave a concentration factor of about 100 for radium, while those of Føyn *et al.*[3] on a phytoplankton sample gave a factor of 1,000 for the same element. The results we have quoted do not enable us to distinguish between the uranium and the radium contributions, but the approximate agreement with the sample of Evans *et al.*[2] is interesting. It would, of course, be easy to match the Føyn *et al.*[3] sample with one of the more active phytoplankton samples from Section III of Table 1. It is perhaps worth stating that Evans *et al.*[2] quoted a content of $0 \cdot 04 \times 10^{-12}$ g radium/g dry zooplankton, while Føyn *et al.*[3] obtained 2×10^{-12} g radium/g dry phytoplankton. If we assume radium-226 to be the head of the uranium series contribution, and assume radioactive equilibrium from there downwards, then 1 pc./g of radium-supported α-activity corresponds to $0 \cdot 2 \times 10^{-12}$ g radium/g dry plankton.

Locality of sample collection. Since relatively few samples have been measured in this preliminary survey, very little emphasis has been placed on the question of sample locality. For record purposes, we can divide the collection sites as follows:

(*a*) All the samples in Sections II and III of the table were obtained at the 'Slangkop Station', situated about 7 miles offshore west of the Cape Peninsula.

(*b*) Samples No. 3, 4, 6 and 'Robben Island' were also obtained in the vicinity of the Cape Peninsula.

(*c*) Sample No. 5 was obtained from Lambert's Bay, about 120 miles north of Cape Town.

(*d*) Sample No. 1 was from Mossel Bay, about 250 miles east of Cape Town.

(*e*) Sample No. 2 and the International Geophysical Year and Indian Ocean Expedition samples in Section IV were obtained off the south-east coast of South Africa between Port Elizabeth and Durban. *NGY* 16 and *NIOE* 7 are the only two of these samples which were obtained at points clearly off the continental shelf. The International Geophysical Year samples in Section IV have been described in detail by Zoutendyk[21].

Table 1. α-ACTIVITIES OF DRIED PLANKTON SAMPLES

Section and type	Sample No.	Date of collection	Total α-activity in pc./g dried material	Thorium series contribution (%)
Section I Zooplankton	1	1899	1·8	20
	2	1904	3·6	28
	3	1947	2·5	32
	4	1950	2·0	46
	5	1957	2·5	14
	6	1962	2·1	22
Section II Zooplankton	*GIL* 526	2/10/63	12·0	17
	GIL 527	29/10/63	3·9	13
	GIL 528	26/11/63	6·7	22
	GIL 537	18/12/63	5·3	31
	GIL 536	29/1/64	5·7	14
Section III Phytoplankton	*GIL* 526	2/10/63	7·7	20
	GIL 527	29/10/63	3·4	34
	GIL 528	26/11/63	3·6	17
	GIL 537	18/12/63	4·4	16
	GIL 536	29/1/64	6·3	29
Section IV Phytoplankton	*NGY* 8	2/3/58	18·3	35
	NGY 16	3/3/58	95·5	8
	NGY 25	18/5/58	16·5	14
	NGY 29	19/5/58	28·6	8
	NGY 33	21/5/58	31·4	13
	NGY 38	22/5/58	5·5	29
	NGY 42	24/5/58	6·3	26
	NIOE 7	8/4/62	4·0	33
	NIOE 18	12/4/62	6·5	36
Section V Phytoplankton	'Robben Island'	7/7/35	3·8	24

It is tempting to associate the higher activities of the Section IV samples with the warmer waters of the eastern side of the sub-continent, but this is not justified on the basis of the data presented here. Additional information is obviously required.

I thank Sir Ernest Marsden for directing my attention to this problem, and Prof. J. H. Day and Messrs. J. G. Grindley, J. Stone, F. J. R. Taylor and P. Zoutendyk for the provision of samples and for their advice. I also thank Mr. L. A. Pasvol and Mr. L. V. Shannon for preparing some of the samples for counting and the Council of Scientific and Industrial Research Oceanographic Research Unit at the University of Cape Town for financial assistance.

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INCONSTANCY OF RENAL CLEARANCE VALUES WITH CHANGING PLASMA CONCENTRATIONS

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ONE of the basic tenets in the concept of renal clearance is that clearance (the calculated volume of plasma which is cleared of a substance per unit time) is independent of plasma concentration, provided that tubular saturation does not occur. In experiments which utilized a fraction collector to obtain frequent urine specimens we have found that clearance of *o*-iodohippurate sodium ('Hippuran') does not remain constant following a single intravenous injection, but on the contrary it declines as the plasma 'Hippuran' concentration declines[1]. Since this finding is in gross conflict with the constant clearance concept which has proved so valuable over the past 30 years, we have sought to develop a mathematical model of the kidney which will reconcile our findings with those of other investigators. At the same time we have attempted to provide explanations for two observations regarding renal clearance which we believe to be thus far inadequately explained. The first is the observation that extraction ratios of substances excreted by tubular secretion rarely exceed 90 per cent. This has been attributed to the passage of 10 per cent of the renal blood through non-excretory renal tissue[2]. Such profligacy in the distribution of myocardial work appears unlikely. The second observation is that of Mitchie and Mitchie[3], who found that following rapid attainment of a constant *p*-aminohippurate plasma concentration about 20 min are required before its clearance becomes constant although it appears in the urine within a much shorter time.

Published data of other investigators indicated that clearance of *p*-aminohippurate and of 'Hippuran' may change with changing plasma concentrations; however, the lack of urinary excretory data permitted these investigators an alternative explanation for their findings. The overall excretion of any substance should follow first order kinetics if clearance is calculated in the usual way.

$$C = \frac{UV}{P}$$

where C, clearance; U, urine concentration; V, volume of urine excreted per unit time; P, plasma concentration. Because of the technical difficulties involved in measuring clearance by the sustaining infusion technique many attempts have been made to determine renal clearance by following the disappearance of exogeneous substances from the plasma after a single intravenous injection. Although these attempts have not been entirely successful in their original purpose, the plasma disappearance curves indicated that something more than simple first order kinetics was involved.

With first order kinetics, assuming extra-renal losses are insignificant and clearance remains constant, the plot of log P versus time should yield a straight line since UV in the equation for clearance can be equated to $V_p\left(-\frac{\mathrm{d}P}{\mathrm{d}T}\right)$, where V_p is the volume of distribution of the substance, and $\frac{\mathrm{d}P}{\mathrm{d}T}$ represents change in plasma concentration with time.

$$C = \frac{V_p\left(-\frac{\mathrm{d}P}{\mathrm{d}T}\right)}{P}$$

$$\int \frac{C}{V_p} \mathrm{d}T = -\int \frac{\mathrm{d}P}{P} + C_1$$

$$\frac{C}{V_p} T = -\ln P + C_1$$

The plot of log P versus T will give a straight line of slope $-2{\cdot}303\frac{C}{V_p}$. Such disappearance curves have been obtained for mannitol[4-6]. Deviations from this equation for inulin have been attributed to a barrier to free diffusion between intravascular and extravascular spaces[7]. Plasma disappearance curves of *p*-aminohippurate[8] and 'Hippuran'[9,10] deviate markedly from this equation, however, and such deviations have been ascribed to a distribution throughout a two-compartment system[8]. Another explanation for these findings is that clearances of *p*-aminohippurate and 'Hippuran' do not conform to the foregoing equations because clearance changes as the plasma concentration declines. More direct evidence for the latter may also be found[11,12].

The requirements for our mathematical model of the kidney may therefore be described as follows:

(1) If plasma concentration remains constant, clearance is independent of plasma concentration for all concentrations (if tubular saturation does not occur) and for all substances whether excreted by glomerular filtration or tubular secretion.

(2) For substances excreted by glomerular filtration alone clearance remains constant even with a varying plasma concentration.

(3) For substances excreted by glomerular filtration alone the plasma concentration will decline logarithmically with time following a single intravenous injection (assuming extra-renal losses are insignificant).

(4) For substances excreted by tubular secretion, clearance will not remain constant if the plasma concentration varies.

(5) For substances excreted by tubular secretion, the plasma concentration will not exhibit a logarithmic decline following a single intravenous injection (assuming extra-renal losses are insignificant).

(6) Experimentally determined extraction ratios must be explained by some mechanism other than passage of renal blood through non-excretory renal tissue.

(7) It must not conflict with the experimental finding that following almost immediate attainment of a constant plasma *p*-aminohippurate concentration clearance will attain a constant value later than the material appears in the urine.

Previous discussions of the kinetics of renal tubular secretion have apparently overlooked an important anatomical fact. Substances which are secreted by the tubules must pass first from the capillaries into the renal interstitial fluid from which the tubule cells may then remove them for transport into the lumina of the tubules. Thus the tubule cells cannot act directly on the material within the capillaries. If a substance were to be completely removed from the capillary plasma in one passage through the excretory tissue of the kidney (the concept at present held for substances with high extraction ratios such as *p*-aminohippurate), the requirements of the diffusion process are such that the tubular excretory processes must maintain the interstitial fluid concentration at virtually zero concentration. The latter appears unlikely. It is probable that active tubular secretion can reduce the interstitial fluid concentration of certain substances to a low, but appreciable, level. This would result, therefore, in incomplete extraction of all substances passing through the renal excretory tissue and would provide an acceptable alternative to our present explanation for maximum extraction ratios of 90 per cent. If this explanation proves correct it will require certain changes in our concept of effective renal plasma flow. Probably all substances which are freely diffusible across the capillary endothelium attain concentration equilibrium between interstitial fluid and capillary plasma before the latter leaves the kidney.

In a model of the kidney six processes which are involved in renal excretion of any substance must be considered: R_1, the rate at which it enters the kidney via the renal arteries; R_2, the rate at which it leaves the kidney via the renal veins; R_3, the rate at which it is filtered by the glomerulus; R_4, the rate at which it diffuses through the capillary endothelium; R_5, the rate at which it is transported from the interstitial fluid into the lumen of the tubule; R_6, the rate at which it is excreted into the ureters. The dimensions of all these rates are $\left(\frac{\text{mass}}{\text{time}}\right)$. These processes are depicted in Fig. 1.

General equations. Let K_1 be the total renal plasma flow (which remains constant under experimental conditions). P is plasma concentration.

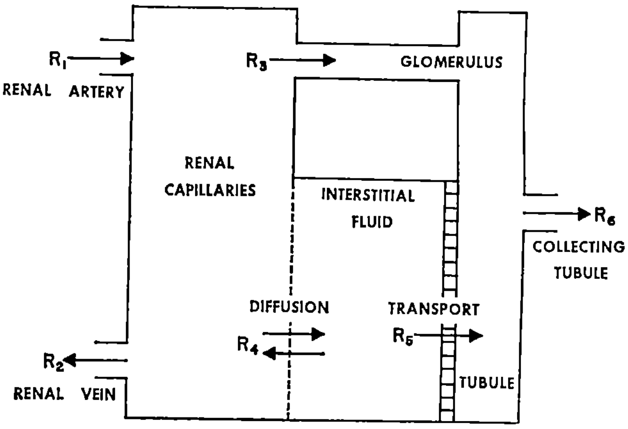

Fig. 1

$$R_1 = K_1P \qquad (1)$$

Let N equal the concentration in renal interstitial fluid. Assuming the concentration in efferent renal plasma is the same as that in the renal interstitial fluid and disregarding that small fraction of renal plasma water which is excreted as urine:

$$R_2 = K_1N \qquad (2)$$

Let K_3 be the glomerular filtration rate (which remains constant under experimental conditions). The rate at which any filtrable substance is removed by glomerular filtration is:

$$R_3 = K_3P \qquad (3)$$

Let Q be the amount of material removed from the plasma at time T through both glomerular filtration and tubular secretion:

$$\frac{dQ}{dT} = R_1 - R_2 = R_3 + R_4 \qquad (4)$$

R_5 is the rate at which a substance is secreted into the tubules from the renal interstitial fluid. Since this is an enzymatic reaction its rate at low substrate concentration should be proportional to the substrate concentration which is the concentration in the renal interstitial fluid:

$$R_5 = K_5N \qquad (5)$$

It is recognized that concentration may not be the same throughout the interstitial fluid, and R_5 and N can represent the mean reaction rate and mean concentration respectively. Let V_n be the volume of the renal interstitial fluid space. The change in interstitial fluid concentration may be defined by the following equation:

$$V_n\frac{dN}{dT} = R_4 - R_5 \qquad (6)$$

Let M be the amount of material excreted into the ureters in time T through both glomerular filtration and tubular secretion.

$$\frac{dM}{dT} = R_3 + R_5 = R_6 \qquad (7)$$

Since clearance is the rate of excretion divided by the plasma concentration:

$$\text{clearance} = \frac{UV}{P} = \frac{\frac{dM}{dT}}{P} \qquad (8)$$

From equations (3), (5), (7) and (8):

$$\text{clearance} = \frac{\frac{\mathrm{d}M}{\mathrm{d}T}}{P} = K_3 + \frac{N}{P}K_5 \tag{9}$$

These equations must fit all situations outlined above in the requirements for a model of the kidney. In the following sections these various situations will be considered.

Clearance values for glomerular filtration without tubular secretion. If there is no tubular secretion $R_5 = 0$ and from equation (9):

$$\frac{\frac{\mathrm{d}M}{\mathrm{d}T}}{P} = K_3$$

The rate of excretion will be proportional to plasma concentration and clearance will remain unchanged at all values for P whether it is constant or changing.

Clearance values for excretion by both glomerular filtration and tubular secretion. If excretion occurs by both glomerular filtration and tubular secretion $R_5 \neq 0$ and:

$$\text{clearance} = \frac{\frac{\mathrm{d}M}{\mathrm{d}T}}{P} = K_3 + \frac{N}{P}K_5$$

The following equations will show in the model that if plasma concentration remains constant throughout the experimental period clearance will be the same for all plasma concentrations. If plasma concentration varies, however, clearance will not remain constant.

From equation (4):

$$R_4 = R_1 - R_2 - R_3 \tag{10}$$

From equations (6) and (10):

$$V_n\frac{\mathrm{d}N}{\mathrm{d}T} = R_1 - R_2 - R_3 - R_5 \tag{11}$$

From equations (1), (2), (3), (5) and (11):

$$V_n\frac{\mathrm{d}N}{\mathrm{d}T} = K_1P - K_1N - K_3P - K_5N \tag{12}$$

$$V_n\frac{\mathrm{d}N}{\mathrm{d}T} = (K_1 - K_3)P - (K_1 + K_5)N \tag{13}$$

$$N = \frac{(K_1 - K_3)P - V_n\frac{\mathrm{d}N}{\mathrm{d}T}}{K_1 + K_5} \tag{14}$$

$$\text{clearance} = \frac{\frac{\mathrm{d}M}{\mathrm{d}T}}{P} = K_3 + K_5\left[\frac{(K_1 - K_3)}{(K_1 + K_5)} - \frac{V_n\frac{\mathrm{d}N}{\mathrm{d}T}}{P(K_1 + K_5)}\right] \tag{15}$$

If plasma concentration is constant throughout the experimental period the interstitial fluid concentration will attain an equilibrium condition in which it also is constant and $\frac{\mathrm{d}N}{\mathrm{d}T} = 0$. In these circumstances:

$$\text{clearance} = K_3 + K_5\left[\frac{K_1 - K_3}{K_1 + K_5}\right] \tag{16}$$

Thus clearance is independent of plasma concentration if the latter remains constant and $\frac{\mathrm{d}N}{\mathrm{d}T} = 0$. If P changes, however, N will vary also and $\frac{\mathrm{d}N}{\mathrm{d}T} \neq 0$. In these circumstances it can be seen from equation (14) that N is not a linear function of P and equation (9) shows that clearance will, therefore, not remain constant.

The model can thus reconcile the observations that, for substances excreted by tubular secretion, if P is constant clearance is independent of P and remains the same for all values of P (provided tubular saturation does not occur). If P varies, however, clearance will not remain constant, but will vary as a function of P and N. This effect is due to the accumulation of material in the renal interstitial fluid, a factor which has not been considered in previous discussions of the kinetics of tubular secretion. It is of interest to note that such accumulation within the renal interstitial fluid may be partly responsible for the 'second phase' in radioisotope renograms[13].

Plasma disappearance curves and slope clearance. We have mentioned that numerous attempts have been made to calculate renal clearances using plasma disappearance curves, and that it was necessary to hypothesize a two-compartment distribution for *p*-aminohippurate and 'Hippuran' to explain the data obtained. The following equations will concern the disappearance curves of substances excreted by glomerular filtration alone and of substances excreted by both tubular secretion and glomerular filtration. In these derivations it is assumed that extra-renal losses are negligible and the material is distributed throughout a single homogeneous plasma compartment. Probably neither of these assumptions is strictly true; however, it will be shown that the two-compartment hypothesis is not necessary to explain why plasma disappearance curves of substances secreted by the tubules fail to show a logarithmic decline.

Q is the quantity of material removed from the plasma at time T. V_pP is the quantity of material remaining in the plasma at time T where V_p is the volume of distribution. Let D be the quantity of material administered at zero time by a single intravenous injection.

$$V_pP = D - Q \tag{17}$$

Differentiation gives:

$$V_p\frac{\mathrm{d}P}{\mathrm{d}T} = -\frac{\mathrm{d}Q}{\mathrm{d}T} \tag{18}$$

From equation (4):

$$V_p\left(-\frac{\mathrm{d}P}{\mathrm{d}T}\right) = R_3 + R_4 \tag{19}$$

From equations (3), (5) and (6):

$$V_p\left(-\frac{\mathrm{d}P}{\mathrm{d}T}\right) = K_3P + V_n\frac{\mathrm{d}N}{\mathrm{d}T} + K_5N \tag{20}$$

If a substance were excreted by glomerular filtration alone very small quantities would diffuse into the renal interstitial fluid and, as the plasma concentration declines, would diffuse back into the plasma. R_4 would be very small in comparison to the other rates and $R_5 = 0$. If $R_4 \simeq 0$, then:

$$V_p\left(-\frac{\mathrm{d}P}{\mathrm{d}T}\right) = R_3 = K_3P \tag{21}$$

$$\frac{K_3}{V_p}\int \mathrm{d}T = -\int\frac{\mathrm{d}P}{P} + C_1 \tag{22}$$

$$\frac{K_3}{V_p}T = -\ln P + C_1 \tag{23}$$

Thus a plot of log P versus T would result in a straight line of slope $-2{\cdot}303\frac{K_3}{V_p}$.

For substances excreted by both glomerular filtration and tubular secretion $R_4 \neq 0$ and $R_5 \neq 0$. Equations (20) and (13) are two simultaneous first-order differential equations of variables P and N. By solving for P we obtain the following second-order differential equation:

$$V_n V_p \frac{\mathrm{d}^2 P}{\mathrm{d}T^2} + \left[(K_1 + K_5) V_p + K_1 V_n\right] \frac{\mathrm{d}P}{\mathrm{d}T} + K_1(K_3 + K_5)P = 0 \tag{24}$$

The general solution will be:

$$P = E_1 e^{S_1 T} + E_2 e^{S_2 T} \tag{25}$$

where:

$$S_{1,2} = \frac{-B_2 \pm \sqrt{B_2^2 - 4B_1 B_3}}{2B_1} = F_1 \pm F_2 \tag{26}$$

$$\left.\begin{aligned} B_1 &= V_n V_p \\ B_2 &= (K_1 + K_5) V_p + K_1 V_n \\ B_3 &= K_1(K_3 + K_5) \end{aligned}\right\} \tag{27}$$

E_1 and E_2 are constants depending on the boundary conditions.

$$P = e^{F_1 T}\left[E_1 e^{F_2 T} + E_2 e^{-F_2 T}\right] \tag{28}$$

$$\ln P = F_1 T + \ln\left[E_1 e^{F_2 T} + E_2 e^{-F_2 T}\right] \tag{29}$$

Since the second term of the right side of equation (29) is not a constant, a plot of log P versus T would not result in a straight line. Equation (28) shows that the declination of P is a double-exponential function of T which conforms to experimental data on the disappearance of *p*-aminohippurate[8] and of 'Hippuran'[10] following a single intravenous injection.

Delay in attainment of constant clearance. The last condition to be explained by the mathematical model is the observation of Mitchie and Mitchie[3] that, following almost immediate attainment of a constant *p*-aminohippurate concentration, a delay is noted before clearance becomes constant.

The material to appear in the ureters first would be that which is excreted by glomerular filtration. There would be some delay, of course, in passage of the material through the collecting tubules, the calyces and the ureters. Tubular secretion will not occur until the material diffuses into the interstitial fluid and $N \neq 0$. Clearance will not attain a constant value until $\frac{\mathrm{d}N}{\mathrm{d}T} = 0$ when the interstitial fluid concentration attains a steady equilibrium concentration. The rate at which this occurs is dependent on the rate at which the material enters the kidney, the rate at which it diffuses into the renal interstitial fluid, the size of the renal interstitial fluid compartment, and the rate at which the tubular secretory processes remove it from the latter compartment. In any event, these processes will require a longer time than the time required for material to be filtered by the glomerulus and to appear in the urine.

The described model of the kidney proposes nothing which is not already known about the actual structure of the kidney. We believe that consideration of the renal interstitial fluid space in the excretory processes can explain several experimental observations which appear to be thus far inadequately explained.

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PLASMA DIAMINE OXIDASE TITRES OF NORMAL AND PREGNANT RATS

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THE rise in the plasma-level of diamine oxidase during human pregnancy was first reported by Marcou *et al.*[1]. Attempts to find a similar change during animal pregnancy have been unsuccessful[2]. Ahlmark[3] reported that rat plasma showed a small rise in diamine oxidase during pregnancy, but this observation was attributed by Anrep *et al.*[2] to bacterial contamination. It has thus been assumed that the rise in plasma-level of diamine oxidase was peculiar to human pregnancy[2,4,5]. With the use of a new assay method for diamine oxidase developed by Okuyama and Kobayashi[6], it has now been found that rat plasma also shows a rise in diamine oxidase during pregnancy.

Female Holtzman rats were used in these investigations. Day one of pregnancy was determined either by the observation of a vaginal plug or by the presence of sperm in a vaginal smear. The rat diet of 'Purina' laboratory chow was supplemented with parsley during the experiment as a source of dietary iron.

2·5 ml. of blood was withdrawn by heart puncture under ether anæsthesia. The blood was oxalated, centrifuged in an International clinical centrifuge at maximum r.p.m. for 15 min, and the plasma removed. The plasma was heated at 50° C for 15 min to activate the diamine oxidase[7]. Then 0·75 ml. of plasma was diluted with 1·25 ml. of saline in a screw-cap culture tube and 5 μg of putrescine labelled with carbon-14 was added. The reaction mixture was incubated in a Dubnoff metabolic shaker at 37° C in air for 2 h, and the reaction product was extracted with toluene twice, according to the method previously described[6]. The amount of end-product was assayed in a liquid scintillation counter (either a Packard Tricarb, model 314 *EX* or a Nuclear Chicago, model 725S).

Table 1. AVERAGE DAILY TITRES OF PLASMA DIAMINE OXIDASE IN C.P.M. OF 12 FEMALE RATS MEASURED FOR 20 CONSECUTIVE DAYS

Day	Mean	± *S.D.*	Day	Mean	± *S.D.*
1	188	66	11	358	214
2	146	79	12	335	160
3	178	81	13	181	99
4	173	96	14	218	161
5	155	122	15	192	103
6	185	99	16	287	130
7	232	90	17	231	112
8	261	73	18	336	137
9	227	85	19	260	79
10	363	167	20	285	128

Table 2. AVERAGE PLASMA DIAMINE OXIDASE TITRE IN C.P.M. OF 12 FEMALE RATS BASED ON 20 CONSECUTIVE DAILY MEASUREMENTS

Rat No.	Mean	±	*S.D.*
1	271		114
2	236		129
3	167		94
4	214		109
5	339		142
6	193		122
7	249		104
8	278		156
9	163		95
10	301		150
11	143		80
12	320		128
Mean	240		119
S.D.	65		

The relative activity of the enzyme was expressed as c.p.m. corrected for background corresponding to putrescine reaction product. The counting efficiency was approximately 50 per cent.

^{14}C putrescine dihydrochloride (New England Nuclear Corp.) was diluted with non-isotopic putrescine dihydrochloride to contain 5 μg putrescine (base)/0·1 ml. with an activity of 20,000 d.p.m./μg.

An estimate of the normal, non-pregnant plasma diamine oxidase titre for female rats was obtained by following the daily plasma titres of twelve rats for twenty successive days. The daily average plasma titres observed are presented in Table 1. It can be seen that the values ranged from 155 c.p.m. to 363 c.p.m. with about a 50 per cent standard deviation. The average diamine oxidase titres of each rat over the twenty-day period are presented in Table 2. These values ranged from 143 c.p.m. to 339 c.p.m., again with a standard deviation of about 50 per cent. These data established a frame of reference for three pregnant rats which was similarly analysed during the gestation period. These data showing the pattern of plasma diamine oxidase titres during the gestation period are presented in Fig. 1. It can be seen that there was a marked rise in the plasma diamine oxidase titre from day 9 reaching a maximum at day 13, 14 or 15, depending on the rat, and then rapidly decreasing towards parturition. Two of the three rats showed a second rise in diamine oxidase at day 19. One rat delivered at day 21 while the other two delivered at day 22. All rats had normal litters.

An analysis of variance of the results in Table 1 showed there was a significant difference among the daily average diamine oxidase titres ($P < 0·001$). A similar analysis of Table 2 showed there was a significant difference between rats ($P < 0·001$). However, in spite of these statistical differences in the normal plasma titres among the rats and the relatively high standard deviation associated with each determination, the maximum change in the plasma titre of this enzyme during pregnancy was 50-100 times the non-pregnant levels. The development of diamine oxidase during rat pregnancy, therefore, is an easily recognized event.

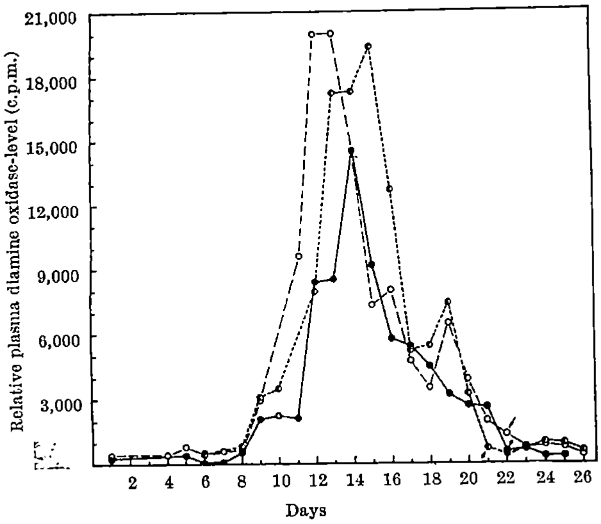

Fig. 1. Pattern of plasma diamine oxidase titres during pregnancy of three rats. Arrows indicate parturition

The pattern of diamine oxidase activity during the rat's gestation period may be correlated with the data presented by Kameswaran *et al.*[8] and Kahlson *et al.*[9]. Kahlson and his group have reported an increase in urinary excretion of free histamine by the mother rat from the fifteenth to about the twentieth day of gestation. At about the twentieth day, there was a sharp drop in the urinary excretion of histamine which was followed by parturition. The urinary histamine has been reported to be of fœtal origin[10]. Kameswaren *et al.*[8] have reported that the pregnant rat's uterus loses its sensitivity to histamine from about the tenth to the fifteenth day of gestation followed by a gradual recovery to full histamine sensitivity at about the nineteenth or twentieth day. If we focus our attention on the last six days of the gestation period, we can postulate that the three events—the drop in plasma diamine oxidase activity, the increasing sensitivity of the uterus to histamine, and the increasing excretion of free histamine (which is indicative of a higher circulating titre of histamine)—may act together to effect a histamine-dependent mechanism associated with parturition. The decreasing circulating titre of diamine oxidase would favour longer biological existence of the circulating histamine of fœtal origin in the mother. When the animal approaches term the sensitivity of the uterus to histamine returns so that the histamine may participate in the parturition process. It is unlikely that histamine and diamine oxidase can explain the complete mechanism of parturition, however, especially in view of the known action of progesterone and oxytocin[11,12]. It has been well established that a fall in progesterone titre during gestation initiates parturition in the rat. It should be recalled, however, that a fall in progesterone alone will not cause uterine tissue to contract and also histamine in the rat antagonizes myometrial contraction. Therefore, in view of the evidence presented here, it appears likely that histamine and diamine oxidase may be participants in the process of parturition in the rat.

Although there is general agreement that the rat placenta is a likely source of diamine oxidase[2,3], the stimulus required for the production and maintenance of plasma diamine oxidase is unknown. In the foregoing discussion it has been suggested that plasma diamine oxidase-levels are somehow associated with the level of circulating histamine. However, it is possible that the observed rise in plasma diamine oxidase may not, in fact, be associated with circulating histamine but with some other diamine.

I thank J. Kupelian and J. C. Dickinson for their assistance.

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SIMULTANEOUS ELECTROMAGNETIC MEASUREMENT OF BLOOD FLOW IN THE MAJOR CORONARY ARTERIES

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CORONARY artery disease is now the leading cause of death in the western hemisphere, and in the United States during the decade 1951–61 its incidence increased from 219·5 to 274·6 per 100,000 population. Furthermore, the percentage of all deaths from major cardiovascular-renal diseases attributable to coronary artery disease increased from 43 to 54 during this period[1].

These facts emphasize the need for a thorough understanding of the hæmodynamics of the coronary circulation. Until recently, however, most direct methods of measuring coronary arterial flow involved cannulation of the artery, the use of anticoagulants and variable trauma to the artery and surrounding tissues. Moreover, the measuring devices themselves often increased the resistance to the flow of blood. These shortcomings were largely overcome by the non-cannulating electromagnetic flowmeter[2], which has, in the past five years, been increasingly used in physiological investigations of the coronary circulation[3]. However, in all the reported work, flow in only one coronary artery at a time has been measured, and so only partial information has been obtained. The simultaneous measurement of flow in all the major coronary arteries would provide data on the total coronary flow and its distribution and the effects of occlusion of one major branch on flow through the other branches could readily be determined. A study of the simultaneously recorded patterns of phasic flow would also provide information regarding the strength and pattern of contraction in different parts of the myocardium. A more complete picture of the effects of drugs of pharmacological and therapeutic importance would also be possible. This article reports the successful use of the gated sine-wave electromagnetic flowmeter[4,6] to measure simultaneously blood flow in the right main coronary artery and in the anterior descending and circumflex branches of the left coronary artery in the anæsthetized dog. Flow in the anterior septal branch of the left coronary artery which carries approximately 15 per cent of the left coronary flow[5] was not determined, and since there were a few small branches proximal to the probes the combined flows measured in this investigation probably represent some 80 per cent of the total coronary inflow.

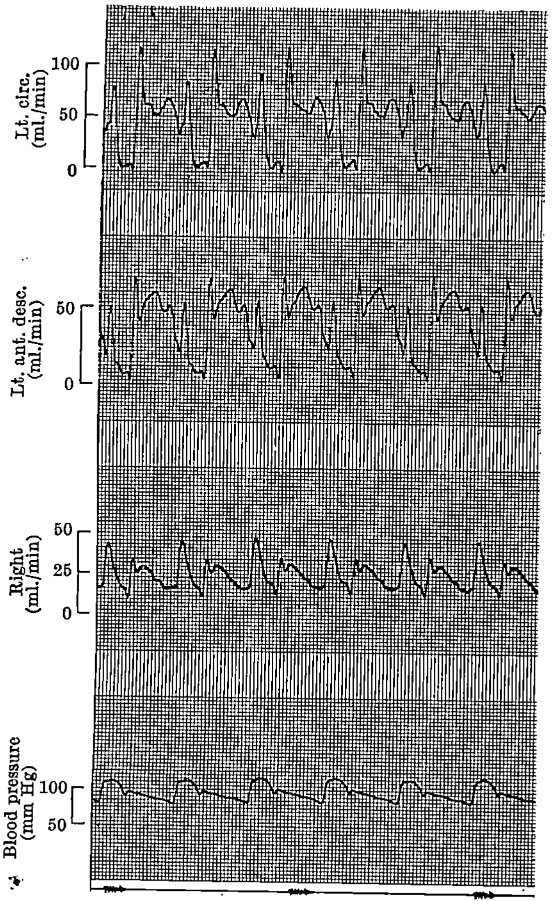

Fig. 1. Phasic blood flow in the left circumflex, left anterior descending and right coronary arteries of the anæsthetized open-chest dog. The lowest trace shows phasic arterial pressure recorded from the aortic root

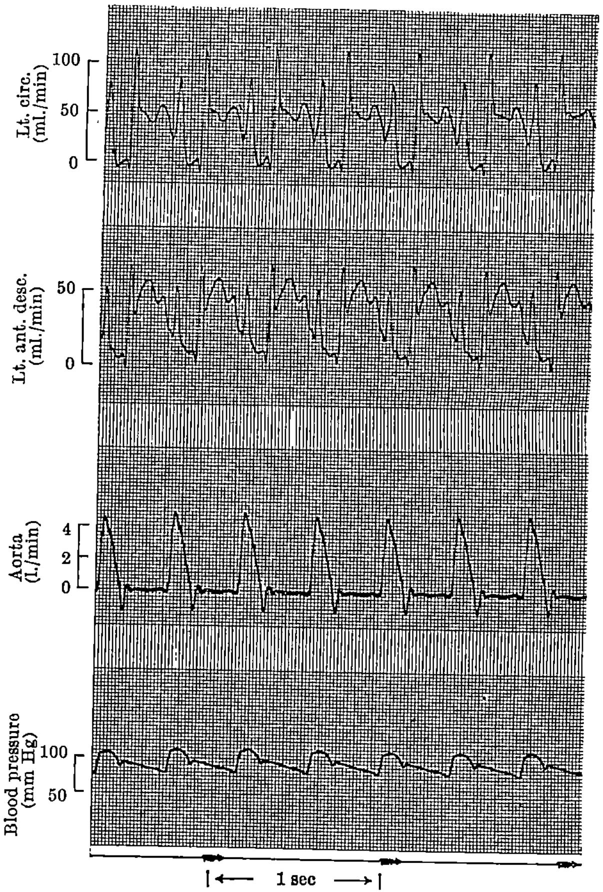

Fig. 2. Phasic blood flow in the left circumflex and left anterior descending coronary arteries and in the ascending aorta of the anæsthetized open-chest dog. The lowest trace shows phasic arterial pressure recorded from the aortic root

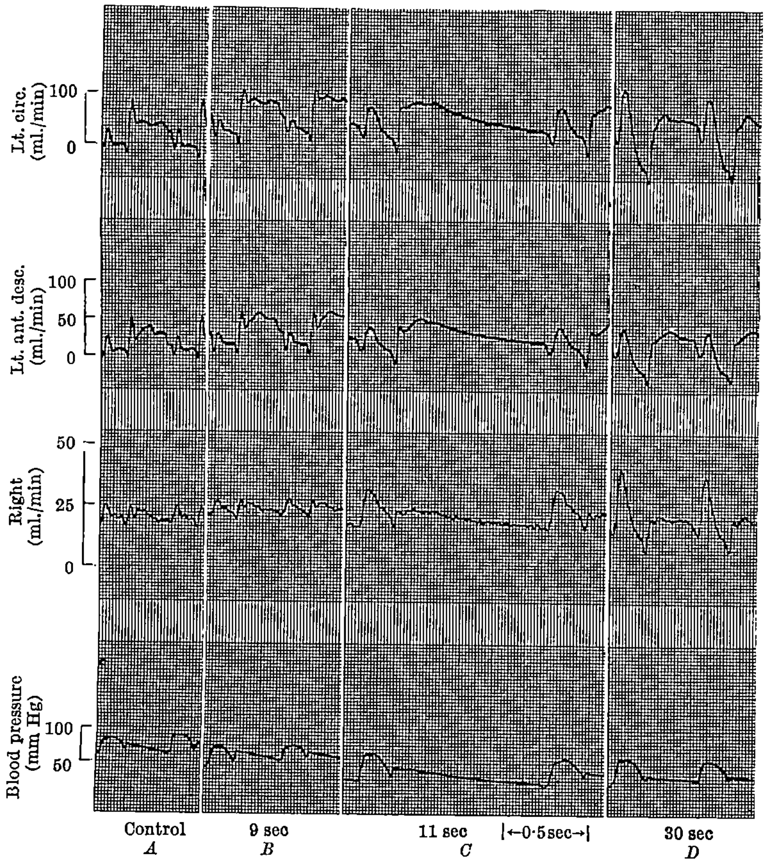

Fig. 3. Phasic aortic root pressure and phasic blood flow in the left circumflex, left anterior descending and right coronary arteries of the anæsthetized open-chest dog. *A*, control records immediately preceding the intravenous injection of 2 mg acetylcholine; *B*, *C*, *D*, record obtained 9 sec, 11 sec and 30 sec after this injection

Animals weighing 20–30 kg were anæsthetized with intravenous pentobarbitone sodium. A median sternotomy was performed and respiration was maintained with a Bird Mark 8 respirator. After opening the pericardium, 1–2 cm of the right coronary and left anterior descending coronary arteries were freed from the surrounding tissue. Access to the left circumflex coronary artery was obtained through an incision in the left fifth intercostal space. Flow probes were then placed on all three coronary arteries. Pressure at the root of the aorta was measured by a Sanborn physiological pressure transducer via a polyethylene tube introduced through the carotid artery. In some experiments a flow transducer was placed on the aortic root and flow in this vessel and in two coronary arteries was recorded. The flow probes and flow channels have been previously described[6–8]. In these experiments all three channels were energized by a common oscillator to avoid the possibility of frequency heterodyning. Calibration was performed at the end of the experiment using saline delivered by a gravity feed.

Table 1. BLOOD FLOW IN THE MAJOR CORONARY ARTERIES OF A 21-KG ANÆSTHETIZED OPEN-CHEST DOG

Heart weight 160 g, rate 150 min. Blood pressure 115/85. Duration of systole, 0·14 sec; duration of diastole, 0·26 sec

	Stroke flow (ml.)		Minute flow (ml.)			Ratio systolic: diastolic flow
	Systolic	Diastolic	Systolic	Diastolic	Total	
Left circumflex	0·07	0·24	10·5	36	46·5	1 : 3·4
Left anterior descending	0·04	0·15	6	22·5	28·5	1 : 3·8
*Total left coronary	0·11	0·39	16·5	58·5	74	1 : 3·5
Right coronary	0·07	0·09	10·5	13·5	24	1 : 1·3
*Total coronary	0·18	0·48	27	72	99	1 : 2·7

* Excluding flow in anterior septal artery.

Fig. 1 shows the phasic flows in the three coronary arteries. The general patterns are similar to those published by Khouri and Gregg[9] and Menno and Schenk[10] except for the more prominent peaks in systole and early diastole. Fig. 2, which shows simultaneous flows in the two main branches of the left coronary artery and at the root of the aorta, indicates that the upstroke of the systolic peak coincides with the beginning of aortic ejection while the upstroke of the diastolic peak corresponds to the beginning of aortic back flow.

These flow peaks are much less prominent in the absence of rigid cuffs around the aorta, such as the flow probe recording blood flow at the aortic root.

Table 1 summarizes some of the data obtained from Fig. 1. It will be noted that, unlike other arteries, flow in the coronary vessels is greater during diastole than during systole due to the impediment to flow through intramural vessels by the contracting myocardium. This impediment varies in different parts of the heart, as shown by the different systolic/diastolic flow ratios.

Fig. 3 shows the profound effect of an intravenous injection of 2 mg acetylcholine on the simultaneously recorded coronary flow patterns. After 9 sec, flow in late diastole is increased in all the coronary arteries, indicating that vasodilatation has occurred. The percentage increase in the stroke systolic flow in the right coronary artery and in the left anterior descending coronary artery is similar to the percentage increase in diastolic flow. A disproportionately large systolic flow increase has ocurred in the left circumflex coronary artery, however, indicating a considerable decrease in systolic extravascular resistance in the vascular bed of this artery. At 30 sec, vasodilatation is lessening but the stroke systolic flows have still further increased. It will also be noted that while late diastolic flow has returned to approximately the pre-injection levels in the right coronary artery and in the left anterior descending coronary artery, it remains elevated in the left circumflex coronary artery. Table 2 summarizes these changes.

The fact that the stroke systolic/diastolic flow ratio varies among the different coronary arteries in the control state and that this ratio varies to a different degree in each artery in response to a drug indicates that the coronary vascular bed is not uniform. This fact is of great importance since it means that flow changes observed in one artery following certain stimuli do not

Table 2. CHANGES PRODUCED BY INTRAVENOUS ACETYLCHOLINE 2 MG

Numbers in parenthesis indicate percentage of control values

		Before injection	9 sec after injection	30 sec after injection
	Aortic pressure	85/65	75/55	55/30
	Heart rate	150	150	143
	Duration of systole (sec)	0·14	0·14	0·14
	Duration of diastole (sec)	0·26	0·26	0·28
Lt. circ. coronary	Stroke systolic: diastolic flow ratio	1 : 13·2	1 : 4	1 : 2·1
	Late diastolic flow (ml./min)	40	80 (200%)	53 (133%)
Lt. ant. desc. coronary	Stroke systolic: diastolic flow ratio	1 : 5·2	1 : 5·6	1 : 3·3
	Late diastolic flow (ml./min)	32	50 (157%)	27 (86%)
Right coronary	Stroke systolic: diastolic flow ratio	1 : 1·8	1 : 1·8	1 : 1·3
	Late diastolic flow (ml./min)	19	24 (121%)	19 (100%)

always reflect the behaviour of the entire coronary circulation as has sometimes been assumed. It is anticipated that the simultaneous recording of flows in the major coronary arteries will lead to greater knowledge of myocardial function and coronary flow behaviour in different parts of the heart.

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ISOLATION OF A SOLUBLE RNA POLYMERASE FROM RAT TESTIS

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AN enzyme which catalyses a DNA-dependent incorporation of nucleotides into RNA, and requiring the presence of all four major ribonucleoside triphosphates, was first detected by Weiss[1,2] in association with insoluble nucleoprotein gels prepared from rat liver cell nuclei. Afterwards, such RNA polymerases were demonstrated in other mammalian[3–5] and plant[6] tissues, and in various micro-organisms[7–10]. But extensive purification of enzymes of this class, and thorough examination of their properties, has been achieved as yet only with certain bacterial polymerases[2,8,9], which can be readily freed from DNA. Most investigations of the DNA-dependent RNA polymerase activity of animal tissues have been conducted with 'aggregate' enzyme[1] preparations derived from cell nuclei. These gels contain firmly bound DNA which cannot be removed from the RNA polymerase protein in a manner which allows unequivocal direction of polyribonucleotide synthesis by exogenous DNA[1,2,11]. There is evidence that RNA synthesis by such aggregate enzyme preparations may be limited more by the priming ability of the bound DNA than by the catalytic activity of the RNA polymerase protein[11]. This complicates interpretation of changes in the RNA polymerase activity of nuclear aggregates from various tissues which have been reported to follow from hormonal[11–14] and other[15,16] treatments.

We report here a simple procedure for isolation of a soluble RNA polymerase from rat testis. The enzyme, which is probably derived from the nuclei of various cellular elements, exhibits an absolute requirement for DNA, and is virtually devoid of DNA polymerase and ribonuclease activities. DNA clearly directs the synthesis of complementary polyribonucleotides by this testicular enzyme. Recently, Furth and Loh[17] described the partial purification of a soluble DNA-directed RNA polymerase from chicken embryos.

Testes were excised from Sprague–Dawley rats (250–400 g) and the capsules and arteries removed. The tubules were homogenized in a Waring blender at low speed with two volumes of ice-cold 0·05 M *tris*(hydroxymethyl)-aminomethane (*p*H 75) containing 0·001 M β-mercaptoethanol. The homogenate was centrifuged at 20,000*g* for 20 min at 2°. The supernatant fluid was centrifuged at 98,000*g* for 1 h. The resulting supernatant fluid was fractionated by addition of solid ammonium sulphate at 0°. The material precipitating between 20 and 42 per cent saturation was collected and dissolved in a solution of the buffered β-mercaptoethanol containing glycerol at a final concentration of 30 per cent (v/v). Further fractionation was carried out on columns (30 cm × 2 cm) of diethylaminoethylcellulose, previously equilibrated against the buffered mercaptoethanol-30 per cent glycerol solution. Approximately 750 mg of protein was run on to the column at 25°, and washed through with the same medium until the absorbancy at 280 mμ of the eluant was less than 0·1. The flow rate was about 30 ml./h, and fractions of 5 ml. volume were collected. A second fraction was eluted with buffered mercaptoethanol-30 per cent glycerol made 0·15 M with respect to ammonium sulphate. This material possessed considerable RNA polymerase activity, but it was contaminated with DNA polymerase. When the optical density at 280 mμ of the eluant resulting from application of the latter fluid reached a value of less than 0·1, further elution was accomplished with buffered mercaptoethanol – 30 per cent glycerol made 0·3 M with respect to ammonium sulphate. This material was pooled, and the protein precipitated at 2° by addition of solid ammonium sulphate to 60 per cent saturation (assuming its solubility in water rather than the aqueous glycerol solution actually present). This protein material was dissolved in 0·05 M *tris* *p*H 7·5, 0·001 M β-mercaptoethanol containing glycerol at a final concentration of 50 per cent (v/v). The ratio of the absorbancy of this protein solution at 280 mμ to that at 260 mμ was in the vicinity of 1·5. The fraction was devoid of DNA polymerase activity[18], and failed to degrade highly radioactive *E. coli* RNA labelled with phosphorus-32 to acid-soluble products[19].

Some features of the RNA polymerase activity of the partially purified testicular enzyme are summarized in

Table 1

Experiment No.	Reaction system	GMP-^{14}C incorporated into RNA (μμmoles)
1	Complete	72·0
	Omit CTP	0·7
	Omit UTP	3·0
	Omit CTP and UTP	0·7
	Omit CTP, UTP and ATP	0·5
	Omit DNA	0·6
	Omit $MnCl_2$	0·0
	Add 2 μmoles spermidine	121·0
	Add 2 μmoles spermine	111·0
2*	Complete	81·5
	Omit 2-mercaptoethanol	37·0
	Add 2·5 μg actinomycin D	11·0
	Add 10 μg actinomycin D	2·0
	Add 2 μmoles phosphopyruvate and 20 μg pyruvate kinase	80·0

Cofactor requirement for soluble testicular RNA polymerase. The complete reaction system contained: 100 μmoles *tris* buffer of *p*H 7·7; 3 μmoles of $MnCl_2$; 5 μmoles of 2-mercaptoethanol; 0·5 μmoles each of ATP, CTP and UTP; 53 μg of calf thymus DNA; 0·078 μmoles of GTP-8-^{14}C ($4·2 \times 10^6$ c.p.m. per μmole); and 280 μg protein of material eluted from the diethylaminoethyl-cellulose column by 0·3 M ammonium sulphate. The final volume was 1·0 ml. The vessels were incubated for 40 min at 37° C.

* The complete system in Exp. 2 was identical to Exp. 1 except that 0·04 μmoles GTP (8×10^6 c.p.m./μmole) and 375 μg protein were added.

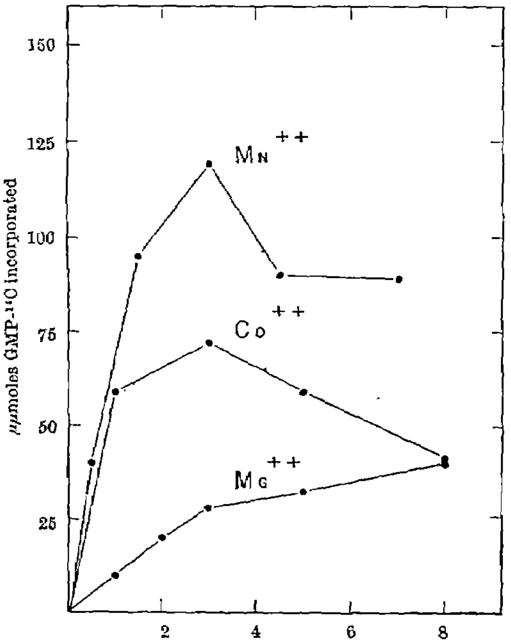

Fig. 1. The requirement of divalent cations for incorporation of GTP-8-^{14}C. The reaction vessels contained: 100 μmoles of *tris* buffer of *p*H 7·3; 0·5 μmoles each of UTP, CTP and ATP; 2 μmoles of PEP; 20 μg of pyruvate kinase; 5 μmoles of 2-mercaptoethanol; 27 μg of salmon sperm DNA; 0·048 μmoles of GTP-8-^{14}C ($8·06 \times 10^6$ c.p.m./μmole); and 470 μg of testicular enzyme. The divalent cations were present in the concentrations indicated. The total volume was 1·0 ml. Incubation proceeded for 40 min at 37°

Table 1. A virtually complete requirement for all four ribonucleoside triphosphates, for DNA, and for Mn^{++} ions was observed. The DNA-primed synthesis of RNA was also supported by Mg^{++} and Co^{++} ions as depicted in Fig. 1, but Mn^{++} ions were the most effective, especially at low levels. The incorporation of nucleotides into RNA was stimulated by β-mercaptoethanol and by polyamines. In experiments of short duration, no enhancement of nucleotide entry into RNA was observed on addition of phosphopyruvate and pyruvic kinase, suggesting that degradation of ribonucleoside triphosphates did not limit the rate of reaction. The RNA polymerase activity was inhibited by low concentrations of actinomycin *D* and inorganic pyrophosphate. The corresponding deoxyribonucleoside triphosphates could not substitute for the ribonucleoside triphosphates. Substitution of one ribonucleoside diphosphate for the corresponding triphosphate decreased the labelled nucleotide incorporation by about 50 per cent. The optimal *p*H for RNA polymerase activity was 7·7. The nucleotide incorporations were linear with respect to enzyme concentration and to time of incubation over an initial period of 40 min. Incorporation of all four labelled ribonucleoside triphosphates into RNA was observed. The system was saturated with all the four nucleoside triphosphates at a final concentration of about 0·12 mM. Maximal rates of reaction occurred at 37°. When DNA isolated from calf thymus, salmon sperm, *Micrococcus lysodeikticus*, and *T*2-bacteriophage was used as a primer, the RNA polymerase activity was optimal in each case at a final concentration of approximately 50 μg DNA per ml. of reaction mixture. Heat-denatured DNA was much less effective as a primer. In marked contrast to the RNA polymerase activity of mammalian nuclear 'aggregate' enzyme preparations, which exhibit highest activity at high ionic strengths[2,3,11], the incorporation of nucleotides into RNA by the soluble testicular enzyme was markedly depressed on raising the ionic strength above 0·1.

That the reaction product with various labelled ribonucleoside triphosphates as precursors was RNA was suggested by the fact that the acid-insoluble radioactive material was rendered acid-soluble by treatment with ribonuclease (but not with deoxyribonuclease), and also after hydrolysis in 0·3 N potassium hydroxide at 37° for 18 h. Radioactivity was detected in all four of the 2′(3′)-mononucleotide products of the base hydrolysis when any single α-^{32}P-labelled nucleoside triphosphate was used as precursor in the presence of the three complementary ribonucleoside triphosphates. When three DNA's of widely different base compositions (*M. lysodeikticus*, calf thymus, and *T*2-bacteriophage) were used as primers with either α-labelled ^{32}P-GTP or uniformly labelled ^{14}C-UTP as substrates, the extent of GMP and UMP incorporation was a reflexion of the composition of the DNA added to the system; and nearest neighbour base frequency investigations[7,10] with ester-labelled ^{32}P-GTP or -UTP provided strong evidence for direction of complementary RNA synthesis by DNAs from several different sources.

Similar testicular enzyme preparations, in a system containing the requisite ribonucleoside triphosphate precursors and Mn^{++}, failed to catalyse any polyribonucleotide synthesis in the presence of either tobacco yellow mosaic virus RNA, polyuridylic, or polycyticylic acids as primer. Such experiments were carried out under conditions where rates of reaction equivalent to 5 per cent of those found with DNA's as primers could have been readily detected. These findings are in marked contrast to those obtained with certain refined bacterial RNA polymerases, which readily catalyse RNA-directed as well as DNA-directed RNA synthesis[19,20].

When rat testis tubules were comminuted in hypotonic solutions as described here, considerable quantities of the RNA polymerase were found in the supernatant fluid resulting from ultracentrifugation of the homogenate. But if the testicles were homogenized in isotonic sucrose containing 1 mM magnesium chloride, then considerable amounts of RNA polymerase, later extractable with hypotonic buffers, were associated with the 'nuclear' fraction which sedimented on centrifugation at 600*g*. Application of the methods recorded here to rat prostate[11] failed to render soluble any appreciable RNA polymerase activity. The factors which permit the facile solubilization of the RNA polymerase from testis and embryonic tissue[17] as compared with certain other mammalian cells[4,11] remain obscure, but the presence of a relatively large number of dividing cells may be important in this respect.

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RESISTANCE OF INFECTIOUS RNA AND TRANSFORMING DNA TO IODINE WHICH INACTIVATES f_2 PHAGE AND CELLS

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THE greater stability and general utility of iodine and certain of its organic derivatives compared to its chlorine counterparts are probably responsible for their use as sterilizing agents in hospitals, surgical areas, and for the disinfection of drinking-water, swimming-pool water, and eating utensils. The effect of elemental iodine on viruses, however, is not consistent. Two apparently opposite results were reported on enteric viruses and on tobacco mosaic virus (TMV). Elementary iodine was found to destroy the infectivity of enteric viruses including polio, coxsackie and echo virus[1], yet TMV[2,3] and, recently, TMV–ribonucleic acid[4] were resistant to iodine.

Since the iodide ion concentration in a reaction mixture significantly influences the rate of iodination of tyrosine in proteins it would not be surprising if the apparent discrepancy in the viricidal action of iodine were due to differences in the iodide concentration in the reaction mixtures.

In the present investigations, the iodide concentration in reaction mixture was appropriately considered as the bactericidal and viricidal action of iodine was examined. Despite its powerful bactericidal and viricidal action in minimal iodide ion concentration, iodine did not produce appreciable destruction of either infectious RNA or DNA.

Infectious RNA from iodinated phage. The RNA bacterial phage was kindly supplied by Dr. N. D. Zinder through Dr. T. Fukasawa. The purification of phage was essentially the same as described by Loeb and Zinder[5]. In order to test the biological activity of infectious RNA from phage inactivated with iodine, partially purified f_2 phage, having 1×10^{13} per ml. of plaque-forming unit, was treated with 0·2 mM of iodine containing 0·24 mM of potassium iodide for 20 min. The phage survival was 0·0018 per cent, or about 5 logs of inactivation. After reduction of the residual iodine by sodium thiosulphate, the RNA was extracted and compared to that extracted from un-iodinated phage. The RNA extraction procedure used was similar to that described by Paranchych[6] with a slight modification. It was found that the infectivity of the RNA extracted from these two preparations was the same, that is, the inactivated phage yielded infectious RNA in quantity indistinguishable from that of normal phage (Table 1). Such a result suggests that either the protein coat of the phage is so compact that iodine cannot reach the RNA or that the RNA is insensitive to iodine, or both.

Iodine resistancy of infectious RNA. To test the sensitivity of infectious RNA to iodine $2 \cdot 6 \times 10^6$ plaque-forming unit of f_2 RNA was treated with 0·4 mM iodine containing 0·48 mM potassium iodide for 10 min at room temperature (27° C). The iodine colour persisted even at the end of the reaction. As shown in Table 2, the infectious RNA activity was completely resistant to treatment with iodine.

From these results it is quite clear that inactivation of phage by iodine in minimal amounts of potassium iodide may be attributed to its action on the protein coat of the phage. The reaction of iodine with protein is well known, and includes oxidation of sulphydryl groups or substitution into the tyrosyl or histidyl moieties[7]. Since f_2 phage has no histidine residue in its protein coat[8], reaction of iodine with the sulphydryl and tyrosyl groups need not be considered, although tryptophanyl residues could be involved. *Para*-chloromercuribenzoate (PCMB), a sulphydryl attacking agent, in 10^{-3} M solution did not inactivate f_2 phage in *p*H 8 *tris* buffer in 60 min contact at room temperature, in contrast to *E. coli* which was inactivated completely within several seconds by the same concentration of this agent. This leaves the reaction of iodine with tyrosyl and perhaps tryptophanyl residues as responsible for the loss of infectivity of f_2 phage.

Table 1. IODINATION OF INTACT PHAGE AND INFECTIVITY OF ITS RNA

Iodination	Infectivity (PFU per ml.) Before iodination	After iodination	Survival (%)	Infectious RNA found
0·2 mM for 20′, at room temperature	10×10^{12}	$1 \cdot 8 \times 10^8$	0·0018	$3 \cdot 25 \times 10^6$
Control	10×10^{13}	10×10^{12}*	100	$3 \cdot 65 \times 10^6$

* Control was treated with thiosulphate only.

Partially purified f_2 phage in *p*H 7 of PBS was iodinated with 0·2 mM (50 mg/l.) of iodine which, containing 0·24 mM of potassium iodide, at room temperature for 20 min, at the end of reaction residual iodine was reduced by sodium thiosulphate. The infectious RNA of both iodinated phage and control of non-treated phage was extracted and assayed for infectivity by the method described in the text.

Table 2. RESISTANCE OF INFECTIOUS f_2 RNA TO IODINE

	Infectivity (PFU/ml.)
Iodination by 0·4 mM I_2 containing 0·48 mM KI at room temperature for 10 min	$2 \cdot 42 \times 10^6$
Control	$2 \cdot 55 \times 10^6$

The infectious RNA extracted from 10^{13} PFU/ml. was diluted with PBS and iodinated with 0·4 mM of iodine containing 0·48 mM of potassium iodide at room temperature for 10 min. At end of reaction, residual iodine was reduced with sodium thiosulphate and activity was measured.

Comparison of iodination of tyrosine to iodine inactivation of phage. Tyrosine is the amino-acid residue most likely to be attacked by iodine at neutral *p*H, and room temperature. In examining the action of iodine on tyrosine, Li[9] noted that the iodide ion retards the reaction. In the present investigation the iodination of tyrosine was performed under conditions comparable to those used for inactivation of phage. L-Tyrosine (Calbiochem) was

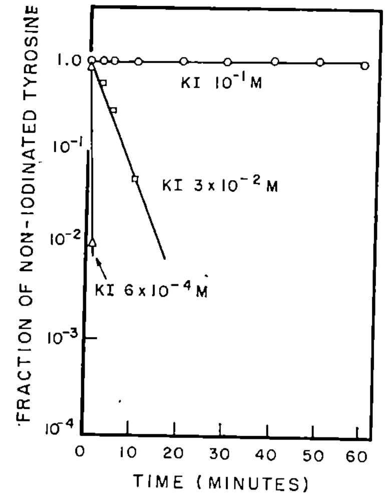

Fig. 1

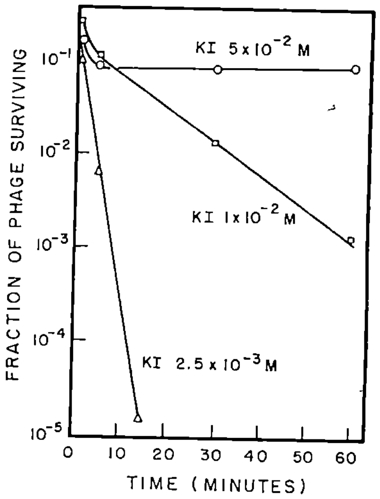

Fig. 2

Fig. 1. Effect of varying amounts of potassium iodide on iodination of tyrosine in 0·05 M PBS (*p*H 7) at room temperature using $1 \cdot 25 \times 10^{-4}$ M of 1-tyrosine and 5×10^{-4} M iodine

Fig. 2. Effect of potassium iodide concentration on rate of phage inactivation with 0·04 mM of iodine in 0·05 M PBS (*p*H 7) at room temperature

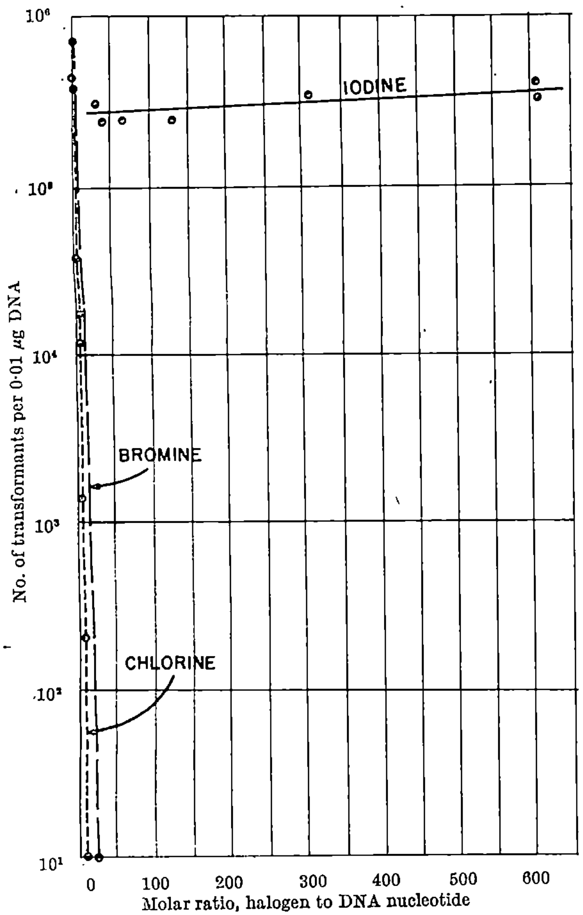

Fig. 3. Effect of iodine, chlorine and bromine on transforming DNA. The number of transformations per 0·01 μg of DNA remaining after reaction with different halogen is plotted against the initial halogen concentration

dissolved in pH 7, 0·05 M phosphate buffer, containing 0·14 M sodium chloride (PBS). Iodotyrosine was determined from the absorbancy at 312 mμ of wave-length[10]. As shown in Fig. 1, iodination of tyrosine was very rapid in 0·6 mM potassium iodide with 0·5 mM iodine, whereas in 100 mM of potassium iodide and the same level of iodine, only 5 per cent of tyrosine is iodinated. Thus the conditions which inhibit iodination of tyrosine (Fig. 1) also inhibit the inactivation of phage by iodine as shown in Fig. 2.

Action of halogen on transforming deoxyribonucleic acid (DNA). The purified transforming DNA containing less than 1 per cent of protein was extracted from strains of *Haemophilus influenzae* resistant to streptomycin (S^r), cathomycin (C^r), erythromycin (E^r), and kanamycin (K^r), and in addition, a strain which utilized protoporphyrin (PP-) in the dark in place of hæmin[11] was used. The DNA was dissolved in 0·1 M phosphate buffer–saline (PBS), and mixed with the halogen under different conditions of time, pH, temperature and concentration. Samples of the reaction mixture were stopped by diluting into 10^{-3} M solution of sodium thiosulphate. The residual transforming activity in these samples was then determined by incubating with competent *H. influenzae*, and then plating in agar containing individual antibiotics[12] or with protoporphyrin instead of hæmin and cultured in the dark.

Summarized in Fig. 3 are the results of a series of experiments in which the number of transformations per 0·01 μg of transforming DNA remaining after reaction with different halogens is plotted against the initial halogen concentration. It is clear from these results that the transforming activity was destroyed by chlorine and bromine, but not by iodine. Even after 4 h of contact at 37° C with 2 mM (500 mg/l.) of iodine in 2·4 mM potassium iodide, all five genetic markers (S^r, C^r, E^r, K^r and PP) were almost fully active. In some experiments DNA survived reaction with iodine at 45° C and pH 8·5. Heat-denatured DNA was also resistant to iodine. Dialysis of DNA after treatment with iodine-131 left no detectable iodine bound to the nucleic acid, so there could have been no addition or substitution reaction of iodine. The iodine also failed to change the ultra-violet absorption of the nucleic acid which was in contrast to the effect of chlorine which flattened the ultra-violet absorption curve.

High concentrations of a commercial organic iodine (Wescodyne) acting for several hours failed to reduce appreciably the transforming activity of DNA.

In a manner resembling somewhat the work on phage, it was found that fully active transforming DNA was extracted from *H. influenzae* which had been treated with 100 lethal doses of iodine (2 mM or 500 mg/l.).

Since iodine and its organic derivatives are commonly used as disinfectants, RNA and DNA resistance to iodine may have special significance. Inactivation of viruses by iodine might merely mean modification of its protein coat, and the genetic properties—particularly the replicability—could be preserved.

Evidence has been presented indicating that f_2 phage inactivation by iodine is due to its action on the protein component. Conditions which affect the phage inactivation also change the iodination of tyrosine in a comparable manner. It was shown by Anson *et al.*[2] that, even in high concentrations of potassium iodide, iodine oxidized —SH groups of protein or viruses. Choppin *et al.*[13] and Allison *et al.*[14] have reported that some viruses are resistant to some —SH reacting agents. The following cases may indicate an explanation for this effect: (1) —SH groups are buried deep in the molecule and are not accessible to iodine. (2) Protein coat of virus does not contain —SH groups such as myosin[15]. (3) —SH groups are not essential for the virus infection and multiplication, as the case of TMV[2].

Only 10 per cent of f_2 phage activity was recoverable after a few seconds of contact with iodine even in 0·5 M concentration of potassium iodide. In this concentration of potassium iodide, iodine reaction with tyrosine would not have been detected for hours. It does not appear likely that 90 per cent loss of activity was due to iodination of tyrosine residues in the protein coat. No explanation for this result can be offered at this time.

These investigations were sponsored by the Committee on Environmental Hygiene and supported in part by the U.S. Army Medical Research and Development Command and a U.S. Public Health Service training grant in environmental science.

I thank Dr. R. M. Herriott and Dr. C. W. Krusé for their advice, Dr. S. Nomura for her co-operation, and Misses Annabel Lindley and Carole Beach for assistance.

Note added in proof. Work at present being carried out in our laboratory by Dr. Nomura indicates that infectious polio RNA is fully resistant to iodine as the f_2 RNA. Also, the disinfection of the polio virus by iodine is inhibited by the presence of the iodide ion.

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Para-O-METHYLATION OF DOPAMINE IN SCHIZOPHRENIC AND NORMAL INDIVIDUALS

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SEVERAL recent publications have concerned the unique occurrence of 3,4-dimethoxyphenethylamine in the urine of schizophrenics[1–3]. Although suggestions have been made that this might be of dietary origin, there remains the possibility that it represents the product of an abnormal metabolism of dopamine, and may be intimately involved in the ætiology of the disease. In the concluding stages of the present work, Friedhoff reported[4] that di-*O*-methylation of dopamine occurs *in vitro* when the substrate is incubated with a liver biopsy homogenate obtained from a schizophrenic patient known to excrete dimethoxyphenethylamine, and further that di-*O*-methylation of administered labelled dopamine can be demonstrated *in vivo*. Thus the question of dietary origin seems to have been settled. There has, however, been no report of results of similar tests made with normal individuals.

Axelrod *et al.*[5] have noted that "successive replacement of phenolic hydroxyl groups of sympathomimetic amines is known to shift the pharmacological activity from the peripheral to the central nervous system"; Michaux and Verly[6] have reported 3,4-dimethoxyphenethylamine to be a more potent catatonic agent than the 3-methoxy-4-hydroxy derivative; and Ernst[7] has noted the catatonic activities of dimethoxy and 3-hydroxy-4-methoxyphenethylamine, finding the 3,5-dimethoxy-4-hydroxy derivative inactive in cats. Methylation in the 3-position represents a normal pathway of catecholamine metabolism *in vivo*, these derivatives (and catabolites) appearing in urine. However, *in vitro*, catechol-*O*-methyl transferase (COMT) affords a mixture of the 3- and 4-*O*-methyl derivatives. The latter, however, have not been found in urine except in the cases of arterenone, adrenalone and dihydroxyacetophenone[8]. It is possible that the presence of 4-*O*-methyl derivatives of epinephrine, *nor*epinephrine and dopamine could have been missed since no chromatographic system has been reported which adequately resolves a mixture of the 3-hydroxy-4-methoxy and 4-hydroxy-3-methoxy compounds. Resolution of the derivatives formed by coupling with any of several diazonium salts did not prove completely satisfactory in our hands[9]. Daly *et al.*[8] have oxidized *normeta*nephrine and *norpara*nephrine to vanillin and isovanillin for identification purposes. The object of the present work was to confirm Friedhoff's initial observations, to look for evidence of 3-hydroxy-4-methoxy catecholamine metabolites in urines, and to examine COMT products for di-*O*-methylated materials.

Using Friedhoff's techniques[1], we were unable to obtain unequivocal evidence of the presence of dimethoxyphenethylamine in schizophrenic urines, due to the presence of other materials in the same region of the papergram, nor did we find two-dimensional systems similar to that described by Takesada[2] as satisfactory as the technique afterwards developed. Further, our methods effect excellent resolution of basic urinary metabolites including the isomeric 3-hydroxy-4-methoxy and 4-hydroxy-3-methoxy analogues of dopamine, epinephrine and *nor*-epinephrine.

Urine samples were hydrolysed with acid and passed over a column of 'Dowex 50(H$^+$)' at *p*H 5. After washing successively with water, 0·1 N sodium acetate and water again, the basic compounds were eluted with 1 N ammonia in 65 per cent ethanol, concentrated, adjusted to *p*H 5 and taken to dryness[10]. The residue, in water, was divided into aliquots equivalent to an amount of urine containing 1 g of creatinine, adjusted to *p*H 10·3 and each aliquot (2 ml.) extracted with four 2-ml. portions of *iso*amyl alcohol–toluene (3 : 2). The organic layer was re-extracted with 0·05 N hydrochloric acid, and the extract dried under nitrogen. The bases in the residue, which include the 3-hydroxy-4-methoxy and 4-hydroxy-3-methoxy analogues of dopamine not separable by Friedhoff's procedure, were chromatographed as described here for separation and identification. To obtain a cleaner chromatogram for dimethoxyphenethylamine *estimation*, the mixture was further purified by extraction into chloroform from an aqueous solution adjusted to *p*H 9·5. The material was applied in methanol to one corner of a 20 cm × 20 cm silica gel ('Silica Gel *G*', E. Merck Acc. Stahl 7731) thin-layer plate, and chromatographed in 1-butanol–acetic acid–water (4 : 1 : 1) and air dried. The plate was then developed in a direction at right angles to the first, using 1-butanol–ethyl acetate–15 N aqueous ammonia (3 : 1 : 1). For estimation of the dimethoxy compound, 200 mg creatinine equivalent of material was used per chromatogram, while half this quantity was used for identification of other metabolites. To aid in identification and quantitation, appropriate amounts of known compounds were applied outside the limits of the solvent fronts. Identity and the almost quantitative recovery of the compounds of interest from urine were established by adding known amounts of these to urine prior to the isolation procedure, and comparison with isolates from unadulterated urine after two-dimensional chromatography. Catecholamine metabolites having a 3-hydroxyl group were detected by spraying first with 0·1 per cent alcoholic 2,6-dichloroquinone-chlorimide, and then with 0·2 per cent ninhydrin in pyridine – acetic acid – acetone (1 : 4 : 95). For quantitation, the first spray alone was used. Dimethoxyphenethylamine was better estimated on plates sprayed only with ninhydrin, 1 μg being readily detectable. As may be seen in Fig. 1, compounds of interest were separated and zones well defined.

Dimethoxyphenethylamine was not detected in any of ten normal urines. Of a group of twelve schizophrenics*, five excreted this compound, and in another group, two of ten†. The higher percentage in the first group may reflect the larger proportion of acute cases. The quantity of this metabolite varied from 10 to 3 μg/g urinary creatinine. The rather lower incidence of this compound in our limited study, as compared with previous reports (57 of 62 (ref. 2), 15 of 19 (ref. 1)), we attribute at least partly to a more selective isolation and chromatographic procedure, eliminating false positive results.

Sen and McGeer[3] found a new urinary metabolite, 4-methoxyphenethylamine, in eleven of twenty-two schizophrenic urines, but no examination of normal urines was

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* We are indebted to Dr. Arnold J. Friedhoff, of the New York University School of Medicine, for these specimens.

† We are indebted to Dr. Nathan Kline, of the Rockland State Hospital, for these specimens.

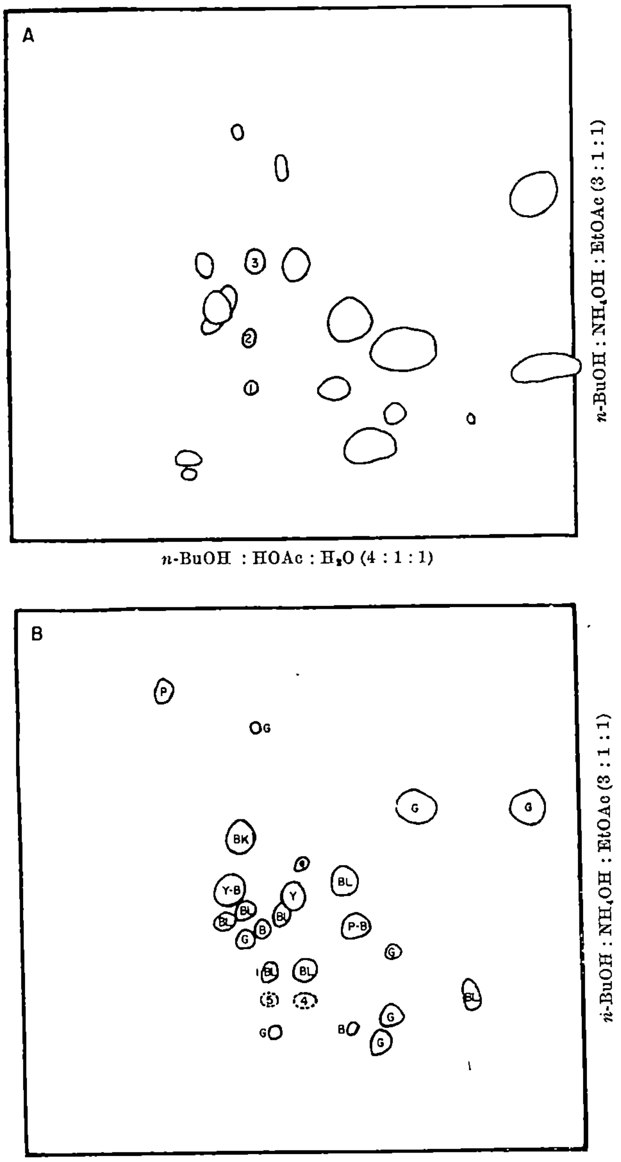
A
1
2
3
n-BuOH : NH₄OH : EtOAc (3 : 1 : 1)
n-BuOH : HOAc : H₂O (4 : 1 : 1)
B
P
G
BK
BL
Y-B
Y
B
P-B
1
4
5
n-BuOH : NH₄OH : EtOAc (3 : 1 : 1)
n-BuOH : HOAc : H₂O (4 : 1 : 1)

Fig. 1. Thin-layer chromatograms of urine extracts

(*A*) sprayed with ninhydrin; (*B*) sprayed with dichloroquinone-chlorimide. In both cases the sample was applied at the lower right-hand corner. Numbered spots are: compound isographic with but not identical to 3-hydroxy-4-methoxyphenethylamine (1), 3-methoxy-4-hydroxyphenethylamine (2), 3,4-dimethoxyphenethylamine (3), *para*nephrine (4), *norpara*nephrine (5). Spots represented as dotted circles did not appear in unadulterated urine extracts. Colours designated are: grey (*G*), black (*BK*), blue (*BL*), brown (*B*), purple (*P*), yellow (*Y*)

reported. We found a compound isographic with 3-hydroxy-4-methoxyphenethylamine, and reacting similarly with dichloroquinone-chlorimide and ninhydrin. However, by eluting this compound from the thin-layer plate and re-chromatographing in methanol-1-butanol–benzene–water (2 : 1 : 1 : 1) on Whatman 3 *MM* paper pretreated with 2·5 per cent sodium carbonate, it was shown that our tentative identification was incorrect. The compound was not further identified, nor was it peculiar to the schizophrenic urines. We were unable to detect the 3-hydroxy-4-methoxy analogues of dopamine, epinephrine or *nor*epinephrine‡ in any of the urines examined.

In our investigations with purified rat liver COMT, the alkaline solvent described in our thin-layer separation was used to confirm earlier findings[9] that dopamine is converted to both 3-hydroxy-4-methoxyphenethylamine and 4-hydroxy-3-methoxyphenethylamine. Both products were visualized by spraying the plate with Folin's reagent and were isographic with the authentic materials both on silica gel and paper. Quantitation was achieved by use of *S*-adenosyl-L-methionine-methyl-^{14}C. The ratio of 3-hydroxy-4-methoxy to 4-hydroxy-3-methoxyphenethylamine was found to be 4 : 1, and this ratio did not change significantly throughout the duration of incubation for 1 h with measurements at 5, 10, 15, 20 and 60 min. Nor was the ratio different when a crude COMT preparation from rat liver or brain was used. This may suggest that a single enzyme gives rise to both products. Although formation of the di-*O*-methyl derivative cannot be completely ruled out, we were unable to find definite evidence for its formation, by rat liver, using either dopamine or one of the mono-*O*-methyl derivatives as substrate. Incorporation of radioactivity occurred when 3-hydroxy-4-methoxy or 4-hydroxy-3-methoxyphenethylamine was incubated with the enzyme and *S*-adenosyl-L-methionine-methyl-^{14}C, more in the case of the latter substrate. In each case, partial conversion to the isomer occurred. Interconversion of some *O*-methyl catechols has been demonstrated *in vivo*, and *O*-demethylation has been demonstrated *in vitro*[8]; however, the interconversion with purified COMT does not seem to have been reported previously.

In conclusion, we have developed an improved method for the separation and identification of some catecholamines, have used it to confirm the unique occurrence of 3,4-dimethoxyphenethylamine in the urine of schizophrenic patients and noted the probable greater frequency of excretion in acute patients. There is no indication in our investigation that 3-hydroxy-4-methoxy analogues of catecholamines are formed *in vivo* by COMT, since no such derivative of dopamine, epinephrine or *nor*epinephrine was detected in urine. Our experiments with rat COMT *in vitro* indicate that this enzyme does not cause di-*O*-methylation. On the other hand, interconversion of the 3- and 4-*O*-methyl group of hydroxy-methoxy phenethylamine by COMT and *S*-adenosyl-L-methionine is reported for the first time, and the absence of 3-hydroxy-4-methoxy metabolites of catecholamines in urine may reflect this interconversion *in vivo*, although there is evidence in rats that administered paranephrine is excreted without change[8].

We must emphasize the fact that our enzyme experiments were performed with COMT from rats. Takesada *et al.*[2] were not able to detect dimethoxyphenethylamine in the urine of rats dosed intramuscularly with dopa, dopamine, or *nor*epinephrine. Friedhoff[11] has commented on this, and has used schizophrenic patients for his metabolite studies[4], but the methodology differs from that of the Japanese authors in that smaller quantities of labelled dopamine were given by intravenous infusion. His *in vitro* experiment also differed from our rat liver experiments in that a homogenate of a biopsy specimen from a schizophrenic known to excrete the corresponding phenethylamine was used, and dimethoxyphenylacetic acid rather than the amine was detected.

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‡ We are indebted to Dr. Marvin D. Armstrong, of Fels Research Institute, and Dr. S. Archer, of Sterling-Winthrop Research Institute, for the samples of *para*nephrine and *norpara*nephrine.

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CYTOLOGICAL DETERMINATION OF THE HOMŒOLOGY OF CHROMOSOMES OF *Triticum aestivum*

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THE 42-chromosome hexaploid wheat of agriculture, *Triticum aestivum*, arose through allopolyploid evolution by the combination of the chromosome sets of the three related diploid species, *Triticum monococcum*, *Aegilops speltoides* and *Aegilops squarrosa* (all $2n = 14$). In the karyotypically primitive forms of *T. aestivum*, like the variety 'Chinese Spring', the chromosomes of the original diploid ancestors are structurally unchanged. Moreover, the original genetical equivalence of corresponding, homœologous, chromosomes derived from different diploid ancestors is still retained to a considerable extent.

This was demonstrated by Sears[1], who showed that all the 40-chromosome nullisomics of *T. aestivum*, deficient for each chromosome pair in turn, have pronounced phenotypic abnormalities. By contrast, the phenotypes are normal for plants that, in certain combinations, are simultaneously nullisomic for one chromosome and tetrasomic for a second. Apparently the chromosome in extra dosage compensates for the disturbances normally caused by nullisomy, indicating similarities in the genetic activities of the two chromosomes concerned. Nullisomic–tetrasomic compensation permits the classification of the 21 pairs of chromosomes into seven groups each of three pairs. Within each group there is good compensation in any nullisomic–tetrasomic combination, but between groups there is little, or no, compensation. Moreover, within each group one pair of chromosomes falls into each genome and was thus derived from a different diploid ancestor of the hexaploid[2]. Pairs of chromosomes in the same group are therefore homœologous, being genetically corresponding derivatives of the ancestral diploid species. This correspondence of homœologues may be presumed to be due to their origin from the same chromosome of a remote common progenitor of all three ancestral diploid species.

Only bivalents are formed at meiosis in *T. aestivum*, and there is disomic inheritance. Each chromosome therefore pairs only with its single fully homologous partner and there is normally no meiotic association between homœologues. The absence of homœologous pairing has been shown to be due to a genetic activity of a single chromosome, number *5B* (refs. 3, 4, 5). In plants deficient for this chromosome homœologous pairing occurs, although the deficiency of no other chromosome produces similar changes in the course of meiosis.

In most hybrids between *T. aestivum* and 14-chromosome diploid species in the genera *Aegilops*, *Secale* and *Agropyron* there is little chromosome pairing at meiosis, presumably because homœologous synapsis is precluded by the unmodified effect of chromosome *5B*. By contrast, in hybrids between *T. aestivum* and the diploid species *Aegilops speltoides* or *Aegilops mutica* there is considerable meiotic pairing, indeed cells occur in which all 28 chromosomes have undergone synapsis and chiasma formation[6]. Bivalents, trivalents and quadrivalents are common, and Riley, Unrau and Chapman[7] were led to propose, from pairing of this nature, that the activity of chromosome *5B* is suppressed in the presence of the genotype of *A. speltoides*. On this hypothesis the pairing is homœologous and the largest configurations—quadrivalents—result from the association of all three chromosomes of some homœologous groups of *T. aestivum* together with, in each case, the corresponding chromosome of *A. speltoides*.

The work recorded here was designed to determine the relationships of the chromosomes of *T. aestivum*, which pair meiotically in the presence of the genotype of *A. speltoides*. It was further anticipated that if, as was expected, the paired chromosomes proved to be homœologous, information would be gained about the structural equivalence of individual arms of the homœologues compared.

Since, at meiosis, it is impossible to distinguish the chromosomes of *T. aestivum* morphologically, it was necessary to mark those examined by introducing distinctive structural conditions. For this purpose use was made of the set of lines, produced by E. R. Sears in the variety 'Chinese Spring', in which each chromosome in turn is represented by a pair of telocentrics for one arm, the other arm being completely deficient. Plants with disomic telocentric conditions for different known chromosomes were crossed together to produce derivatives in which two pairs of chromosomes each consisted of one normal and one telocentric member. These derivatives were pollinated by *A. speltoides* to produce 28-chromosome hybrids which were selected for the presence of two telocentric chromosomes. Such *T. aestivum* × *A. speltoides* hybrids carried telocentrics of two different, and known, chromosomes of *T. aestivum*.

Emphasis in this first exploration of the technique was concentrated on simultaneously marking two chromosomes of homœologous group 5, or one of the chromosomes of group 5 and one of those of either group 3 or group 6. Before naming the chromosomes concerned it should be interpolated that, in the designation of the chromosomes of *T. aestivum*, the number represents the homœologous group and the letter the genome to which each belongs. The chromosomes of homœologous group 5 are thus *5A*, *5B* and *5D*, while those of groups 3 and 6 are respectively *3A*, *3B*, *3D* and *6A*, *6B*, *6D*. *T. aestivum* × *A. speltoides* hybrids were produced with the following two *Triticum* chromosomes simultaneously marked by being telocentric:

(*a*) Within homœologous group 5

5A–*5B* *5B*–*5D*

(*b*) Between homœologous groups

3A–*5A*	*3B*–*5B*	*6B*–*5B*
3A–*5B*	*3D*–*5D*	*6D*–*5B*
3B–*5A*	*6B*–*5A*	*6D*–*5D*

The behaviour of the two telocentrics, relative to each other, was investigated at first metaphase of meiosis in all these hybrids using permanent Feulgen-propionic orcein squashes of pollen mother cells. In hybrids, in which the telocentrics were in different homœologous groups, the marked chromosomes never participated in the same configuration. Either one or both of them occurred in bivalents, trivalents or quadrivalents in the many cells examined, but they were always associated with complete chromosomes and never with each other (Fig. 1*a*).

By contrast, in the hybrids in which chromosomes *5A* and *5B* or *5B* and *5D* were simultaneously marked, the telocentrics associated together in the same configurations. In some cells both telocentrics were not paired, and in others both paired with a complete chromosome in separate bivalents (Fig. 1*b*). They also participated together in configurations that were similar whether the

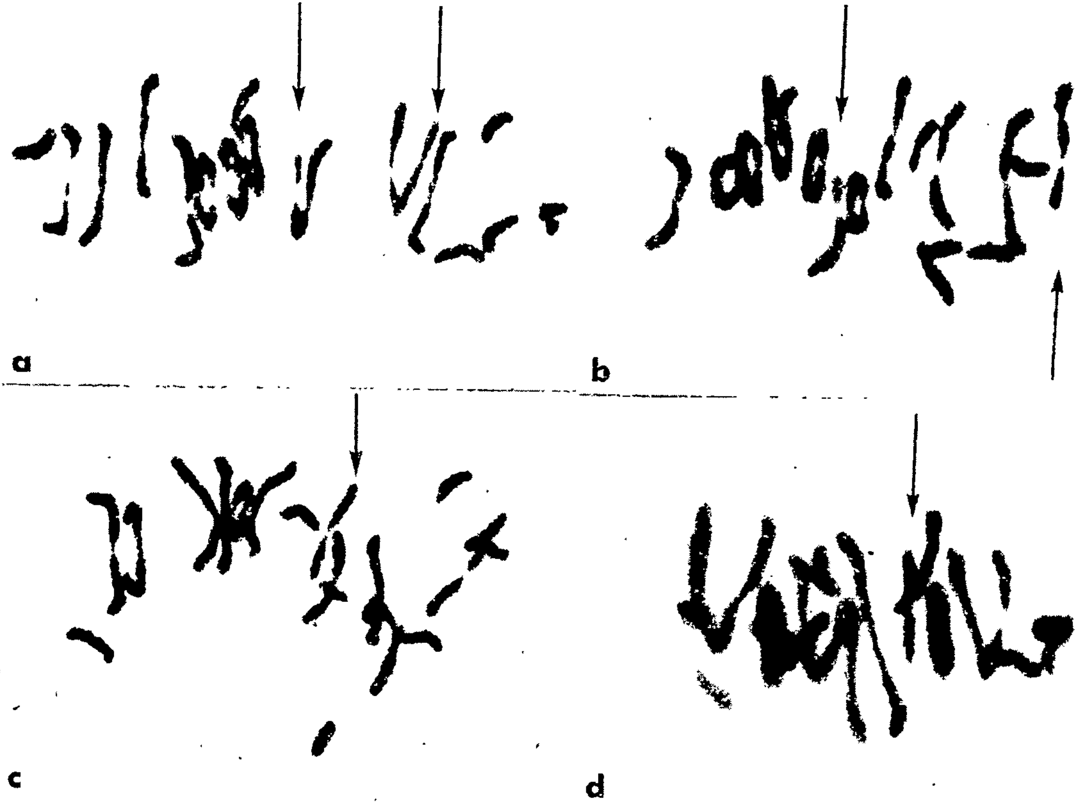

Fig. 1. First metaphase of meiosis in *T. aestivum* × *A. speltoides* hybrids with two chromosomes of *T. aestivum* simultaneously telocentric. (*a*) Three trivalents, seven bivalents and five univalents, telocentrics of 5*D* and 6*D* in different trivalents (arrowed); (*b*) one trivalent, eleven bivalents and three univalents, telocentrics of 5*B* and 5*D* in separate bivalents (arrowed); (*c*) one trivalent, ten bivalents and five univalents, telocentrics of 5*B* and 5*D* paired together in a bivalent (arrowed); (*d*) cell partly out of focus, but in focus a trivalent containing the telocentrics of 5*A* and 5*B* and a complete chromosome (arrowed)

telocentrics were 5*A* and 5*B* or 5*B* and 5*D*. The following patterns of association occurred:

(*a*) The two telocentrics paired together to form a rod-shaped bivalent (Fig. 1*c*).

(*b*) The two telocentrics were associated with a complete chromosome to form a triradial trivalent (Fig. 1*d*).

(*c*) The two telocentrics were associated with two complete chromosomes to form a quadriradial quadrivalent.

(*d*) The two telocentrics associated with two complete chromosomes to form a chain-of-four quadrivalent, in which the telocentrics were at opposite ends of the chain.

If the initial classification into homœologous groups, by the use of genetic criteria, is accepted, these observations can be interpreted as confirming that in the presence of the genotype of *A. speltoides* homœologous meiotic pairing occurs, presumably because of the suppression of the restrictive activity of chromosome 5*B*. Alternatively the capacity of chromosomes 5*A*, 5*B* and 5*D* to pair at meiosis may be regarded as demonstrating their cytological homœology independently of any preconceived notions of their relationships. By the determination of the chromosomes, which although not strictly homologous are capable of meiotic pairing, an unequivocal recognition of homœology is possible in cytological terms.

Moreover, the use of the present technique permits a further refinement in the understanding of the cytogenetic structure of *T. aestivum*. The telocentrics used, of chromosomes 5*A*, 5*B* and 5*D*, are revealed to represent equivalent arms of all three homœologues, because they are capable of pairing together directly. The arm of 5*A* present as a telocentric carries the genes *Q*, causing speltoid suppression, and b_1 causing awning; the arm of 5*B* carries the gene p_{5B}, causing the suppression of homœologous pairing; while the arm of 5*D* carries the prime factor for spring habit of growth. The characteristics associated with these genes, or their alleles, are now shown to be determined by equivalent arms of homœologous chromosomes. The acquisition of similar information about the arm relationships of the chromosomes of other homœologous groups would clearly provide a useful extension in the understanding of the genetic structure of wheat.

The aspect of this first investigation, however, that may offer the most promise for further work is the observation of differential pairing between the homœologues examined. Thus, when chromosomes 5*B* and 5*D* were simultaneously marked they paired together, usually as a rod bivalent, in about 50 per cent of cells. By contrast, when chromosomes 5*A* and 5*B* were simultaneously marked they paired together comparatively rarely, in only about 5 per cent of cells, and then often as a triradial trivalent which also incorporated a complete chromosome (Fig. 1*d*). These distinctive behaviours indicate a closer relationship between chromosomes 5*B* and 5*D* than between 5*A* and 5*B*, which may imply relatively greater evolutionary divergence in detailed structure and possibly in gene content between some homœologues than others. The extension of evidence of this kind to other homœologous groups, and its quantitative evaluation, should permit a reassessment of the evolutionary adjustment of *T. aestivum* to its polyploid status.

This first description of a technique for the cytological evaluation of chromosome homœology has outlined the possibilities made available in *T. aestivum*. Clearly the technique is feasible and offers considerable opportunities for the further genetic and cytogenetic investigation of the species; but its use need not be confined to intra-specific examinations. Provided that, in alien chromosome addition lines, one wheat and one alien chromosome can be simultaneously marked, the determination of the precise relationships between *Triticum* chromosomes and those of the alien species becomes possible through the suppression of the 5*B* effect by the *A. speltoides* genotype. Knowledge of these relationships would not only be of considerable interest in evolutionary investigations of the wheat group but might assist in shaping programmes for the improvement of wheat by the introduction of alien genetic variation.

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MITOCHONDRIAL MORPHOGENESIS

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ALTHOUGH mitochondrial morphogenesis has interested many workers in cell biology, including Roullier[1], Faucett[2], Hoffman and Grigg[3], Green[4], Ross[5], Kilarski[6], McAlear[7], Luck[8], Robertson[9] and, more recently, Berger and Sjostrand[10], no such compelling evidence has been forthcoming that the presentation of still another perspective on this complicated problem is precluded.

In a series of earlier publications[11–13] we described a sequence of morphological steps which seem to characterize the assembly of 'mitochondria-like organelles'. These bodies are formed in the follicular cells surrounding the early oocytes of the hen. They are of about the same size as conventional mitochondria (about $0{\cdot}5\mu \times 2{\cdot}0\mu$). They possess a double external envelope with membranes of about 50 Å each (the dimensions of the membranes in conventional mitochondria is also about 50 Å) and what appear to be rudimentary cristæ (Fig. 1). They are synthesized in large numbers at the surfaces of the follicular cells and bud into the intercellular spaces whence they pass into the oocyte. Here they appear to participate in the organization of yolk (at least they are present in groups within the developing yolk granules), an association that is fulfilled by conventional-appearing mitochondria in the oocyte of the frog[11] and the turtle. It is not known if they contain succinic dehydrogenase or other enzymes associated with the Krebs cycle.

The earliest feature which has been definitely linked with their morphogenesis is the swelling of a vesicle (adjacent to the plasma membrane, suggesting that it has newly entered the cell) and the elaboration from this vesicle of a number of sub-vesicles which eventually spread about its entire inside surface. These sub-vesicles then stand out more darkly (in osmium-fixed material) and while in this state elaborate 'micro-vesicles' about their surfaces which fuse or elaborate further to form the two unit membranes of the envelope of the 'mitochondrion-like organelle' and also the rudimentary cristæ which are spaced about one sub-vesicle apart[13].

We feel we should be remiss if we did not at this point give some indication of our ideas regarding the origin and the nature of the vesicle that serves as a precursor for the formation of this organelle.

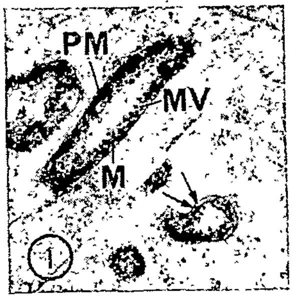
Our hypothesis is that the vesicle, or at least some of the material in it, may have originally arisen in the nucleus. Particularly in the oocyte, the nuclear membrane is heavily studded with so-called 'annular pores'. In the early chicken oocyte these 'pores' are, however, not wide open holes. An electron-dense granule appears to be firmly fixed in the centres of most of these[14] and it has been found in *Drosophila* that the magnitude of the potential across the nuclear membrane is independent of the number of 'pores'[15].

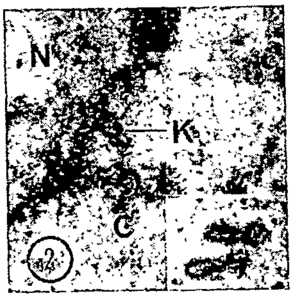
One way in which larger units appear to traverse these 'pores' is by reduction in size and in the case of vesicles by reassembly of micro- to sub- to 'mature' vesicles on the cytoplasmic side of the 'pore' (Fig. 2). The 'mature' vesicles very closely resemble the 'kinetosomes' of unicellular organisms. Although in *Paramecium* their origin has not been shown, Ehret and De Haller[16] and others have indicated that they appear to be involved in formation of cilia. They further point to their presence in certain vacuolated mitochondria of this organism[16]. Close inspection of the vesicles both in *Paramecium* and the oocyte of the hen (Fig. 2) reveal that they are bounded by single 'membranes' with bristled surfaces and that, at least in some cases, they contain centrally located rods or granules. Although they vary considerably in size and shape, the mature vesicles, when spherical, measure about 1500–2000 Å in diameter. They possess an inherent density which is characteristic of nucleic acid. Their failure to bleach on application of periodic acid and silver methenamine further attests to such a character. They (or vesicles resembling them) travel freely from cell to cell, going through the aforementioned process of dissolution and reassembly in order to traverse the two plasma membranes. Often, very similar appearing vesicles (commonly referred to as pinocytotic- or absorption-vesicles) form by a process of simple invagination of the plasma membrane. Our view is that the latter may be essentially similar in nature to the vesicles derived directly from the nucleus—that at least some of their materials are products of the nucleus and that the vesicles, whatever their immediate origin, carry some sort of information or scheme of organization provided by the genetic centre and destined for different expressions in the cytoplasm.

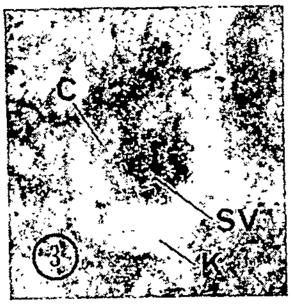
Some of the factors leading to our suspicion that the mitochondria-like organelles are formed in association with such nuclear precursors include: (*a*) a cohesive parcel of repeating organizational and/or informative units would appear to be a logical precursor for the synthesis of a radially organized structure such as a 'mitochondrion-like organelle'; (*b*) sub-vesicles and micro-vesicles are seen to contribute to—and elaborate from—the precursor vesicles, processes that are strongly reminiscent of the character of the 'kinetosomes' elaborated from the nuclear 'pores' (Fig. 2); (*c*) the precursor vesicles often present bristled exterior surfaces (micro-vesicles ?) relating them to 'kinetosomes'; (*d*) 'kinetosomes', including central granules, are observed within the fluid interiors of many mature 'mitochondria-like organelles'.

Although conversion of 'mitochondria-like organelles' into more conventional appearing mitochondria has been entertained by us, we are now of the opinion that this occurs rarely.

Observations have recently been made on mitochondria appearing in cells of early chicken embryos (18–24 h incubation) and on mitochondria of developing livers. As in the case of the 'mitochondria-like organelles' of the follicular cells, the ontogenesis of these bodies is intimately related to vesicles that exhibit at least two of the charac-







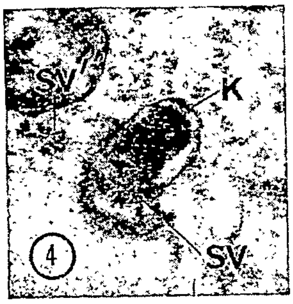


Fig. 1. Mitochondria-like organelles from an intercellular sinus between follicular cells. Note closely adherent plasma membrane (*PM*), double membraned mitochondrial envelope (*M*) and rudimentary cristæ (arrows). A rudimentary crista at point labelled (*M V*) consists of 2 micro-vesicles. (× *c*. 28,350)

Fig. 2. This vesicle (or kinetosome) (*K*) is being assembled on the cytoplasmic side of the nuclear pore from a number of smaller vesicles. Chicken oocyte: *N*, nucleus; *C*, cytoplasm. Inset shows fine structure of more fully differentiated vesicle. Note dense centre streaks. It is possible that the 'bristles' are actually the joined surfaces of micro-vesicles. (× *c*. 21,750)

Fig. 3. Cytoplasmic bud (*C*) with sub-vesicles (*SV*) projecting into lumen of expanded 'kinetosome' in chicken embryo cell. This is the earliest form in which a prospective mitochondria can be recognized at present. (× *c*. 39,000)

Fig. 4. 'Dense body' stage of mitochondrial morphogenesis in chicken embryo liver cell is depicted here. Sub-vesicles (*SV*) are present at base and to side (*SV'*). Observations indicate that some of sub-vesicles, elaborating from the sides of the kinetosome (*K*) and spilling out into the cytoplasm (*SV'*), are destined to form endoplasmic reticulum. No distinct membrane or envelope has formed about the bud. (× *c*. 28,350)

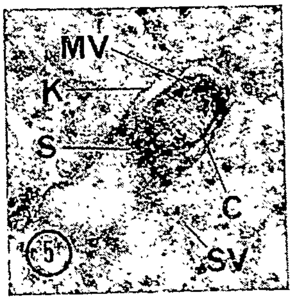
teristics of the above-mentioned 'kinetosomes', namely, bristled or 'fuzzy' limiting membranes and a propensity to convert to—and from—sub-vesicles and micro-vesicles.

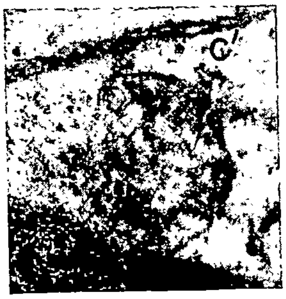
An initial event appears to be the expansion of a 'kinetosome' into a larger vesicle and the projection into it of a bud consisting of: (*a*) vesicular membrane; (*b*) sub-vesicles; (*c*) cytoplasm, including ribosomes (Fig. 3). This step in morphogenesis has also been observed by Berger[10], who makes the identification at the bases of the photoreceptors in the guppie where there is very little chance that it can be mistaken for another organelle. Thus, in contrast to the mode of formation of the rudimentary cristæ of the 'mitochondrion-like organelle', elaboration of an extensive system of cristæ apparently requires the presence of a substantial quantity of cytoplasmic material.

A later stage in the development of a mitochondrion is presumably represented by a bud that more nearly fills the lumen of the original vesicle (Fig. 4). Note that, except for the sub-vesicles at its base, it has become darker but retains a single limiting membrane. In some cases the bounding vesicle will dissolve away into smaller vesicles or the bud will break through it to lie in the ground substance of the cytoplasm. In this form it is recognizable as a 'dense body' long claimed by Roullier[1] to be a precursor form for mitochondria. It is, of course, possible that all mitochondria do not pass through this stage. However, elaboration of micro-vesicles is often, if not always, accompanied by a darkening in osmium-fixed tissues.

By the time the bud has become less dark again the beginning of cristæ formation appears to have taken place (Fig. 5). Some micro-vesicles can be discerned in it (arrows) and the large vesicle in which it lies has usually begun to dissociate into smaller vesicles some of which contribute to the newly forming endoplasmic reticulum in the case of chicken embryo and liver cells. Berger[10] indicates that the double membrane which constitutes the limiting envelope is formed by delamination from the original vesicle. That this is not so in all mitochondria described by us is indicated in Fig. 6. Here it appears that the limiting envelope of the mitochondrion, as well as its cristæ, is being formed by a fusion of—or elaboration from—micro-vesicles which have formed from the sub-vesicles of the bud. Further support for the existence and role of micro-vesicles in giving rise to cristæ is given in Fig. 7, which we think is an example of a mitochondrion which has for some reason failed to fuse—or keep fused—its micro-vesicles into cristæ (note that the organelle is bounded by a vesicular external envelope). A summary of proposed steps involved in morphogenesis of a mitochondrion in the tissues examined is given in Fig. 8.

It is, of course, precarious to select morphogenic steps from a series of photographs not sequentially related and in tissues where morphogenesis, though rapid, includes various organelles. The extent to which artefact may obtain is another consideration. However, the foregoing





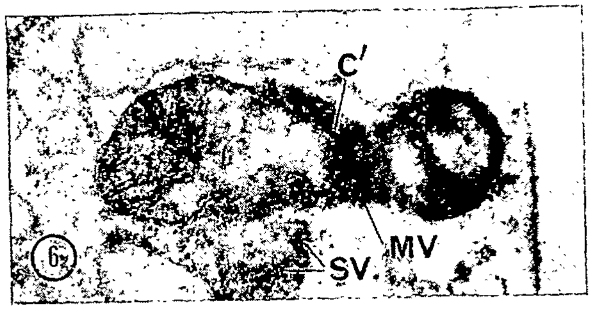


Fig. 5. In this later stage the prospective mitochondrion has become lighter again. The original vesicle (*K*) is dispersing into sub-vesicles (*SV*) (at several points) which do not appear to be contributing to the new mitochondrion. The bud is limited by a single membrane and is seen to contain sub-vesicles (*S*) and micro-vesicles (*M V*) which have contributed to the partial formation of at least one crista (*C*). The sub-vesicles at the lower right (*SV*) will form endoplasmic reticulum. The limiting membrane of the bud is thickened, suggesting that it may be doubling. (× *c*. 28,350)

Fig. 6. A nearly mature mitochondrion in chicken embryo cell suggesting that elaboration of limiting envelope as well as cristæ (*C'*) is by virtue of dissemination of micro-vesicles (*M V*) from sub-vesicles originating in cytoplasmic bud (Fig. 3). Although it is possible that these micro-vesicles are artefacts due to degeneration, osmotic—or other—factors, the general manner in which 'guidance' of detailed morphogenesis is accomplished is still indicated. Sub-vesicles labelled (*SV*) are from original vesicle. They are probably forming endoplasmic reticulum here since they are spaced the usual distance from the mitochondrion for such formation. (× *c*. 33,000)

Fig. 7. This body observed in a somatic cell from an early chicken embryo is thought to be a mitochondrion that has failed to assemble its cristæ (*C'*) from the micro-vesicles seen here or the cristæ of which have disassociated into such micro-vesicles. It is possible that it is a cistern of a Golgi apparatus, but such usually contain large sub-vesicles with well-defined centre granules. These, too, elaborate membranes by dissemination into micro-vesicles. (× *c*. 28,350)

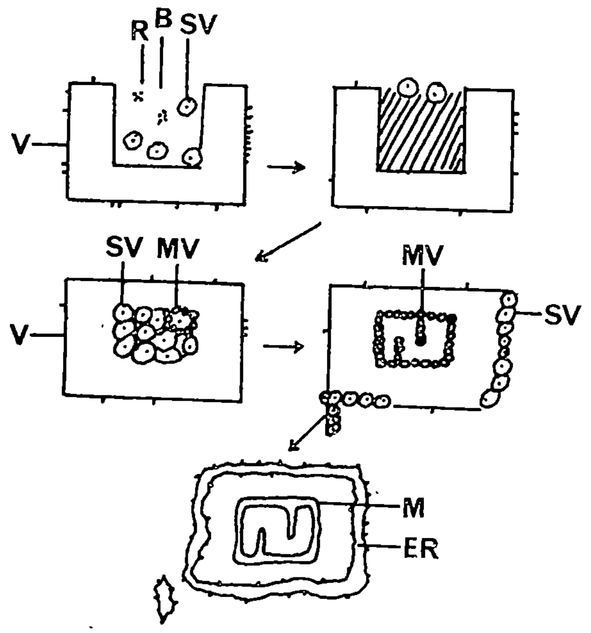


Fig. 8. Self-explanatory figure summarizing proposed morphogenic steps in mitochondrial development. *V*, vesicle; *R*, ribosomes; *B*, bud; *SV*, sub-vesicle; *MV*, micro-vesicle; *ER*, endoplasmic reticulum; *M*, mitochondrion

concepts are based on a large number of observations of different organelles; they develop a logical scheme of nuclear control over cytoplasmic differentiation and thus provide some new perspectives that may prove useful in this regard.

Although we have not attempted to demonstrate the existence of the micelles advocated by Sjostrand[17] as constituting the unit membranes of mitochondria, his concept is in complete accord with a scheme of morphogenesis by vesicular assignment. Some of the phenomena which may be associated with the transition of micro-vesicles to membrane sheets, including the micelles, may be revealed by future electron microscopy at higher resolution.

Before the wider concept of vesicle complexes originating in the nucleus, passing to the cytoplasm and there aiding in (or actually directing) the elaboration of mitochondria (or other organelles) can be unconditionally accepted, supporting data of an experimental nature must be forthcoming. It should also be shown to apply to other species[18,19].

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EXERCISE OF MATING PREFERENCE BY A MERINO RAM

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MATING preference has long been postulated as one of the mechanisms concerned in the development of new species[1,2]. It does not appear, however, to have been observed in operation in wild-type or quasi-wild-type populations, although Thoday and Gibson[3] and Parker[4] have inferred that it occurred during the course of their experiments.

It is the purpose of this article to describe what appears to have been the exercise of mating preference by a Peppin Merino ram.

During the past twenty-five years a number of families of inbred Merinos have been developed on the C.S.I.R.O. McMaster Field Station at Badgery's Creek, New South Wales, Australia. Each family was founded by mating a single ram with a number of ewes. He was then mated to his own daughters and, provided that he survived, to his inbred female offspring from them. On his death or retirement for senility, his most inbred son replaced him as head of the flock, and the breeding programme was continued.

Two families of Peppin Merinos in particular, designated *B*323 and *B*464, were founded by sons of a sire known as *A*991 in 1942 and 1944 respectively. Our immediate concern is with the behaviour of two sires in these families, namely, *K*68 and *K*504, during the mating seasons of 1959, 1960 and 1961. Including *A*991 and *K*68 six generations of sires were used in the *B*323 family (Fig. 1), the pedigree of the sires themselves being shown in Fig. 2. The ancestry of *K*504 follows a similar pattern.

No selection was practised in these families except that each sire used was the most inbred young animal of mating age available at the particular time. All females born were introduced to the breeding flock at one and a half years of age and, provided that they remained physically sound, were retained therein as long as they lived. During mating each ram and his particular family of ewes were run together in a small paddock as a breeding group. The ram was marked on the brisket with coloured grease raddle, some of which he transferred to the rump of any ewe with which he mated. The flock was inspected daily, the ear-tag numbers of raddled ewes recorded, and fresh raddle applied to the brisket of the ram. On the seventeenth day the colour of the raddle was changed. The duration of each mating period was two clear œstrus cycles (34 days). Except at mating time all ewes on the Station were run together as a single flock. During lambing the flock was inspected daily, and new-born lambs were caught, ear-tagged and identified with their mothers.

Numbers of ewes in the two families were never great, and their reproductive efficiency declined as the level of inbreeding increased. Two young rams, *K*68 and *K*504, were brought in to head the flocks in 1959, by which time ewe numbers had become so low that recourse was had

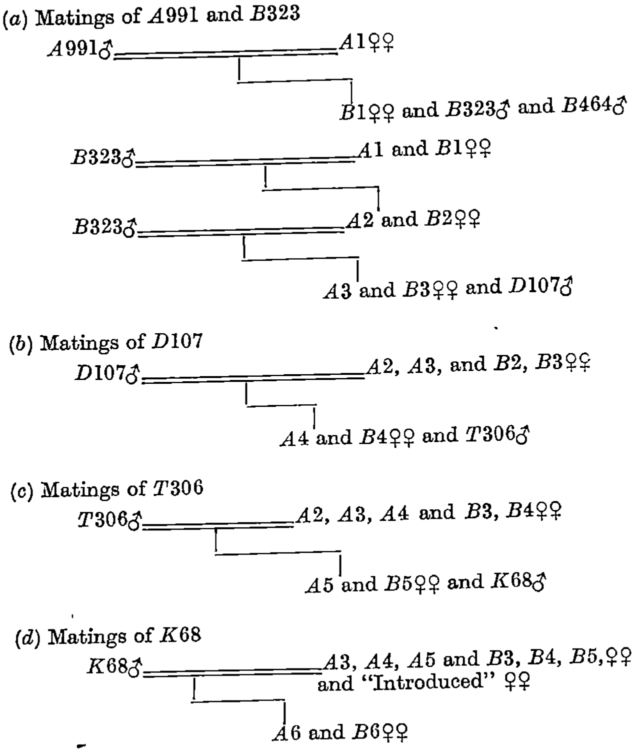

Fig. 1. Matings in the *B*323 family. *A*1, foundation ewes; *B*1, ewes by *A*991 ex *A*1 females; *A*2, ewes by *B*323 ex *A*1 females; *B*2, ewes by *B*323 ex *B*1 females; *A*3, ewes by *B*323 ex *A*2 females; *B*3, ewes by *B*323 ex *B*2 females; *A*4, ewes by *D*107 ex *A*2 and *A*3 females; *B*4, ewes by *D*107 ex *B*2 and *B*3 females; *A*5, ewes by *T*306 ex *A*2, *A*3 and *A*4 females; *B*5, ewes by *T*306 ex *B*3 and *B*4 females; *A*6, ewes by *K*68 ex *A*3, *A*4 and *A*5 females; *B*6, ewes by *K*68 ex *B*3, *B*4 and *B*5 females

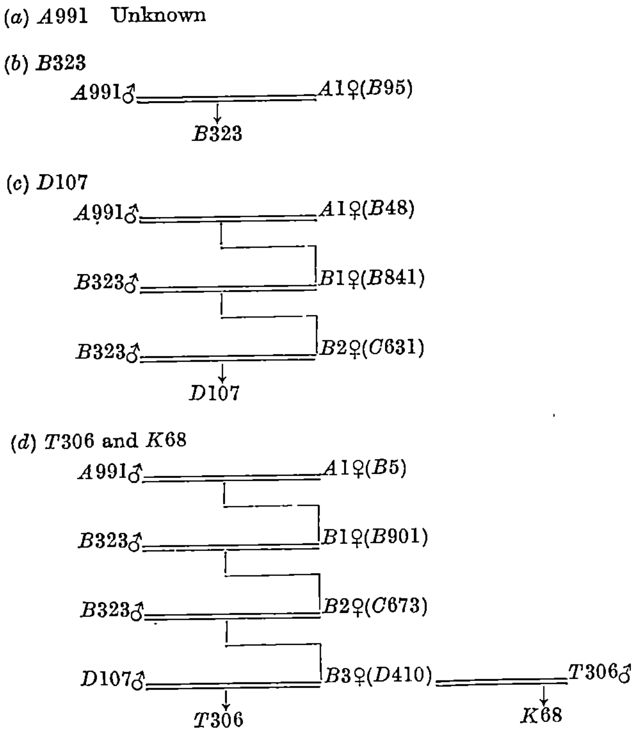

Fig. 2. Pedigrees of sires used in family *B*323

to the Peppin flock from which the foundation stock was originally purchased, and forty cast-for-age ewes were obtained. These were divided between the two families at random, twenty to each, and were described as the 'introduced' ewes to distinguish them from the inbred ewes already present.

The mating performance of each ram with both inbred and introduced mates for the seasons 1959, 1960 and 1961 are shown in Table 1.

Table 1. ANNUAL SUMMARY OF MATINGS IN THE *B*323 AND *B*464 FAMILIES DURING 1959–61 INCLUSIVE

Family	Sire	Year	Type of mate Inbred Mated	Not mated	Total	Introduced Mated	Not mated	Total
*B*323	*K*68	1959	22	0	22	5	14	19
	*K*68	1960	20	6	26	4	14	18
	*K*68	1961	24	3	27	4	14	18
	Totals, 3 years		66	9	75	13	42	55
*B*464	*K*504	1959	12	2	14	19	1	20
	*K*504	1960	11	4	15	20	0	20
	*K*504	1961	29	0	29	16	0	16
	Totals, 3 years		52	6	58	55	1	56

The results show no heterogeneity between years, and the behaviour of the two rams may be compared on their total number of matings over the three-year period.

The comparisons show that differences between the two sires in their matings with inbred ewes were not statistically significant ($\chi^2_1 = 0{\cdot}89$, $P < 0{\cdot}50 > 0{\cdot}30$), but that they differed greatly in their behaviour towards their introduced mates ($\chi^2_1 = 62{\cdot}454$, $P < 0{\cdot}001$). Thus *K*68 favoured his inbred mates above his introduced ones ($\chi^2_1 = 39{\cdot}462$, $P < 0{\cdot}001$), while *K*504 exhibited no preference ($\chi^2_1 = 3{\cdot}662$, $P < 0{\cdot}10 > 0{\cdot}05$).

Further, as shown by the results in Table 2, *K*68 consistently favoured a few females among his introduced mates, to the almost complete exclusion of the others.

Table 2. RECORDS OF MATINGS OF INTRODUCED EWES TO *K*68 DURING 1959–61 INCLUSIVE

Ewe No.	Year 1959	1960	1961
325	+	+	+
327	+	–	–
333	+	+	+
334	+	+	+
339	+	–	–
340	*	+	+

* Ewe No. 340 was in-lamb when purchased and was not able to mate for this reason.
+ Ewe mated by ram.
– Ewe not mated by ram.

The results strongly suggest that mating preference has been displayed by *K*68. Both rams were maidens at their first joining in 1959, and their behaviour could not have been conditioned by previous experience. The introduced ewes, being cast-for-age from a breeding flock, were known breeders themselves; they were divided between *K*68 and *K*504 at random, and it is most unlikely that those joined to *K*68 were in any way incapable of mating with him. It has already been stated that all ewes were run together as a single flock except at mating time, so there is no possibility that *K*68 avoided his introduced mates because they differed from the inbred ewes as the result of some artefact.

Three possible physiological reasons appear to exist for this mating phenomenon. *K*68 failed to recognize the usual olfactory signs of œstrus in the introduced ewes; he recognized them and avoided them; or the ewes avoided him. The last possibility is considered improbable as ewes in œstrus are known actively to seek the ram (ref. 5 and our own general observations). Of the two possibilities remaining, the first is considered most likely. Merino rams possess considerable libido and will occasionally try to mate ewes whether they are in œstrus or not; in fact, the raddling of ewes number

327 and 339 by $K68$ in 1959 could have been due to such attempts at mating, as neither ewe was recorded as either becoming pregnant or returning to service after 17 days. Because of this, it is concluded that he failed to recognize when the majority of the introduced ewes were in season.

If it is accepted that the mating preference exercised is under physiological control, it is necessary for the control itself to have a genetic basis, and that isolation and selection should have operated before the behaviour of $K68$ in relation to his inbred and introduced mates can be considered in relation to speciation. The common origin of the inbred families and the introduced ewes, together with the presence of a proportion of animals among the latter with which $K68$ could regularly mate (and from which he produced offspring), support the proposition that there is a genetic background. Isolation was provided by several generations of inbreeding in his relatively small one-sire family, such conditions being optimal for the increase of homozygous recessives. The phenomenon being considered is one in which, if the homozygous recessive is characterized by mating preference, natural selection against the heterozygote and homozygous dominant would be complete and rapid.

If $K68$'s mating preference was due to his having been a homozygous recessive for some physiological peculiarity which caused him to respond selectively to certain of the olfactory stimuli provided by ewes in œstrus, the fact that he mated with virtually all his related ewes indicates that one of his near ancestors was also homozygous.

For this explanation of the origin of the mating behaviour shown by $K68$ to be correct, three stages of mating behaviour should be evident in the $B323$ family records. During the first period they should be normal, during the second many ewes should appear to have aberrant records, and the third period should be characterized by a return to normal. A preliminary examination of the mating records for the entire family since its inception suggests that this course of events may have happened. Results of the detailed examination of the data will appear elsewhere.

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IMPORTANCE FOR RADIO-PROTECTION OF THE REACTION OF CELLS TO SULPHYDRYL AND DISULPHIDE COMPOUNDS

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CHEMICAL protection by certain sulphydryl compounds and their disulphides against the lethal effects of ionizing radiations is a well-established phenomenon seen in nearly all biological systems. Evidence that radio-sensitivity can be increased by treatment with substances capable of reacting rapidly with SH groups under physiological conditions (called 'thioloprives' by Bacq[1,2]) is being found for a variety of cells[3] and some indications for sensitization have been found even with intact mammals[4]. Since experiments with isolated cell constituents such as proteins and nucleic acids as well as with viruses have shown that radiation damage of vital macromolecules can be protected against by SH compounds it seemed that protection *in vivo* could readily be accounted for by the fact that the SH protector diffuses into the cell and thereby raises the intracellular SH level[5]. Conversely, sensitization by thioloprives could be seen as the direct consequence of the reduction of the number of intracellular SH groups by direct chemical combination with reactive chemicals[6]. Recent investigations have shown that the actual situation in mammals (and certain types of isolated cells) is much more complex and that the SH protectors and the thioloprives may exert their action by shifting physiological processes within the cell. These agents exert a rather specific 'biochemical shock' which initiates a sequence of intracellular changes that then alter the radio-resistance of cells in either direction. It remains probable that the changes in radio-sensitivity are due to differences in the extent of protection offered by SH compounds to essential macromolecules—as demonstrated in model experiments[5]—but the level within the cell of SH and —S—S— protectors is determined *in vivo* by biological factors.

Intracellular concentration of protective agents. Pihl and Eldjarn[7] showed an impressive correlation between radio-protection in mice and the ability of some thirty —SH and —S—S— compounds to combine *in vitro* with proteins by a disulphide exchange reaction. As a consequence of this, and possibly other reactions, protective sulphydryl and disulphide compounds become bound to cellular components in tissues extremely rapidly. In rats injected with the —SH or S—S protectors one sees exactly the same pattern, as was found by Pihl and Eldjarn[7] *in vitro*, namely, that the protectors are bound to proteins in a few minutes. This rapid combination at the cellular level has been most clearly demonstrated for cysteamine and its disulphide, but related substances behave similarly. A number of investigations[8,9] have shown that the concentrations of non-protein —SH and —S—S— substances* within cells are not raised immediately after injection of SH protectors, but that at this time these substances are found bound to protein in the radio-sensitive organs. On standing, the non-protein SH levels rise in these tissues and part of this increase has been found to be due to the reappearance of the radio-protector itself (Fig. 1). In mice, Shapiro *et al.*[10] observed that ~ 20 min after injecting mercaptoethylguanidine (MEG) all this substance is no longer bound to protein, but that some of it is present in a free form. The physiological state of the cells must have undergone a profound change to make it possible for these reactive sulphydryl compounds (that is, MEG and cysteamine) to remain uncombined with proteins. Revesz and Bergstrand[11]

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* The action of reductases and the occurrence of accidental oxidation make it technically very difficult to differentiate whether the non-protein bound protective agents are present in the —SH or —S—S form within the cell and for this reason they are expressed as the combined value.

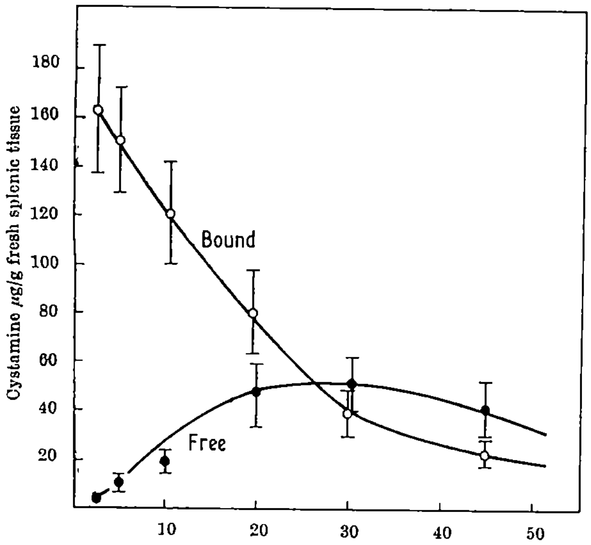

Fig. 1. Free and protein-bound cysteamine–cystamine in fresh splenic tissue of rats after intraperitoneal injection of 100 mg/kg of cystamine (drawn from results of Betz *et al.*, ref. 8)

find with ascites cells *in vitro* that the concentration of intracellular non-protein SH substances is considerably increased at 15 min after exposure to a solution of cysteamine. It seems likely that the protector will be bound rapidly even to isolated cells suspended in cysteamine-containing medium and the increase in intracellular free —SH substances may also here be partly indirect. The 'biochemical shock' produced by thioloprives may also cause a delayed change in the level of free intracellular —SH substances. Many of the thioloprives combine extremely rapidly under physiological conditions with —SH groups[1,2], yet changes in cellular —SH levels are only seen some time after injection[12].

If the protective action of the —SH and —S—S— substances relies on producing physiological changes in the cell, then optimum protection should not be seen immediately after irradiation. In mice, maximum protection with cysteamine and cystamine is found within 10 min of administration[13], but in rats Patt *et al.*[14] found cysteine to be fully effective 1 h after administration. Smoliar[15] showed that cysteamine is almost ineffective in rats 2 min after injection and that maximum protection against X-rays occurs 45 min post injection. The optimum period of protection does not coincide either with the time when binding of the protector to the proteins is at a maximum (which is very soon) or with the time when the protector exists free in the cell following a physiological release process (Fig. 1).

For protection against alkylating agents such as nitrogen mustards it has been generally observed[9,16,17] that the maximum effect is seen if the sulphydryl compounds are given a considerable time before the poison and the protection cannot therefore arise as a direct consequence of binding to proteins. The necessity for the long time interval also makes it most unlikely that the injected sulphydryl substances protect by themselves competing for the alkylating agent and it seems most plausible that the changes consequent on the 'biochemical shock' (possibly release of free SH substances) are also the actual cause of the protection against chemicals.

Nature of the 'biochemical shock'. The concept that the injection of the —SH and —S—S— protectors produce profound physiological alterations does not depend only on the finding that there are changes in the proteins of the cell which allows cysteamine (or its disulphide) and MEG to reappear and to remain unbound. Evidence for a 'biochemical shock' is also revealed by the observations that, within minutes after injection of cysteamine, ascorbic acid and other unidentified reducing substances are liberated in the plasma[18], glycogenolysis occurs in the liver[19] and the glutathione content falls in the liver and kidney[20]. Cystamine has an immediate and pronounced effect on the respiration of cells[21].

A series of observations by Neubert and Lehninger[22] may help in the elucidation of the mechanism by which the protective agents produce the 'biochemical shock'. Various —SH and —S—S— compounds induce a discharge or leakage from rat liver mitochondria of contraction factors which cause the mitochondria to undergo a peculiar type of swelling which is not reversed by the addition of adenosine triphosphate. There is a correlation between the ability of substances: (1) to bring about this change in mitochondria; (2) to protect against radiation *in vivo*; (3) to undergo disulphide exchange (for example, cysteine, cysteamine and glutathione are active while penicillamine, ergothionine and mercaptoethanol are inactive). Mercaptoethanol is particularly interesting since this substance protects bacteriophage and isolated nucleoprotein—presumably by a direct physicochemical mechanism[5]—but is quite inactive as a protector in mammals and its disulphide reactivity as well as its action on mitochondria is also poor.

As a result of disulphide exchange, the activity of intracellular enzymes will be affected and bound —SH compounds will be set free. This, however, can only be a first step since the delayed release of protein-bound protective agents is indicative of extensive alterations of some proteins in the cell. One might speculate about changes following disulphide exchange in the activity of glutathione reductase and peroxidase which displace the —S.S→SH equilibria and increase the concentration of —SH groups in the cell available for physico-chemical methods of protection. Inhibition of enzymes by thioloprives could act in the opposite direction and via metabolic processes decrease the concentration of —SH groups available for protection. The place at which the injected protectors and sensitizers act in the cell need not therefore be the same as the site of the radiation induced primary lesion which initiates the processes that lead to the death of the cell. It is also quite possible that some treatments which change the radio-sensitivity of mammals and which do not initially involve reactions with —SH or —S—S— groups may bring about changes in intracellular —SH levels. In this way quite unrelated compounds may alter radio-sensitivity through a common pathway. The failure[23] of animals which have received repeated injections of cysteine over a period of weeks to be protected by a further dose given before irradiation can be explained as inability (possibly by exhaustion) to respond in the normal way to the insult of an injection of a massive dose of —SH or —S—S— substance.

On general biochemical grounds it is obvious that the —SH ⇌ S—S— equilibrium (free and protein-bound) in the vegetative cell is a carefully balanced system. If disturbed by the introduction of extraneous —SH and —S—S— substances the physiologically active cell is bound to respond to this change. In retrospect it is clear that a direct extrapolation to the whole mammal of mechanisms clearly identified in less complex systems is illogical. We[24] still incline to the view that the mechanism of protection of mammals at the molecular level involves interaction of SH groups with radicals and that processes such as instantaneous repair of the primary lesion by hydrogen transfer[5] are ultimately responsible for the observed increase in radio-resistance. The —SH groups responsible for such reactions are, however, not necessarily directly related to the protective agent that has been injected. The possibility that the 'biochemical shock' produced by the protective agents raises the post-irradiation repair capacity of the cell cannot be excluded, but the 'universality' of chemical protection makes this mechanism rather unlikely. Before any of the suggestions about the mechanism of protection at the molecular level can be considered as more than speculation, much more basic information must be obtained about the biochemical changes which follow on the intoxication of mammals with —SH and —S—S— substances.

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LIPID DEPOSITION IN INTERCELLULAR SPACE

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THE physiological anatomy of wound healing by suberization and cork formation was investigated comprehensively by Haberlandt[1]. The wound hormone postulated as the stimulating agent has been isolated, analysed and tested physiologically in recent years[2–4]. The experimental work of Priestley indicated that causal factors in cork formation include the presence of oxygen in the air and a supply of food material[5]. In higher plants the development of intercellular spaces and deposition of lipid material on the limiting cell walls appear to confirm this theory. The breaking of plasmodesmata during cell expansion is virtually a wounding of the protoplast. Air enters the intercellular space, and apparently simultaneously lipid deposition begins on the wounded cell. The lipid is extruded presumably through the plasmodesmata. The elaboration of traumatin and of lipid is presumably the first reaction of the wounded protoplast and is followed by the microscopically visible deposition of lipid.

In the past 30 years the structure and composition of cellulose, the principal component of the plant cell wall, have been intensively investigated by light and electron microscopic techniques, and by chemical and physical methods[6–10]. Chemical analyses of other wall components, the cutin and the suberin of epidermis and cork respectively, also of lignin and the non-cellulosic polysaccharides, indicate variation in different species. In the present article, the deposition of a non-cellulosic lipid layer on the cell walls limiting the intercellular spaces and extending therefrom to the walls of adjoining idioblasts is discussed.

The term 'internal suberization' was first used to connote a non-cellulosic lipid layer which lines all intercellular spaces in the leaf of the orange, *Citrus sinensis*[11]. This layer resembles cutin and suberin in its microchemical behaviour in testing for cellulose by treatment with $I_2KI-H_2SO_4$. The cellulose swells, turns blue and eventually disappears, but the suberin remains intact, or fragmented *in situ* if in young tissue. The reaction may be slowed or stopped for recording on 'Kodachrome' by irrigation of the section with glycerin. Since later work showed that all intercellular spaces in stem and root apices and in other meristematic tissues are also lined with a pellicle similar in microchemical reaction to that seen in mature leaves, the term lipid layer appears preferable to suberin layer[12].

In the higher plant the meristematic cells of shoot and root tip, of leaf, flower and fruit primordia, are polyhedral and approximately isodiametric. All cell faces are pitted ubiquitously. During growth and cell expansion the cell corners round off, certain of the marginal plasmodesmata are broken and these broken ends contract into the cell wall. The first intercellular spaces, minute but visible under the light microscope because air-filled, occur within about 20 microns of stem and root apices[12].

The cell walls limiting these intercellular spaces are already lined with a thin film of non-cellulosic lipid material. As differentiation of parenchyma such as cortex and pith proceeds, the polyhedral cells become spherical, ellipsoidal or cylindrical[13]. The intercellular space forms a continuum, a network throughout the entire axis, also through leaves, flowers and fruits. On the end walls of the elongate cells, the pit pattern remains as in the meristematic cells, the pits small and ubiquitous. In contrast, on the lateral walls, the pits are larger in diameter, fewer in number and are aligned in vertical series. Through them the plasmodesmata maintain connexion with the surrounding cells, 5–7 in number. During growth the lipid pellicle either thickens to a well-defined layer around the now conspicuous intercellular spaces, or it may remain tenuous. The thickness varies in different species, is conspicuous in xerophytes, while in the hydrophytes examined it is thin as in meristematic tissues.

It seems probable that the long-lasting quality of flowers may depend on the thickness of the lipid layer in the intercellular spaces. In the petals of *Camellia* sp., in orchids such as *Cypripedium* sp., and *Cymbidium* sp., also in the spathe of *Anthurium* sp., the lipid layer is thick as in xerophytic leaves. In contrast, in *Primula* sp., where the flowers, cut or uncut, last for a day or less, the pellicle is tenuous as in the hydrophytes.

It is generally stated in text-books that intercellular spaces are absent in the vascular tissues of the higher plants. In *Ricinus communis*, however, in living material, minute air-filled spaces occur around differentiating spiral vessels[14]. These spaces are lined with lipid pellicles visible under light and electron microscopes. In vessels which have reached their full diameter, but in which the walls are not yet lignified, the cylindrical cellulose wall and the spiral thickening disintegrate during microchemical treatment with $I_2KI-H_2SO_4$. A cylinder, or in sectioned material a ring, remains which is marked by projecting ridges, equal in number to the intercellular spaces between the surrounding cells. This lipid cylinder is evidently formed during vessel differentiation by the lateral extension of lipid pellicle from the intercellular spaces along the plane of the middle lamella.

The endodermis has long been recognized by the suberization and, generally also, the lignification of the Casparian strip. Lipid-lined intercellular spaces occur between the inner zone of the cortex and the outer face

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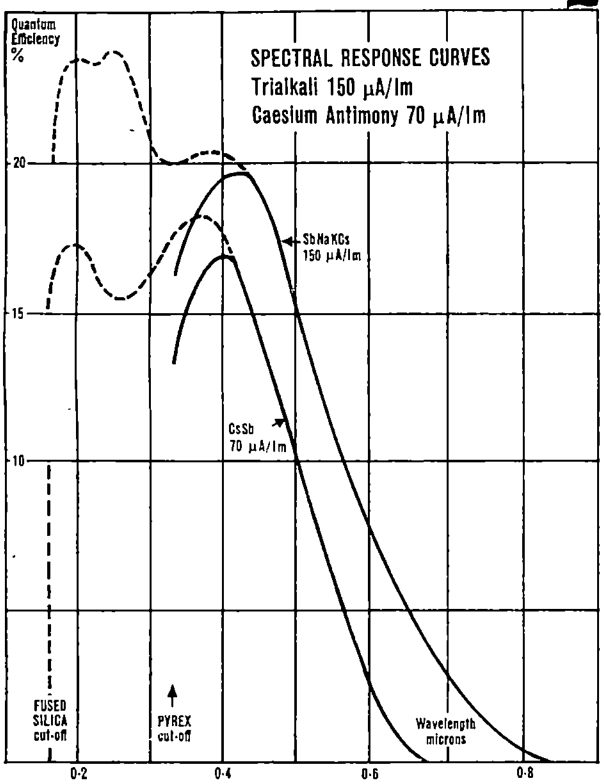

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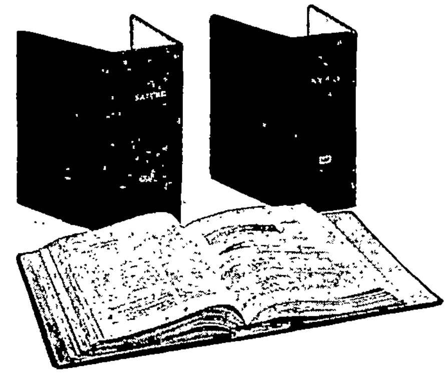

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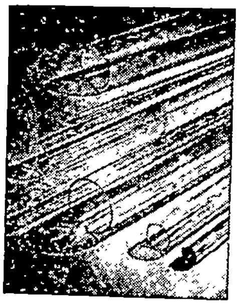

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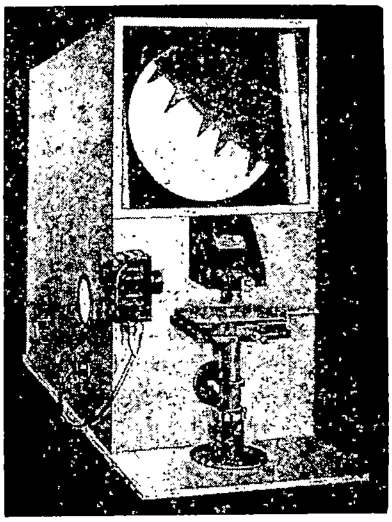

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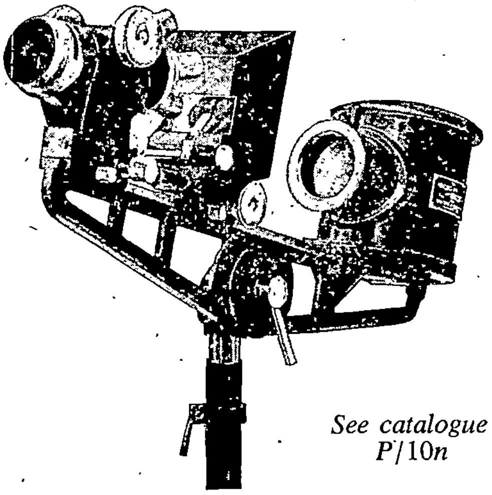

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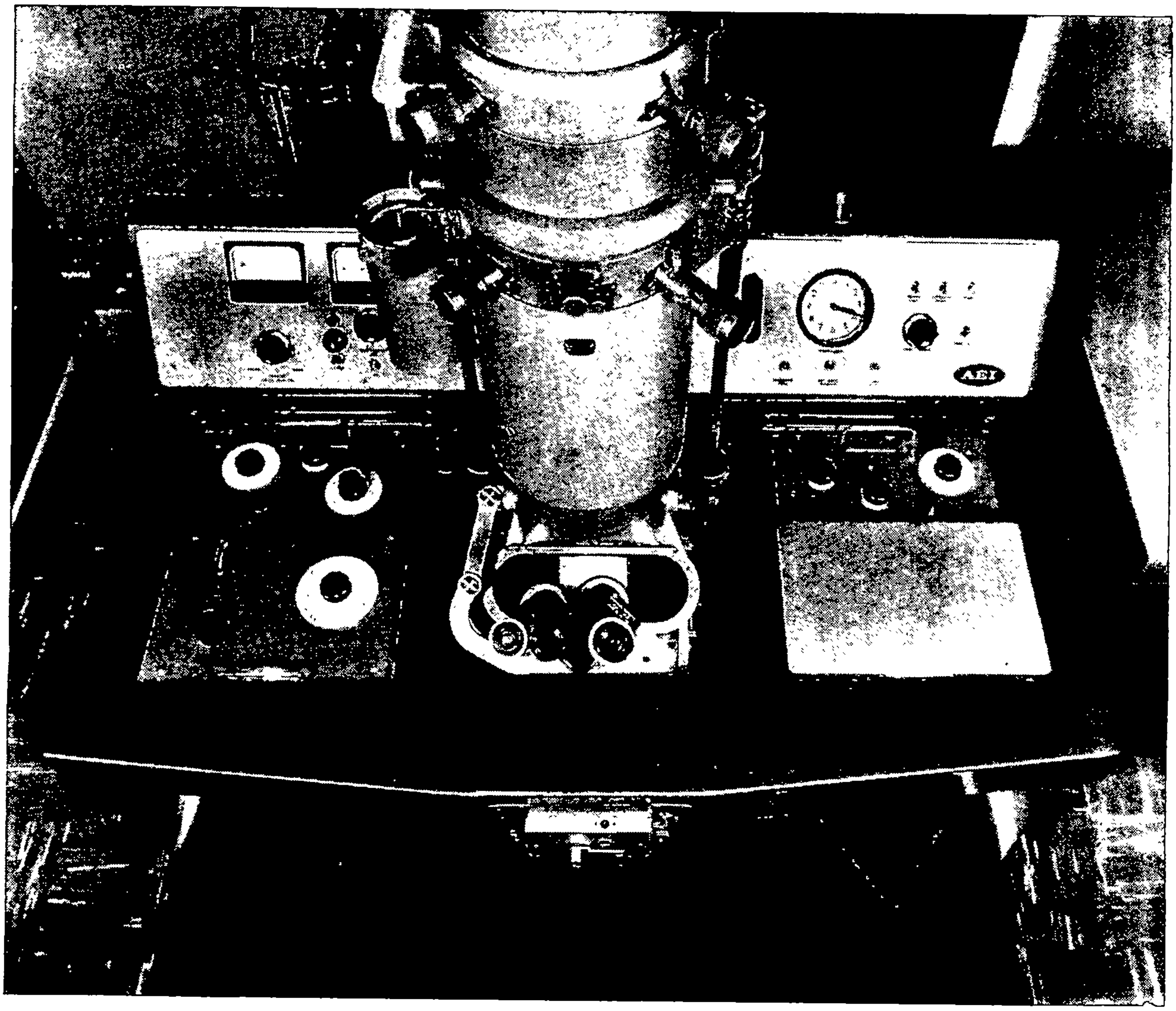

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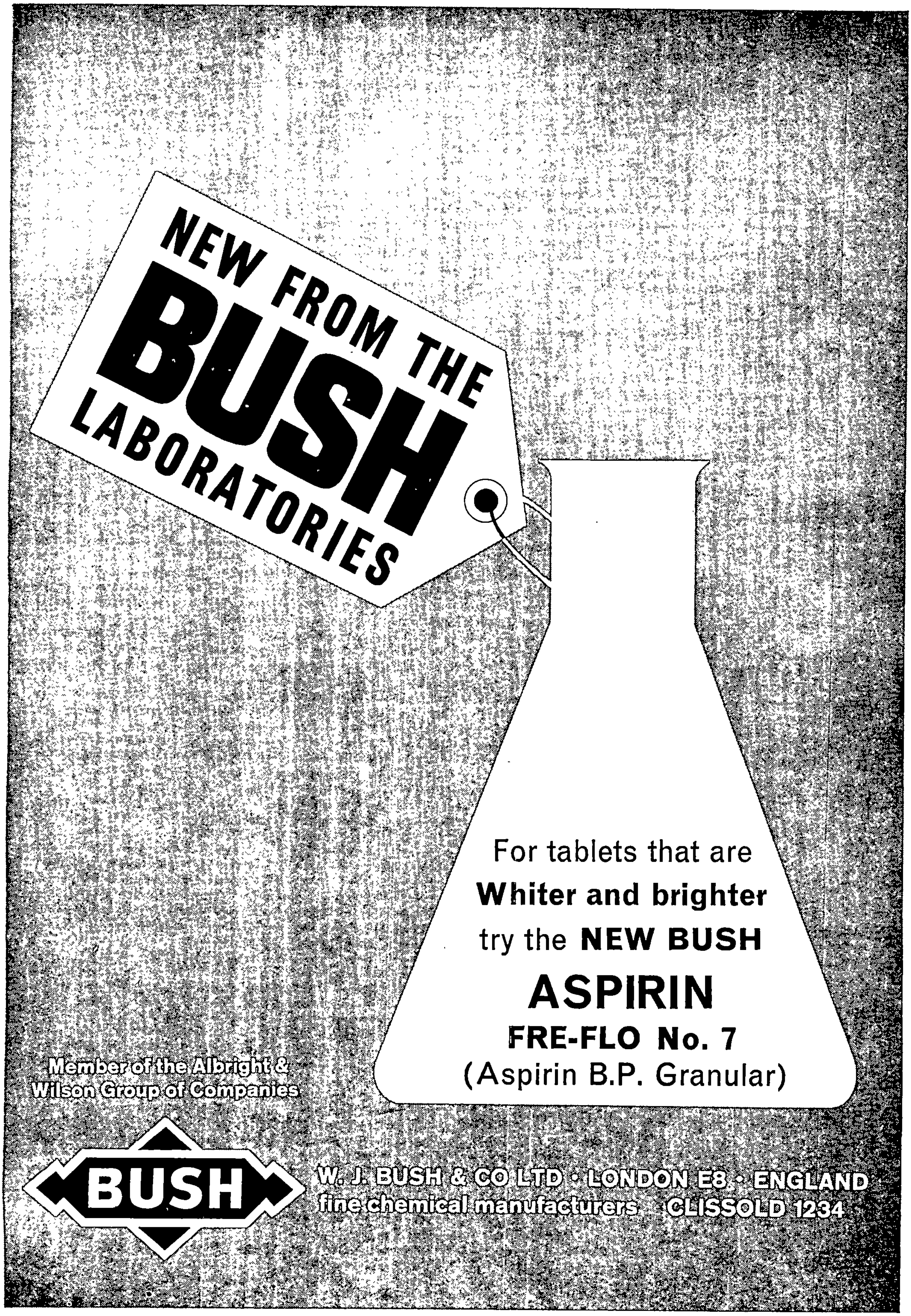

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of the endodermis. Minute intercellular spaces are also seen along the inner face of the endodermis and the adjacent vascular parenchyma. Suberization of the Casparian strip probably begins at the time when the air enters the abundant intercellular spaces formed during the expansion of the cortical and other cells. Suberization of the strip frequently more or less blocks the pits in the radial walls of the endodermal cells, as is seen under the electron microscope[15]. To what extent this suberization interferes with intercellular transport in the young root is not known.

The ovary of the dicotyledons, in its early stage of development, consists of a papilla of meristematic cells in plasmodesmatal connexion. During growth, as seen, for example, in *Ricinus*, differential expansion occurs in the inner wall of the carpel and the outer integument of the seed. The differentiated epidermal walls of the carpel and the seed become separated by an air space and the cutinized surfaces of both are already stainable with sudan 3.

The embryo of the higher plant is at first surrounded by nucellus and endosperm. When plasmodesmatal connexions between embryo and nutritive tissues eventually break, the epidermis of cotyledons, radicle and plumule becomes cutinized. It is probable that, by this time, a film of air surrounds the embryo. The cutinization of the epidermis is retained in the primary root[16].

In the seed coat of the xerophyte, palo verde (*Cercidium macrocarpa*, family Leguminosae), the epidermal cells, palisade-like in outline, are enveloped in a lipid sheath[17]. In the same seed, the mucilage zone of the inner integument consists of stellate cells. In the living material, examined at various stages of growth, no air has been observed in the intercellular spaces at any time. The spaces between the radiating cell arms are filled with an amorphous mass of mucilage. Nevertheless, in these spaces a thin pellicle of lipid material occurs which parallels the cellulosic pitted arms at a distance of a few μ. It is considered probable that, in the mucilage mass, a gradient in oxygen content exists which stimulates the localized deposition of the lipid layer.

Intercellular spaces, lipid-lined, are ubiquitous in the mesocarp of the avocado, *Persea americana*, fruit. They appear between parenchymatous cells, and around the characteristic oil idioblasts[18]. Differentiation of the oil idioblast includes the thickening and suberization of the pitted wall and the impregnation of the delicate cellulosic oil sac membrane with a certain amount of lipid material. The penetration of aqueous stains such as neutral red, Janus green, thionin, and anilin blue into the developing idioblasts is retarded at an early stage of growth and is eventually inhibited in the ripe fruit. To what extent, if at all, the suberization of the pitted idioblast wall affects the transport of precursor reserve materials through the plasmodesmata into the oil sac is unknown.

Under the electron microscope, the layers of suberin isolated from *Cercidium*, *Ricinus* and *Persea* are similar in appearance. They resemble in texture a very fine, porous, artificial rubber or plastic sponge. With the isolation techniques used at present, clearing and ultrasonic treatment, these layers vary in thickness in younger and older tissues but are indistinguishable morphologically[14,17,18].

The deposition of lipid on cell walls bordering intercellular spaces appears to be similar to the reaction of the epidermis on wounding, for example, in the fruit of the orange, *Citrus sinensis*[19]. The epidermis of *Citrus* fruits of various species consists of tabular cells, polygonal in outline, interrupted by stomata and sub-epidermal oil glands. In young fruits, 1–3 cm in length, the epidermis may be strained to cracking point and minute slits torn between adjacent cells. The plasmodesmata are broken at least temporarily. The wounds appear to be healed simultaneously by cutin deposition, indicated by irregular thickenings in the radial cell walls, which are stainable with sudan 3. These occur occasionally near stomata or oil glands, but more generally between unspecialized epidermal cells. The inherent weakness of the epidermis of *Citrus* is a factor in the pathology of water spot. In the ripe fruit, cracks are caused presumably by physiological variations of internal pressure, and at this time the epidermal cells are no longer able to keep pace with the elaboration of cutin necessary to heal the cracks. The ensheathment of the epidermal cells of the seed coat of *Cercidium* may originate in a similar way by the healing of epidermal cracks which develop during seed growth.

The causal factors of the intracellular deposition of lipid material, cutin or suberin, in calcium oxalate crystal idioblasts appear to differ from those hitherto discussed in this article. Calcium oxalate crystals develop in living cells and the form of the crystal, solitary or druse, depends on the pH of the cell[20]. The crystals are enclosed in a cellulose sheath. In mature idioblasts the crystals may fill almost the entire cell cavity, while the protoplasm with nucleus and organelles, although now parietal in position, appears normal as seen under the light microscope. The cellulose sheath, however, is now impregnated with lipid, and a similar layer lines the inner surface of the idioblast. At no stage in the development of these idioblasts has air been observed within the cell cavity[21].

In conclusion, lipid distribution on the cell walls which bound the intercellular space system of the higher plant may be considered to be a wound reaction. The wounding of the protoplast occurs during cell expansion when the interconnecting plasmodesmata are broken. The protoplast reacts by elaboration of wound hormones and lipid precursors. The lipid layer deposited on the cell wall is the first microscopically visible reaction. The presence of air is, in general, essential for the deposition of lipid. During growth, the lipid layer thickens, and in many cases may extend in any direction from the initial intercellular space along the plane of the middle lamella of adjacent cells. The factors which control this extension are unknown at present.

The deposition of lipid in mucilage-filled intercellular spaces of the inner integument of the seed of *Cercidium* is interpreted, at present, by postulating an oxygen gradient in the amorphous mucilage mass which is extruded from the many armed cells of the tissue.

The intracellular cutinization or suberization of calcium oxalate crystal idioblasts apparently follows a pattern different from that in the intercellular spaces since the presence of air has not been observed in any of the material examined.

It is expected that, in the near future, the techniques of ultra-thin sectioning will clarify some of the innumerable problems of lipid deposition on, and in, the cell wall.

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ISOLATION FROM SPINACH CHLOROPLASTS OF PARTICLES CONTAINING DIFFERENT PROPORTIONS OF CHLOROPHYLL *a* AND CHLOROPHYLL *b* AND THEIR POSSIBLE ROLE IN THE LIGHT REACTIONS OF PHOTOSYNTHESIS

By Dr. N. K. BOARDMAN and Dr. JAN M. ANDERSON
C.S.I.R.O. Division of Plant Industry, Canberra, Australia

EVIDENCE in favour of the concept of two distinct primary photochemical reactions in the process of photosynthesis in the green plant has been reviewed recently[1]. These primary reactions are thought to be driven by light absorbed by two pigment systems which have been denoted system 1 and system 2. System 2 absorbs more light of the shorter wave-lengths than does system 1, but the pigment composition of the two systems is unknown[2]. There is evidence from spectrophotometric investigations to support the hypothesis that light absorbed by system 1 causes the reduction of NADP and the oxidation of a cytochrome, while light absorbed by system 2 causes the reduction of the cytochrome and the oxidation of water to oxygen. The products of the light phase of photosynthesis, $NADPH_2$ ATP and O_2 result from the co-operation of the two systems.

The photoreduction of NADP by isolated chloroplasts coupled to oxygen evolution is inhibited by 3-(*p*-chlorophenyl)-1 : 1-dimethyl urea (CMU)[3,4], which is thought to act on system 2, and prevent the reduction of the cytochrome[1]. The inhibition of NADP reduction can be overcome by the addition of the electron donor, sodium ascorbate plus an indophenol dye, suggesting that reduction under these conditions results from light absorbed solely by system 1.

The aim of the work reported in this communication was to fractionate the chlorophyll–protein complex of the spinach chloroplast in an attempt to obtain a physical separation of the two pigment systems.

Spinach chloroplasts, prepared in an isotonic sucrose medium, were fragmented by incubation at 0° in 0·05 M phosphate buffer, *p*H 7·2, containing 0·01 M potassium chloride and 0·5 per cent digitonin. The resulting chlorophyll-containing particles were separated by differential centrifugation. The first centrifugation was for 10 min at 1,000*g* and this was followed by centrifugations at 10,000*g* for 30 min, 50,000*g* for 30 min and 144,000*g* for 60 min. The pellet from each spin was resuspended in 0·05 M phosphate buffer, containing 0·01 M potassium chloride. The chlorophyll content of each fraction was determined by taking suitable aliquots, making them 80 per cent with respect to acetone, clarifying by centrifugation and analysing spectrophotometrically. The equations of Vernon[5] were used to calculate the contents of chlorophyll *a* and chlorophyll *b*. Each fraction was tested for the following photochemical activities: (1) reduction of *o*-chlorophenol-2,6-dichloroindophenol (TCIP); (2) reduction of NADP; (3) reduction of NADP with sodium ascorbate and TCIP as the electron donor couple.

Previously, Wessels[6] had reported that chlorophyll-containing fragments, prepared by incubation of spinach chloroplasts with digitonin, followed by centrifugation between 5,000*g* and 50,000*g* were capable of cyclic phosphorylation, and of NADP reduction if provided with a suitable electron donor.

Table 1. CHLOROPHYLL CONTENT OF FRACTIONS ISOLATED FROM SPINACH CHLOROPLASTS

Fraction	Chlorophyll content (μg)	$\frac{A663}{A647}$	$\frac{\text{Chl. } a}{\text{Chl. } b}$
Chloroplast suspension	3,120	2·31	3·0
1,000*g*	580	2·12	2·4
10,000*g*	1,374	2·14	2·5
50,000*g*	373	2·68	4·6
144,000*g*	259	2·91	6·2
144,000*g* supernatant	336	2·80	5·4

Table 2. PHOTOCHEMICAL ACTIVITIES OF FRACTIONS ISOLATED FROM SPINACH CHLOROPLASTS

Fraction	Rate of reduction (μmoles/mg chlorophyll/h) TCIP*	NADP†	NADP† (ascorbate plus TCIP as electron donor)
Chloroplasts in phosphate buffer	195	109	58
Chloroplasts in digitonin	92	13	35
1,000*g*	116	20	14
10,000*g*	56	14	12
50,000*g*	4	0	39
144,000*g*	0	0	117
144,000*g* supernatant	0	0	84

* The reaction mixtures contained (in 3 ml.) chloroplast fraction equivalent to 7 μg chlorophyll and (in μmoles) *tris*-HCl buffer *p*H 7·8, 40; sodium chloride, 70; TCIP, 0·06. The reaction rates were measured by the decreases in absorbancy at 620 mμ after illumination for 45 sec at 40,000 lux.

† The reaction mixtures contained (in 3 ml.) chloroplast fraction equivalent to 25 μg chlorophyll and (in μmoles) *tris*-HCl buffer *p*H 8·0, 40; sodium chloride, 70; magnesium chloride, 10; NADP, 0·6; and spinach PPNR sufficient to saturate the system. Where sodium ascorbate and TCIP were added, the amounts were (in μmoles) sodium ascorbate, 6·0; TCIP, 0·02. The reaction rates were measured by the increases in absorbancy at 340 mμ after an illumination time of 2 min at 40,000 lux.

The results of an experiment in which the incubation time in digitonin was 30 min are shown in Tables 1 and 2. A significant finding was the different proportions of chlorophyll *a* and chlorophyll *b* in the various fractions. The (0–1,000*g*) fraction (later designated 1,000*g* fraction) and (1,000*g*–10,000*g*) fraction (10,000*g* fraction) had lower chlorophyll *a*/chlorophyll *b* ratios than the chloroplasts whereas the (10,000*g*–50,000*g*) fraction (50,000*g* fraction), the (50,000*g*–144,000*g*) fraction (144,000*g* fraction), and the high-speed supernatant (144,000*g* supernatant) had higher ratios. The difference in the proportions of chlorophyll *a* to chlorophyll *b* between the 10,000*g* and 144,000*g* fractions was evident also from absorption curves of the fractions (Fig. 1). The *in vivo* absorption band of chlorophyll *b* at 650 mμ (ref. 7) was clearly visible in the 10,000*g* fraction. The main absorption maximum of the 10,000*g* fraction was shifted slightly to the blue (about 2 mμ) as compared with the 144,000*g* fraction.

A comparison of the figures in Table 2 for the photoreduction of NADP and TCIP by intact chloroplasts and chloroplasts treated with digitonin shows that there was a greater percentage loss in the rate of NADP reduction.

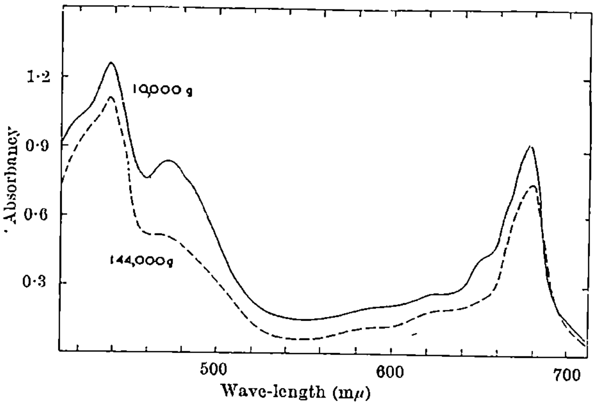

Fig. 1. Absorption spectra of fractions obtained from spinach chloroplasts by incubation with digitonin and differential centrifugation. —— 10,000*g* fraction; ---- 144,000*g* fraction

Table 3. EFFECT OF ADDING FERREDOXIN OR A CRUDE PREPARATION OF PPNR ON THE RATE OF NADP REDUCTION

Fraction	Addition	Rate of NADP reduction (μmoles/mg chlorophyll/h)
Chloroplasts in phosphate buffer	None	1
,, ,,	Ferredoxin	88
,, ,,	PPNR	97
50,000–144,000*g*	None	0
,,	Ferredoxin	9
,,	PPNR	110

Reaction mixtures as given for Table 2. Saturating amounts of ferredoxin or PPNR were added. Sodium ascorbate and TCIP were added to the 144,000*g* fraction only.

The 1,000*g* fraction reduced both TCIP and NADP at a faster rate than the digitonin-treated chloroplasts, the 10,000*g* fraction was less active than the 1,000*g* fraction and the other fractions were either inactive or had very low activity. Sodium ascorbate and TCIP had an inhibitory effect on the rate of NADP reduction by untreated chloroplasts or by the 1,000*g* and 10,000*g* fractions, but it stimulated greatly the rate of reduction by the 50,000*g* and 144,000*g* fraction and the 144,000*g* supernatant. In fact, the rates of reduction of NADP by the 144,000*g* fraction and the 144,000*g* supernatant in the presence of ascorbate and TCIP were of the same order as the reduction rate by intact chloroplasts with NADP alone.

Impure photosynthetic pyridine nucleotide reductase (PPNR) as obtained by an acetone fractionation of a spinach leaf extract[8] may be separated by chromatography on DEAE cellulose into a flavoprotein enzyme, and a non-hæm, iron-containing protein, named ferredoxin[9]. Plastocyanin[10], a copper-containing protein, also may be separated from ferredoxin on columns of DEAE-cellulose. In the present work, it was found that NADP reduction by the 144,000*g* fraction was restored by adding crude PPNR, but not by ferredoxin, whereas with chloroplasts, crude PPNR or ferredoxin restored activity (Table 3). It is assumed, therefore, that the flavoprotein or plastocyanin[11] or both had been lost from the small particles.

After incubating chloroplasts for 30 min in 0·5 per cent digitonin, about 25 per cent of the total chlorophyll of the chloroplast was associated with chlorophyll-containing particles which were capable of photoreducing NADP at a high rate if they were supplied with an electron donor. This approximate estimate was obtained by summing the chlorophyll contents of the 144,000*g* fraction and the high-speed supernatant and adding one-third of the chlorophyll content of the 50,000*g* fraction. (The 50,000*g* fraction is only one-third as active as the 144,000*g* fraction.) Increasing the incubation time in digitonin to 21 h resulted in a small increase only (to 30 per cent of the total chlorophyll) in the amount of chlorophyll associated with particles which were able to reduce NADP at a high rate. After 72 h in 0·5 per cent digitonin, the amount was 37 per cent, but after 72 h in 2 per cent digitonin, the figure was 49 per cent.

Irrespective of the time of incubation or concentration of digitonin, the chlorophyll *a*/chlorophyll *b* ratios of the 144,000*g* fraction and the high-speed supernatant remained about the same, whereas the ratios of the 1,000*g* and 10,000*g* fractions decreased slightly with time of incubation. Thus, in an experiment where the incubation time in 2 per cent digitonin was 72 h, the chlorophyll *a*/chlorophyll *b* ratios of the 1,000*g* and 10,000*g* fractions were 2·18 and 2·08, respectively.

These experiments provide evidence for the suggestion that the chlorophyll of the spinach chloroplast is associated with two different particles. One type of particle with a chlorophyll *a*/chlorophyll *b* ratio of approximately 6 is rendered soluble by digitonin. The other particle apparently is attached much more strongly to the structural proteins of the chloroplast grana, since it is not 'solubilized' by the digitonin treatment. It has a chlorophyll *a*/chlorophyll *b* ratio of approximately 2, compared with 3·0 for intact chloroplasts. The total chlorophyll of the chloroplast appears to be divided about equally between the two particles.

Since the chlorophyll-containing particles which are rendered soluble by digitonin have a high proportion of chlorophyll *a*/chlorophyll *b* compared with intact chloroplasts and are able to photoreduce NADP only if provided with an electron donor, it is tempting to speculate that these particles belong to system 1, whereas the chlorophyll which is not rendered soluble by treatment with digitonin for long periods is an integral part of system 2. If this hypothesis is correct, then chlorophyll *a* and chlorophyll *b* are associated with both pigment systems.

Arnon *et al.*[12] have suggested that the pigment of system 2 is chlorophyll *b*, since an action spectrum for oxygen evolution by chloroplast fragments, using an indophenol dye as oxidant and in the presence of ferricyanide, showed a peak of 644 mμ. Müller, Fork and Witt[13] isolated system 2 from system 1 in a similar way to Arnon *et al.*, but instead of oxygen evolution they measured absorption changes at 515 mμ, which are thought to be due to the reaction of plastoquinone with the pigments of system 2. Their action spectrum showed a peak at 674 mμ and a shoulder at 650 mμ, implicating both chlorophyll *a* and chlorophyll *b* in system 2.

Further work is in progress to determine the structure, composition and function of the chlorophyll-containing particles.

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INCIDENCE OF THETA RHYTHM PRIOR TO AWARENESS OF A VISUAL STIMULUS

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IN the course of an investigation of the EEG correlates of awareness for, and recognition of, verbal stimuli (Dixon and Lear)[1], we carried out an independent analysis of changes in the theta component of the EEG.

In this investigation the EEG was recorded from the occipital area during the gradual increase in intensity of visually presented words. Four emotional and four neutral control words of equal length and frequency, selected

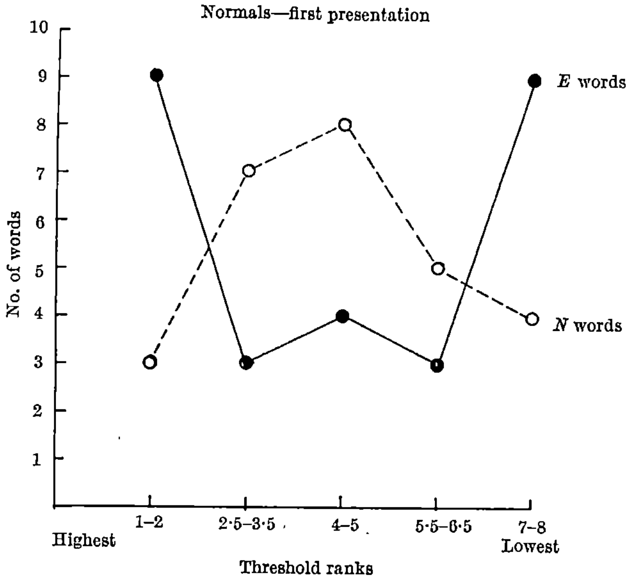

Fig. 1. Distribution of threshold ranks for emotional (*E*) and neutral (*N*) words

on the basis of results from a word association test, were presented twice each in a random order to each of fifteen subjects. Of these subjects eight were psychiatric patients of University College Hospital, and seven normal adults of approximately the same age and intelligence quotient levels as the patients. Of the patients four presented with predominantly paranoid features while four were predominantly depressed.

EEG and threshold data were recorded on magnetic tape prior to frequency analysis by a Muirhead low-frequency analyser. The EEG was quantified over 6-sec epochs by passing the output from the analyser through a low-inertia motor-driven counter.

Three general hypotheses were investigated: (1) Thresholds for emotional words would be higher or lower than those for neutral words. (2) Differences in theta abundance between emotional and neutral word presentations recorded prior to awareness of these words would exceed those recorded for a control group to whom words were not in fact presented. (3) Changes in theta abundance between stimulus onset and awareness would be greater during those presentations in which high or low thresholds were recorded than in those for words in the middle of the threshold range.

Stimulus emotionality and awareness threshold. The eight words shown to each subject were ranked in order of the intensity at which they first became perceptible.

The following curve shows the distribution of rankings for emotional and neutral words in respect of normal subjects on the first presentation. Similar curves were obtained for patients and for normals on the second presentation. The main finding that thresholds tended to be high or low for emotional words and clustered round the centre of the threshold range for neutral words is significant on the first presentation, for normals and patients combined, at the 0·6 per cent level of confidence (sign test). On the second presentation results for normals are significant at the 0·8 per cent level of confidence and for normals and patients together at the 5 per cent level. They may be taken to imply some discrimination of meaning prior to awareness of the stimulus items.

Experimental versus control group. To control against the possibility that observed differences in EEG, as between presentation of emotional and neutral words, might be artefactual, recordings from the experimental group were compared with those from a group of six normal controls. These subjects were given a treatment identical with the others except in so far as no words were presented. As with the experimental EEG's, each control record was broken down into 6-sec epochs and each trial designated 'emotional' or 'neutral' according to the paradigm ENNE NEEN. Each trial was approximately 1 min in length, equivalent in the case of the experimental subjects to the period between stimulus presentation and verbal recognition. The following mean differences in integrator scores between 'emotive' and 'neutral' word trials were obtained for the two groups of subjects over all eight trials.

Theta abundance and the awareness threshold. The following curves, for patients on the first presentation,

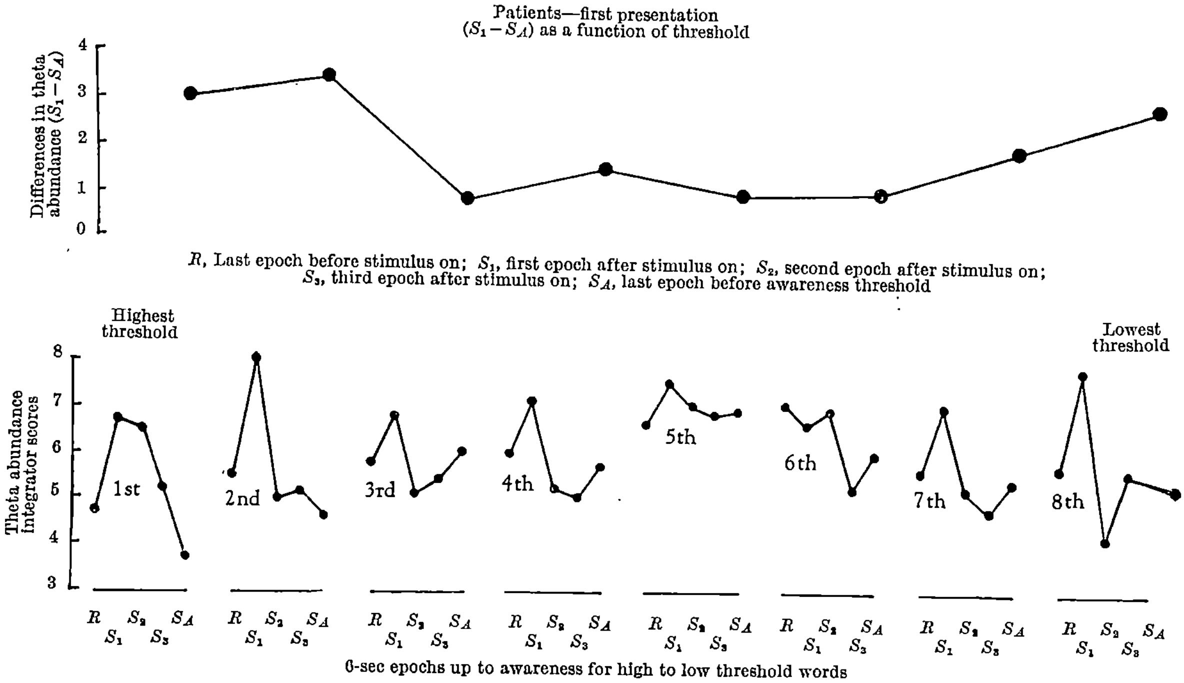

Fig. 2. Theta abundance as a function of threshold

show the incidence of theta, in 6-sec epochs between onset of the stimulus and awareness, as a function of the awareness threshold. Comparable curves showing the same generally 'U'-shaped relationship between theta activity and threshold were obtained for the patients' second presentation and for normals on the first presentation.

(Since the length of time between stimulus onset and awareness varies from subject to subject the convention was adopted of labelling the first 6-sec epoch after stimulus onset as S_1 and the last epoch before awareness as S_A; S_2 and S_3 represent means over two 6-sec periods of the intervening epochs.)

From these results the following observations may be made:

(1) For the first 6 sec after presentation of the stimulus there was a general tendency for theta to rise. For the patients in the first presentation this rise in theta, shown by all but one subject, was significant at the 2 per cent level of confidence by a two-tail test ($t = 3{\cdot}22$).

The normals as a group showed the same general effect but to an insignificant extent. However, a breakdown of the normal group into those with higher and those with lower thresholds for emotional as opposed to neutral words revealed an interesting fact. Whereas the high-threshold group showed a large rise in theta, the low-threshold group showed an almost equally large fall. The difference between these groups was significant at the 2 per cent level of confidence by a two-tail test ($t = 3{\cdot}64$). A breakdown of the patient group into high- and low-threshold subjects revealed the same tendency at work though not to a statistically significant extent.

As with the patients, the normals showed a greater rise for the four words for which they had the highest threshold. For normals that word for which they had the highest threshold evoked a rise in theta which was significant by a two-tail test at the 2 per cent level of confidence ($t = 3{\cdot}49$). For normals and patients combined this result was again significant at the 2 per cent level of confidence by a two-tail test ($t = 2{\cdot}86$).

(For both patients and normals this rise in theta during the first 6 sec of the stimulus contrasts with a fall in abundance of the alpha component. In the case of the patients this difference between alpha and theta change is just not significant at the 5 per cent level by a two-tail test ($t = 2{\cdot}3$). Like the patients the normals also showed a fall in alpha which was greater for the low-threshold words than for the high-threshold words.)

(2) Between the first epoch after the stimulus was presented and the awareness threshold there was a fall in theta (S_1–S_A), which was greater for the two words with the highest threshold and the two with the lowest threshold than for those four words in the middle of the threshold range.

Over all subjects on the first presentation this finding is significant at the 5 per cent level of confidence by a one-tail test ($t = 1{\cdot}78$). Nine out of the fifteen subjects showed this effect.

Table 1. DIFFERENCES IN THETA ABUNDANCE BETWEEN EMOTIONAL AND NEUTRAL WORD PRESENTATION

First presentation		Second presentation	
Experimental subjects	Control subjects	Experimental subjects	Control subjects
23·5	5·7	10·3	5
6·3	0·7	10·0	6
7·5	4·1	13·0	0
22·5	7·1	11·7	6·3
45·0	0·4	8·1	0·9
6·9	0·7	11·4	0·3
9·3		19·0	

By the Mann–Whitney 'U' Test these scores differ significantly from each other at high levels of confidence ($P_1 < 0{\cdot}002$, $P_2 < 0{\cdot}001$).

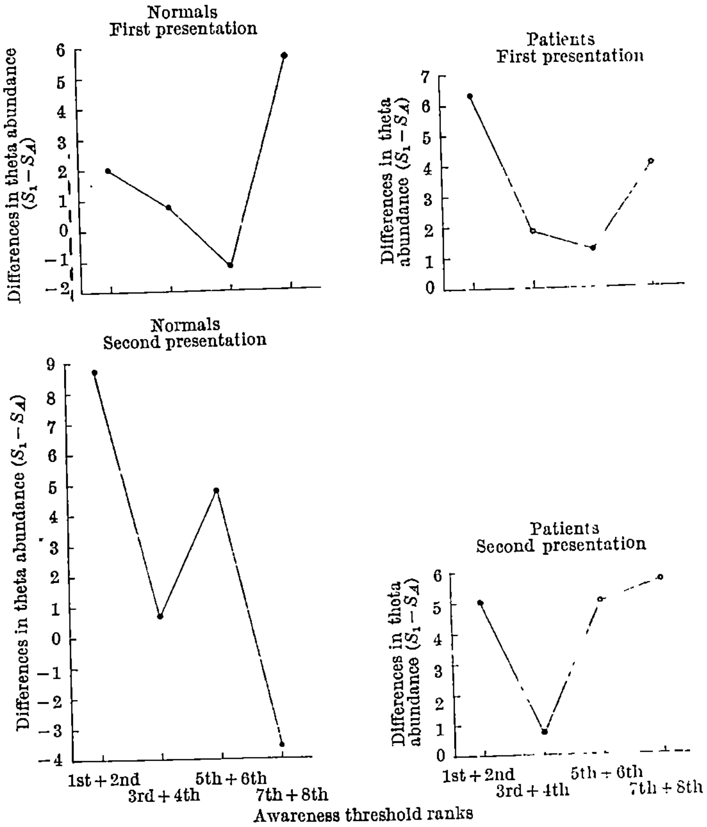

Fig. 3. Differences in theta abundance between first epoch after stimulus presented and last epoch prior to awareness as a function of the awareness threshold

(3) On the second presentation, six of the eight patients showed the same tendency. This result did not reach statistical significance.

The normals, however, on this presentation did not, as they had previously done, show a 'U'-shaped relationship between theta and threshold. Whereas they showed a fall in theta for high-threshold words they showed a progressive rise for the two low-threshold words. Within this group the difference in theta change between high- and low-threshold words was significant at the 5 per cent level of confidence by a two-tail test ($t = 2{\cdot}7$).

The difference between patients and normals in this respect on the second presentation was also significant at the 5 per cent level of confidence by a two-tail test ($t = 2{\cdot}27$).

(4) As with the analysis of the alpha component reported in a previous paper, subjects in each group were divided into sub-groups depending on whether or not their average threshold was higher for *E* words as opposed to *N* words. Applying this breakdown to the relationship between theta change from stimulus onset to awareness (S_1–S_A), and thresholds for the individual words, it is apparent that the a/m 'U'-shaped relationship holds most markedly for those normals and patients who had lower thresholds for '*E*' words than for '*N*' words. For those subjects with higher thresholds for '*E*' words, however, there is the suggestion of a difference between patients and normals. Whereas the former again showed a 'U'-shaped relationship between theta fall and threshold the normal group showed a relationship between theta and threshold that was negatively correlated (rho = − 0·79) with that recorded for the normal low-threshold group.

It is interesting to note that this difference between the normal subjects with higher thresholds for '*E*' words, and all other subjects, was very much more marked in the case of the alpha component. In the case of the high-threshold normals only, there was a significant tendency for alpha to fall more during the presentation of medium-threshold words than for those words for which these subjects had either a high or a low threshold ($t=5\cdot17$ $P<0\cdot05$ 2-tail). In this respect these subjects differed significantly from both the high- and low-threshold patient groups at 1 and 5 per cent levels of confidence respectively ($t=5\cdot6$ and $3\cdot11$) by two-tail tests.

First and second presentations. (1) Whereas the patients showed the same 'U'-shaped relationship between theta fall (S_1–S_A) and awareness threshold on both presentations, the normals only showed this tendency on the first presentation.

(2) For both normals and patients there was a significant relationship between thresholds on the first presentation and theta abundance on the second presentation.

It is apparent from Table 2 that those subjects with a higher mean threshold for emotional than for neutral words on the first presentation showed a greater theta abundance for emotional words than for neutral words, in the last epoch prior to awareness, on the second presentation.

Table 2

First presentation Awareness threshold	Second presentation Abundance prior to awareness	
	$\theta E>\theta N$	$\theta E<\theta N$
$AE>AN$	6	1
$AE<AN$	0	8

Similarly, those with a lower threshold for emotional words on the first presentation showed less theta for these same words than for neutral words on the second presentation.

By the Fisher-Yates test this finding is significant at the 1 per cent level of confidence by a two-tail test (computed P for a two-tail test $=0\cdot0028$).

The recognition threshold. Above the awareness threshold stimulus intensity continued to rise until the subject reported recognition of the stimulus word. An analysis was made of:

(1) The relationship between the recognition threshold and the emotionality of the stimulus. For the normals a 'U'-shaped relationship similar to that for the awareness threshold still held. On both presentations emotional words tended to be recognized earlier or later than neutral words. For the patients, however, the emotional words appeared to result in high thresholds only. Over both normals and patients the tendency for the highest emotive word threshold to exceed the highest neutral word threshold and the lowest emotive word threshold to be lower than the lowest neutral word threshold was significant at the 2·5 per cent level of confidence.

(2) The relationship between the recognition threshold and theta abundance in the epoch immediately prior to the recognition threshold. Here, there was a general tendency for theta to be less for an emotional word than for a neutral word, irrespective of the actual threshold value. In this respect theta behaved differently from alpha. For alpha it was found that whereas those subjects with higher thresholds for emotional than for neutral words showed more alpha for emotional than for neutral words, subjects with lower thresholds for emotional words showed the opposite effect. This finding is significant at the 5 per cent level of confidence by a one-tail test. It supports our main hypothesis that threshold regulation depends on control of cortical activation.

Conclusions. The findings described in our previous article provided some support for the hypothesis that high or low thresholds for visually presented words depend, respectively, on decreased or increased activation of the occipital cortex. This conclusion was based on the fact that subjects with raised thresholds for 'emotional' words showed less desynchrony in the pre-awareness EEG than did those with lowered thresholds for emotive material. Implicit in this finding was the fact that at some level of stimulus intensity below the awareness threshold a differential response to the meaning of the material must have occurred. It was tentatively suggested that short-term changes in alpha abundance observed immediately after onset of the stimulus reflected this initial discrimination.

Our present analysis of changes in slow-wave abundance suggests that stimulus emotionality and slow-wave activity bear a 'U'-shaped relationship to both the awareness and recognition thresholds. In the light of the relationship between slow waves and emotional arousal reported by other workers it is thought that the rise and fall of theta abundance prior to awareness of high- and low-threshold words reflect the emotional arousal occasioned by these words. The problem remains as to why this arousal should sometimes initiate cortical activation with consequent lowering of the visual threshold and at other times a lack of activation with a heightened awareness threshold.

One possible explanation is that these results reflect successive stages in what is really a single process.

The reception of an emotionally arousing stimulus initiates an immediate burst of slow-wave activity accompanied by suppression of alpha. With this suppression there occurs a momentary lowering of the cortical discrimination threshold and possibly awareness of the stimulus.

If the awareness threshold is not reached, however, because of insufficient emotional arousal, a second stage of reception occurs wherein the temporarily heightened cortical discrimination brings about a progressive inhibition of the recticular activating response. The direct consequence of this would be a raising of the visual threshold. In terms of the EEG this raising of the awareness threshold would be accompanied by a return of synchronous alpha activity and, along with the lowering of emotional arousal, a disappearance of slow waves.

(These hypotheses are consistent with the curvilinear relationship between threshold and anxiety found by Bruner and Postman[3], De Lucia and Stagner[4], and McClelland and Liberman[5].

They are also consistent with relationships between the EEG and emotion that have been described by Hoagland[6], Jost[7], Sherman and Jost[8], and Grinker and Serota[9], while data on EEG changes in pre-conscious stages of the perceptual process have been provided by Ricci and Collatina[10].

Finally, some evidence for the fact that a momentary lowering of the visual threshold precedes its raising by a visual stimulus comes from a previous experiment[11], in which it was found that during the 30-sec subliminal presentation of an emotive stimulus to one eye the threshold of the other eye fell before rising to a level above that occasioned by the presentation of neutral stimuli.)

However, these are mere conjectures which anyway fail to account for several of our *post hoc* results such as the differences between patients and normals, and between first and second presentations of the stimulus.

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

Detection of Fine Structure in the Crab Nebula at 38 Mc/s

SEVERAL observations of the distribution of radio brightness across the Crab Nebula have been reported over a wide range of frequencies[1,2,4-6]. The results indicate that the distribution, while slightly elongated, has an approximately Gaussian profile. There is some evidence that the angular diameter increases at the lowest frequencies[1], but no fine structure has been detected. Kuz'min and Salamonovich[6], observing at a wave-length of 8 mm, noted a small source slightly displaced from the centre of the nebula which they ascribed to an independent source of thermal origin.

During the course of an investigation of the scattering of radio waves in the solar corona and the interplanetary medium[3], new information has become available on the angular diameter of the Crab Nebula at a frequency of 38 Mc/s. These results show that fine structure is present which has not been detected at higher frequencies but give no evidence of a significantly increased angular diameter. Further results derived from a recent lunar occultation of the nebula support these conclusions (see following communication).

The observations at 38 Mc/s were made with phase-switching interferometers on an east–west baseline. The arrangement of the aerials for spacings of 200 and 560 wave-lengths has been described elsewhere[3]. For spacings of 903 and 1,290 wave-lengths a system of higher sensitivity was devised which incorporated grating arrays to produce a reception pattern having a triple response. One array was a corner reflector 3,000 ft. in length containing dipole elements spaced by 1·4 wave-lengths and the other was a similar array 800 ft. in length. Signals from the distant array were conveyed to the receiver by a direct cable link. The Crab Nebula was observed at meridian transit, corresponding to an east–west aerial separation of 1,290 wave-lengths, and also at times displaced by ± 3h 20m from the meridian transit, which gave projected spacings of 903 wave-lengths.

The different systems were calibrated with reference to the source 3*C* 123, which is known to have an angular diameter $< 12''$. At the larger aerial spacings 3*C* 123 and the Crab Nebula yield comparable fringe amplitudes so that calibration is simple and direct. No attempt was made to determine the absolute phase of the fringes.

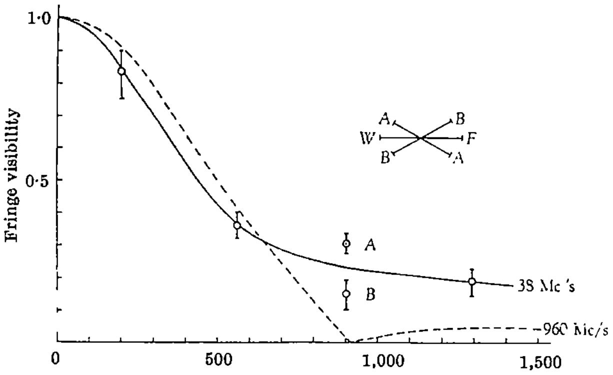

Fig. 1. The fringe visibility curve derived from observations of the Crab Nebula at 38 Mc/s. The broken line indicates corresponding results at 960 Mc/s

The fringe visibility curve derived from our measurements is shown in Fig. 1. The two points at a spacing of 903 wave-lengths correspond to different position angles of the fringes as indicated. By comparison with the results of Maltby and Moffet[4], also shown in the figure, it is clear that observations at 38 Mc/s indicate fine structure which is not present at higher frequencies. The simplest model which may be chosen to fit the observed visibility curve comprises two Gaussian sources having east–west angular diameters of $3'\cdot 7 \pm 0'\cdot 5$ and $30'' \pm 15''$. The flux densities of the two components are in the ratio of approximately 4 : 1. This result has been confirmed by recent observations of a lunar occultation of the nebula at 26 Mc/s which suggest that the small diameter component becomes even more pronounced at lower frequencies (following communication).

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Radio Observations of the Crab Nebula during a Lunar Occultation

ON April 16, 1964, a lunar occultation of the Crab Nebula was observed at Cambridge at 408, 178, 81·5 and 26·5 Mc/s. East–west interferometers were used at all frequencies and strip brightness distributions were obtained across the nebula in two directions.

The path of the radio source relative to the Moon is shown in Fig. 1. Immersion and emersion of the optical centre of the nebula occurred at position angles, measured on the Moon from north positive to east, of 43° and 295° respectively. The directions *OB* and *OA* (Fig. 1) correspond approximately to the major and minor axes of the optical object, the size of which is about 6′ arc by 4′ arc in these two directions. The Moon crossed the source at the rate of 22″ arc per minute of time perpendicular to the limb during immersion and emersion.

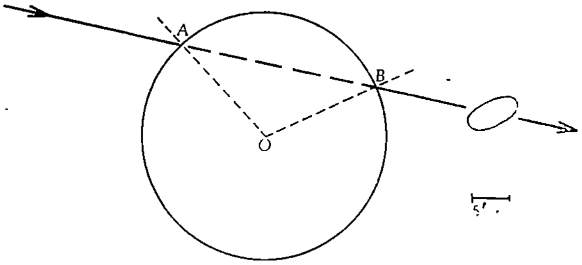

Fig. 1. Path of the Crab Nebula relative to the Moon

At 408 Mc/s the source was tracked continuously using two 60-ft. diameter paraboloids at a separation of 225λ. The 178-Mc/s observations were made with two 8λ corner reflector aerials separated by 130λ. It was necessary to move these aerials in azimuth between immersion and

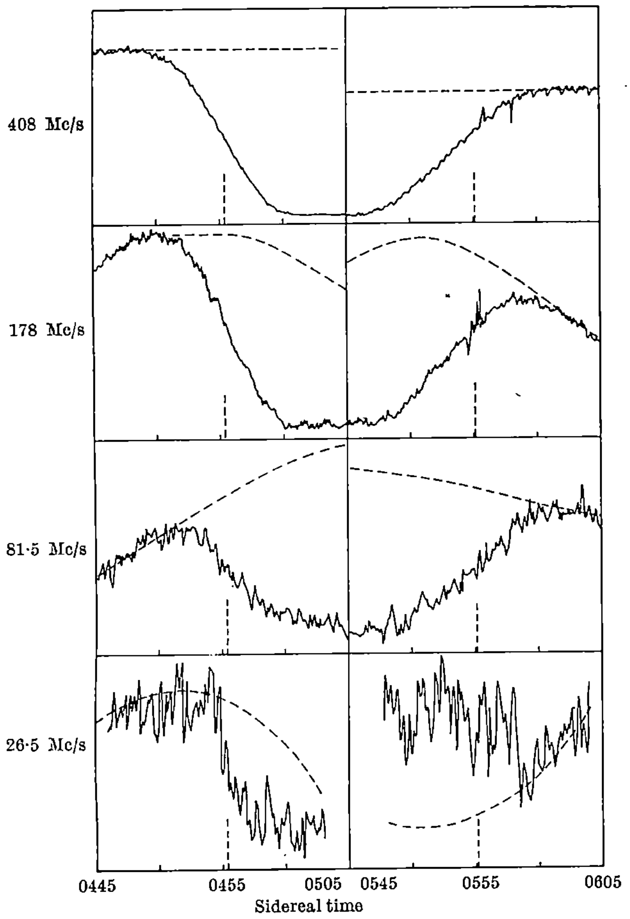

Fig. 2. Records obtained at immersion and emersion

emersion. At 81·5 Mc/s, two $2\lambda \times 2\lambda$ broadside arrays 130λ apart were used to observe both events, and the 26·5-Mc/s aerial consisted of two arrays of 30 full wave dipoles at a separation of 5λ.

At the three higher frequencies information was recorded on punched tape for computer processing, while the 26·5-Mc/s records were analysed by hand. Receiver time constants of 2 sec were used at all frequencies and the systems were calibrated by trial runs on at least one other day.

The immersion and emersion records are shown in Fig. 2; the 26·5-Mc/s records have been smoothed with an effective time constant of 12 sec. The predicted times of disappearance and reappearance of the optical centre of the Crab Nebula are shown by vertical dotted lines; comparison records for the unocculted source are also shown, indicating the variation of receptivity with those systems using fixed aerials.

The occultation curves were analysed to obtain strip brightness distributions along the two directions OX and OY (Fig. 3). The curves in Fig. 4 show the variation along XOX' and YOY' of the relative intensity of the slightly curved strips perpendicular to these directions. The analysis was based on strips 11″ arc in width. The curves are normalized so that each encloses the same area.

At immersion the strip brightness distributions at 408 Mc/s and 178 Mc/s are similar, but the 81·5 Mc/s distribution shows a greater central condensation (Fig. 4*a*). The 26·5 Mc/s curve is significantly different with a much more localized source, as is evident from the faster disappearance of the source (Fig. 2). At emersion, the three higher frequency strip distributions are noticeably flattened across the central region (Fig. 4*b*). This fact, when combined with the immersion distributions, suggests emission from a cylindrical or ellipsoidal shell. At 26·5 Mc/s much of the radiation is again seen to originate in a small region of the nebula.

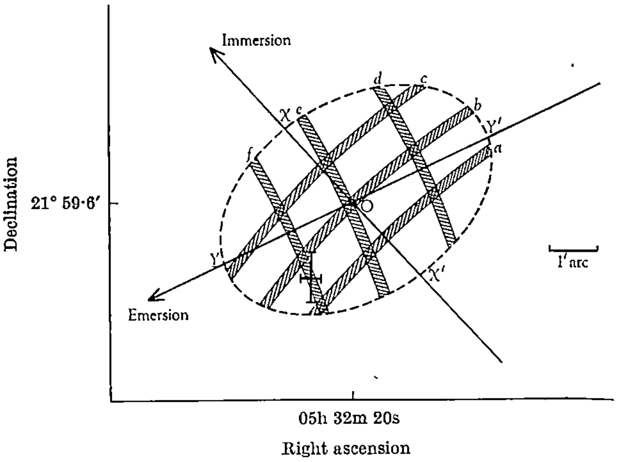

Fig. 3. Outline of Crab Nebula showing directions of travel of the Moon's limb. The position is given in 1964·3 co-ordinates

It is seen from Fig. 4*b* that there is a systematic movement of the radio centre along the major axis towards the east with decreasing frequency; this movement is too great to be attributed to refraction in the lunar atmosphere[1,2]. The position of the localized region at 26·5 Mc/s is 1·2′ arc east of the optical centre along strip *b* (Fig. 3). This position is near a prominent filament in the nebula.

The localized strip of intense emission observed at 26·5 Mc/s on both immersion and emersion indicates the presence of an area having an angular diameter of about 20″ arc which contributes 30–50 per cent of the total emission from the nebula at this frequency; its brightness temperature must exceed 10^9 °K.

In order to examine the spectrum of the emission from different parts of the source, the contributions from each

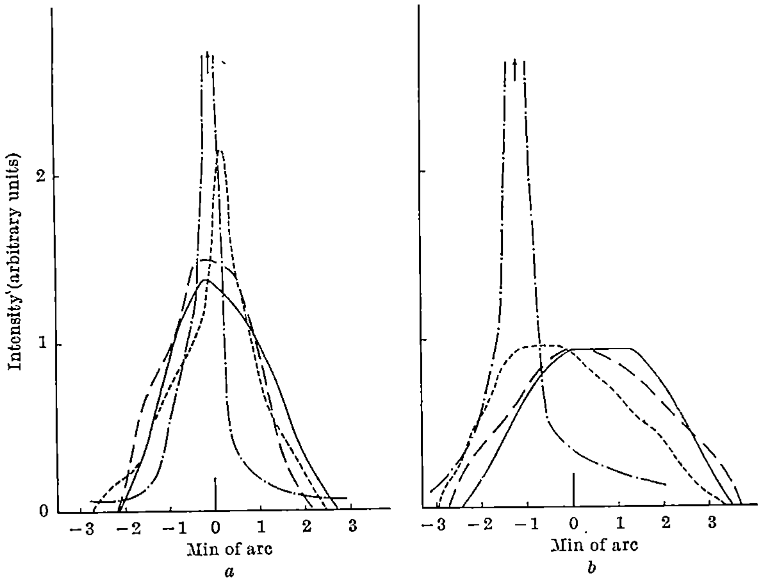

Fig. 4. Strip brightness distributions across the Crab Nebula. (*a*) Immersion; (*b*) emersion. ——, 408 Mc/s; – –, 178 Mc/s; - - -, 81·5 Mc/s; —·—, 26·5 Mc/s

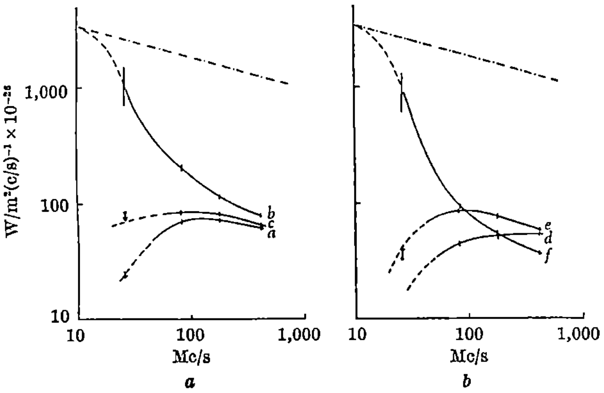

Fig. 5. Spectra of the strips *a* to *f* shown in Fig. 3. (*a*) Immersion; (*b*) emersion

of the six strips shown in Fig. 3 have been determined at each frequency and plotted in Fig. 5; the spectrum of the emission from the whole source can be approximated very well by a straight line having a gradient of – 0·27 (refs. 3 and 4), and this is shown by the dotted line. It can be seen that except for the strips (*b*) and (*f*), which contain the localized low-frequency source, there is little variation in the spectrum from different parts of the nebula; the well-defined reduction in the emission at frequencies below 50 Mc/s suggests that there may be a cut-off in the electron energy spectrum.

A further investigation of the nebula was made at 408 Mc/s using an interferometer with spacings up to $1{,}450\lambda$; these observations showed the presence of a localized source having an intensity of about 2 per cent of the total.

The position of the source is shown in Fig. 3, and agrees within the limits of error with the position obtained at 26·5 Mc/s.

The spectrum of the localized source is shown in Fig. 6. The 26·5-Mc/s points were obtained from the strip brightness distributions (Fig. 4) at immersion and emersion. At 81·5 Mc/s the intensity of the central condensation at immersion is shown together with the limit detectable at emersion for a source of similar diameter. At 178 Mc/s and 408 Mc/s upper limits for the emission from the localized source are shown. Also included are the 38-Mc/s point obtained by Hewish and Okoye (preceding communication) and the 408-Mc/s value of 2 per cent discussed above. It is seen that the spectrum of the small region is straight with the very steep gradient of – 1·2.

The physical nature of this small source is unknown. Woltjer[5] has shown that models for emission from the Crab Nebula cannot be constructed in terms of electron energy spectra uniform throughout the source. It seems significant that a source containing two regions with such unusual spectra should have a resultant spectrum which is so remarkably straight. A possible interpretation could be that electrons with a differential energy spectrum of the form:

$$N(E)\,dE \propto E^{-\gamma}\,dE$$

are produced, but the low-energy electrons are trapped in the localized region while the high-energy electrons escape into the rest of the nebula.

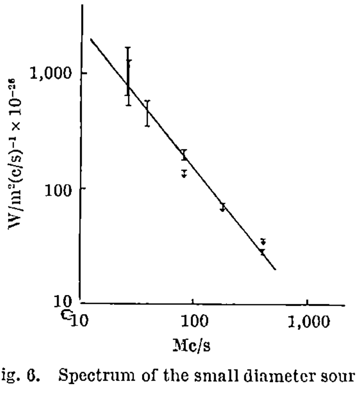

Fig. 6. Spectrum of the small diameter source

The general form of the observed radio spectra for the main body of the nebula resembles that produced by an electron energy spectrum with a low-energy cut-off. Using the formulæ given by van der Laan[6], and assuming a magnetic field of 10^{-3} gauss[7], the spectra of the nebula suggest a value of $\gamma \sim 1\cdot6$ and a low-energy cut-off at ~ 100 MeV.

We thank Prof. M. Ryle and Dr. P. F. Scott for their assistance with the observations, Miss J. A. Bailey for undertaking the numerical analysis, and Mr. W. Nicholson of H.M. Nautical Almanac Office for providing details of the circumstances of the occultation.

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RADIOPHYSICS

Spectrum of the Galactic Radio Emission between 1·5 and 10 Mc/s as observed from a Satellite

THE *Alouette* satellite (1962 β α 1) carries an ionospheric sounder in a nearly-circular orbit at a height of about 1,000 km. This experiment provides data on the electron number density at and below the satellite, and on the local magnetic field strength[1]. In addition, since the automatic-gain control voltage of the sweep-frequency receiver is telemetered to the ground, simultaneous measurements are also made of the background radio noise. (The receiver is inactive during the pulse transmission, and the included integration circuit essentially removes any effects of the echo pulses from the recordings of the automatic-gain control voltage.) Accordingly, for frequencies below those which can penetrate the ionosphere, these background noise data contain information on extra-terrestrial radio sources; the accompanying ionosonde data help to describe the propagation and reception of such cosmic radio waves by the satellite. In this communication the results of the galactic noise measurements as a function of frequency are outlined; they will be discussed in more detail elsewhere, as will the observations of other extra-terrestrial sources.

Essential features of the receiving system are the two orthogonal dipoles on the satellite, of length 150 ft. and 75 ft. Impedance matching networks broaden and shape their frequency response, and the two outputs are applied, in parallel, to the receiver. In the range 1·5–12 Mc/s the transmission losses in the antenna system vary between about 1 and 9 decibels (for the antennæ in free space), but below 1·5 Mc/s the system response drops very rapidly. In the presence of an ionized plasma this antenna system response is altered somewhat; nevertheless, 1·5 Mc/s is still a practical lower limit for observations of the background noise. A practical upper limit for such observations is set by the F-layer critical frequency at the particular time and location.

The receiver gain was calibrated before launch, but absolute power calibration could not be provided in flight. Equipment voltages in the satellite are stabilized, as is the vehicle temperature to a considerable extent, so that changes in the gain of the receiver are expected to be minor. In fact, the data for the 19-month period since launching are consistent enough to suggest that no significant gain changes have yet occurred. Moreover, the computed absolute brightness temperatures for frequencies greater than 4 Mc/s are in fairly good agreement with ground-based measurements when allowances are made for differences in antenna apertures.

In-flight frequency calibration of the records is carried out automatically, and for each recording (one sweep through the frequency range takes approximately 12 sec) an ionogram is available to describe the surrounding plasma. The plane of the satellite orbit processes about ·1 degree per day in space, and records are now available that cover all regions of the celestial sphere. Records are also available for a variety of ionospheric conditions and the antenna performance can be assessed quantitatively by comparing data obtained under these differing conditions.

In scaling records, data are not used below 1·5 Mc/s because of poor sensitivity, nor are they used for frequencies that lie too close to the ionospheric resonances[2]. This means that those spectral regions are avoided in which the propagation conditions and antenna performance are most difficult to assess: this procedure also limits the effective aperture to half-cone angles greater than 50°. In general, records have been selected so as to omit the regions toward the centre of the Galaxy, although with the broad effective antenna aperture used this distinction is not always meaningful at the higher frequencies. The relative received power as a function of frequency was scaled from the recordings for a wide range of local electron number densities and for different local magnetic field strengths. No apparent dependence on the orientation of the satellite antennæ was found in the data, nor was there any significant dependence on the local magnetic field strength except when the observing frequency approached close to the ionospheric resonances. A dependence on the local electron number density, however, was noted in the data. Accordingly, the values of the received power for a particular observing frequency were compiled and plotted as a function of the local electron density. This process was repeated for a number of other frequencies throughout the range of interest. A family of 'best fit' curves—one curve for each particular frequency—was drawn through these plotted data and extrapolated to zero electron density. Since each curve was drawn with a shape closely similar to that of its adjacent member, the errors involved in the extrapolation process are thought to be relatively small in at least half the cases. The resulting intercept values of the relative power for the particular frequencies are taken as corresponding to free space conditions; when combined with the known free space impedance for the antenna system they give values of the relative galactic brightness temperature. The relative temperature values so obtained are plotted in Fig. 1 as a function of frequency, together with error limits that allow for scatter in the observations as well as for uncertainties in the extrapolation process. This curve is to be taken as representative of medium and high galactic latitudes, and any fine detail in these regions has been smoothed out because of the large antenna aperture.

From Fig. 1 it can be seen that the most accurate results are obtained for the lower half of the frequency range. Above about 5·5 Mc/s only one accuracy limit can be given for each data point, and the indicated curve has been extrapolated from lower frequencies with a gradual increase in slope such as would bring it into agreement with the results of ground-based measurements at frequencies greater than about 15 Mc/s. It should also be noted that the indicated uncertainties do not include a provision for changes in the receiver characteristics after launch, if any occurred.

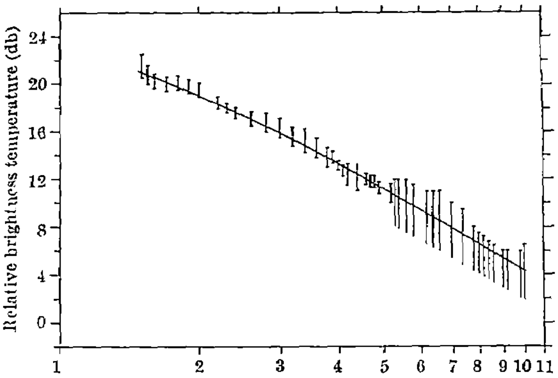

Fig. 1. Galactic noise spectrum obtained with the swept-frequency receiver in *Alouette*

A comparison of this spectrum with that obtained by Ellis, Waterworth, and Bessell[3] from ground-based equipment shows a significant difference, as may be seen in Fig. 2. Here the solid curve represents the results of Ellis *et al.* for high galactic latitudes, and the dashed curve has been obtained from Fig. 1 by converting relative brightness temperatures to relative flux densities. The two curves have been normalized at 4·8 Mc/s, where the present data are thought to be quite reliable. Between 4 and 10 Mc/s there is reasonable agreement between the two curves, but this is seen not to be so for lower frequencies; the discrepancy increases with increasing wave-length. This dependence on wave-length, together with the fact that the solid curve has a terminal slope of about +2 as one would expect for radio wave absorption, suggests that absorption occurs below the satellite height—that is, below 1,000 km. In other words, it is thought that a large part of the discrepancy between the two curves of Fig. 2 can be attributed to ionospheric absorption. Since there is no description as to how Ellis *et al.* evaluated and removed the effects of ionospheric absorption from their data, it has been assumed that they underestimated the effects. Generally accepted values for the D-region electron number density are such that there should always be

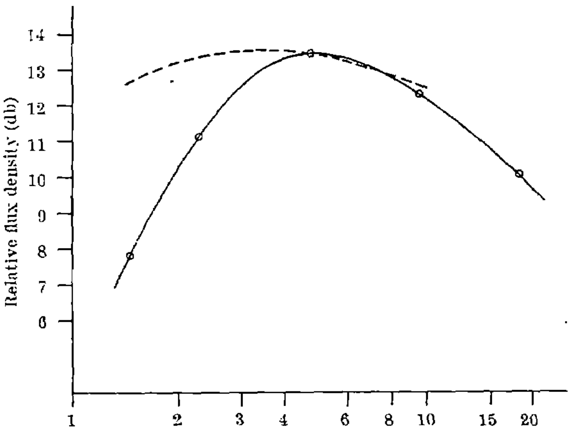

Fig. 2. The relative flux density for the galactic noise as a function of frequency; as determined from the *Alouette* data (dashed curve); and as obtained by Ellis *et al.* (solid curve)

observable absorption at these frequencies. Differences in antenna aperture in the two cases may also be important in so far as iris effects and deviative absorption are concerned in the ground-based measurements.

The importance of radio wave absorption in the Ellis *et al.* results has already been recognized by Hoyle and Ellis[4], but they attributed all the absorption to the galactic gas. This interpretation led them to a 'surprisingly high value' for the electron density in the Galaxy. If, as suggested here, the Ellis *et al.* galactic noise spectrum still includes a contribution from ionospheric absorption, the computed galactic electron densities should be reduced accordingly.

Because of the lack of in-flight equipment calibration, absolute data have been considered somewhat less reliable than relative data. Nevertheless, values for the brightness temperature have been obtained for representative regions of the celestial sphere; these suffer from the poor antenna resolution, and only the very crudest mapping is presently possible. The brightest region in the Galaxy is centred approximately on the south galactic pole and at 2·3 Mc/s has a temperature of approximately $1·8 \times 10^7$ °K. The comparable region containing the north galactic pole has a temperature at this same frequency of $1·1 \times 10^7$ °K. This differential, if it can be substantiated by further observations at such low frequencies, would seem to substantiate the asymmetrical location of the solar system with respect to the plane of the Galaxy. The lowest temperature, approximately $5·0 \times 10^6$ °K at 2·3 Mc/s, has been obtained for a region centred on R.A. 9 h, dec. + 75°.

The contributions of the many people and agencies that were involved in the various stages of the *Alouette* project and who continue to gather and reduce the data are gratefully acknowledged.

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ASTRONOMY

Gravity and Rotation

It is possible to show mathematically that a particle which is located on a rotating object is attracted by all the particles of the object according to the inverse square law. Several important consequences follow as a result of this theorem. One of the most important is that Newton's second law of motion and the law of universal gravitation are not laws apart; one can be deduced from the other. Another is that gravity is due to the rotation of the Galaxy, and from this the following verifiable conclusion is obtained.

If ρ is the average density of the Galaxy, ω its angular velocity and γ the constant of universal gravitation, then

$$2\pi\gamma\rho = \omega^2$$

The present values for ρ and γ are in C.G.S. units 7×10^{-24} and $6·8 \times 10^{-8}$. Using the foregoing formula ω is $1·7 \times 10^{-15}$ radians/sec. The present value for the angular velocity of the Galaxy is given by astronomers as 10^{-15} radians per sec.

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PHYSICS

Fracture of Liquids in Shear

In a recent article on melt fracture of silicone polymers under shear[1] Hutton reports that the critical shear stress for fracture in a cone and plate viscometer is independent of polymer molecular weight but dependent on cone diameter. He further suggests a criterion for predicting the onset of melt fracture. It is the purpose of this communication to discuss these points.

(1) The phenomenon of extrudate distortion in capillary flow, originally observed by Spencer and Dillon for polystyrene[2], was ascribed to 'melt fracture' by Tordella[3–5] on the basis of visual observations of polymer flow into the capillary. In their original work Spencer and Dillon found that the critical shearing stress for melt fracture, τ_d, was dependent on molecular weight, the product of τ_d and weight average molecular weight, for a series of polystyrenes, being constant. This constant was later shown to be proportional to the product of temperature, polymer density and recoverable shear strain at fracture[6]. It was found empirically that the recoverable shear strain at fracture is always about 7 ± 2 units for linear polymers, independent of polymer type. Thus the critical shear stress measurement can be used as a means for determining molecular weight[6–8]. The results entered in Table 1 of Hutton's paper[1] are thus seen to be at variance with those earlier reported results.

The results in Table 1 of ref. 1 suggest a dependence of the critical shearing stress for melt fracture on the cone geometry, a point which is elaborated in the author's theoretical approach to the melt fracture phenomenon. Here again inconsistency with earlier capillary flow data seems evident, since in this earlier work it was established[9,10] that the critical shearing stress for onset of melt fracture in extrusion investigations is independent of the geometry of the extrusion system. The geometry of the system does, however, influence the macroscopic effects of melt fracture[8,9], thus often leading to a conclusion which we believe to be erroneous that the two factors are fundamentally interdependent. Certainly the interpretation of the melt fracture phenomenon given in reference 6, and implied also by Spencer and Dillon[2], would require that τ_d be independent of system geometry.

The explanation of these discrepancies may lie in the possibility that the effects observed by Hutton are not due to melt fracture but to some other phenomenon. Certainly in capillary extrusion of polymer melts effects are noted which are not attributable to melt fracture as usually defined[11,12] but to other complex flow characteristics. This suggestion is reinforced by the fact that only two of the silicone fluids used by Hutton have molecular weights above the critical molecular weight for entanglement of this polymer[13] (~30,000). Except at very high rates of shear, non-Newtonian flow would not be expected for polymers with molecular weights lower than the critical weight for entanglement[14,15]. Further, at these low molecular weights it has been suggested that elastic effects should not be observed either[16]. The results reported by Hutton for samples *MS*200/12.500 and *MS*200/30,000 therefore raise the question whether unknown effects are not complicating the experimental evidence, and contributing to an incorrect assessment of the critical shearing stress in terms of polymer molecular structure.

(2) In addition to these purely experimental points of difference some discussion is warranted of the model postulated by Hutton, wherein the fracture will occur only when the total elastic energy is at least sufficient to supply the energy requirements of the new surface formed. This is the criterion applied by Griffith[17] to brittle fracture of glasses, where the work to form the fracture surfaces is a function of the surface tension of the material. Thus the fracture stress, σ, is related to

E, α and c, the modulus of elasticity, specific surface free energy and defect length in the sample, by:

$$\sigma \geqslant \sqrt{\frac{E\alpha}{c}} \qquad (1)$$

Application of this criterion in reference 1 leads to the prediction that surface work and shear stress for melt fracture are independent of polymer molecular weight. It is unlikely, however, that the surface tension term is at all significant in fracture of liquids, or even of metals. In most cases of fracture the work of forming new fracture surfaces is probably dependent primarily on the plastic work occurring in the brittle fracture zone ahead of a growing crack[18–22]. This plastic work must depend on a viscosity term and hence on molecular weight.

By analogy with the brittle fracture of steels one may suppose that there are three stages in the process of melt fracture[23–25].

(i) The formation of a crack of atomic dimensions is the initiation phase. The initial crack may be pre-existing, such as dirt, microgel or agglomerates of filler or pigment, or it may form initially as a result of shear stress on the flowing melt[26,27]. We are unable to distinguish between these alternatives on the basis of our present information.

(ii) The initial microcrack must grow to the critical dimension for cleavage fracture. This is the growth phase. Persistence of the crack at the dimensions required for stability could result from the piling up of minor cracks or voids at an obstacle such as a very long, entangled polymer chain. Detailed speculation must await further relevant experimentation. However, the polymer melt stream does fracture and a flaw must grow through the dimensions corresponding to the crack initiation and growth phase.

(iii) The final phase is crack propagation. The modified Griffith criterion for crack propagation in a polymer melt is that the elastic energy, E, released by the melt as the crack advances is at least equal to the work, P, to form two new crack surfaces. This work involves at least two terms: (*a*) the surface-energy work; (*b*) the plastic work. The surface-energy work will be minor compared with the plastic work. Further, the plastic work, which will depend on viscosity of the melt and the velocity of crack propagation, should depend markedly on molecular weight if only because of the viscosity dependence of molecular weight.

The elastic energy per unit volume at fracture is given by[28]:

$$E = \frac{\tau_d^2}{2\mu} \qquad (2)$$

where μ is the shear modulus of the melt. This relation is valid for a polymer which obeys Hooke's law in shear and which relaxes, after melt fracture, to zero shear stress. The problem in applying the criterion $E \geqslant P$ is to calculate P, the non-recoverable fracture work. This should be given by the product of a viscosity and a shear rate, both of which may depend on the velocity of crack propagation and crack size and geometry. It is worth noting, however, that an interesting result is obtained if the plastic work is taken as given by:

$$P = \eta_{\max}\dot{\gamma} \qquad (3)$$

where $\eta_{\max}$ is the "inelastic viscosity" and $\dot{\gamma}$ the shear rate. This $\eta_{\max}$ is defined by[6]:

$$\eta_{\max} = \frac{\tau}{\dot{\gamma}}\sqrt{1 + \frac{S_R^2}{4}} \qquad (4)$$

where S_R is the recoverable shear strain.

The condition for the Griffith criterion for melt fracture thus becomes:

$$\frac{\tau_d^2}{2\mu} \geqslant \eta_{\max}\ \dot{\gamma}_d \qquad (5)$$

It is interesting to observe in Fig. 1 that a plot of τ_d versus $\sqrt{\mu\ \eta_{\max}\ \dot{\gamma}_d}$ is linear for a wide range of both linear and branched polyethylenes, and that the slope of the curve is $\sqrt{2}$. The data are therefore consistent with the Griffith criterion, when the plastic work is given by equation (3).

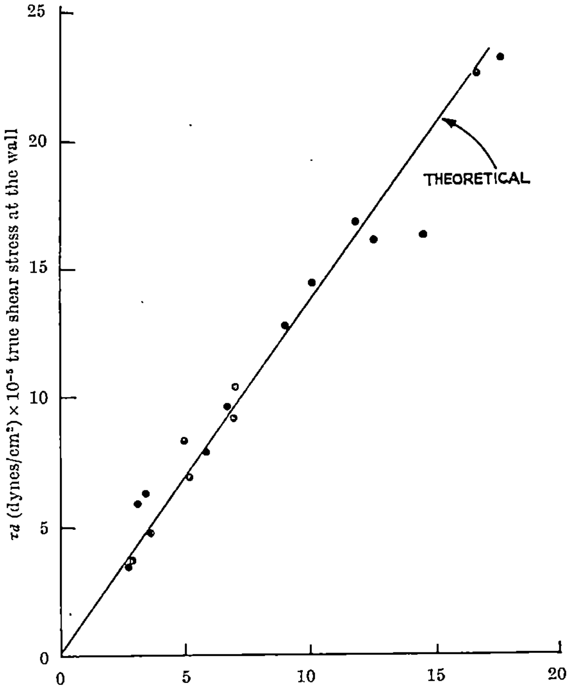

Fig. 1. The critical shearing stress for fracture, τ_d, versus $\sqrt{\mu\ \eta_{\max}\ \dot{\gamma}_d}$

The linearity of the plot in Fig. 1 is, however, to be expected. For $\frac{S_R^2}{4} \gg 1$ equation (5) can be rewritten as:

$$\frac{\tau_d^2}{2\mu} \geqslant \tau_d\,\frac{S_R}{2} \qquad (6)$$

which for equality is Hooke's law in shear. It was already known from end correction investigations of the resins in Fig. 1 that this was true, so that Fig. 1 is merely a re-statement of our results.

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THE first and major point raised by Bagley, Rudin and Schreiber is that when they compare my results[1] for the onset of flow instability in cone-and-plate viscometers with those for the onset of flow instability in capillary extruders they observe discrepancies. For example, the critical shear stress in the first type of instrument is independent of molecular weight and dependent on geometry, whereas in the second type it is dependent on molecular weight and independent of geometry. They have chosen the conclusion that what I have observed and measured is some other phenomenon than the one observed in capillaries. While this may be a correct conclusion it certainly does not follow from their argument about effects of 'geometry', and I think it is an incorrect conclusion for the following reasons.

An increase in the radius of a capillary tube is, in a sense, a change of geometry, but it is not the same sort of change as that between a capillary and a cone-and-plate viscometer: and, because it has been observed that the shear stress at the onset of melt fracture is independent of tube radius, it does not follow that the stress is independent of other sorts of geometrical changes. In the theory I give for fracture I take into consideration the total elastic energy in the sheared liquid. This is the integration of elastic energy through all variations in shear field and throughout the volume of liquid. For the cone-and-plate viscometer, in which the rate of shear is everywhere constant, this integration is very simple. Since the final expression contains certain dimensions of the cone-plate system, geometrical factors are important, and I stated this clearly in the final sentence of the article. A similar calculation for a capillary tube extruder cannot possibly contain the same geometrical factors, and a facile transference of critical shear stress data from one geometry to another is therefore not permissible. On the other hand, a theory which, when applied to geometrically completely different shearing devices, gives results in agreement with experiment will probably be correct. The application of the total elastic energy concept to the case of a capillary tube does in fact lead to the prediction that the shear stress at the onset of flow instability for silicone fluids and other 'power law' liquids is independent of tube radius. This and similar calculations are to be published.

The second major point concerns the approach to fracture through the elastic energy concept. I agree that the concept is a direct descendant of Griffith's theory[2], but it is considerably modified to avoid a crack length in the final expression and to find application to liquids. Griffith's own discussion of liquid fracture is more to do with cavitation than fracture, and the method of calculating the elastic energy from the components of stress was not available to him. A puzzling feature of the analysis given by Bagley, Rudin and Schreiber is the failure to introduce a fracture criterion. There seems to be no reason why their final expression, equation (4), should not apply at any pair of values of shear stress and rate of shear and not just at the values at melt fracture. In fact the whole analysis appears to comprise a set of dimensionally consistent identities, and agreement with experiment, as they state, is to be expected.

A further point should not go unanswered. Bagley, Rudin and Schreiber, using a dubious argument about entanglements, conclude that some of the silicone fluids I studied 'would not be expected' to have non-Newtonian properties and 'should not' have elastic properties. However, these theoretical predictions are not borne out by my own measurements on silicones in the 10^2–2×10^3 poise range or by the measurements of J. J. Benbow and E. R. Howells[3] in the 10^2–3×10^5 poise range, of A. J. Barlow and J. Lamb[4] in the 8–$2 \cdot 6 \times 10^2$ poise range, of A. J. Barlow and G. Harrison[5] in the 1–10^3 poise range, and of C. C. Currie and B. F. Smith[6] in the 30–10^3 poise range.

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'Hook Effect' in the Analysis of X-ray Diffraction Line Profiles

IN the analysis of X-ray diffraction line profiles by the Warren and Averbach method[1] a hook has often been observed in the particle size coefficients (A_L^P) versus distance (L) plots. In a recent work[2] on the analysis of X-ray diffraction line profiles due to cold-worked silver obtained by Geiger counter X-ray diffractometer, no such hook was observed, while in another work[3] on the analysis of X-ray diffraction line profiles of cold-worked molybdenum by a photographic method, the hook was quite pronounced. In order to investigate as to why the hook is produced in the photographic method and not in the diffractometer method, the 111 and 222 reflexions of cold-worked aluminium were examined by photographic as well as diffractometer method.

The aluminium examined was spectroscopically pure and was supplied by Johnson Matthey and Co., Ltd., London. Spectroscopic examination revealed that aluminium rod contained an estimated amount of 0·0002 per cent of silicon, 0·001 per cent of magnesium, 0·0005 per cent of iron and copper and less than 0·0005 per cent of tin, cadmium, sodium and calcium. Filings from an aluminium rod were allowed to pass through a sieve of 325 mesh/in.^{2} and the samples for the Geiger counter diffractometer were prepared in the form of flat-faced briquettes with a binder. The X-ray diffraction patterns of the cold-worked and annealed materials were measured with a Geiger counter diffractometer. To avoid the asymmetric broadening and shift of the peaks by rate meter recording the line shapes were recorded manually and fixed-count measurements were made of the diffracted intensity, at intervals in 2θ varying from 0·5° in the background to 0·01° in the lines using an electronic scaling unit and an EIT electronic counter. For a photographic method, an 11·46 cm diameter camera was used. All the films were processed under the same conditions. The line intensity measurements were carried out by an Adam Hilger non-recording type of microphotometer. The intensity scale was calibrated through suitably prepared standard wedges.

The Fourier coefficients for 111 and 222 reflexions were determined by Beevers–Lipson strips. For comparison of the Fourier coefficients of the line profiles determined by two methods, the coefficients of pure X-ray diffraction line profiles were determined by eliminating the geometrical broadening by the method of Stokes[4]. For eliminating the instrumental broadening the line profiles from fully annealed specimens were used as standard.

The high-order Fourier coefficients obtained by film as well as diffractometer method agree within 6 per cent. The agreement in the case of low-order Fourier coefficients is, however, very poor. The values of the low-order Fourier coefficients obtained by film method are less than those obtained by counter method by 12 per cent. The decrease of Fourier coefficients is perhaps due to the assumption of a high background in the film method which amounts to taking the lower area of the peaks. This decrease in area essentially affects the low-order Fourier coefficients while the effect on high-order coefficients is within the limits of experimental accuracy. The appearance of the hook in the A_L^P versus L curves in the analysis of X-ray diffraction line profiles, therefore, seems to be due to the inability of the photographic method to record properly the tailings of the X-ray diffraction line profiles.

I thank Prof. H. N. Bose, head of the Department of Physics, for the provision of laboratory facilities. I also thank Dr. G. B. Mitra for his advice, and the Director-General, Council for Scientific and Industrial Research, for the award of a senior research fellowship.

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GEOPHYSICS

A Possible Interpretation of the Fast Regular Fading observed on Satellite Transmissions

Frihagen and Tröim[1] have already observed a deep and nearly sinusoidal type of fading on fast amplitude records at 108 Mc/s. The effect only lasted for a few periods and a time delay between similar features on records taken at two spaced aerials indicated that it was introduced in the ionosphere. They proposed that this phenomenon was caused by diffraction at a single 'blob' in the ionosphere and that it is similar to that which was observed in radio-star scintillation by Wild and Roberts[2]. Apparently the same kind of amplitude variations were observed on several occasions at Kiruna (67·8° N., 20·4° E.) (ref. 3) on records of *Transit IV A* at 54 Mc/s which showed intense scintillation. The phenomenon was usually more persistent than that observed by Frihagen and Tröim and often lasted up to a hundred periods. The fading periods varied between 50 and 500 msec, but there was a predominance of periods of about 100 msec. Fig. 1 gives an example of persistent regular fading lasting about 10 sec. Some cases of the phenomenon of short duration (about 1 sec) which exhibit monotonically increasing (or decreasing) amplitude and period may be explained by diffraction at single 'blob' in the ionosphere. However, it is difficult to explain long lasting, regular fading in this way.

The phenomenon was intensively investigated at Kiruna during the second half of 1962 using recording equipment described by me[3]. It was found that it occurs most frequently at rather large zenith distances, with a maximum between 40° and 50°.

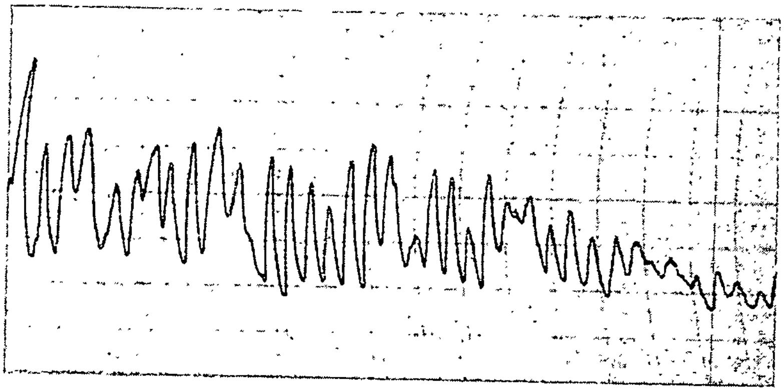

Fig. 1. An example of fast regular fading observed on rev. No. 7192. One division of the scale is 0·5 sec

The plot of sub-satellite positions at times when the regular fading was observed at Kiruna (Fig. 2) shows that in most cases the phenomenon occurred to the east or west of the observing station. Measurements made using spaced receivers usually exhibited a time delay between patterns observed at different aerials.

The following explanation of the phenomenon is proposed. The frequency of the satellite signal received at the aerial is different from the transmitted frequency, f, by an amount Δf, given by:

$$\Delta f = -\frac{f}{c}\dot{\rho} + \frac{f}{c}\frac{d}{dt}\int (1-n)dr \qquad (1)$$

where c is the velocity of light in a vacuum, and $\dot{\rho}$ is the time derivative of the geometrical length of ray path. The first term represents the Doppler effect in a vacuum and the second term is a 'correction term' which takes account of the effect of the dispersive medium between the transmitter and the receiver and where n is the refractive index of the medium and the integration is performed along the propagation path. When the transmitted frequency is high, then, using the usual notation

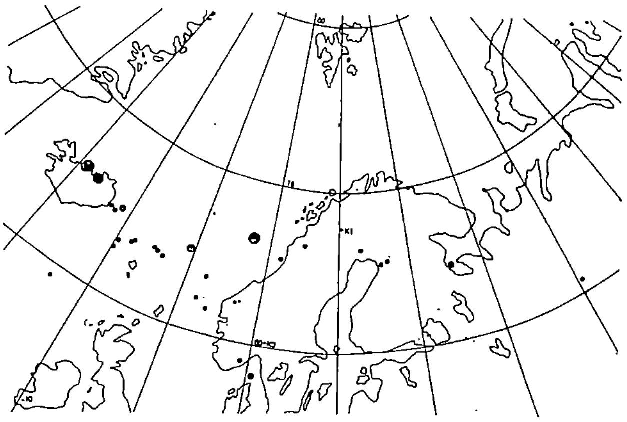

Fig. 2. Sub-satellite positions at times when the regular fading was observed at Kiruna. The diameter of circles is proportional to the duration of the fading

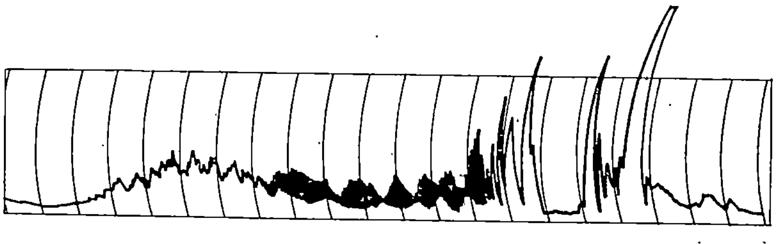

Fig. 3. An example of a record where three closely spaced frequencies are present (rev. No. 1208 of 1963–49C). One division of the scale is 5 sec

of the magnetoionic theory and rationalized M.K.S. units, the second term of equation (1) may be rewritten:

$$\frac{40{\cdot}25}{cf}\cdot\frac{\mathrm{d}}{\mathrm{d}t}\left(\int_0^{r_s} N\mathrm{d}r\right) \tag{2}$$

where the integration is performed along the propagation path up to the satellite. When the satellite signal propagates along two different paths 1 and 2, then at the aerial these two components differ in frequency by an amount:

$$F = -\frac{f}{c}\left[\dot{\rho}_1 - \dot{\rho}_2\right] + \frac{40{\cdot}25}{cf}\left[\left(\frac{\mathrm{d}n_s}{\mathrm{d}t}\right)_1 - \left(\frac{\mathrm{d}n_s}{\mathrm{d}t}\right)_2\right] = -\frac{f}{c}\,\Delta\dot{\rho} + \frac{40{\cdot}25}{cf}\cdot\Delta\left(\frac{\mathrm{d}n_s}{\mathrm{d}t}\right) \tag{3}$$

where $\Delta\dot{\rho}$ is the difference between radial velocities and $\Delta\left(\frac{\mathrm{d}n_s}{\mathrm{d}t}\right)$ is the difference between the time derivatives of the slant electron content for each propagation path. If these components have amplitudes of the same order of magnitude, the resultant signal received will be modulated by the frequency F given by equation (3). Thus, for a frequency of 54 Mc/s:

$$F = -\,0{\cdot}18\Delta\dot{\rho} + 2{\cdot}485 \times 10^{-15}.\Delta\left(\frac{\mathrm{d}n_s}{\mathrm{d}t}\right) \tag{4}$$

It is clear that both terms contribute significantly to F even if the propagation paths are only slightly different. The only condition is that both propagation paths must exist for a period large compared with $1/F$. It is obvious that larger frequencies F are more probable for large zenith distances when the influence of ionospheric refraction and slant electron content is greater. On the other hand, the amplitude of any scintillation which may obscure the regular fading also increases with zenith distance. The observed distribution of the fast regular fading is a result of these two effects.

The path difference between the two components reaching the observer, and the difference between their temporal variations when the satellite moves, may be caused in two different ways: (*a*) passage through irregularities in the total electron content; (*b*) reflexions between ionospheric layers or irregularities in the presence of sufficiently large gradients of electron density. It may also be caused by other, not purely ionospheric, effects, as abnormal tropospheric refraction or ground backscatter. There is still no experimental evidence about the magnitude of the difference in arrival angles of the two rays, so that many mechanisms are possible.

The result shown in Fig. 2 suggests that the effect occurs mainly in the auroral zone. Furthermore, radio-interferometer measurements made in Kiruna show that the effect is purely an amplitude modulation.

In one case, probably three propagation paths were present on the transmission from 1963–49*C* at 54 Mc/s. The observation was made on March 4, 1964, during a very disturbed period. The amplitude record given in Fig. 3 shows the presence of three closely spaced frequencies beating together. The fade-out of signals at the end of the period of fast fading is probably due to the interference between two waves of almost the same frequency in antiphase.

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GEOLOGY

Origin of Lake Bosumtwi (Ghana)

MR. S. O. BAMPO's recent report on the "Kumasi Conference on the Lake Bosumtwi Crater"[1] raises a few questions.

In the summary of his own talk, which is part of the report, the author refers to certain deposits near Morontuo as 'brecciated conglomerates', 'conglomerates' and 'breccia'; but, in fact, only the term breccia is appropriate. Morontuo, sometimes spelled Morawtuo, is a village south-west of the Lake. The rocks concerned mainly consist of angular and subangular fragments of vesicular glass and of the surrounding Birrimian country rock with its intrusives. Junner[2] identified these deposits, which are evidently considerably younger than the underlying Precambrian rocks, as pyroclastics. This has been the main argument for the assumption by him and Rohleder[3] that the Bosumtwi depression is of (crypto-) volcanic origin. Mr. Bampo seems to consider a slight difference in composition between the glassy material from the Lake Bosumtwi breccias and dacitic pitchstone from Scotland as an argument against the volcanic theory for the first. However, variations in composition between similar volcanic rock-types from different areas are normal and form no criterion in the present discussion.

Although the passage is not very clear, it seems that Mr. Bampo suggests that only large deposits of volcanic material around a structure of the type under review provides sufficient evidence for a volcanic origin of the structure. This is not so, for any deposit of this kind, however small, indicates volcanic activity and, if clearly confined to the area of the structure, represents at least a very strong argument for a belief in such an origin. Explosive volcanic activity without lava outflow, but resulting only in the ejection of pyroclastics, is well known in geological history. In the case of Lake Bosumtwi its isolated position outside areas of contemporaneous volcanic activity is indeed a problem, but the main problem is an alternative interpretation of the 'volcanic breccias and tuffs' near the depression as products of meteorite impact ('impactites'). This suggestion has been raised after the discovery of coesite in the glassy constituents of the breccias[4]. At present much attention is being directed to this particular question.

It may be mentioned that during the post-conference excursions Prof. Gentner and Dr. Lippolt of the Max Planck Institut für Kernphysik, Heidelberg, Prof. Monod of the Institut Français d'Afrique Noire, Dakar, and I examined Junner's 'type'-localities and some of the glass containing material was collected. This material is now being investigated.

It is not clear what relevant conclusion Mr. Bampo wants to draw from his statement that there is "an elliptical distribution of alluvial carbonado type diamonds in Ghana which are associated with recent conglomerate deposits and also with deep valleys, including the Bosumtwi Crater". As emphasized by the director of the Geological Survey of Ghana, Mr. Cudjoe, during the discussion at the conference, the alluvial diamond occurrences in Ghana are known to be river concentrates from Precambrian source rocks. Diamonds have been found in the Birrimian. It is difficult to see how these occurrences could be related to the origin of the relatively young Bosumtwi structure. So far, no diamonds are known from the structure itself and its immediate vicinity.

Mr. Bampo suggests that attention be directed to the changes in level of the Lake. These changes have been considerable in the past as can be seen from the occurrence of terraced lacustrine deposits up to about 150 ft. above the present Lake level. It seems likely that climatic variations are the cause of these changes[2,5]. Further investigation into this matter is desirable, as it may contribute to the solution of the problems of the age and origin of the Lake.

Mr. Bampo mentions the numerous iron slags which occur in Ghana, including the Lake Bosumtwi area, as well as in many other places in Africa. They always seem to be associated with archæological sites in laterite-covered regions and it is beyond doubt that they are produced by human activity[6]. If it is suggested that the slags are the result of meteorite impact, then it is unlikely that they would have resisted weathering since the early Pleistocene, which is probably the age of the Bosumtwi structure.

The dense vegetation and scarcity of outcrops make it difficult to trace Rohleder's 'Drucksuturen'-locality, which is the only place where features in rocks have been interpreted as percussion marks[7]. The few outcrops of quartzite examined by us in the area during the post-conference investigations did not show anything that responded to Rohleder's description. So far as I know, no undoubted shatter cones have been found by any geologist who has searched for them in recent times, and I have never seen anything that could be interpreted as shatter cones in my visits to the site.

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New Zealand Mid-Tertiary Stratigraphical Correlation

DR. D. G. JENKINS recently averred[1] that, consequent to the discovery of *Globorotalia kugleri* in rocks of Otaian age in New Zealand, our[2] correlation between the mid-Tertiary stages of New Zealand and the Cipero Formation globigerinid zones of Trinidad is 'completely wrong'.

We agree with Dr. Jenkins that *G. kugleri* is an important short-ranging species in the well-documented Trinidad Oligo-Miocene section, but his assumption that it is so in New Zealand remains to be confirmed. The period of overlap of '*Catapsydrax*' *dissimilis* and *Globoquadrina dehiscens*, also well documented, is, in the Trinidad succession[3], indicative of the *stainforthi* zone, two zones later in time than *Globorotalia kugleri*; in New Zealand '*C.*' *dissimilis* and *Globoquadrina dehiscens* are known to occur together in the Otaian[4] and underlying Waitakian (that is, U. Landon to L. Pareora). In Trinidad the overlap of these two species is within the range of *Globigerinoides 'trilobus'*[3] which, in New Zealand, ranges down to the lower Pareora; hence our approximate equation (a triple concurrent-range zonal equivalence) of the Pareora with the *stainforthi* zone.

This triple concurrence of ranges occurs, in Trinidad, after the extinction of *Globorotalia kugleri*, so Dr. Jenkins's record of this species "from Otaian samples from North Auckland" raises problems surprisingly ignored in his communication. If it is assumed that the planktonic zonal sequence is the same and that the ranges of the species are effectively isochronous in Trinidad and New Zealand, then either: (*a*) the samples from North Auckland containing *G. kugleri* are older than the *stainforthi* and *dissimilis* zones and hence pre-Pareora in age, or (*b*) the samples are Otaian and another species has been misidentified as *G. kugleri*. On the other hand, if it is accepted that the planktonic zonal sequence is similar, but that ranges of all species are not essentially identical in Trinidad and New Zealand, then either (*a*) *G. kugleri* ranges up to the *stainforthi* zone (as delimited by the *dissimilis*/*dehiscens* overlap), or (*b*) *G. dehiscens* ranges down to the *kugleri* zone, or (*c*) the ranges of both *kugleri* and *dehiscens* are such that they produce mutual overlap in the *dissimilis* zone in New Zealand.

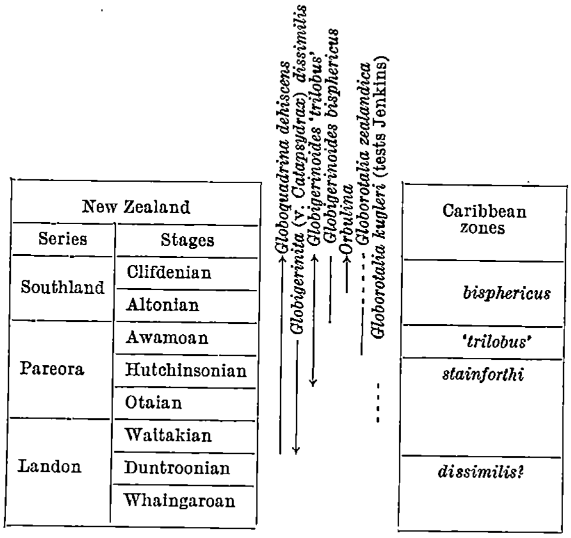

Fig. 1

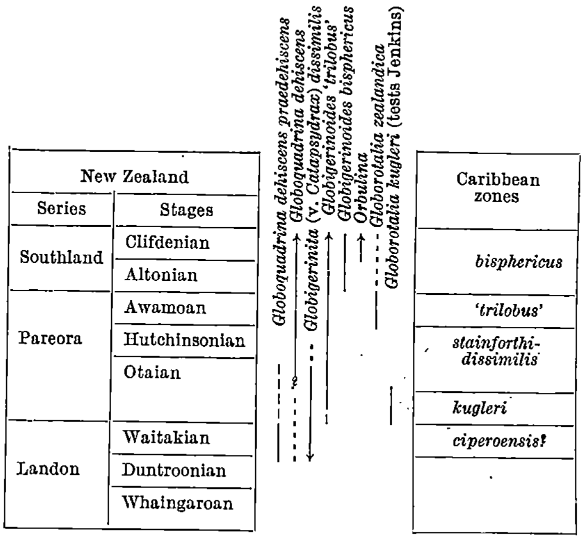

Fig. 2

Dr. Jenkins's simple assumption that his record of *G. kugleri* (unfigured) in samples attributed to the Otaian stage (but the age of which he does not confirm on faunal evidence) shows our suggested correlation to be "completely wrong" is clearly not warranted; instead his equally simple equation of the New Zealand stages and Trinidad zones in his Fig. 1[1], far from correcting a previous mid-Tertiary correlation, only poses more problems, the solution of which has yet to be advanced. In this context it is unfortunate that Dr. Jenkins, so quick to quote our suggested correlation, seems to have overlooked our conclusion in the same paragraph[2] that the faunas of the interval concerned were not sufficiently known to give more than an approximation.

The published occurrences and known ranges of the planktonic foraminifera[1–4] indicate a correlation on the lines set out in Fig. 1, in which, however, *Globorotalia kugleri* appears to be misplaced. It is possible, on the other hand, that Hornibrook's 1958[4] interpretation of *Globoquadrina dehiscens* includes the sub-species *G. dehiscens praedehiscens*, which is known to occur from the *ciperoensis* to low *stainforthi* zones in Trinidad, and which we have recorded from New Zealand[2] in samples believed to be Waitakian. Taking this into account, and accepting

Jenkins's record of *G. kugleri* as being from low in the Otaian, a scheme of correlation such as that set out in Fig. 2 would be possible: its confirmation by the observations of New Zealand workers would, of course, be necessary.

We note that his more recent work[5] has served to indicate that the type Aquitanian at Moulin de Bernachon, near Soucats, includes *kugleri* and/or *stainforthi* zones, as shown by the fauna of his sample from 3 metres above the exposed base of the section at that locality. This is especially interesting, since in 1962 we[2] demonstrated the presence of the *Globigerina ouachitensis ciperoensis* zone in the type Aquitanian and had, *inter alia*, assigned an Aquitanian age to the *kugleri* and *stainforthi* zones.

The foraminiferal faunas of the five younger samples from Aquitaine studied by Dr. Jenkins are relatively undiagnostic: of the nine identified species in them none is restricted to any particular zone or zones within the broad *kugleri-fohsi robusta* zonal interval and, with the exception of *Cassigerinella chipolensis* (only in sample 4), all range up to younger zones. The absence, from these poorly fossiliferous samples, of diagnostic, planktonic, zonal species tends to vitiate his correlation of these younger samples with the planktonic zonal scheme of Bolli; his very selective emphasis solely on the absence of one form (*Orbulina*) as being significant appears highly tendentious, as would be the suggestion that Jenkins's samples 5–8 are post *C. chipolensis*, and, therefore, post *fohsi robusta* zone[3], an equally valid assumption on the inadequate evidence!

Further work on the Tertiary planktonic Foraminifera is still required to clarify their zonal equivalences and in New Zealand to "establish a more accurate correlation of the Tertiary with rocks elsewhere".

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In a previous communication[1] I provided evidence for correlating at least part of the Otaian Stage rocks in New Zealand with the *Globorotalia kugleri* zone of Trinidad and concluded that Eames *et al.*[2] had incorrectly correlated the Waitakian–Awamoan Stages with the *Catapsydrax stainforthi* zone of Trinidad.

After the discovery of *Globorotalia kugleri* Bolli from the North Auckland rocks, specimens were sent to Dr. H. M. Bolli for examination. He confirmed the identification in a private communication and it was then decided to publish the information. This should satisfy Eames *et al.*, who suggest in their reply that *G. kugleri* has been misidentified.

Eames *et al.* have apparently missed a previous publication by me[3] in which I attempted to explain the discrepancy in the stratigraphic ranges of *G. dehiscens* between Australia and Trinidad. In Australia[3] and New Zealand[5] the initial stratigraphic appearance of *G. dehiscens* is well before that of *G. trilobus*, whereas in Trinidad Bolli[6] records its initial appearance after that of *G. trilobus* and *G. kugleri*. It is assumed that the initial appearance of *G. trilobus* was simultaneous in the two areas (that is, within the range of *G. kugleri*) and that *G. dehiscens*, which was first described from the Miocene rocks of Australia, originated in the Australasian region and later spread into the Trinidad area.

The quadrate nature of the early form of *G. dehiscens* from Australia, soon after its initial appearance, has already been illustrated[3].

In a more recent publication Blow and Banner[2] have suggested that *G. praedehiscens* was the ancestor of *G. dehiscens*. This hypothesis is difficult to follow because *G. dehiscens*, which is a much more quadrate species, existed in Australasia at the time its supposed ancestor, *G. praedehiscens*, existed in Trinidad. Therefore, the initial appearance of *G. dehiscens* should not be used in any attempt to correlate the Australasian rocks with the Trinidad sequence.

In their reply Eames *et al.* have had two further attempts at correlating the New Zealand Stages with the Trinidad zones. It is noted that in their original discourse Eames *et al.*[2] stated "but we know from our own observations that *G. trilobus* occurs at the base of the Pareora and ranges up" and this fact is repeated in their reply (Fig. 2). The exact locations of these basal Pareora rocks in New Zealand need to be published so that they may be examined for '*G. trilobus*' by other workers.

Recent detailed investigation by Mr. N. de B. Hornibrook (personal communication) on New Zealand species has now shown that: (1) *Orbulina suturalis* (Blow emend, 1956)[4] starts at the base of the Lillburnian Stage and not at the base of the Clifdenian Stage as previously published[5]; (2) The level of extinction of *G. dissimilis* in New Zealand is still uncertain because it occurs rather sporadically. A previous record of *dissimilis* in the Altonian Stage[5] was based partly on its occurrence in rocks which are now known to be Otaian. The New Zealand Geological Survey has only one or two records of *dissimilis* in rocks definitely of post-Otaian age, but they are in fault zones and the specimens are possibly allochthonous; (3) The one known occurrence of *G. insueta* in New Zealand is near to the boundary of the Altonian–Clifdenian Stages in Hawkes Bay. An up-to-date range chart is given of 6 species of planktonic Foraminifera in the New Zealand rocks and a correlation is attempted with the Trinidad zone (Fig. 1). It should be noted that these ranges are based on the work of the New Zealand Geological Survey and differ from those given by Eames *et al.* in their reply (Figs. 1 and 2).

It is gratifying to note that Eames *et al.* in their reply have not reasserted the "considerable disconformity" originally proposed by them between the Duntroonian and Waitakian Stages[2]. The overall faunal succession of planktonic Foraminifera in New Zealand from this interval shows no evidence for this "considerable disconformity".

My recent remarks[7] concerning the post-Burdigalian entry of *Orbulina* in the European Tertiary rocks were intended to be tendentious, as Eames *et al.* suggest. The conclusion that "the initial appearances of *Globigerinoides bisphericus* Todd – *Orbulina universa* d'Orbigny lineage

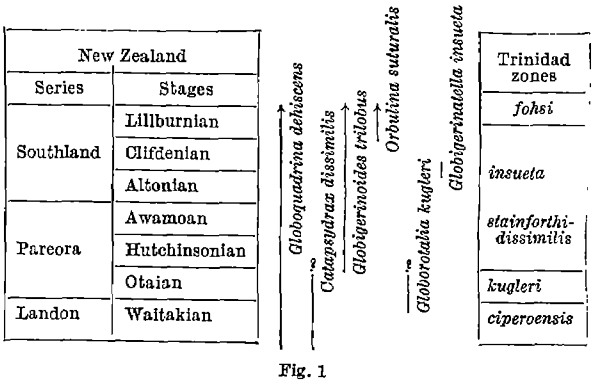

Fig. 1

must occur in post-Burdigalian rocks"[7] was reached after an examination of the type Aquitanian–Burdigalian planktonic foraminiferal fauna. Eames *et al.* state that the type samples are poorly fossiliferous, but this is not true. More than 100 specimens of planktonic Foraminifera have been extracted from each of the 5 youngest samples and the uppermost Burdigalian sample has innumerable planktonic foraminiferal specimens.

My "more recent work" on the type Aquitanian–Burdigalian fossils was undertaken in order to try and break the deadlock of opinion regarding the entry of *Orbulina* in European rocks which has retarded efforts at mid-Tertiary stratigraphic inter-continental correlation. The 'British School'[2] advocate the entry of *Orbulina* in the Upper Aquitanian and the "Continental School"[8–10] have apparently proved its entry in the Helvetian–Tortonian.

If Eames *et al.* maintain that the initial stratigraphic appearance of *Orbulina* occurs in the Upper Aquitanian, then more convincing evidence must be presented to substantiate this theory. If *Orbulina* were found in the type Upper Aquitanian–Burdigalian rocks it would pose the problem of the double entry of the lineage in European Miocene rocks and two well-separated *Orbulina* datum lines.

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OCEANOGRAPHY

Evidence of a South Equatorial Countercurrent in the Atlantic Ocean in July 1963

AN eastward-flowing current of speeds of 4–7 cm/sec has been indicated in the Atlantic Ocean between 5° and 12·5° S. at about 14° W. longitude.

The evidence lies in the calculations of geostrophic flow at the sea surface with respect to the 1,000-decibar surface. The results for these calculations were collected during June 29–July 7, 1963, aboard the *Argo* on the seventh leg of the *Lusiad* expedition. A line of 13 oceanographic stations was made extending from 14° 54·5′ S., 019° 22′ W. to 05° 0′ N., 012° 39·5′ W. It is the first such section made across the equator in that season. The eastward flow between 5° S. and 12·5° S. averaged 4·5 cm/sec; the greatest speed of the eastward flow was about 7·4 cm/sec and was observed between the two stations at 12·5° S. and 10° S.

Flow to the east at this latitude is not indicated by any of the various atlases in any season[1,2]; instead, the flow in the area south of 6° S. is indicated as westward but very much weaker than that in the area north of 6° S. (ref. 1). It is not impossible that a weak eastward flow (4–7 cm/sec) could prevail in this season in all years without being detected from the ordinary set and drift observations. The set from such a current might easily be masked by the effect of the south-easterly trade winds that average more than Beaufort Force 4 (5·5–8 m/sec) in June and July[3]. Furthermore, the major shipping routes do not cross this area, and the few transits are on nearly north–south courses[4]; vessels would spend only a short time in the area of this eastward flow; they would be subject to a total current-drift of only about 7·8 km even if they required 48 h to cross the area. This is within the error of celestial navigation, and is probably smaller than the effect of the winds in that area.

There is no other sort of evidence available to examine the existence of this current. The other hydrographic data in the area are few and isolated and are not enough to confirm or deny such a current[5]; those measurements of density that lie near the *Argo* data are in agreement with them, however, and suggest that the *Argo* was not observing an unusual condition. It is interesting that this easterly flow was observed at the same season when the (North) Equatorial Countercurrent prevails in the Atlantic, and at the same season that the South Equatorial Countercurrent has been most clearly observed in the Pacific[6].

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CHEMISTRY

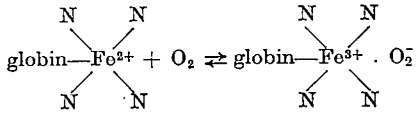
Nature of the Iron–Oxygen Bond in Oxyhæmoglobin

J. J. WEISS[1] has proposed that oxyhæmoglobin is a hæmoglobin peroxide, with the iron atom in the ferric state and the oxygen molecule present as an O_2^- ion, which is then taken up in the co-ordination shell of the ferric ion. He states that such an assumption accounts for the properties of oxyhæmoglobin.

Oxyhæmoglobin is, however, diamagnetic[2], and the generally accepted definition of oxidation number[3] requires that the iron atom in any diamagnetic molecule or complex containing one iron atom (no iron–iron bond) has an even oxidation number. It is accordingly not possible for an iron atom in oxyhæmoglobin to be described as ferric (oxidation number +3) unless a definition of oxidation number different from the usually accepted one is used.

The problem discussed by Weiss, the nature of the iron–oxygen bond in oxyhæmoglobin, was given a thorough discussion by me in two communications published about 15 years ago[4,5]. It is now possible to amplify the discussion somewhat, in part because of further development of the theory of the chemical bond[6] and in part because of the increased knowledge about the structure of oxyhæmoglobin and related molecules that has been obtained by the X-ray diffraction investigations of Kendrew[7] and Perutz[8].

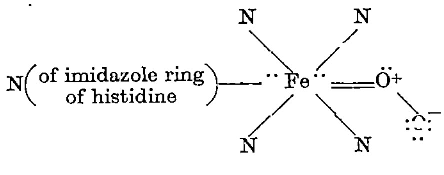
Weiss has illustrated his proposal by writing the following equation:



$$\text{globin—}\overset{\text{N}\ \ \text{N}}{\underset{\text{N}\ \ \text{N}}{\text{Fe}^{2+}}} + O_2 \rightleftarrows \text{globin—}\overset{\text{N}\ \ \text{N}}{\underset{\text{N}\ \ \text{N}}{\text{Fe}^{3+}}} \,.\, O_2^-$$

In the formula at the right, the dot represents not an electron but some sort of bond.

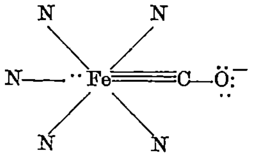
The structural formula which I proposed for oxyhæmoglobin[4,5] is the following:

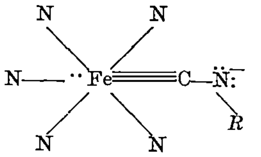


In this structural formula the iron atom is shown as forming a double bond with the first oxygen atom, which forms a single bond also with the second oxygen atom. (The iron atom then has two unshared electron pairs in its outer d^5sp^3 shell.) There is a formal charge $+1$ on the first oxygen atom and a formal charge -1 on the second oxygen atom. The difference in electronegativity of iron and oxygen is 1·7, which corresponds to 51 per cent of ionic character[9]. The electric charge assigned to the first oxygen atom is accordingly reduced to 0, whereas that of the outer oxygen atom remains -1. Except for the outer oxygen atom, the assigned structure is accordingly in good agreement with the principle of electroneutrality[10].

The negative charge assigned to the outer oxygen atom of oxyhæmoglobin can now be accounted for by the structure of myoglobin, as determined by Kendrew[7]. In the myoglobin molecule there is a residue of histidine adjacent to the iron atom on one side of the hæm group, and another residue of histidine constrained by the configuration of the polypeptide chain to a position a few Ångströms removed from the iron atom on the other side of the hæm. These two residues of histidine are those that were proposed originally by Conant[11], in order to account for the observed hæm-linked acid groups of hæmoglobin, and their positions relative to the iron atom are those postulated by Coryell and Pauling[12] in their detailed discussion of the mechanism of change in acid strength of hæmoglobin on oxygenation. It has been pointed out that the positive electric charge of the imidazolium side-chain of the second histidine residue probably serves, through its electrostatic interaction with the iron atom, to stabilize the bipositive state and assist in preventing oxidation of hæmoglobin to ferrihæmoglobin[13]. We see, also, that for oxyhæmoglobin the structure assigned above places an oxygen atom with a negative charge in close approximation to the positively charged imidazolium group and, indeed, in such a position as to permit hydrogen bonding with the negatively charged oxygen atom, thus further stabilizing the structure.

Similar structures, involving iron–carbon double bonds, were formerly assigned to carbonmonoxyhæmoglobin and the ferrohæmoglobin alkylisocyanides[4,5]. We may now recognize, however, that the arguments of the preceding paragraph strongly suggest that these molecules have structures in which the iron atom forms a triple bond with the carbon atom, and the outer oxygen atom or nitrogen atom has a negative electric charge:





I conclude that oxyhæmoglobin and related hæmoglobin compounds are properly described as containing ferrous iron, rather than ferric iron, that their electronic structure involves essentially the formation of a double bond between the iron atom and the near-by oxygen atom in oxyhæmoglobin (with the iron–oxygen–oxygen bond angle about 120°) and a triple bond to the carbon atom in carbonmonoxyhæmoglobin and the hæmoglobin alkylisocyanides (with iron–carbon–oxygen or nitrogen bond angle 180°). The iron–oxygen and iron–carbon interatomic distances in these compounds are predicted to have the values corresponding to these multiple bonds.

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The essence of my suggestion was that there is a charge transfer from the hæmoglobin to the oxygen molecule, that is, some delocalization of one of the d-electrons of the ferrous towards the oxygen. This was meant as a starting approximation, the details to be settled by some physical measurements concerning the electronic structure. There are no obvious grounds why the resulting structure could not be diamagnetic. If this would not agree with a certain conventional definition of oxidation number then this would only show that this definition is no longer useful in this case. This is perhaps not surprising, because the O_2^- ion has itself an unpaired spin unlike the common anions such as OH^-, CN^-, which are all diamagnetic. This does, therefore, involve the more complicated problem of the interaction of the two magnetic ions when they come close together.

The charge-transfer complex and the structure proposed by Prof. Pauling have two prominent features in common as, according to Pauling, there also is (1) a charge of -1 on the oxygen, and (2) the iron–oxygen bond has a large percentage of ionic character. The difference lies essentially in the disposition of the third positive charge, which Pauling does not associate explicitly with the iron. It might be expected, in any event, that there will be some delocalization of the positive charge over the porphyrin ring. It will be of interest to see whether Prof. Pauling's much more specific proposals can be confirmed.

The reasons for my suggestion were given previously[1], one of these being the observation that oxyhæmoglobin can act as a strong oxidizing agent; for this it would be difficult to give a simple explanation on the basis of the conventional structure.

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Molecular Orbital Evidence for Weiss's Oxyhæmoglobin Structure

We have read with interest the recent paper by Weiss[1] concerning the electronic structure of oxygenated hæmoglobin. From a consideration of: (1) magnetic properties, (2) absorption spectrum and (3) acid dissociation constant of oxygenated hæmoglobin, Weiss has proposed that the iron has a $+3$ charge. Therefore, iron is essentially in the ferric state. His paper has prompted us to give additional evidence in support of his idea.

The charge on the iron atom can be derived from the coefficients of the molecular orbitals of the complex. The molecular orbitals used were calculated for a general examination of the structures and properties of oxygen metal complexes. The calculations included a total of 17 atomic orbitals with 18 electrons in these orbitals. The orbitals considered were the $2s$ and three $2p$ on each oxygen atom and the five $3d$, one $4s$ and three $4p$ orbitals on iron. The general method of the calculation is that of Ballhausen[2], but the details and a discussion of the results will be given at a later date. Equation 1 (ref. 3) was used to calculate the charge on iron:

$$q_j = \sum_r \sum_j n_r C_{rj}^2 + \sum_r \sum_j \sum_{i \neq j} n_r C_{rj} C_{ri} S_{ji}$$
$$q_{\text{atom}} = Q - \sum_j q_j \qquad (1)$$

where q_{atom} = the signed charge of the atom in question; Q = number of electrons on the unperturbed atom; q_j = electron density in the j^{th} atomic orbital; n_r = number of electrons in the r^{th} filled energy-level; C_{rj} = wave function coefficient of the j^{th} atomic orbital in the r^{th} energy-level; S_{ji} = overlap integral value between the i^{th} and j^{th} orbitals of the system. The index j is over the orbitals of the atom in question, whereas index i is over all the orbitals of the system. Index r denotes only the filled energy-levels.

The result of our calculation is $q_{\text{iron}} = +\,2{\cdot}02$ based on the Pauling structure[4] for oxygenated hæmoglobin. The Pauling theory of the oxygen–iron complex is that an electron pair (probably sp^2) is donated from oxygen to an empty d^2sp^3 orbital of iron as a normal octahedral chelate is formed. Then there is back donation from a $d\pi$ orbital of iron into a higher π_g orbital of oxygen. Essentially Weiss predicts that this back donation is quite large. If the first electron pair is shared equally between ferrous iron and oxygen, and if there is no back donation, the calculated charge would be $q_{\text{iron}} = +\,1$. However, if back donation is so large that the iron is essentially ferric, and if the first electron pair is again equally shared, q_{iron} would be $\sim +\,2$. Thus the calculated value of $q = +\,2{\cdot}02$ shows that back donation is very largely in agreement with the theory proposed by Weiss.

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Sequence Specificity in Synthetic Polydeoxyribonucleotides

Kornberg has shown that enzymatic synthesis of polydeoxyribonucleic acids from mononucleotides is possible even in the absence of a primer DNA molecule[1,2].

When mixtures of the two deoxyribonucleoside-5′-triphosphates, the heterocyclic bases of which were thymine and adenine, were used, Kornberg found that the polynucleotide formed after an initial time-lag was copolymer (Poly dAT) in which the bases alternated[1]. When the same procedure was carried out with the corresponding nucleotides containing the bases, guanine and cytosine, the product was a mixture of the two homopolymers, Poly dG and Poly dC, not always in equal amounts.

In both cases, in which exactly equimolar amount of the two mononucleoside triphosphates were used, there was evidence for double helix formation with base-pairing of the Crick–Watson type[2].

In order to determine how far this sequence specificity is a result of enzyme stereochemistry or simply a result of the molecular configurations of the nucleotides themselves, we have repeated the polymerization using equimolar amounts of the thymine and adenine deoxyribonucleoside-5′-phosphates, the thymine derivative being labelled with phosphorus-32 and a chemical condensing agent (di*cyclo*-hexylcarbodi-imide)[3].

In syntheses leading to deoxyadenosine polynucleotides, Khorana found it necessary to acylate the 6-amino group of the purine[4]. In our work, however, we have avoided the use of this blocking group in order that the molecular configurations should be as close as possible to those in the unprimed enzymatic synthesis. Parallel experiments are planned with systems incorporating the acylated nucleotide to determine the influence of these protecting groups on the sequence specificity.

The nearest neighbour analysis method was used on the polymeric product which had been freed from small molecules by dialysis and afterwards degraded by the action of spleen phosphodiesterase and micrococcal DNase producing nucleoside-3′-phosphates. Thus the labelled phosphate which enters the polymer as thymidine-5′-phosphate appears in the hydrolysate as the 3′-phosphate of the nucleoside which was next to the thymidine in the polymeric chain.

The nucleotides were separated by paper chromatography; the spots were cut out and their activity measured.

If homopolymers are formed (for example, of the Poly dG–Poly dC type), all the activity should be found in the originally labelled nucleoside, thymidine, in the form of the 3′-phosphate. If, however, there is co-polymerization with a perfect alternation of bases, all the activity should be transferred to the initially unlabelled nucleoside, deoxyadenosine. Co-polymerization with a random sequence of bases should lead to both nucleoside-3′-phosphates having equal labelling.

In our experiments, we have found that the polymeric material, on degradation, yields a mixture of nucleoside-3′-phosphates in which approximately 20 per cent of the labelled phosphate has been transferred to the 3′-position of the deoxyadenosine.

Thus it is clear that there is some co-polymerization, but that there is neither a perfectly alternating sequence nor a completely random sequence.

Further experiments, in progress, using labelled deoxyadenylic acid should indicate how far the result may be explained by loss of deoxyadenylic acid from the polymerizing system as a result of the unprotected amino group.

We thank the Department of Scientific and Industrial Research for a grant for equipment and Unilever, Ltd., for a research studentship (G. R. B.). We also thank the Radiochemical Centre, Amersham, for a gift of ^{32}P-labelled thymidine-5′-phosphate.

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A Flow Method for Determining the Thermal Conductivity of Gas Mixtures

The thermal conductivity of a mixture of two gases does not, in general, vary linearly with the composition of the mixture. For two gases of similar molecular weight the degree of non-linearity is small, while for systems of widely different molecular weight, linearity rarely extends beyond 0·05 mole fraction from either pure component.

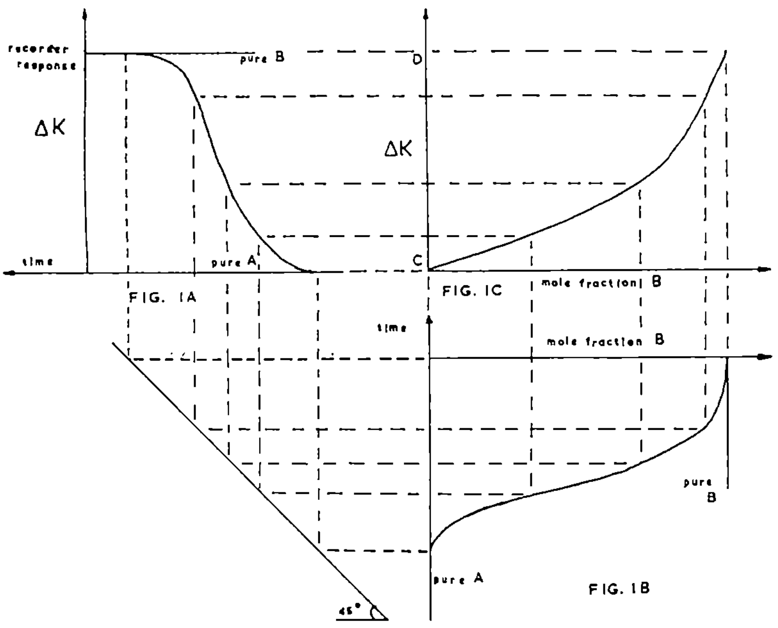

Fig. 1

Mixtures containing appreciable amounts of both components are of interest experimentally, and in the theory of transport phenomena. The collection of such conductivity data is usually time-consuming.

During some experiments on the dispersion of gases in laminar flow through a circular tube, anomalous results were obtained, as expected, for pairs of gases of widely differing thermal conductivity when a katharometer was used to determine the tracer gas concentration profile. It is possible, however, to explain quantitatively the discrepancies between the theoretical predictions and the experimental results in terms of the non-linearity described previously. This provides a method of obtaining, in a few minutes, a continuous curve of the variation of thermal conductivity with composition.

Assuming that the operation of the katharometer depends only on thermal conductivity[1], and that the recorder trace is a linear representation of the thermal conductivity difference, ΔK, then the introduction of a step change from gas A to gas B will produce a plot of the thermal conductivity of the mixture, ranging from pure A to pure B, as a function of time. Such a trace, which will be a distorted concentration profile, is given diagrammatically in Fig. 1A.

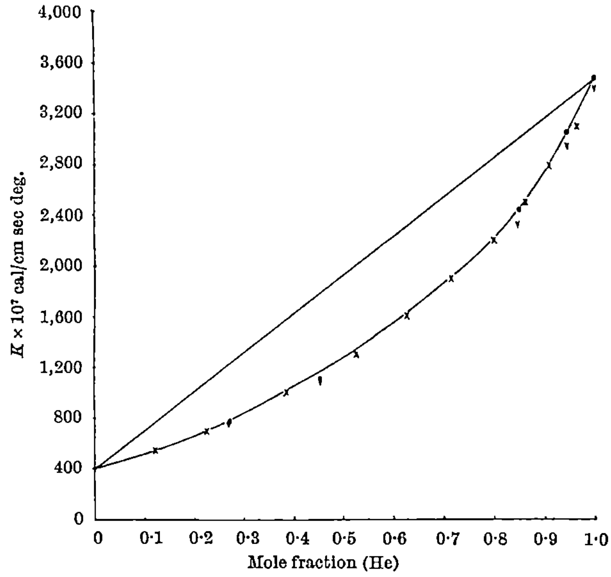

Fig. 2. Thermal conductivity of argon/helium mixtures. ×, this work; ●, calculated (ref. 5); V, experimental (ref. 5)

For a given mean velocity, $\bar{u}$, which may be found from the volume flow rate, and the molecular diffusion coefficient for the system, D_{AB}, the true concentration profile corresponding to Fig. 1B, may be calculated from equations (1) and (2), since for a tube, radius a, the effective dispersion coefficient is D_e (ref. 2).

$$D_e = D_{AB} + \frac{a^2\bar{u}^2}{48D_{AB}} \qquad (1)$$

This has been verified by us to hold to a high order of accuracy, and details will be published shortly. The mean concentration across the tube, as a function of time, t, is:

$$\frac{C_m}{C_0} = \frac{1}{2}\left\{1 - erf\left[\frac{L - \bar{u}t}{2\sqrt{D_e t}}\right]\right\} \qquad (2)$$

when the tracer has travelled a length L.

These equations imply that the value of the molecular diffusivity is independent of concentration. To a first approximation this is true.

Equation (2) may be plotted on the same time-scale as the recorder trace, as in Fig. 1B. By projection, given by the broken lines, as many points as may be required can be found for the curve of thermal conductivity against composition (Fig. 1C).

The method was tested experimentally by injecting a step change of helium into flowing argon. The apparatus consisted of a straight steel tube 17·29 m long, mounted horizontally in a room maintained at 19·5° ± 0·5° C. A twin cell katharometer, forming part of a Wheatstone bridge, monitored the concentration profile. The use of two hot wires eliminated the effect of small velocity fluctuations, and presented, on the recorder, the profile in terms of the difference ΔK, in thermal conductivity between carrier gas A with tracer B, and pure A. The katharometer is specially designed to have a negligible volume (0·5 ml.), and to present as little disturbance to gas flow as possible. Tracer injection is carried out by means of an 'O' ring, annular valve[3].

The results are given in Fig. 2, in which are also plotted theoretical values, and some experimental results of Wachsmith[4]. The literature values refer to 0° C, whereas the experimental values were obtained at 20° C. Since the difference between the thermal conductivities of the pure components at these two temperatures is relatively slight, the conductivities of the pure components at 0° C were assigned (for purposes of comparison) to positions C and D in Fig. 1C.

It can be seen that the degree of fit is good. In an accurate determination, the thermal conductivities of the pure components would have to be known at the mean system temperature.

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Acid-labile Ribose as an Indicator of Ribonucleic Acid Base Composition

The lability to acid of purine ribomononucleotide N^9-glycosidic linkages, as opposed to the stability of pyrimidine ribomononucleotide N^3-glycosidic linkages, is well documented (for example, ref. 1). It has, however, been noted that the latter linkage is not completely resistant, in the cases of cytidylic and uridylic acids, under the conditions of the Bial reaction of their pentose moieties with orcinol[2].

The acid lability of purine mononucleotides has been used to estimate the purine/pyrimidine ratios of yeast whole-cell ribonucleic acid[3] by recalculation from the molar ratios of acid-labile ribose/nucleotide phosphorus. The validity of this method depends on the freedom of the examined RNA from extraneous, non-nucleic acid phosphorus and the hydrolytic release only of purine ribose and its reaction with orcinol. The first condition was satisfactorily achieved; an examination of the second is reported here.

Approximately 1 mM aqueous solutions of mixed 2′ and 3′ isomers of GMP, AMP, CMP and UMP (Sigma) were prepared. Quadruplicate aliquots of 1 ml. were used in estimating acid-labile ribose by a modification[3] of the method of Ceriotti[4]. The results, presented as the molar ratios of acid-labile ribose/orthophosphate (estimated by the method of Boltz and Mellon[5]), are shown in Table 1.

Table 1. MOLAR RATIOS OF ACID-LABILE RIBOSE TO ORTHOPHOSPHATE IN RNA MONONUCLEOTIDES

Nucleotide	Molar ratio Expected	Found
GMP	1·0	0·985 ± 0·011
AMP	1·0	1·000 ± 0·020
CMP	0	0·0097 ± 0·003
UMP	0	0·062 ± 0·003

Under the conditions of ribose estimation (final concentration of hydrochloric acid, 5 N) hydrolysis of GMP and AMP is virtually complete. Only UMP of the pyrimidine nucleotides contributed significant colour.

Acid-labile ribose was similarly estimated in artificial mixtures of the four nucleotides. Ribose/phosphate ratios, recalculated to purine/pyrimidine ratios, are shown in Table 2. 'Expected' ratios were based directly on molar proportions of nucleotides, given 100 per cent purine mononucleotide hydrolysis and no pyrimidine mononucleotide hydrolysis, while 'corrected' ratios included allowances for 98·5, 100, 1 and 6 per cent hydrolysis, respectively, for GMP, AMP, CMP and UMP.

Table 2. PURINE/PYRIMIDINE RATIOS OF ARTIFICIAL MIXTURES OF RNA MONONUCLEOTIDES

Molar proportions				Purine/pyrimidine ratio		
GMP	AMP	CMP	UMP	Expected	Corrected	Found
24·7	26·0	26·8	22·5	1·03	1·08	1·08
29·6	29·2	20·4	20·7	1·43	1·49	1·49
24·0	21·0	25·0	30·0	0·82	0·88	0·88

Agreement between corrected and experimental values of 'purine/pyrimidine' ratios was good. Consistent overestimate of the true value, of the order of 0·05–0·06 unit, is apparent, so that precise estimates of purine/pyrimidine ratios must still depend on separation of the four nucleotides in hydrolysates of RNA. However, the method provides a useful guide before the more time-consuming separations are undertaken.

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Oxidation of β-Carotene by Hydroperoxides

It is generally believed that bleaching of β-carotene concurrent with lipoxidase catalysed oxidations of fixed oils occurs by reaction with intermediates of the autoxidation process. This view, which appears to be based originally on the observation that loss of colour proceeds only very slowly in the presence of previously peroxidized fat[1], has led to a general conclusion that hydroperoxides do not oxidize carotenoids readily[2]. Recent investigations of the action of tetralin hydroperoxide, concerned with product analysis, have again emphasized that the reaction proceeds only very slowly[3].

We have now been concerned with rates of oxidation of β-carotene by *tert*-butyl hydroperoxide over a series of concentrations—using hydroperoxide/pigment molar ratios of 5–200/1.—and find that loss of β-carotene occurs in a regular manner for each amount of hydroperoxide used.

To 0·1 per cent (0·0186 mole per cent) solution of β-carotene in chloroform (2 ml.) was added the required amount of pure *tert*-butyl hydroperoxide and the mixture, diluted to constant volume (2·5 ml.) with more chloroform, kept at 25° in the dark. The small flasks were flushed with nitrogen and access of oxygen prevented so far as possible during handling. Residual β-carotene was separated from oxidation products by chromatography on alumina using light petroleum (b.p. 60°–80°) for development, and estimated spectrophotometrically. Results are illustrated in Fig. 1.

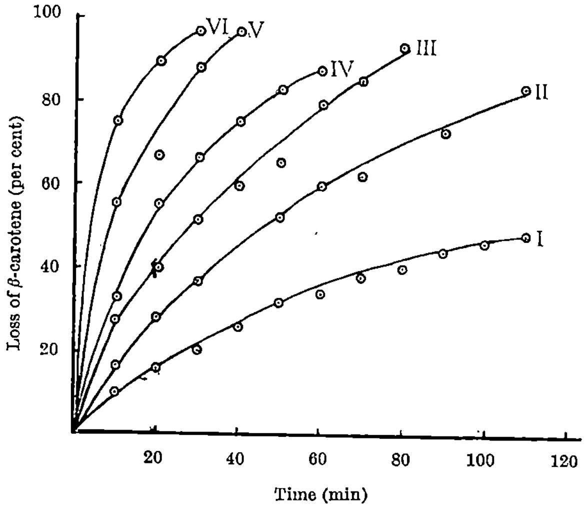

Fig. 1. Oxidation of β-carotene by *tert*-butyl hydroperoxide in chloroform at 25°. Moles hydroperoxide per mole of pigment: I, 5; II, 10; III, 20; IV, 50; V, 100; VI, 200

It will be observed that even with the lowest concentration of hydroperoxide used (5 molar ratio), loss of β-carotene is recognizable after 10 min. Indications are that the initial reaction is first order with respect to the β-carotene but stepwise increase in the concentration of hydroperoxide used does not lead to a proportional increase in the rate of loss of pigment. Although all solutions ultimately are completely bleached, due to the continued action of hydroperoxide on the initial products, nevertheless the early losses of β-carotene are masked by a darkening and change in shade of colour of the solution. This is easily visible to the eye, but the trends of change are better revealed by the Lovibond readings of Table 1.

While the intensity of colour precludes use of the readings other than as indicating the general trend of change, nevertheless they provide a more adequate representation of the process than vaguer descriptions of appearance visually. In particular the introduction, rise and gradual subsequent fall in blue components should be noted.

Table 1. LOVIBOND TINTOMETER READINGS DURING OXIDATION OF β-CAROTENE BY *Tert*-BUTYL HYDROPEROXIDE (1/20 MOLAR RATIO) IN CHLOROFORM AT 25°

Time (min)	Lovibond units Yellow	Red	Blue	Time (min)	Lovibond units Yellow	Red	Blue
10	18	14·3	0·0	100	26·0	14·5	7·0
20	18	14·3	1·0	110	27·0	13·3	6·0
30	19	19·3	1·0	120	19·0	11·8	4·8
40	21	21·3	2·2	130	19·0	11·8	4·8
50	24	22·3	5·0	140	25·0	10·8	4·8
60	24	22·3	5·0	150	26·0	9·9	4·2
70	24	19·3	6·0	160	26·0	9·9	4·2
80	24	18·3	7·0	170	26·8	9·6	4·0
90	26	14·5	7·0	180	27·8	8·6	3·0

Over the first 60 min there is an increase in red and yellow components together with the introduction of a not inconsiderable blue component, which is reflected by a change in visual appearance to slight off colour from the cleaner reddish-yellow of the original solution. As oxidation proceeds over the next 60 min a change in the balance of colour occurs, with increases, at a reduced rate, in the yellow and blue components coupled with loss of red. At this stage the solution darkens considerably to the eye, reflecting the continued increase in blue values. The outstanding feature of the third hour is the maintained loss of red units now accompanied by loss of blue, that is, visually the solution becomes a cleaner yellow of reduced overall depth of colour.

It is not unlikely, therefore, that similar effects in fats may have been responsible for early statements, which appear to be based on colour observation alone, suggesting that no immediate reaction occurs.

Explanations offered for the ultimate bleaching action of linoleate hydroperoxide appear in the main to have been concerned with the initiation of radical decomposition[4] without consideration of the possible superimposed more direct effects of low concentrations in the early stages of the process. Present results therefore indicate that, for oily solutions, more attention might be given to the possible role of preformed fatty acid hydroperoxide in directly attacking β-carotene.

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BIOCHEMISTRY

A New Steroid found in the Urine of Children but not Adults

DURING a longitudinal investigation of the changes with age in steroid excretion in healthy children, we have found a new steroid substance[1]. It was present in 11 out of 19 healthy children aged 8–12 years; in all of 7 healthy 3-year-old children; and in none of about 100 adult men and non-pregnant women. Twelve of the 8–12-year-old children have been examined repeatedly at six-monthly intervals, for 18 months–2 years. Six were initially excretors and six non-excretors, and none showed any change in status. Four adults with Cushing's syndrome were all non-excretors, but both of two pregnant women tested excreted the substance. Two children with the rare disease of lipodystrophy and accelerated growth and one 3-year-old child with progeria showed a markedly greater excretion than anybody else we have seen.

The substance does not appear to be dietary in origin since it was excreted by three children under strict dietary control in the metabolic ward of the Hospital for Sick Children. Furthermore, we have followed two non-excretors and one excretor during ACTH response test given for other reasons. Both non-excretors produced the substance on stimulation together with a general increase in oxosteroids and corticosteroids. The excretor failed to respond to ACTH, and the excretion of the substance remained unaltered. It seems, therefore, that this steroid may be regarded as a normal constituent of healthy children's urine, at a certain stage of growth.

The substance was excreted as a glucuronide salt only. After extraction of the urine, hydrolysis with β-glucuronidase and preliminary separation on Whatman 3 *MM* paper, it appeared as a Zimmermann-staining spot near to the 11-oxo-17-ketosteroids in the modified two-dimensional system of Edwards[2].

In the modified Bush *B*3 system[3] it ran with 11-hydroxyandrosterone but was distinguished from it by absorbing ultra-violet light. It was eluted from the modified *B*3 run on No. 2 paper, as used in our usual procedure[4], and isolated by re-running on a slower-moving chromatogram for 24 h. It was then purified by passage through a 'Sephadex' column[1].

It then showed the following reactions: (1) under ultra-violet light a peak at 223 mμ; (2) blue colour with Zimmermann reagent; (3) ultra-violet fluorescence when treated with sodium hydroxide and a small amount of triphenyl tetrazolium chloride; (4) after reaction with 20 per cent orthophosphoric acid a weak pinkish colour after dipping, and strong ultra-violet fluorescence; (5) a pink colour when refluxed with selenium dioxide in acetic acid and benzene (Fieser reaction)[5]; (6) sulphuric acid spectra in various modifications[6–8] gave peaks at 410, 408 (470) and 370 (480) mμ respectively whereas an authentic sample of a Δ^5-3-hydroxy compound (dehydro*epi*androsterone) gave peaks at 410, 408 (490) and 390 (470) mμ (the figures in parentheses indicate minor peaks); (7) Menini–Norymberski[9] reaction with *tert*-butyl chromate on paper[10] positive with strong ultra-violet fluorescence; (8) reaction with phosphomolybdic acid gave a similar speed to dehydro*epi*androsterone. Reactions 5–8 indicate that the substance has a Δ^5 structure: (9) R_M value of $+0 \cdot 72$ on a modified Bush *B*3 system paper chromatogram (approximately equal to 11-OH-androsterone). R_M values of formates, acetates and propionates in several modified Bush *B*3 systems were compared with similar esters made from mono-, di- and tri-hydroxy reference steroids. From this it was evident that the substance contained one hydroxyl group, most likely at C3; (10) R_M of the acetate and its CrO_3 oxidant product and the $NaHCO_3$ hydrolysate of the oxidation product were all the same; (11) the infra-red spectrum (carried out by the Cambridge Instrument Co.) revealed the presence of single free OH group. Reactions 9–11 indicate a single hydroxyl group, at C3: (12) reaction with blue tetrazolium reagent positive, but much slower than cortisol, deoxycorticosterone or compound *S*; (13) positive reaction with silver diamine reagent[11]; (14) reaction with 2,4-dinitrophenyl hydrazine and thiosemicarbazine was positive; (15) after treatment with Girard reagent, the substance was recovered from the ketonic fraction with R_M value unchanged; (16) reaction with hydroxylaminic acid reagent and $FeCl_3$ gave a pink colour; (17) Schiff's reaction positive; (18) infra-red absorption at 1,676 cm^{-1}; reactions 12–18 strongly suggest an aldehyde group, at C17.

The ultra-violet spectrum and selenium dioxide reaction suggest a diene structure, and treatment with osmium tetroxide gave a compound with a much larger R_M value than the original substance. The placement of the second double bond must await further study of synthetic compounds, but the infra-red spectrum and ultra-violet absorption suggest that it may be at C16. Oxidation with CrO_3 and $NaBiO_3$ and reduction with a borohydride were not very successful. The first gave 'streaking' on paper, the second 'streaking' and several Zimmermann-reacting materials with R_M values of 0·72, 0·31 and −0·36 in modified Bush $B3$ system.

The pattern of the infra-red spectrum indicates a steroid structure. Though large numbers of reference standards including Δ^{16}-androsternol, 4-6-dienes, 16-OH-pregnenolone, Δ^{16} pregnenolone, 6-OH testosterone, 11-ketotestosterone, 2-OH testosterone, 7-keto and 7-OH dehydro*epi*androsterone, kryptogenin, diosgenin, 3β-ol-chol-5-enic acid were compared with the substance none of them showed an identical pattern.

From the results so far obtained, we may conclude that the new substance is probably a steroid with a hydroxyl group at C3, one double bond at C5, another possibly at C16, and an aldehyde group at C17.

The substances observed in the urines of new-born infants by Dr. Bierich[12] and Dr. Cathro *et al.*[13], may well be the same compound as ours. At present its physiological significance is unknown. Possibly it represents an intermediate or alternative product of the adrenal androgen system, characteristic of some or all children at a stage prior to the maturation of some unidentified enzyme system. As it seems likely to be something which occurs in children's urine, disappearing at some time, yet to be determined, before maturity, we suggest 'childhood steroid' as a strictly temporary descriptive label until its full chemical structure is known.

We thank Prof. I. E. Bush and Dr. R. W. H. Edwards for their advice, Prof. W. Klyne for a supply of reference steroids, Dr. Barbara Clayton, Dr. John Davis, Dr. Eades and Dr. D. Shaw for allowing us to study their patients, Dr. Chamberlin, of Merck, Sharpe and Dohme for a gift of ergosterol-D, Dr. B. W. L. Brooksbank for a gift of androst-16-en-3-ol, Mrs. Anna Cole for assistance and critical suggestions, and Mrs. A. V. Bagnall for the collection of urine specimens. This work was supported by a grant from the Medical Research Council.

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Pure Chlorogenic Acid not Allergenic in Atopy to Green Coffee: a Specific Protein Probably is involved

Recent articles[1–5] have reported that chlorogenic acid (3-caffeoylquinic acid) is the principal allergen in green coffee, oranges, other fruits and vegetables, and castor beans. Figley and Rawlings[6] and Coulson *et al.*[7] have shown that apparent cross-reactions to green coffee and castor beans are due to traces of castor bean proteins present as a contaminant in commercial green coffee. Layton *et al.*[8] have shown that primary allergy to castor beans involves neither chlorogenic acid nor any other natural constituent of the coffee bean. Recent reports by Freedman, Sehon *et al.*[4,5] have reaffirmed their view of the allergenic nature of chlorogenic acid and of its involvement in atopic hypersensitivity to coffee, castor beans and oranges. Chlorogenic acid is a ubiquitous component of higher plants, and if it should prove to be an allergen then this becomes a matter of major concern to both medicine and agriculture.

We were conducting clinical and laboratory investigations on several hundred French patients with allergy to castor beans and had found 29 patients who showed strong skin reactions to green coffee. Hence, we decided to investigate this newly reported aspect of atopy to a common component of plant materials by utilizing the allergic serum transfer test in monkeys[8,9] and relating the results to the clinical tests on the serum donors.

50 g of green coffee beans were freed of dust by rinsing with cold water. The coffee was dried at room temperature and then ground in a coffee mill. The ground green coffee was extracted with 250 ml. of distilled water at room temperature; the lyophilized crude extract weighed 9·4 g. 2 g of the dried crude extract was dissolved in 40 c.c. of water and dialysed for 18 h through 23/32 Visking* seamless cellulose tubing against two changes of water of 500 c.c. each. The lyophilized diffusate weighed 1·57 g, and the non-diffusible 'retentate' weighed 0·4 g. Ultra-violet absorption spectra indicated that almost all the chlorogenic acid was in the diffusate, and very little in the retentate.

'Crude' chlorogenic acid was prepared from green coffee by the method of Sondheimer, Szymanski and Corse[10,11], which involves extraction of the green coffee with 70 per cent isopropanol (aq.) followed by several recrystallizations of the potassium caffeinate–chlorogenic acid complex. A sample of chlorogenic acid was also obtained from Nutritional Biochemicals Corporation*. Pure chlorogenic acid was prepared from the crude acid by counter-current distribution between ethyl acetate and 10 per cent sodium chloride (aq.) in a 200-tube counter-current distribution extraction apparatus.

Clinical skin-scratch tests were conducted on 200 French patients most of whom were allergic to castor bean dust and house dust. Twenty-nine of the patients tested showed a high degree of sensitivity (2+ to 4+ reaction)[8] to a 1 per cent solution of crude green coffee extract. None of the patients showed significant sensitivity to a 1 per cent solution of chlorogenic acid. We used blood sera from the 29 French patients, and Dr. S. O. Freedman of Montreal, Canada, provided sera from 12 of his coffee-allergic patients. All these coffee-allergic sera were tested in macaque monkeys by the allergic serum transfer tests of Layton[8,9]. The monkeys were intradermally sensitized in skin sites with all 41 of the French and Canadian sera. 48 h after passive sensitization the monkeys were given intravenous injections of Evans blue dye solution. Each was challenged at 20-min intervals by intravenous injections of 25-mg samples of each of the materials being tested for allergenicity, beginning with the most highly purified and ending with a 5-mg injection of the crude retentate of green coffee. The blue lesions (extravasation of dye) resulting from reaction between reagin and green-coffee atopen were evaluated and photographed in colour.

Of the French patients clinically tested with coffee components, all 29 reacted to crude extract of green coffee but none reacted to chlorogenic acid. In the passive transfer tests, none of the 41 reaginic sera reacted with chlorogenic acid purified by counter-current distribution. All the Canadian sera reacted with 25 mg of one preparation of crude chlorogenic acid, but few of the French sera reacted with this material. 25 mg of another sample of crude chlorogenic acid prepared in our laboratory caused all sera to react. When the passively sensitized animals were challenged with 25 mg of 8-h and 18-h dialysates

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* Reference to a company and/or product name does not imply approval or recommendation of the product by the U.S. Dept. of Agriculture to the exclusion of others that may be suitable.

(diffusates) of green coffee there were no reactions; however, diffusate from a 72-h dialysis of green coffee caused reactions in one French and one Canadian serum-sensitized site. The 5-mg sample of non-diffusible retentate of green coffee contained very little chlorogenic acid, but caused all 41 sites to react strongly (3+ to 5+ lesions). Coffee beverage prepared from roasted coffee was found to contain considerable chlorogenic acid but to be non-allergenic when tested in the passively sensitized monkeys.

From these results of clinical tests and passive transfer in monkeys, it appears that the 12 Canadian patients, all of whom work with green coffee, are more sensitive than are the French patients whose sources of green coffee-sensitization are as yet unknown. The clinical tests clearly indicate that chlorogenic acid is not a significant factor in allergy to green coffee among the French patients. The passive transfer tests indicate that the atopic reagins of both the Canadian and French patients react primarily or exclusively with a higher-molecular-weight non-diffusible component of green coffee. This atopen is probably a protein. Chlorogenic acid is obtained from green coffee and most samples probably are contaminated by traces of protein from the coffee. Coffee beverage prepared from roasted coffee is non-allergenic.

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Utilization of Adenine by a Mutant of *Candida utilis*

Previous work has shown that *Candida utilis* (strain *IMI* 23311) efficiently utilizes exogenous adenine for the synthesis of RNA[1]. During the course of subsequent investigations[2], fresh cultures obtained from the Commonwealth Mycological Institute were found to have undergone mutation. The mutant had a longer generation time than the original strain when grown in rich medium, and failed to grow satisfactorily under conditions used in tracer experiments. Since no culture afterwards supplied by the Commonwealth Mycological Institute was found to have the characteristics of the original strain, experiments were continued with *C. utilis* strain *NCYC* 321. This strain had been maintained since 1951 from an original culture of strain *IMI* 23311 (ref. 3) and was found to have similar growth characteristics to those of the original culture of strain *IMI* 23311 used in these laboratories. The mutant has now been found to differ from strain *NCYC* 321 with respect to the utilization of exogenous adenine, and it is desirable to record that strain *IMI* 23311 no longer has the metabolic characteristics of the organism used in our previous work.

C. utilis strain *NCYC* 321 had a generation time of 120 min under the conditions previously described[1]. Under the same conditions, the generation time of the mutant of strain *IMI* 23311 varied between 7 and 12 h. Growth of the mutant was somewhat stimulated by addition to the medium of biotin (0·01 mg/l.), thiamin hydrochloride (1 mg/l.) or myo-inositol (1 mg/l.), the generation time ranging from 3·5 h to 4·5 h under these conditions. Addition of all three of the above vitamins to the medium produced no greater increase of growth than that produced by each added singly and no precise growth requirement for the mutant can be given. Addition of the above vitamins to the medium did not stimulate the growth of strain *NCYC* 321.

Cells were allowed to grow logarithmically for one generation in presence of adenine labelled with carbon-14, collected, and RNA was isolated as previously described[1]. In cultures of *C. utilis* strain *NCYC* 321, 98·7 ± 3·6 per cent of the radioactivity of the added adenine was recovered in the RNA; in cultures of the mutant of strain *IMI* 23311 recovery amounted to only 37·6 ± 1·9 per cent. This was raised to 53·4 ± 0·87 per cent by addition of biotin, thiamin hydrochloride and myo-inositol to the medium, presumably due to the greater requirement for adenine under conditions of higher growth-rate.

Cells collected towards the end of logarithmic phase were suspended in buffer at *p*H 7·5 (ref. 4), and broken in the Mickle disintegrator. The cell-free extract was centrifuged at 0° at 20,000*g* for 1 h and aliquots of the supernatant (2 ml.) were incubated at 37° with ^{14}C-adenine (0·15 μc.; specific activity 0·15 mc./mg), tetrasodium fructose 1,6-diphosphate (0·3 ml., 10 per cent w/v) and protamine sulphate (30 μg). After varying intervals of time, enzymic reactions were stopped by the addition of 10 per cent (w/v) trichloroacetic acid and, after removal of protein by centrifugation, the supernatant was freed from trichloroacetic acid and submitted to paper electrophoresis at *p*H 3·5. Radioactive spots were located either by autoradiography or by means of the radioactive chromatogram counter (Baird and Tatlock, Ltd., London). Radioactive material was eluted from appropriate areas of the paper and identified by paper chromatography in two solvent systems. After incubation for 30 sec, highly radioactive spots corresponding to adenine and hypoxanthine were identified in extracts of both strains of *C. utilis*, no other radioactive materials being present on the paper. After incubation for 5 min or longer, extracts of strain *NCYC* 321 resulted in radioactive spots corresponding to adenosine and inosine in addition to adenine and hypoxanthine. With extracts of the mutant of strain *IMI* 23311, a radioactive spot corresponding to adenosine was produced after incubation for 5 min or longer, but no spot corresponding to inosine was identified up to incubation for 30 min. It was estimated that radioactivity as low as 0·05 per cent of that present in the adenine- or hypoxanthine-containing spots would have been detected by autoradiography under the conditions used.

These results indicate that, in both strains, hypoxanthine is the earliest recognizable metabolite of adenine. The absence of detectable amount of radioactive inosine from cell-free extracts of the mutant strain is believed to be due to its formation from hypoxanthine being blocked; the alternative explanation, namely, that inosine can be utilized more rapidly than it is formed, is not in agreement with the poor utilization of the radioactive adenine by this strain. On the basis of the present experiments, it cannot be decided whether adenosine and inosine arise directly from the corresponding bases or whether they are formed by dephosphorylation of nucleotides. In either event, it appears probable that the conversion of adenine into hypoxanthine is the first reaction in the major route in the utilization of exogenous adenine for RNA synthesis and that loss of ability to form nucleotides from hypoxanthine is responsible at least in part for the poor growth of the mutant of *C. utilis* in simple medium.

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Some Physical Properties of Interfering Substances associated with the Alkaline Hydrolysis of RNA from Plant Leaf Tissue

Previous investigations[1] in our laboratory indicated that treatment of de-fatted, dechlorophyllized leaf tissue with alkali would hydrolyse the RNA into spectrophotometrically measurable nucleotides. Other studies[2] showed that RNA was preferentially eluted with 0·12 N hydrochloric acid from a column of 'Dowex 1–8X' on which mixtures of RNA and DNA had been adsorbed. This shortened exposure to alkali and recovery of RNA from mixtures of nucleic acid suggested the possibility of direct estimation of RNA in neutralized leaf hydrolysates by passage through a column of Dowex and subsequent elution with 0·12 N hydrochloric acid. Such eluates, however, invariably possessed greater absorption at 260 mμ than comparable material treated according to Diener and Lasheen[3]. The lower maxima/minima (*Mx*/*Mn*) ratios (A_{260}/A_{234}) for the neutralized material indicated that this higher absorbance was due to substances other than RNA. It was found that acidification of these alkaline hydrolysates followed by addition of an equal volume of ethanol and 2 drops of 1·0 M magnesium chloride would precipitate these substances absorbing at 260 mμ.

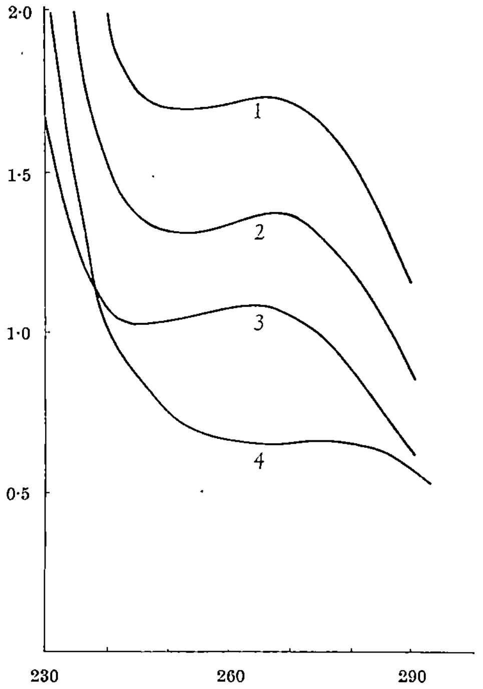

Fig. 1. Absorbance of 0·33 N potassium hydroxide digest. Area between curves 1 and 4, water-soluble impurities, *p*H 7·0; area between curves 2 and 4, 50 per cent ethanol soluble impurities, *p*H 7·0; area between curves 3 and 4, 50 per cent ethanol plus Mg^{++}-soluble impurities, *p*H 7·0; area between curves 4 and base, absorbance of hydrolysate adjusted, *p*H 5·0

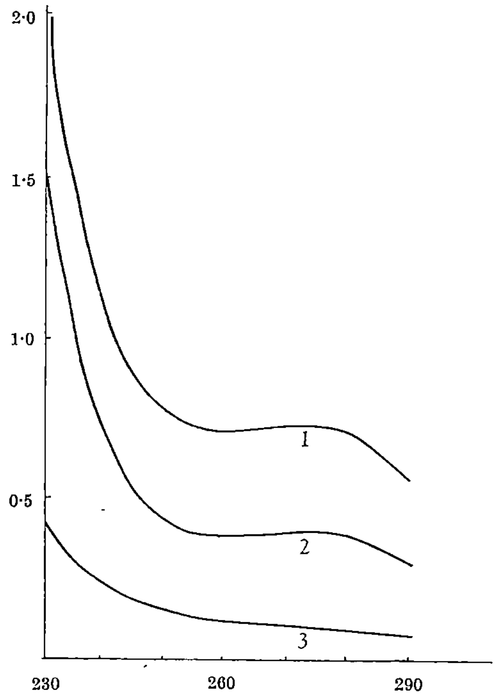

Fig. 2. Absorbance of impurities not adsorbed by 'Dowex' resin. Area between curves 3 and base, absorbance of acid alcohol impurities, *p*H 5·0; area between curves 1 and 3, absorbance of water-soluble impurities, *p*H 7·0; area between curves 2 and 3, absorbance of alcohol-soluble impurities, *p*H 7·0

Several portions of the hydrolysates, after removal of the extraneous material by centrifugation, were adjusted to *p*H's of 5·0, 6·0 or 7·0. Equal volumes of water, alcohol, and alcohol plus 2 drops of magnesium chloride were added to duplicates at each *p*H and stored for at least 2 h at 2° C. These were then centrifuged to remove the precipitated substances and brought to volume with water and then 10-ml. portions were passed through the column. After washing the column with 5 ml. of double distilled water, the RNA was eluted with 0·12 N hydrochloric acid. The results of a typical experiment are given in Table 1.

These data showed good reproducibility in the recovery of RNA for *p*H 5·0 for the treatments involving ethanol and ethanol plus magnesium chloride. *Mx*/*Mn* ratios for both treatments were greater than 2, values similar to those observed by Reddi[4] for solutions of tobacco mosaic RNA. Nearly as good recoveries of RNA were found in the other alcohol treatments, although those involving magnesium chloride indicate some loss at *p*H 6·0 and 7·0. Whenever water was added, the results were erratic and showed *Mx*/*Mn* ratios less than 2, indicating the presence of material other than RNA. It appeared that substances contributing to the high absorbance of the neutralized hydrolysates were water-soluble but were eliminated by the addition of alcohol. This is depicted in Fig. 1, where absorbance of the hydrolysates at 7·0 and of the alcohol-treated solutions at *p*H 5·0 are shown.

As indicated in Fig. 1, the addition of water to the neutralized hydrolysate solubilized substances with absorbance at 260 mμ of about 1·75. At this same *p*H

Table 1. EFFECT OF *p*H AND WATER, ALCOHOL OR ALCOHOL AND $MgCl_2$ ON THE RECOVERY OF RNA IN ALKALINE HYDROLYSATES OF LEAF TISSUE

(Absorbance of purified yeast RNA at 260 mμ—reference standard)

Sample	*p*H	Solvent	RNA (mg/g* dry wt.)	*Mx*/*Mn*
1	5·0	Ethanol	7,742	2·12
2	5·0	Ethanol and $MgCl_2$	7,743	2·09
3	5·0	Water	7,299	1·70
4	6·0	Ethanol	7,637	2·22
5	6·0	Ethanol and $MgCl_2$	7,442	2·22
6	6·0	Water	7,822	1·61
7	7·0	Ethanol	7,694	2·14
8	7·0	Ethanol and $MgCl_2$	7,317	2·02
9	7·0	Water	7,834	1·66

* 1 μg RNA = 0·0225 *A* at 260 mμ.

the treatments involving ethanol and ethanol plus magnesium showed absorbances at 260 mμ of about 1·4 and 1·1 respectively. All except the magnesium chloride had high absorbance at 230, part of whichw as contributed by the column. The differences in the alcohol and alcohol plus magnesium chloride seem to be due to another fraction which is not adsorbed on the column (Fig. 2). The base curve (3) represents the absorbance of the alcohol–*p*H 5·0 treatment and is identical with the absorbance of the alcohol plus magnesium chloride at *p*H 7·0. The area between curves 2 and 3 in Fig. 2 is roughly equal to the area between curves 2 and 3 in Fig. 1.

These observations indicate the presence of interfering substances that are soluble in water but precipitated by 50 per cent alcohol in hydrolysates of leaf material to be analysed for RNA. These substances, if not removed by alcohol precipitation, give high absorbance at 260 mμ. Further, the addition of magnesium ion as magnesium chloride and adjustment of the *p*H to 5·0 aid in the precipitation of these and other substances which do not pass through the column, thereby decreasing its efficiency.

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PHYSIOLOGY

Effect of Season and Environment on the Retinal Pigments of Two Fishes

DARTNALL *et al.*[1] have shown that in the paired-pigment species *Scardinius erythrophthalmus* (visual pigments based on vitamin A_1 and on vitamin A_2) the retina is dominated by the vitamin A_2-based pigment in the winter and by the vitamin A_1-based pigment in the summer (in England at latitude 52° N.). The present report concerns a similar investigation on two species of fishes living in their natural habitats at lat. 25° N. in Florida, U.S.A. One species, the poeciliid *Balonesox belizanus* Kner, was caught in an area of saline canals (average salinity about 10 parts per thousand), while the other, the cyprinid *Notemigonus crysoleucas boscii* Valenciennes, was caught in two environments separated by some 60 miles from each other. One of these, the 'Cutler Rd. location', was an area of drainage canals completely isolated from any other body of water throughout the period of the investigation (18 months)[2], whereas the other, the 'Loop Rd. location', was part of the canal, lake and swamp system covering most of south Florida and known as the 'Everglades'. Both these environments were fresh water (average salinity below 1 part per thousand).

Methods of extraction and analysis have been fully described elsewhere[2,3]. *Notemigonus* has pigments with λ_{max} at 529 (vitamin A_2-based) and at 502 mμ (vitamin A_1-based)[2]; *Balonesox* has pigments with λ_{max} at 521 (vitamin A_2-based) and at 498 mμ (vitamin A_1-based). The results of analyses of retinal extracts from these fishes taken during October 1962–March 1964 are summarized in Fig. 1. (It is now known that the proportions of vitamin A_1- and vitamin A_2-based pigments may vary considerably from fish to fish[2]; consequently the data of this figure refer to the mean composition of separately analysed extracts from individual fish in each batch.) Common to all three sets of data (those of *Balonesox* and those of *Notemigonus* from the two locations) is the result that the proportion of vitamin A_2-based pigment is at a minimum during the summer and at a maximum during the months of January and February. These positions virtually correspond to those found for *Scardinius* by Dartnall *et al.*[1]. Corresponding extract λ_{max} for the August minimum and for the January–February maximum are listed in Table 1, showing that, according to the time of year, mean extract maxima may change by as much as 13 mμ in the Cutler Rd. *Notemigonus*, 11 mμ in the Loop Rd. ones and 14 mμ in *Balonesox*.

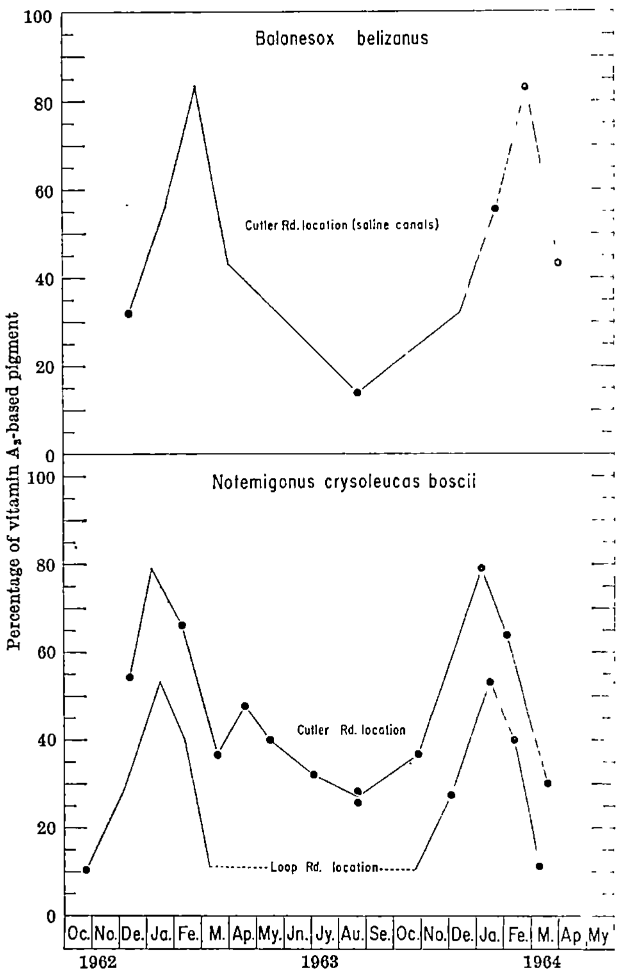

Fig. 1. Seasonal variation in the proportion of vitamin A_2-based pigment in the retinæ of *Balonesox* and of *Notemigonus* batches from two different locations. Where no points are shown at the angles in the diagrams, the figures from other years have been transposed

Whatever the physical causes of this variation (see, for example, refs. 1 and 4), it must be assumed that it has very considerable consequences from the point of view of the fishes' visual sensitivity. These consequences are likely to be even greater at the red end of the spectrum than a consideration of the λ_{max} alone would suggest. At 660 mμ, for example, the vitamin A_2-based pigment of *Notemigonus* has a photosensitivity some 25 times that of its vitamin A_1-based pigment.

The function of individual variation has been dealt with elsewhere[2]: that is, it may serve to 'broaden' the spectral sensitivity of fish schools. The consistent difference found between groups of *Notemigonus* from the two locations may be related to the observation that the highest proportion of red-sensitive (vitamin A_2-based) pigment occurred in the fish inhabiting waters which were

Table 1

Date	*Notemigonus* λ_{max} (mμ) Cutler Rd.	Loop Rd.	*Balonesox* λ_{max} (mμ)
August 1963	508* (506–512)†	—	501 (498–503)
January 1964	521 (518–526)	514 (511–521)	508 (504–515)
February 1964	517 (514–522)	511 (507–514)	515 (510–519)
March 1964	509 (505–515)	505 (503–509)	506 (501–512)

* Mean λ_{max} of extracts (corrected 0–2 mμ for D_{min}/D_{max}).

† Range of λ_{max} observed in the extracts of each individual fish in the group.

generally turbid. The other group, from the Loop Rd., were always found in clear waters. Thus we find, in a paired-pigment freshwater species, a possible parallel with the results of Munz[5], who reported that the fishes of turbid coastal waters possessed pigments with λ_{max} displaced towards the red end of the spectrum when compared with those from fishes of more clear marine waters.

With regard to seasonal variation, there is a striking similarity between the visual pigment changes observed in a cyprinid living in temperate English fresh water at lat. 52° N. (ref. 1), a poeciliid living in subtropical Florida saline water at lat. 25° N. and a cyprinid living in subtropical Florida fresh water also at lat. 25° N. Clearly, it is necessary to seek some varying requirement of visual sensitivity which is common to fishes living in these very different environments.

In conclusion, it should be pointed out that no direct measurements of the spectral sensitivities of these fishes have been made. While it may be justifiably supposed that scotopic sensitivity approximates to the summed light-absorbing properties of the two extracted pigments mixed in their appropriate proportions, it is pertinent to enquire whether the photopic sensitivity of winter fishes is near 620 mμ (as in the vitamin A_2-dominated retina of *Tinca*[6]) and whether that of summer fishes is near 550 mμ (as in animals possessing vitamin A_1-dominated retinæ[6]). Information of this kind would contribute considerably to our knowledge of rod and cone photochemistry.

This work was supported by the U.S. National Science Foundation (grant *GB* 186) and the U.S. National Council to Combat Blindness (grants *G*–268 and *G*–268 (*C*2)).

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Free α-Amino-N Content of Peripheric and Hepatic Venous Blood of Normal Adults

ALTHOUGH the α-amino-N content of human peripheric venous blood in health and liver disease is well known, the amino-acid content of the hepatic venous blood has been investigated only in the lower animals. Comparative quantitative analyses of peripheric and hepatic venous blood of human beings have not been carried out. Such analyses could clarify the protein synthesis in the liver. The differences between the inflow of amino-acids by the portal vein and hepatic artery, and as an analogue to this, peripheral circulation on one hand and the excretion of amino-acids by the hepatic vein on the other, are a partial measurement for the protein synthesis from amino-acids by the liver.

The quantitative determination of free α-amino-N was carried out according to the colorimetric ninhydrin method of Müting and Kaiser[1,2]. The advantage of this method is that one needs only 0·1 ml. of serum or plasma. The sensitiveness of amino-acids is very high at a concentration of 0·5γ. As a standard we used a 0·1 per cent aqueous solution of glycine (5γ–100γ). The normal range of error of this method is about ±2·0 per cent.

In 60 healthy adults the v. femoralis was punctured. Over a leading wire a 'Teflon'-catheter was inserted forward for about 10 cm. At this position the sample of peripherous venous blood was obtained. Then a catheterization of the v. hepatica was carried out. With sufficient practice the catheterization of the v. hepatica from the v. cava inferior is relatively easy. In most cases the right lobe of the liver was catheterized. Then the blood-sample of v. hepatica was taken. No complications were observed in any of these cases. Most of our patients suffered from congenital or acquired heart diseases. For the pre-operative diagnosis, a catheterization of the heart was necessary. In these patients no signs of cardial decompensation were found. Clinical and biochemical investigations revealed no damage of liver parenchyma.

For those 60 adults not suffering from liver disease, the average free α-amino-N in the peripheral venous blood was 4·15 ± 0·12 mg per cent. This result is similar to most of the values found in the literature by colorimetric or gasometric ninhydrin-method[1]. In contrast, the free α-amino-N of the hepatic venous blood of these patients was 3·45 ± 0·11 mg per cent. There is a difference of 0·61 mg per cent, which has been proved statistically ($P < 0·0001$).

Normally, the liver circulation is about 1,530 ± 300 ml./min (ref. 3) to 2,500 ml./min (ref. 4). Taking the first value of 1,530 ml./min and a hæmatocrit of 40 per cent, the daily protein synthesis from amino-acids in the liver $= 0·61 \text{ mg} \times \frac{1,530}{100} \text{ ml.} \times 60 \text{ min} \times 24 \text{ h}$ in blood or in serum $\times \frac{60}{100} = 8·04$ g nitrogen. Supposing a liver circulation of 2,500 ml./min, the daily protein production from amino-acids in the liver is about 14 g nitrogen. This value corresponds with the daily excretion of urea-nitrogen in urine (7–15 g in normal adults). Therefore, it will be supposed, the difference from 0·61 mg per cent α-amino-N is caused only by the protein synthesis from amino-acids.

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Electrical and Mechanical Activity of Isolated Vascular Smooth Muscle of the Rat

SPONTANEOUS electrical and mechanical activity of smooth muscle from the portal vein of the rat were recorded simultaneously for the purpose of determining the relationship of membrane potential to tension development.

A helical strip, 0·5 mm wide and 3 mm long, was cut from the portal vein of a rat, exsanguinated after being stunned by a blow on the head. Smooth muscle of these strips was found by one of us (S. F.) to show conspicuous spontaneous contractions when suspended in a physiological salt solution (NaCl, 118·9; KCl, 4·7; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $NaHCO_3$, 14·9; dextrose, 5·6; $CaCl_2$, 1·5; sucrose, 49·9; and calcium versenate, 0·026 in mM/l.) at 35°–37° C. The muscle strip was mounted in a narrow 2-c.c. bath connected at either end, via polyethylene tubing, to a 20-c.c. syringe. The two syringes were synchronously driven so that one would withdraw bath solution at the same rate (2 c.c./min) that it was injected by the other, thus keeping the surface of the bath at a constant level. High-resistance micro-electrodes (50–70 M) filled with 3 M potassium chloride solution were used to record trans-membrane potentials; an electro-mechanotransducer (Grass, model *FT* 0·03) recorded muscle contractions.

The insertion of micro-electrodes into the smooth muscle fibres was carried out with a Peterfi-type micro-

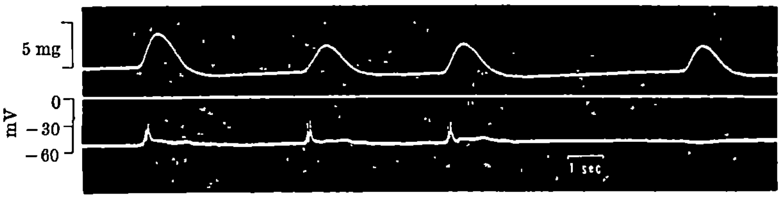

Fig. 1. Simultaneous records of spontaneous electrical and mechanical activity. Upper, middle and lower records are of changes in muscle tension, zero potential and electrical activity, respectively. Note double spike potentials. The particular muscle cell impaled failed to fire at the last contraction

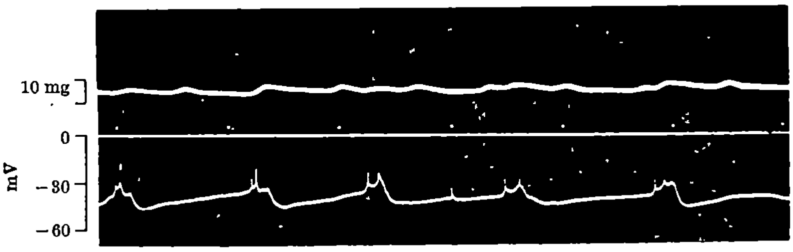

Fig. 2. This record illustrates the irregularity of spike potentials based on slow waves fluctuating at higher amplitude during higher muscle activity. Note the prolonged after-hyperpolarization of the slow waves and the single spike arising from the trough between two waves. Time marks, 5 sec

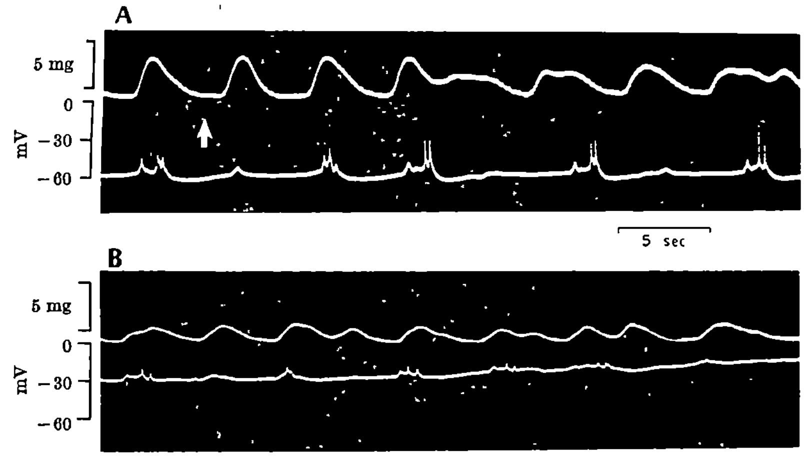

Fig. 3. Effect of adrenaline on electrical and mechanical activity. Arrow in *A* indicates administration of adrenaline (20 μg/l.). *B* is 4·5 min after the administration of adrenaline. Note progressive depolarization of the membrane and change in muscle tension

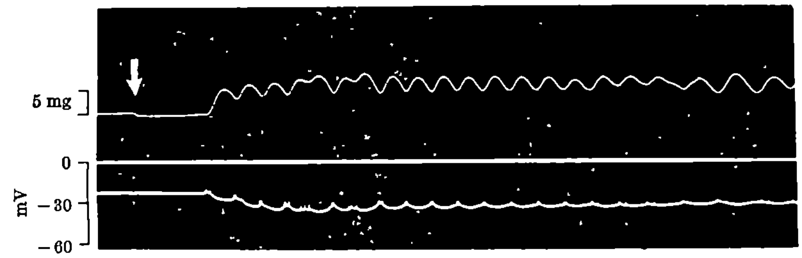

Fig. 4. Effect of acetylcholine on an inactive muscle and the transmembrane potential. Arrow indicates administration of acetylcholine (50 μg/l.), which caused a remarkable hyperpolarization and a summation of contractions. Note the low resting potential of a muscle cell in the inactive muscle. Time marks, 5 sec

manipulator, under binocular microscope with incident illumination. When the tip of the micro-electrode entered a single muscle cell the transmembrane potential always showed a negativity against the external medium, ranging from 30 to 65 mV. The potential sometimes showed slight rhythmic fluctuations or slow waves at various intervals, rarely with an after-hyperpolarization, which resembled those in visceral smooth muscle[1,2]. Transmembrane potentials were measured at maximum polarization during both spontaneous activity and the intervening pause, because, as in visceral smooth muscle, the membrane potential is constantly changing and this is the most readily identifiable value. During rhythmic contractions the changes in muscle tension were preceded by single or double spike potentials arising from the shoulders or summits of slow potentials (Fig. 1). The mechanical activity often formed regular sine waves, especially when contractions occurred at a high frequency (Fig. 4). Individual contractions of the muscle developed about 5 mg tension and lasted only 1–3 sec. This contractile response is much more rapid than any we have observed in other vascular smooth muscle.

Slow waves having an amplitude of 20 mV or more and a duration of 5–10 sec (Fig. 2) were observed. Spike potentials were common on the crests of these slow waves but occasionally occurred in the troughs. Spike duration was 50–100 msec, much shorter than that observed in vascular smooth muscle of the turtle[3]. When spikes occurred in pairs the interval was at least 90 msec. Spike potentials varied from 8 to 30 mV and never displayed a reversal of the membrane potential (overshoot). This again differs greatly from the large potentials, with overshoot, observed in amphibians[3,4]. Using an *in situ* preparation of rat mesenteric vessels, Trail[5] observed single and paired spike potentials, followed by after-hyperpolarization, similar to those that we have seen in the isolated bath.

During the present work the effects of certain agents on the muscle's activity were examined (Fig. 3). Administration of adrenaline, in concentrations of 1–50 μg/l., always increased muscle activity; this effect was accompanied by progressive depolarization of the membrane and increase in spike frequency, in proportion to the concentration of the stimulant. In the initial stage of the process there was an increase in spike potentials associated with an increase in both frequency and amplitude of individual contractions. As depolarization progressed the spike potentials became smaller and more irregular and there was a gradual reduction in tension development.

Acetylcholine in concentrations of 10 μg–200 mg/l. had a diphasic effect on the smooth muscle activity. It caused an increase in membrane potential for a period of 20 or 30 sec up to 2 min, depending on its concentration, accompanied by an increase in spike height and a slight increase in spike frequency. This period of hyperpolarization was followed by the second phase of gradual depolarization, during which there was an increase in spike frequency and the mechanical activity formed regular sine waves.

Some preparations were studied in which membrane potentials were abnormally low and which displayed no action potentials or mechanical activity. Acetylcholine administration to such a preparation caused a remarkable hyperpolarization and initiated frequent rhythmic action potentials and a summation of contractions (Fig. 4). The increase in membrane potential and the initiation of action potentials are probably due to two different actions of acetylcholine.

These observations indicate that in vascular smooth muscle there is a direct relationship between the spike potential and the individual contraction.

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PATHOLOGY

Transformation of Embryonic Human Cells by Rous Sarcoma Virus

IT was established in 1957 and 1958 that Rous sarcoma virus (RSV) is pathogenic for mammals and induces hæmorrhagic cysts and sarcomas in rats and fibromas in rabbits[1–4]. These results have been confirmed and expanded by numerous authors, and the pathogenicity of RSV for many other species of mammals has been demonstrated in various laboratories.

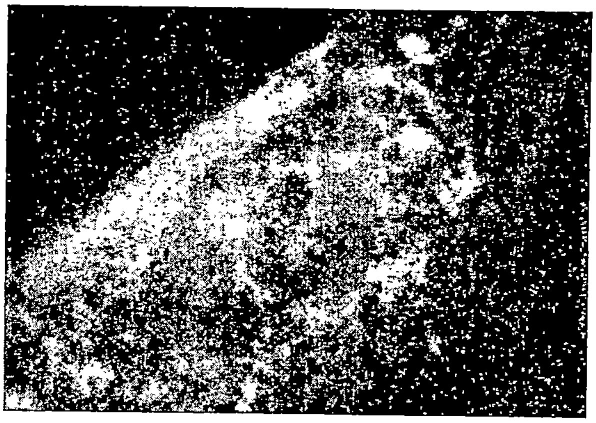

Fig. 1. Cell of infected culture treated with labelled antibodies to Rous virus: specific luminescence of cytoplasm; vacuolization of cytoplasm; enlargement of nucleus (× 720)

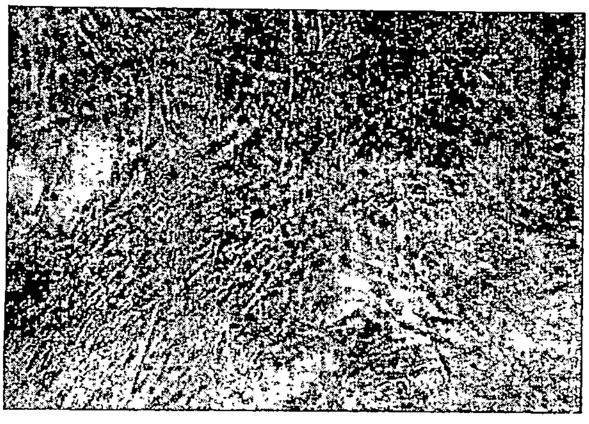

Fig. 2. Foci of multi-layered growth in infected culture after 65 days following infection (× 80)

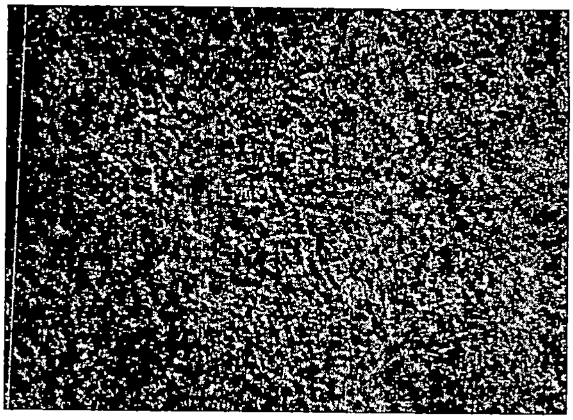

Fig. 3. Infected culture on the seventh day; multilayered growth of cells (× 80)

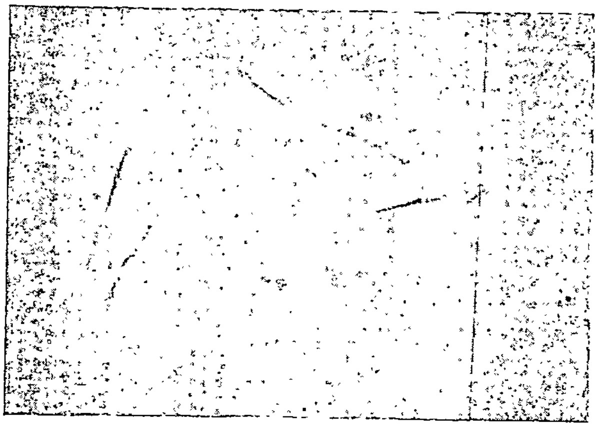

Fig. 4. Control culture during the same period as Fig. 3 (× 80)

The neoplastic transformation of cells by RSV (strain Prague) was also successfully reproduced in tissue culture of rat fibroblasts. The transformed culture was inoculated into rats in which tumours developed. These tumours can be transplanted into chicks by the cells but not by the filtrates[5]. Similar results were obtained with the Bryan strain[6].

In other investigations, the formation of foci of round granulated cells in cultures of rats, mice, guinea pigs fibroblasts infected with Schmidt–Ruppin strain was demonstrated[7,8].

We have examined the possibility of transformation of embryonic human tissue by RSV. For this purpose, we used the Schmidt–Ruppin strain which Prof. Åhlström kindly sent us since it proved to be more oncogenic in comparison with others.

Our technique was as follows. Extracts of Rous sarcoma tissue were added to the fresh suspension of embryonic cells of a human embryo in relation 1 : 2. This mixture was placed for a night on a magnetic stirrer at temperature + 4° C. The activity of this viral extract was tested by titration in 2–3 day chicks, and it was equal to $ID_{50} = 5 \times 10^{-3.5}$ in 1 ml. The same suspension, but without the virus, was placed under the same conditions also for a night.

The next day the examined suspension was washed out with nutrient medium under double centrifugation, and explanted in flasks with the medium of the following composition: 40 per cent Medium 199, 15 per cent bull serum, 15 per cent human serum and 30 per cent bovine amniotic liquid. The control suspension was washed out in the same manner. Renewal was carried out twice a week. Cultures were cultivated at 37° C.

One-and-a-half months later, the cultivation was continued on Medium 199 with the addition of 20 per cent bull serum and 0·1 per cent yeast extract. The passages of cells were made at intervals of 2–3 weeks.

The character of cell growth in the examined and control cultures was identical for 2 months, and the cells in these cultures did not differ from one another under the microscope. Infectious virus was not detected in nutrient medium on the twelfth, twenty-eighth and thirty-third days of cultivation by infection of chicks.

On the thirty-fifth day of cultivation we discovered in infected cells the viral antigen with the help of antibodies to RSV-labelled isothiocyanate fluorescein using Coons's method.

We observed very clear fluorescence of cytoplasm of infected cultures and also considerable vacuolization of cytoplasms and enlargement of nucleus in cultures infected by RSV (Fig. 1). We did not observe such a picture in control cultures not infected with RSV.

At the beginning of the third month of cultivation the foci of intensive cell multiplication in the flask were noted in the infected culture.

Fig. 2 demonstrates the foci of multilayered growth appearing during the following days. Gradually, the cell growth became irregular, forming the multilayered cell mass that grew in different directions (Fig. 3). In control cultures we did not observe any signs of transformation; their growth remained monolayered and became very poor (Fig. 4).

The same results were obtained by Koprowski, Jensen and Girardi (personal communication).

The afore-stated character of transformation gives some evidence to suppose the malignization of our cultures, but further experiments are needed to draw a definite conclusion.

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Acidophilia of Aortic Elastin in the Child

In a previous note[1] some results were presented to show that if 5–7-μ sections of human aorta were exposed to 0·025 per cent bromphenol blue (B.D.H. indicator) in *p*H 8·2 Sorensen buffer, then left in distilled or ordinary tap water, aortæ from young subjects retained the dye in the elastic laminæ considerably better than those from the old.

As the maximum resistance to leaching appeared in the young child, a further series has been collected, concentrating on this particular age group. In all, 259 aortæ of children under 7 and 261 aortæ of older individuals, a total of 520, have now been examined. In each case, a portion of descending thoracic aorta was fixed in 10 per cent formol-saline buffered with chalk, embedded in paraffin and cut at 5–7μ.

The method of reading the results has been simplified, and is now more accurate and reproducible. We stain for 20 min in the dye solution, leave overnight (20 h) in water, and grade the residual laminar colour in water under the low power of the microscope: no colour = 0, detectable colour = +, definite colour = + +, strong colour = + + +. The overnight reading seems to be the most consistent as readings at shorter intervals are more affected by minor variations in technique.

In aortæ from subjects older than 7 years, the bromphenol blue is invariably leached by overnight immersion in water. Up to 7, resistance of the dye to leaching develops (Table 1) and regresses (Table 2).

Table 1. Analysis of 184 Aortæ under 1 Month Somatic Age

Weight of infant in grams	Aortæ arranged in groups according to laminar colour remaining after overnight leaching in water			
	0	+	+ +	+ + +
60–500	5	10	1	—
500–1,000	5	19	3	4
1,000–2,000	4	14	11	16
2,000–5,000	2	9	13	68

Table 2. Analysis of 336 Aortæ from Subjects aged 1 Month to 95 Years

Somatic age of subject in years	Aortæ arranged in groups according to laminar colour remaining after overnight leaching in water			
	0	+	+ +	+ + +
1/12–2	—	—	—	59
2–3	—	—	1	2
3–4	—	5	—	2
4–7	2	4	—	—
7–20	17	—	—	—
20–95	244	—	—	—

The results may be summarized as follows. During gestation and the neonatal period, bromphenol blue retention in aortic laminæ is roughly proportional to the weight of the subject, reaching a maximum level at about the age of one month. That level is retained until the age of 2 years, when it begins to fall. There is little left at 4 years, and the maximum somatic age which has shown any degree of bromphenol blue retention is 6 years 10 months.

We thank the City Morgue, the Royal Children's Hospital, the Royal Melbourne Hospital and the Royal Women's Hospital for help in collecting the aortæ used for this investigation.

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Metabolic Activity of Neural Crest Tumours in Tissue Culture

Numerous reports have described the raised urinary excretion of metabolites of norepinephrine, and its precursors, by children with tumours of neural crest origin[1–7]. It has also been observed that many of these patients show increased norepinephrine production but produce little or no physiological evidence of increased secretion of this potent catecholamine. Some possible explanations for this apparent paradox are as follows: (1) There might be an adaptation to increased blood-levels of norepinephrine. (2) The norepinephrine might be inactivated within the tumour before release into the blood. (3) A combination of the foregoing two mechanisms might be acting. (4) The rate of inactivation of norepinephrine in tissues other than the tumour (liver, kidney, etc.) might be increased. In a previous investigation, La Brosse and Karon have presented evidence that neural crest tumours contain catechol-*O*-methyltransferase (COMT) activity[8]. Since the 3-*O*-methylated product of norepinephrine has been shown by Evarts *et al.*[9] to be physiologically inactive in comparison with norepinephrine, the finding that COMT activity existed within the tumour indicated that, at least in some cases, norepinephrine may be inactivated before being released into the blood.

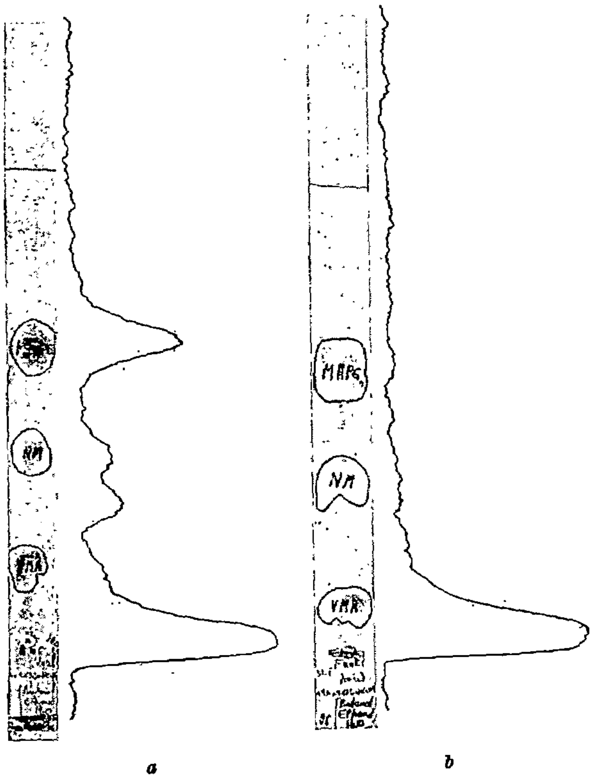

Fig. 1. Radiograms of culture media 48 h after adding 7-^{3}H-noradrenaline hydrochloride. *a*, Radiogram of medium from tube containing a neural crest tumour; *b*, radiogram of medium from tube containing only medium and plasma clot. Abbreviations of reference compounds are: VMA, 3-methoxy-4-hydroxymandelic acid; NM, normetanephrine; MHPG, 3-methoxy-4-hydroxyphenylglycol

In an attempt to obtain further information bearing on these questions, tritium-labelled norepinephrine was incubated with isolated, living neural crest tumours in tissue culture.

Tumour tissue was obtained at operations from 12 patients with neural crest tumours; on histological examination eight tumours were diagnosed as neuroblastomas, three as ganglioneuroblastomas and one as a mixed tumour. The tumour tissue was explanted to plasma clot in tubes containing Eagle's medium[10] and 30 per cent pooled human plasma (or a mixture of 15 per cent pooled human plasma and 15 per cent calf plasma). A mixed cellular outgrowth appeared usually within the first 48 h after explantation. Nerve cells emitting typical neuritic filamentous ramifications were accompanied by fibroblast-like cells and some macrophages. D,L-Norepinephrine-7-^{3}H (^{3}H-NE) (7·5 μc., 1·7 μg) was added to the culture medium (about 1·5 ml.) at 2–6 weeks after explantation; the cultures have always shown the presence of differentiated nervous cells at the time of addition of the labelled compound. For comparison, the labelled norepinephrine was also added to tubes containing plasma clot and culture medium but without tumour. The tubes were incubated at 37° C for 48 h, the culture medium was removed and portions of it were chromatographed on strips of Whatman No. 3*MM* filter paper in the following solvent systems: (1) *n*-butanol/acetic acid/water (4 : 1 : 1), (2) *n*-butanol/ethanol/water (4 : 1 : 1), (3) *n*-butanol/ethanol/water/acetic acid (80 : 20 : 20 : 1), (4) benzene/propionic acid/water (1,000 : 700 : 41), (5) isopropanol/1·4 N ammonia (4 : 1) and (6) butanol/ethanol/ammonia (4 : 1 : 1). Prior to chromatography, reference compounds were added to each strip. After development, the strips were dried and scanned for radioactivity. Finally, they were sprayed with diazotized *p*-nitroaniline. The radiograms of culture medium alone and of culture medium from a tube containing one of the tumours are shown in Figs. 1 *a* an *b*. Comparison of these chromatograms reveals that the are three radioactive areas on the chromatogram of tl medium from the tube containing the tumour whic are not present on the chromatogram of the cultu medium alone. Areas of radioactivity were found correspond to 3-methoxy-4-hydroxyphenylglycol ar normetanephrine in all the solvent systems teste In addition, hydrolysis studies on eluates of the materi which has an R_F of 0·24 in the No. 2 solvent syster already indicated here that this compound is the sulpha conjugate of normetanephrine. In the case of one tumo there was also a tritium-labelled metabolite whi migrated farther than MHPG; the migration characte istics suggest that this compound may be 3-methox 4-hydroxymandelaldehyde.

The formation of the metabolites mentioned he indicates that these explants of tumour contained catech *O*-methyltransferase (COMT), monoamine oxidase (MA aldehyde reductase (AR) and sulphate conjugase (S activities. The presence of COMT activity in tumou of this type has been previously reported[8], and t other enzymatic activities are being investigated.

When D,L-3-methoxy-4-hydroxymandelic acid-7- or D,L-3-methoxy-4-hydroxyphenylglycol-7-^{3}H were add to the culture media as above, there was no evidence further metabolism.

Further work is in progress to establish, beyond reaso able doubt, the identity of the metabolites of ^{3}H- which appeared under the conditions described he additional investigation is also required before it is possi to state whether the metabolic transformations wit the tumour are stereospecific since only the race norepinephrine-7-^{3}H was available to us.

These results indicate that neural crest tumours tissue culture, particularly those from patients who not show signs of excessive norepinephrine secreti but who excrete elevated amounts of norepineph metabolites in the urine, are capable of metaboliz D,L-norepinephrine-7-^{3}H to 3-*O*-methylated compoun This finding provides additional evidence that metab ism as well as synthesis of norepinephrine may oc within neural crest tumours.

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IMMUNOLOGY

Rejection of Isogeneic Murine Skin Grafts following Exposure to Allogeneic Ribonucle Acid

TISSUE grafted between non-identical individual ordinary circumstances is recognized as foreign rejected by the host. This rejection is an immunolo phenomenon which is determined by multiple domi histocompatibility genes. The genes are ultimately responsible for the synthesis of lipoprotein histocompatibility antigens[1]. This probably occurs by means of a mechanism which transcribes coded information to messenger ribonucleic acid (RNA), and the subsequent translation of this information determines the specific proteins[2].

The experiments described here were based on the hypothesis that messenger RNA contains the information for the determination of specific histocompatibility antigens; thus the introduction of allogeneic RNA into the cells of a graft could theoretically determine a new protein foreign to the graft. It has previously been reported that cells exposed to foreign RNA can synthesize protein not ordinarily produced by these cells[3,4]. Since multiple genetic differences exist in the usual donor–host homograft relationship, these experiments did not attempt to alter all the incompatibilities. Rather, a system was selected that is sensitive enough to detect any change in one or more histocompatibility antigen(s). This was done by observing the fate of isogeneic mouse skin grafts following their exposure to RNA derived from another inbred strain.

The *C3H/TB* and *BALB/c/TB* inbred strains were used throughout our experiments. They are genetically different at a minimum of 12 histocompatibility loci including Histocompatibility-2. Induction of acquired tolerance between the strains by neonatal inoculation of allogeneic cells has not been possible. Both factors indicate a wide genetic disparity between the lines[5].

RNA was isolated from the livers of the inbred mice by a modification of the phenol method[6]. The interphase of the phenol extract was re-extracted with 0·5 per cent sodium dodecylsulphate containing 0·001 M K_3PO_4 and afterwards extracted with phenol. This was done because the experiments of Georgiev *et al.*[7] and Sibatani *et al.*[8] suggest that messenger RNA is retained in the interphase. All extractions were carried out at 5° C. The final RNA solution was made up in 0·9 per cent sodium chloride. Concentrations of RNA and proteins used in our system are listed in Tables 1 and 2. RNA concentration was determined by the orcinol method[9] and the protein concentration by the method of Lowry *et al.*[10].

Table 1

No. of animals	Host strain	Skin donor strain	Source of liver RNA in incubation media	Conc. RNA (μg/c.c.)	Conc. Prot. (μg/c.c.)	No. rejected	%
4	*C3H*	*C3H*	*C3H*	260	0·3	0	0
10	*BALB/c*	*BALB/c*	*C3H*	260	0·3	9	90
5	*BALB/c*	*BALB/c*	*BALB/c*	700	13·5	0	0

Grafts were incubated in RNA plus balanced salt solution at *p*H 6·5 for 4 h at 37° C prior to grafting.

Table 2

No. of animals each with two grafts	Host strain	Donor skin strain	Composition of media	No. of grafts rejected	%
5	*BALB/c*	*BALB/c* (graft No. 1)	Balanced salt solution	0	0
		BALB/c (graft No. 2)	*C3H* liver RNA in balanced salt solution*	4	80
5	*BALB/c*	*BALB/c* (graft No. 1)	Hydrolysed *C3H* liver RNA in balanced salt solution†	0	0
		BALB/c (graft No. 2)	*C3H* liver RNA in balanced salt solution*	4	80

* RNA concentration = 813 μg/c.c. and protein concentration = 8·1 μg/c.c.

† RNA hydrolysed in 1 N hydrochloric acid at 100° C for 1 h. *p*H re-adjusted with 1 N sodium hydroxide to 6·5.

Donor mice were anæsthetized, skin for incubation was taken from their abdomen, and the adhering fat was removed. The pelts measured approximately 2·5 cm × 3·5 cm and provided skin for 2 grafts after their flotation on the incubation media in sterile glass Petri dishes. The incubation media was either Hanks's or Earle's balanced salt solution. RNA was added after the *p*H of the media had been lowered to 6·5 with concentrated hydrochloric acid. Incubation was carried out for 4 h at 37° C. The grafts were then removed from the solution, placed on filter paper to drain excess fluid, and sutured on to a full thickness bed on the dorsum of host animals. The animals were caged individually, observed for gross signs of graft rejection, and several grafts biopsied on day 7.

Table 1 shows the results obtained in the first experiment. Nine of ten grafts incubated in the allogeneic RNA were destroyed while all control grafts were accepted. The observation that *BALB/c* skin incubated in RNA from *BALB/c* was not rejected by *BALB/c* hosts indicates that a non-specific sensitivity of *BALB/c* skin to RNA was not a factor in the outcome of the experiment. *C3H* grafts incubated in RNA from *C3H* mice were accepted by *C3H* hosts, which indicates that a non-specific toxicity did not influence these results. This does not exclude the possibility of specific toxicity of RNA from *C3H* mice on *BALB/c* skin.

Table 2 shows results from two groups of 5 *BALB/c* mice, each animal with two grafts. The first group of mice had one of its grafts incubated in balanced salt solution without any RNA and the other graft was incubated in *C3H* liver RNA. The second group of animals had one of its grafts incubated in acid hydrolysed *C3H* liver RNA and its second graft was incubated in *C3H* liver RNA. This was done to eliminate the possibility of rejection of grafts on the basis of incompatibility due to factors other than our incubation mixtures. In each of these groups, four of five RNA-incubated grafts were destroyed while all control grafts were accepted.

In both experimental groups, a total of 20 animals showed 17 (85 per cent) that rejected their isogeneic grafts over a period of 10–27 days. Eighteen days was the median and modal time of complete rejection. Gross evaluation prior to the complete destruction and necrosis of the tissue was difficult, but the end result of scarification was obvious. Biopsy of the grafts incubated with allogeneic RNA on day 7 showed an œdematous dermis heavily infiltrated by mononuclear cells and some degree of epidermal atrophy, but no epidermal destruction, at that time. The grafts incubated with isogeneic RNA showed little cellular infiltration. Four *BALB/c* animals that rejected their *C3H*–RNA-incubated isogeneic skin grafts were regrafted with *C3H* skin, and these grafts were grossly rejected by day 7 in two cases and by day 14 in the other two cases. This suggests induction of strong immunity in 2 of the 4 animals. The mean rejection time determined by total graft destruction between the *C3H* and *BALB/c* strains is 12·5 days. Other controls included the subcutaneous injection of RNA from *C3H* mice into 4 *BALB/c* graft sites followed by *BALB/c* skin grafts, and this failed to affect the take of the grafts. Also, the intraperitoneal injection of RNA from *C3H* mice failed to induce accelerated rejection of *C3H* allogeneic skin grafts on *BALB/c* hosts.

These results indicate that it has been possible to alter the antigenic specificity of skin following incubation in allogeneic RNA, when measured in a system of isogeneic grafts. Abolition of the effect by acid hydrolysis of the extract suggests that RNA itself is responsible; however, the presence of specifically active polypeptides is not excluded.

The evidence that the observed effect was due to a change in histocompatibility antigens is suggested by isogeneic graft rejection, accelerated rejection in 2 of 4 animals regrafted with allogeneic skin, and a histological picture at 7 days resembling a homograft reaction. The possibility that the isogeneic rejection is due to the alteration or induction of proteins other than histocompatibility antigens is also recognized. If, however, the change in antigenicity is due to new histocompatibility antigens, it will be important to determine whether or not these replace or add to the existing antigenic complex. This appears to be a fundamental problem when considering the possible clinical applicability of this approach.

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Gamma-globulin Synthesis in Hormonally Bursectomized Chickens

IN previous investigations it has been found that the normal development of the bursa of Fabricius in chickens is essential if the bird is to develop fully its potential antibody-forming capacity[1-3]. In these investigations[2,3] the development of the bursa was partially or completely inhibited by the intra-embryonic injection of testosterone propionate. When the inhibition of bursal lymphoid tissue was complete, no antibody production could be detected after challenge at 4–6 weeks of age with 5 different antigens[3]. Providing the thymus in these chickens was morphologically normal, there was no detectable reduction in splenic lymphoid follicles, peripheral blood lymphocyte-levels, or homograft immunity to solid grafts[3]. A marked reduction in cells of the plasmacytic series has been observed in the spleens of both hormonally[3] and surgically[4] bursectomized chickens. As these chickens resembled patients with agammaglobulinæmia[5] in their inability to produce circulating antibodies, physicochemical studies on the serum immunoglobulins were commenced and form the basis of this presentation.

Long and Pierce[6] have previously reported that there was a reduction in the γ-globulin level of a pool of sera from hormonally bursectomized chickens, as determined by electrophoretic and immunoelectrophoretic analysis. Similar work on the sera of thymectomized rats[7] with an impaired antibody response to bovine serum albumin has failed to reveal any abnormality in the β-2M or γ-globulin bands, although a third immunoglobulin line termed *IgX* (perhaps analogous to β-2X-globulin) was absent or reduced in most of the rats.

It is necessary to raise birds to an age of at least 4–5 weeks or older before assessing their globulin patterns, since previous studies[8] have shown that the γ-globulin level declines in normal young chickens, reaching a minimum at about 4 weeks of age, and it is, therefore, impossible to make a statement on newly synthesized γ-globulin prior to this age. Since complete absence of the bursa seems to be incompatible with survival beyond 3–4 months of age, the majority of birds were between 1 and 2 months of age.

All chickens used were outbred White Leghorn 'Australorp' hybrids, and hormonal bursectomy was performed by the intraembryonic injection at 12 days of incubation of 4 mg of testosterone propionate (B.D.H.) in corn oil. The ability to produce circulating antibody was tested by intramuscular challenge with 8 mg of bovine serum albumin after 4 weeks of age. Serum titres were determined 7 days after challenge by the tanned cell hæmagglutination technique. Microimmunoelectrophoresis was performed with a 0·025 M veronal buffer at *p*H 8·2, the precipitation lines being produced with a rabbit anti-whole chicken serum. Analytical ultracentrifugation was performed at 20° C in a Spinco model *E* ultracentrifuge, using a schlieren optical system equipped with phase plate.

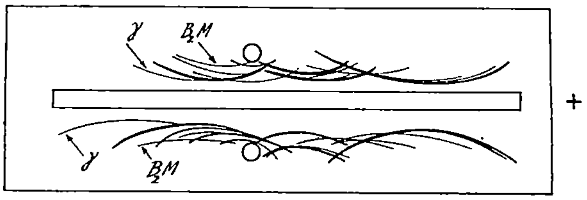

Fig. 1. Immunoelectrophoretic patterns of sera from two birds producing no detectable antibody to bovine serum albumin. The lower pattern shows a normal line while the upper pattern shows a reduced γ-globulin line

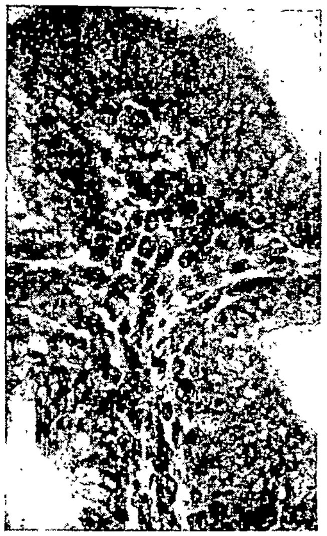

Fig. 2. Bursal remnant of a 5-week-old hormonally bursectomized chicken showing sub-epithelial plasma cell accumulation (Unna Pappenheim stain, × 400)

The sera from 28 1–2-month-old hormonally bursectomized chickens were taken one week after immunization with bovine serum albumin and 17 of the birds were also killed for histological examination. The results in Table 1 show that the γ-globulin line was apparently normal in 14 birds in which antibody production could not be detected, and in 5 birds producing low but significant antibody titres. The β *2M*-line was present in all but 3 of the 28 sera. A typical immunoelectrophoretic analysis is shown in Fig. 1 of two sera from birds producing no detectable antibody to bovine serum albumin, one with a normal line, and one with an apparent reduction of the γ-globulin line.

Ultracentrifugal analysis of 4 normal sera and 7 sera from non-antibody-producing bursaless chickens was performed. The calculated values of 5*S*, 7*S*, and 19*S* fractions (Table 2) revealed a significant reduction of 7*S* material, and a slight reduction of 19*S* proteins. As most of the bursaless birds used in this group were 1–2 weeks younger than the normals, this difference in 7*S* proteins could be actually less for birds of exactly comparable ages. Since each of these two fractions contains several components the reduction could be due to a slight fall in all or a more marked reduction of a single component. In the light of the immunoelectrophoretic result it is probable that the reduction in 7*S* is due to γ-globulin.

Tissues from 17 of the birds were examined histologically for the presence of plasmacytic cells. In 12 birds no antibodies to bovine serum albumin could be detected and in all but one of these birds no lymphoid follicles were seen in

Table 1. COMPARISON OF ANTIBODY TITRE WITH γ-GLOBULIN PRODUCTION IN HORMONALLY BURSECTOMIZED BIRDS

γ-Globulin titre	Antibody titre (normal mean 640)	
	< 10	10–1,280
Normal (8–16)	14	5
Low (4–8)	7	2

Table 2. ULTRACENTRIFUGAL ANALYSIS OF NORMAL AND BURSALESS CHICKEN SERA

	5*S*	7*S*	19*S*
Normal	2·70 (2·41–2·91)	1·44 (0·98–1·77)	0·35 (0·22–0·58)
Bursaless	2·53 (2·04–3·21)	0·53 (0·19–0·83)	0·20 (0·13–0·32)

The values are the mean calculated amounts of protein sedimenting in the respective fractions expressed in g per cent. The range of values is shown in parentheses.

any of several sections examined of each bursal remnant. However, in all cases, variable numbers of plasma cells were found in the bursal remnants, cæcum, and occasionally in spleen. The numbers in the latter two tissues were below normal levels, although in some birds, as shown in Fig. 2, plasma cells were plentiful in the bursal remnant, and were nearly always located directly beneath the epithelial lining. In the remaining 5 birds, low but significant antibody titres to bovine serum albumin were detected after challenge, and in all cases at least a few typical bursal lymphoid follicles were seen in the bursal remnant, and plasma cells were plentiful in the spleen, cæcum and bursal remnant. No correlation was evident between the intensity of the γ-globulin line and the approximate numbers of plasma cells present. Although it is unlikely, other cell types may be responsible, at least in part, for the γ-globulin production observed.

The basic finding presented in this article is that, although antibody production has been depressed to at least below detectable limits, in most hormonally bursectomized chicks, γ-globulin production and plasma cells are quite readily detectable. Studies with sera from normal 1–2-month-old chicks at dilutions of 1/4, 1/8, 1/10 and 1/16 indicate that the γ-globulin line is not usually detectable at dilutions greater than 1/10. Since the sera from most of the non-antibody-producing bursaless chickens contain detectable γ-globulin in dilutions of at least 1/4 (in many cases 1/8), it can be stated that the bursaless chickens synthesize at least 40 per cent of the normal amount of γ-globulin at this particular age. A comparison of antibody titres in normal and 14 bursaless chickens immunized with bovine serum albumin (Table 1) shows that no more than 1 per cent of the normal level of antibody has been produced in the bursaless chickens.

These results imply that in the absence of the bursa of Fabricius, although specific antibody production is not detectable, some form of γ-globulin synthesis is still occurring. Either most of this γ-globulin is 'non-antibody' γ-globulin or else it represents a summation of small amounts of many antibodies which are present individually in amounts too low to detect.

The question of the possible formation of non-antibody γ-globulin has arisen previously in regard to the small amounts of γ-globulin formed by patients with agammaglobulinæmia[5] and in several experimental systems. It has frequently been noted that stimulation of specific antibody production is accompanied by a rise of γ-globulin production which is well in excess of all detectable antibody synthesis[10]. Furthermore, in rabbits subjected to 400 r. whole-body irradiation 24 h prior to immunization with bovine serum albumin, even though no specific antibodies could be detected, an increased production of γ-globulin similar to that found in immunized unirradiated rabbits was observed[11]. In both these situations such an increase has been attributed to non-specific γ-globulin, the nature of which is quite unknown. The possibility has recently been raised that this may represent the non-specific stimulation of many other antibody-producing clones[12].

At the present time we can only speculate as to the precise defect in the chain of specific antibody synthesis in these bursaless birds. The development of the specific receptor site for the specific antigen, the correct mechanism of antigen binding to this site, or correct handling of the antigen by macrophages may in some way be under bursal control, and therefore render these birds incapable of specific antibody production. The rapid restoration of immunological competence to bursectomized birds by a humoral bursal product would be strong supportive evidence for these types of explanations, and is at present under investigation.

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RADIOBIOLOGY

Long-life Radioactivity in Occasional Thyroids

IN January and February 1964 radioiodine-131 in cattle and sheep thyroids of the northern hemisphere reached the lowest concentrations (< 0·5 μμc./g) and for the longest time in the past 10 years (see ref. 1 for previous results). The lack of interference by iodine-131 and improved instrumentation have permitted γ-spectroscopy of small amounts of long-life radioactive substances in thyroids and some of these could mistakenly be considered iodine-131. In the first two months of 1964, 1–10 per cent of 520 cattle thyroids were detectably radioactive; the glands were received, unselected, from slaughterhouses in several countries, and the sources reported here were (number radioactive/number not detectably radioactive): Cali, Colombia (10/95); Tokyo, Japan (6/94); South Central United States (2/250).

Specimens were screened for radioactivity by a single-channel pulse height analyser which accumulated the 0·3–0·4-MeV γ-radiation, detected by a 2-in. diameter sodium iodide (Tl) crystal with a sample well 1 in. diameter; this was shielded by 4 in. of lead. The samples, preserved with paraformaldehyde, weighed 15–18 g each and radioactivity was measured for 60–200 min. Occasionally radioactivity was found to be 50–100 per cent greater than the 4·5 c./m background. Repeated measurements of samples containing the most activity showed there was no detectable reduction in the radioactivity after two to four months, therefore the radioactive source was not iodine-131 (half-life = 8 days). Two or three samples from each of the above countries were investigated by a 200-channel pulse height analyser

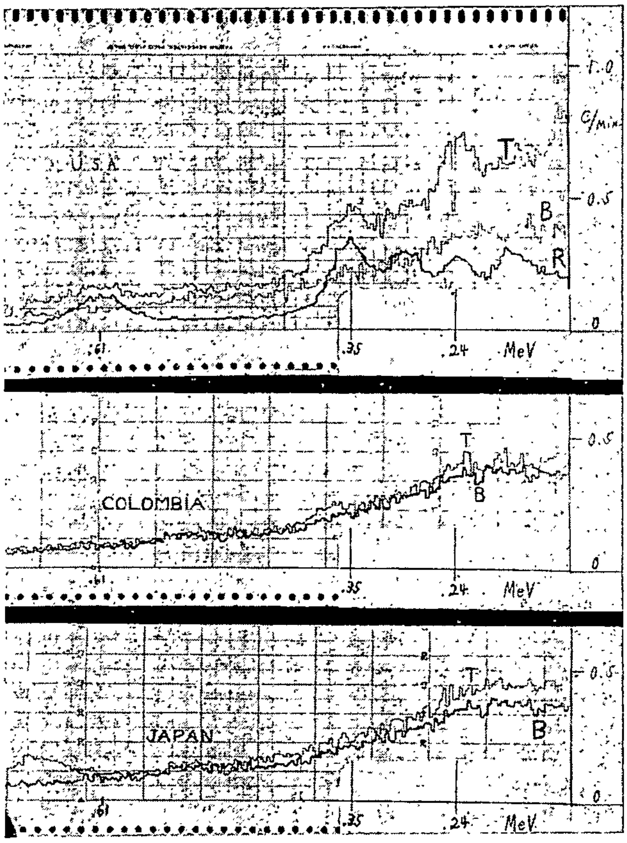

Fig. 1. Output of multichannel γ-spectrometer; 180 channels are shown, covering equivalent γ-energies of 0·13–0·70 MeV. Measurements were made for 10^3 min (16·66 h). The letter *T* is adjacent to the curves obtained from thyroid glands, *B* is adjacent to a 10^3-min background measured immediately before or after the sample, *R* in the top graph shows a spectrum obtained from a radium dial watch shielded by 1 cm water and measured for 10 min; therefore, for *R* curve, the ordinate represents 10^2 greater numbers than for other samples. A technical artefact appears on U.S.A. curve *T* at 0·24 MeV

connected to the 2-in. sodium iodide well scintillation detector. Radioactivity of each sample was accumulated for 16·66 h and Fig. 1 compares three of the results with background and with the radiation from radium (for calibration).

The thyroids from Japanese cattle showed a continuous distribution of γ-energies from the lowest energy to somewhat greater than 0·45 MeV; such a phenomenon is consistent with the Compton continuum to the maximum Compton edge, 0·48 MeV (ref. 2), which should appear in a thin sodium iodide crystal exposed to the 0·66 MeV radiation emitted by the Japanese glands. The glands from Colombia showed a small increase above background in 15 channels between 0·35 and 0·40 MeV; this was analysed by comparing the differences between the means of the 15 paired observations of sample and background counts in the 15 channels; the difference was highly significant ($t = 4{\cdot}1$ and $P \ll 0{\cdot}01$). The energy coincides with the predominant γ-radiation of iodine-131, but the half-life in excess of two months excludes the possibility of iodine-131. The thyroid from the United States had well-defined γ-energies near 0·35 and 0·24 MeV, both of which were suggestive of lead-214, which has a 14-min half-life and is a radioactive daughter of long-life radium-226 (which emits a small amount of 0·19 MeV γ-radiation).

Three thyroids from Colombia had γ-spectra lines like the gland from the United States in Fig. 1, but muscle from the same animals showed only background. Two samples of cattle muscle from Japan contained easily measured 0·66 MeV γ-radiation (probably cæsium-137), but no characteristic γ-peak at 0·3–0·4 MeV was observed.

At least three types of long-life radioactivity in thyroids of grazing animals are reported here: two of them are tentatively identified as cæsium-137 and radium-226; the third is still unidentified. Radium may concentrate in the thyroid and cæsium-137 is widely distributed, but their interference between 0·3 and 0·4 MeV can give a false indication of the 0·364 MeV γ-ray of iodine-131. A serious mistake could result if 0·3–0·4 MeV γ-radiations were measured many months after exposure to iodine-131 and if the observed activity were erroneously 'corrected' for radioactive decay of iodine-131. For example, if the gland from Colombia in Fig. 1 were tested 80 days after slaughter, the small amount of radioactivity above background would be multiplied by 1,024 to 'correct' for ten half-lives of iodine-131 decay. Logically, it follows that several different observations might be necessary to prove the existence of iodine-131 in thyroid glands.

Thyroids were kindly sent to our laboratory every 3–14 days from Dr. Palyo Correa, Cali, Colombia, R. Tanaka, Ibaraki-Ken, Japan, and Mr. John Anthony, Memphis, Tennessee. This investigation was supported by research grant *At*(40-1)-1643 from the U.S. Atomic Energy Commission and *Am-K*6-40 from the U.S. Public Health Service.

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Modification of the Radiation Effect on Ascitic Tumour Cells by Pharmacological Agents injected intravenously

THE modification of the effect of radiation on a murine ascitic tumour irradiated *in vivo* by pharmacological agents has been previously reported[1–3]. In those investigations the effect of radiation on the reproductive capacity of ascitic tumour cells was modified by direct injection of the pharmacological agent into the peritoneal cavity, the site of tumour growth. The experiments reported here were performed with intravenous injection of the agent to utilize physiological processes in the host (tumour bearing) animal to transport the pharmacological agent to the site of the tumour. It was our belief that although the concentration of the agent at the site might be limited, the technique would more closely simulate clinical situations where it is frequently not possible to achieve high concentration of a drug at the site of the tumour. The modifications of the effect of radiation on the tumour cells could be measured quantitatively by changes in radiation survival curve parameters.

The tumour used was the ascitic *P*-388 murine lymphocytic leukæmia of the *DBA/2JN* strain. Female mice 3–4 months of age were used exclusively for the routine serial weekly transplants of 10^6 cells injected intraperitoneally in 0·1 ml. volume of Tyrode's solution. The donor mice used in the experiments were also females injected in the same manner; the mice used for the bioassay of the cells after any given treatment were either male or female but of one sex only in each individual experiment.

The irradiations consisted of whole-body exposures of cobalt-60 γ-rays in an enclosed cylindrical irradiation chamber. The techniques have been described previously[4]. The exposure rates during the period of time over which the experiments were performed ranged from 266·3 r./min to 246·5 r./min.

The tumour-bearing donor mice were routinely irradiated 48 h after the intraperitoneal inoculation of the tumour. At this stage of growth the radiation survival curve parameters ($Do = 195 \pm 15{\cdot}2$; $n = 2{\cdot}2 \pm 1{\cdot}1$) (ref. 5) correspond reasonably well with an oxygenated population, therefore oxygen diffusion gradients and transport mechanisms across the peritoneal membrane are assumed to be physiological. Peritoneal invasion by tumour is not demonstrable at this stage of growth.

Three types of pharmacological agents were tested: (1) an alkylating agent (nitrogen mustard); (2) a combination of two sulphydryl compounds (AET + glutathione); (3) a halogenated pyrimidine (BUdR). The injections were made into the tail veins of the tumour-bearing mice with a tuberculin syringe through a 26-gauge needle, and the volumes injected ranged between 0·05 to 0·20 ml. The nitrogen mustard was injected 24 h prior to irradiation of the donor mice in doses of 3·0 mg/kg, 0·1 mg/kg, and 0·03 mg/kg. The AET (*S*, 2-aminoethylisothiuronium · Br · Hbr) + glutathione mixture in a 1 : 3 ratio was injected 10–15 min prior to irradiation in doses of 45 mg/kg of AET and 135 mg/kg of glutathione. This dose frequently caused convulsions and loss of consciousness but was followed within 3 min by apparent full recovery. Higher doses (60 mg/kg of AET and 180 mg/kg of glutathione) caused rapid death following intravenous injection. The powdered AET and glutathione were dissolved in distilled water and neutralized with sodium hydroxide to a *p*H of 7·4 immediately prior to injection which converts the AET to MEG[6]. The BUdR was injected in two doses of 200 mg/kg each at approximately 44 and 24 h before irradiation. The bioassay was performed immediately after the irradiation of the donor mouse using the dilution techniques described previously[7,8], and the recipient mice developing ascitic tumours within 40 days were scored as positive.

Fig. 1 illustrates the radiation survival points obtained after combined treatment with the pharmacological agent and radiation exposure. The survival curve of 2-day-growth tumour following radiation alone[5] is included for comparison. The survival points following treatment with BUdR as well as low. doses of nitrogen mustard (0·1 and 0·03 mg/kg) do not deviate significantly from the control curve. No cytotoxic effect was demonstrated due to drug alone with these low dose-levels of nitrogen mustard or with the BUdR. The radiation survival points following treatment with a greater dose, 3·0 mg/kg of nitrogen mustard, fall at lower levels of survival than the control curve, but do not indicate a significant change in slope from that of the control curve, which suggests additivity of effect. The survival points obtained after irradiation of the cells treated with AET and glutathione are located above the control curve at the higher radiation exposures, indicating a radioprotective effect. The limited amount of the data, particularly at the lower exposures, makes it difficult to ascertain whether the effect is expressed by a change in slope (less steep than the control), by a change in extrapolation number (greater than the control), or a combination of both.

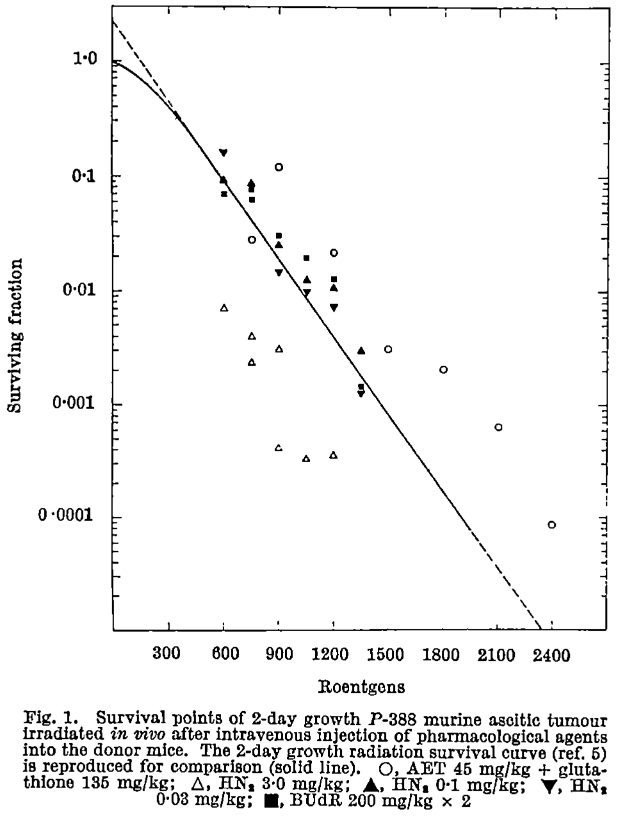

Fig. 1. Survival points of 2-day growth *P*-388 murine ascitic tumour irradiated *in vivo* after intravenous injection of pharmacological agents into the donor mice. The 2-day growth radiation survival curve (ref. 5) is reproduced for comparison (solid line). ○, AET 45 mg/kg + glutathione 135 mg/kg; △, HN$_2$ 3·0 mg/kg; ▲, HN$_2$ 0·1 mg/kg; ▼, HN$_2$ 0·03 mg/kg; ■, BUdR 200 mg/kg × 2

Potentiation of radiation effect has been readily demonstrated with 5-bromodeoxyuridine *in vitro*[9,10]; however, it has been difficult to demonstrate similar effects *in vivo* in spite of direct injection of the compound into the site of tumour growth, the peritoneal cavity, and over a longer period of exposure to the drug than was feasible in our experiments[1,3]. On the other hand, previous pharmacological investigations with halogenated pyrimidines including BUdR have shown that these compounds undergo rapid dehalogenation, principally in the liver, and only a small amount is incorporated into the DNA of normal tissue cells or tumour cells[3,11,12]. We assume this to be the reason for the fact that no potentiation of radiation effect was demonstrated with BUdR administered intravenously in our experiments.

The use of *P*-388 murine lymphocytic leukæmia in the ascitic form has been used to explore a system for quantitatively investigating the effects of combinations of pharmacological agents injected intravenously and irradiation while also demonstrating modification of the radiation effect. The system which we have used has the potentiality for development into such a quantitative combined therapy evaluation system. An additive effect with nitrogen mustard and a protection against radiation effect by AET + glutathione were demonstrated. No potentiation of radiation effect was observed with BUdR.

We thank Miss Marion Matthews, Mr. Bruce Weed, and Mr. Claudius Walker for their assistance in performing these experiments.

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BIOLOGY

Graft Transmission of the Flowering Stimulus from a Wild *Beta* Species to a Line of Beet selected for Resistance to Bolting

Successful transference of the flowering stimulus from a bolting beet stock to a scion bred for resistance to bolting has been reported previously[1]. In the work recorded here grafts of similar bolting-resistant scions were made to other species of *Beta* ready to flower. The plants were grown in a glasshouse where long days (16 h) and controlled temperatures (21°C day, 10°C night) were maintained.

In November 1963 early cotyledon stage scions of a line inbred for bolting resistance were inserted into leaf-axils of six-inch flowering scapes of *Beta trigyna*. Six *B. trigyna* stocks, grafted with three scions each, were kept in a humid atmosphere for three weeks, by which time the scions on three stocks had established themselves. In one, the lowermost scion (at the third node) showed stem elongation ten weeks after grafting (Fig. 1) and flowered eight weeks later. The uppermost scions remained dormant and were removed in the tenth week. In the other two stocks all scions prospered to produce large vegetative crowns with swollen stems about two to three centimetres in diameter. Adventitious budding in the stock was suppressed. Control scions inserted in vegetative stocks of *Beta vulgaris* and *B. trigyna* gave no indication of bolting under these growing conditions.

In an experiment in 1959, 65 seedling scions of a bolting resistant *B. vulgaris* line were grafted to members of the *Patellares* group (33 *B. patellaris*, 20 *B. webbiana* and 12 *B. procumbens*). No scions flowered even though the plants were kept from April to December in long-day conditions. Similarly, in 20 reciprocal grafts (10 *B. webbiana*, 7 *B. procumbens* and 3 *B. patellaris* grafted on *B. vulgaris* stocks), although the scions prospered and flowered freely, no flowering was induced in the stocks (Fig. 2).

Any internal flowering stimulus, if such exists, in *B. trigyna* must be the same as, or very similar to, that in *B. vulgaris*, but although the *Patellares* group are annuals in that they flower freely after producing four or five leaves, the stimulus to do so is either unnecessary and presumably lacking, or is incapable of being substituted for that in *B. vulgaris*.

Flowering can be induced in scions which are actively assimilating[1]. It was suggested that the difference in physiological age of the stock and scion might account for the difference between this and previously published observations. In the case of the grafts of *B. vulgaris* on *B. trigyna* there is further evidence that the degree of dominance of stock over scion may be important. In those grafts where the shoot of the stock became dormant, the scions remained vegetative. In another graft of bolting-resistant *B. vulgaris* on flowering *B. vulgaris* the scion flowered only after complete defoliation. Six days after the operation flower buds had formed and were beginning to open, although no stem elongation took place (Fig. 3). The last observation is in agreement with Stout[2], who assumed that the flowering stimulus could only affect scions where the *translocation* stream ran from stock to scion. In view of the flowering of actively growing scions, however, it seems that the dominance need not be related to the direction of translocation stream. On the other hand, it supports the theory of the production of a flowering suppressor in the leaves of the scion.

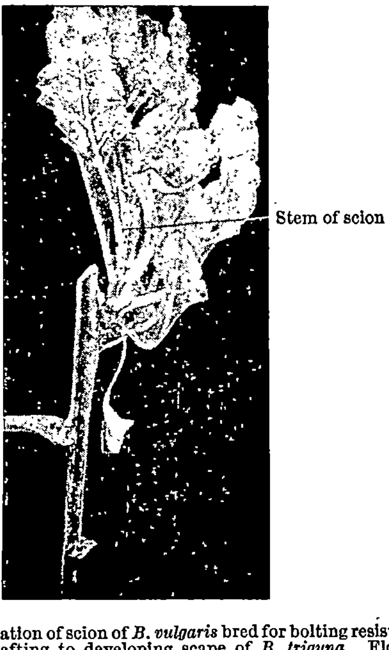

Fig. 1. Stem elongation of scion of *B. vulgaris* bred for bolting resistance ten weeks after grafting to developing scape of *B. trigyna*. Flowers formed eight weeks after photograph was taken

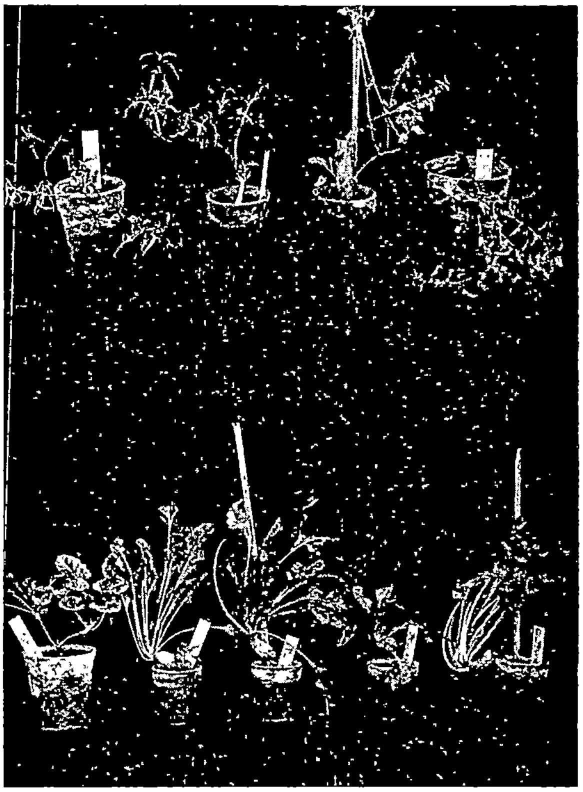

Fig. 2. Inter-specific grafts of wild species on *B. vulgaris* stock (top) and *B. vulgaris* on wild species stock (bottom)

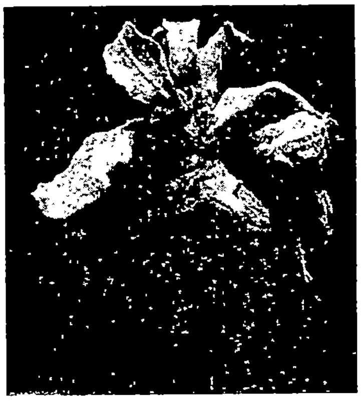

Fig. 3. Flowers produced 6 days after defoliating a scion which had not bolted

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Infection of Wheat Roots by Ascospores of *Ophiobolus graminis*

In 1933, Samuel and Garrett[1] published their observation that ascospores are violently ejected from the perithecia of the fungus *Ophiobolus graminis* (Sacc.) Sacc. and thus become air-borne; it had previously been supposed that these spores were merely washed down into the soil by rain. Samuel and Garrett, therefore, suggested that the association between outbreaks of the take-all disease of wheat in its whiteheads form and seasons of good spring rainfall in South Australia might be connected with the occurrence of suitable conditions for liberation and dispersal of ascospores, and for subsequent infection of wheat roots by them. Later, however, Garrett[2] reported that he had been unable to secure infection of wheat seedling roots by inoculation with ascospores when the seedlings were growing in unsterilized soil or sand. Infection was readily obtained, however, by inoculation of seedlings growing in completely sterile soil or sand. Garrett explained his results by postulating that the food reserves of an individual ascospore were insufficient for establishment of a progressive root infection, unless supplemented by the microbial nutrients liberated by soil sterilization, or by those provided by root excretions, which would be wholly available to the germinating ascospores in sterile, but not in unsterile, sand.

Garrett, therefore, concluded that infection by ascospores of *O. graminis* was unlikely to play any significant part in the epidemiology of the take-all disease. He pointed out that this disease can be satisfactorily controlled by starving the fungus out of the soil through the practice of crop rotation; this result would not be expected if crops grown on clean land could be regularly and widely infected by air-borne ascospores. In 1946, however, Garrett[3] was shown outbreaks of take-all in wheat crops grown on the recently drained soils of the Noord-Oost polder of the former Zuider Zee in the Netherlands. As described by Bosma[4], the freshly drained soil of a new polder is at first colonized by a sward of grass (and especially by species of *Agrostis*, which are known to be susceptible to infection by *O. graminis*). After ploughing up the grass sward, several crops of wheat are taken in succession; the first may show some patches of the take-all disease, and the second and third crops are often quite severely affected. It is virtually certain that the soil of any new polder, when first drained, is free from the presence of *O. graminis*, so that these observations by Bosma on the Noord-Oost polder once more raised the question as to whether the take-all disease could be spread to clean land by air-borne ascospores.

Although the negative results reported by Garrett in his ascospore inoculation trials seem convincing, several queries can be raised in the light of subsequent work. The dose applied by Garrett was generally 3×10^4 ascospores, applied in 1 ml. suspension; this suspension was either poured on to the surface of the soil around an established seedling, or added to the planting hole before sowing. Although this may seem a large dose, it is now known[5] that some fungal spores have individually a very low probability of infection, so that a massive ED_{50} is required. Secondly, it is known that soil exercises a strong filtering effect on spore suspensions, and it is also possible that placement of spores and timing of spore germination in relation to root emergence and growth may be critical for the occurrence of ascospore infection. With these considerations in mind, it was deemed worth while to make some fresh attempts at obtaining ascospore infection, directing particular attention to placement of spores and timing of spore germination in relation to root emergence.

Perithecia of *O. graminis* for these experiments were obtained both from naturally infected cereal stubble and from the roots of wheat seedlings grown over maizemeal–sand inoculum of the fungus in glass jars exposed to diffuse light. Wheat seedlings (var. 'Cappelle Desprez') were planted on the surface of moist, unsterilized soil or quartz sand in covered glass containers. After 6 days, the first three seminal roots of each seedling had emerged and were beginning to penetrate the soil; at this time the exposed surface of the proximal part of each root was inoculated with one drop (0·024 ml.) of a suspension containing approximately 16×10^4 ascospores/ml. After a further period of 7 days, the exposed and inoculated parts of the seedling root systems were covered with unsterilized soil or sand similar to that on which the seeds had been sown. After 3 weeks from the time of inoculation, all seedlings were washed out for examination of the root system. Most of the inoculated roots showed infection characteristic of the take-all disease; the vascular cylinder was discoloured, and the surface of the root carried dark-coloured runner hyphæ of *O. graminis*. From these inoculated roots, *O. graminis* was isolated in pure culture.

These experiments have shown that, at least under optimal conditions, ascospores of *O. graminis* can infect the roots of wheat seedlings. This finding, taken in conjunction with the observations of Gregory and Stedman[6] on the occurrence of these ascospores in the air spora, suggests the most probable explanation for the appearance of the take-all disease in wheat crops grown on the newly drained polders of the former Zuider Zee.

I thank Dr. S. D. Garrett for suggesting this problem to me. The work was carried out during my tenure of a postgraduate studentship given by the Agricultural Research Council.

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Gynodiœcy in New Zealand *Gentiana*

In March 1964 the junior author noticed that populations of *Gentiana bellidifolia* Hook. f., a perennial alpine species, were heterœcious in several places in South Island, New Zealand, mountains. Closer investigation revealed that the populations were gynodiœcious. We sampled a population in Rough Creek basin, Arthurs Pass, at 5,000 ft. by taking one flower from each plant along a transect of some 500 ft. and found the following numbers and proportions of the sexes.

	Hermaphrodite		Female
Number	82		27
Proportion	3	:	1

Some 'intersexes'—flowers with one stamen and four staminodes—were also present. There is no doubt of the functional gynodiœcy of these populations. The proportions of hermaphrodite flowers setting seed were not established because the collection was made too early in the season; but some individuals, both females and hermaphrodites, bore ripening fruit full of seed. Indications are that most hermaphrodites set seed, although a few hermaphrodite flowers with withered gynœcia were seen. No conclusions can yet be made about the mechanism for maintenance of the proportions of the sexes found in the sampled population (see ref. 1 for a brief discussion of the significance of the gynodiœcious breeding system).

The differential expression of sex in flowers of gynodiœcious species is often accompanied by size differences and other heteromorphic differences in floral parts. This pattern is followed by *Gentiana bellidifolia* although there is overlap in flower size between plants of different sex. Fig. 1 illustrates typical flowers of both types and Fig. 2

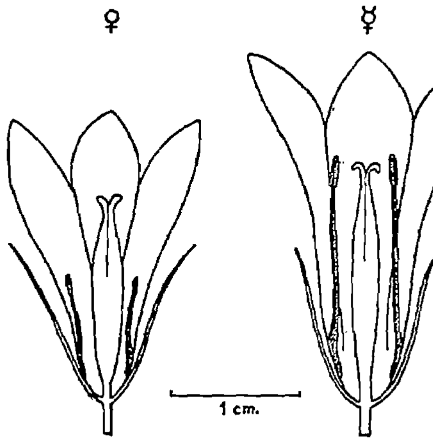

Fig. 1

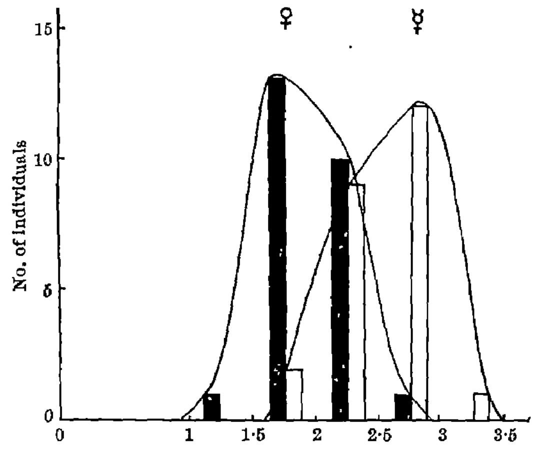

Fig. 2

shows the measurements of corolla length for 25 individuals of each sex.

So far as we are aware, this is the first time that gynodiœcy has been reported for members of the family Gentianaceae. (New Zealand gentians are not close congeners of northern hemisphere species. They belong to a natural group, possibly a distinct genus, found in New Zealand, Tasmania, Australia and South America.) Investigation of possible heterœcy in other members of the family is warranted, since most Floras describe the perfect flower as a family characteristic in the Gentianaceae.

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The observations of Burrows and Hobbs (preceding communication) direct attention again to one of the peculiarities of the New Zealand flora. As far back as 1880, Thomson[1] had observed that "species, genera and families which are characterized by hermaphrodite flowers in other parts are frequently unisexual here". Later work has brought to light a number of examples of gynodiœcy and other sex forms in New Zealand plants that were unknown to Thomson. In December 1961 I noted another in *Ranunculus*, a genus in which gynodiœcism had not hitherto been reported. A large colony of *R. limosella* F. Muell. ex Kirk consisting of many thousands of plants occupying several acres of the dried-up bed of Lake Forsyth on the Banks Peninsula was inspected and a pad of the lake mud was removed to observe the plants in detail. Some of the flowers in this sample were without stamens, and observations over the next few days on freshly opened flowers established that some purely female plants were present with the normal hermaphrodite form. A week later, the site was revisited with Dr. E. J. Godley and a search was made for female plants. None was found until the site of the original sample was rediscovered. At this point there was a small colony of female plants. It is evident that the proportion of female plants in the Lake Forsyth population as a whole was extremely small. A search for female plants in other localities established their presence over a wide area in Canterbury so that gynodiœcism appears to be a normal feature of this species.

According to Parsons[2], the proportion of diœcism in the South Australian flora is significantly greater than in European floras. This he attributed to climatic factors. Comparison with New Zealand, which has a widely different climate and a high incidence of sex forms, does not suggest that climate is of major importance. It is more probable that the important factors are biological and concerned with the pollinating organisms. In Australia brilliantly coloured flowers are abundant and are associated with a high frequency of bird pollination and a numerous fauna of large Lepidoptera. The few brilliantly coloured flowers in New Zealand are bird pollinated, while the majority are small, white or pale yellow in hue and adapted for pollination by flies and beetles; large Lepidoptera are scarce. One factor in common between Australia and New Zealand is the absence of any native large colonial Hymenoptera. Colonial bees in the northern hemisphere are a major factor in effecting cross-pollination, and many floral mechanisms in hermaphrodite plants help to ensure that outbreeding takes place. In the absence of colonial bees, the advantages of outbreeding can be obtained to various degrees by partial or complete separation of stamens and pistils. Conditions in Australia may be expected to favour the evolution of such sexual forms, while in New Zealand the small number of species of bird pollinators and of large Lepidoptera should tend to shift the balance even more in their favour.

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Free Sugars in Spores of *Pithomyces chartarum*

Very few analyses of free sugars have been reported in fungal spores. Owens *et al.*[1] found 9·3 per cent hot-water soluble carbohydrate in conidia of *Neurospora sitophila* but only 0·06 per cent free sugars, principally glucose. Other authors[2,3] have found higher amounts of free sugars in fungi. Sussman and Lingappa[4] found 10 per cent trehalose in ascospores of *Neurospora tetrasperma*, while other fungi[5] and yeasts[6] also contain trehalose, often as the main carbohydrate reserve. The conidia of *Neurospora sitophila*, however, apparently contain none[1]. Irani and Ganapathi[2] found free glucose, ribose and a trace of galactose in the mycelium of *Penicillium chrysogenum*. This report gives results of a study of the free sugars in the spores of *Pithomyces chartarum* (Berk. and Curt.) M. B. Ellis.

Spores were collected from rye–corn cultures[7] after drying at 40° for several days. Extraction of sugars from the spores by the usual method with hot 80 per cent (v/v) ethanol was found to be unsatisfactory. Despite thorough desalting, all paper chromatograms streaked badly. Extracts were therefore prepared by disrupting a suspension of spores (1 g in 5 ml. water) in a Hughes press[8]. After centrifuging the resulting homogenate, the cell debris was resuspended in water and recentrifuged, and the combined supernatants were ultrafiltered[9]. A small portion (*A*) of the ultrafiltrate was retained and the remainder (*B*) desalted with 'Biodeminrolit' (Permutit Co.) and concentrated.

The extracts were analysed by paper chromatography, using the solvent systems[10] (1) ethyl acetate–pyridine–water (2 : 1 : 2, top layer), (2) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), and the sprays[10] (1) silver nitrate–sodium hydroxide, (2) aniline hydrogen phosphate, (3) *p*-anisidine hydrochloride, (4) diphenylamine–aniline–orthophosphoric acid, (5) urea–orthophosphoric acid, (6) naphthoresorcinol–hydrochloric acid, (7) diphenylamine–urea–orthophosphoric acid[11]. The monosaccharides were best resolved by two-dimensional paper chromatography in solvents 1 and 2. Sugars on the chromatograms were measured quantitatively by the method of Wilson[12] using aniline hydrogen phosphate spray reagent. Fructose was estimated by comparing the intensity of its silver nitrate sport with those of known amounts of fructose.

Of the silver nitrate-reducing substances present in (*B*), seven were chromatographically identical with glucose, galactose, mannose, fructose, ribose, arabinose and xylose. Concentration ranges (mg/g) were: glucose 5–14, ribose 0·5–2·0, mannose 0·7, fructose 0·3, galactose 0·1–0·15, arabinose and xylose trace only (< 0·1). These sugars were present in approximately the same proportions in two extracts of spores grown on rye-corn and in two extracts of samples of spores grown on glucose and xylose respectively as sole carbon source. The only oligosaccharide present in measurable quantity was identified chromatographically as trehalose, which occurred up to about 0·25 mg/g spores. Its identity was confirmed by its non-reducing reaction with silver nitrate, and the appearance of a substance chromatographically indistinguishable from glucose as its sole hydrolysis product. Other neutral reducing substances present in the extracts did not react with specific sugar spray reagents and were not examined further.

Chromatography of the undesalted extract (*A*) revealed several silver nitrate-reducing substances, but only one was present in significant quantity. This latter substance was acidic, and gave colour reactions typical of sugars. Its constitution is as yet undetermined.

The present report extends the number of free sugars known to occur in fungal spores. In total, the sugars identified constitute approximately 1 per cent of the dry weight. In contrast to findings in some other fungi[4–6] trehalose was found to be only a minor constituent of *P. chartarum* spores. Studies on the polysaccharides are in progress and will be reported elsewhere.

Failure of the ethanol extraction, the classical method of extracting sugars from plant material was entirely unexpected and is as yet unexplained. I thank Dr. R. W. Bailey for his advice.

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Effect of Hydrogen Sulphide on *Bullia* (Gastropoda)

THE toxicity of hydrogen sulphide to mammals and certain other vertebrate animals is well known, and it is generally considered that the lethal effects of this substance are due in part to direct combination with the animal's hæmoglobin, leading to the formation of choleglobin and sulphæmoglobin, as well as to the inhibition of several enzyme systems associated with heavy metals. Invertebrate animals do not appear to have been investigated in this respect, excepting only *Arenicola marina*, the hæmoglobin of which does not combine with hydrogen sulphide but is a very active catalyst of sulphide oxidation[1]. By means of this, and possibly other, mechanisms, the worm is able to tolerate high concentrations of sulphide for lengthy periods.

The production of sulphides in marine sediments has been examined by Bruce[2], Galliher[3] and other workers, while a review of the subject is provided by Perkins[4]. Marine ecologists have noted the absence of macro-organisms from regions so affected and have tended to link this with the lack of oxygen in the 'black layers'. The present observations on *Bullia* were initiated by the disappearance of *B. laevissima* and *B. digitalis* from the Harbour Beach and the North Beaches respectively of Hout Bay, beaches on which these sand-burrowing prosobranchs were previously abundant (*vide* Brown[5]). The gradual disappearance of these molluscs coincides with the increasing organic pollution from the outlet of a fish-meal factory to which the Hout Bay beaches have been subjected in recent years. At the present time the northern beaches of the bay are virtually devoid of macro-organisms between tide-marks, the sand is blackened and disturbance of the substratum releases small quantities of hydrogen sulphide gas. Conditions are not, however, as extreme as have been observed in many relatively unpolluted South African estuaries[6,7].

Individuals of *B. laevissima* and *B. digitalis* were kept in jars of sea-water through which hydrogen sulphide was bubbled. From time to time individuals were removed and placed in fresh, aerated sea-water. All those removed after 0·5 h or longer of this treatment appeared dead and did not recover. Animals removed within 15 min all recovered, though movement had ceased. Of the animals removed at intermediate stages some recovered completely, others did not, and some appeared to recover but were found dead the next day. In a subsequent series of observations, jars of sea-water were bubbled with hydrogen sulphide, an animal placed in each, and the jars then closed without trapping any air. The controls were five individuals of each species placed in identical jars and similarly protected from the air, in water through which nitrogen gas had been bubbled. The test animals ceased all movement within 0·5 h, but those removed within 1 h and 10 min recovered completely. Six individuals removed after 2 h were already dead and of eight removed after 1·5 h only one recovered completely. The control animals were still active after 2 h and were only released the following day, after some 18 h. All showed complete recovery despite having been deprived of oxygen. Hydrogen sulphide is thus lethal to *Bullia* quite apart from oxygen lack, though the animals may be more resistant than are mammals. They do not, however, display a resistance comparable with the polychæte *Arenicola* which, according to Patel and Spencer[1], can survive up to 24 h under the foregoing conditions.

Hæmolymph was extracted from the cephalopedal sinuses of three *B. laevissima* and after being allowed to stand for a moment was placed in a Thunberg tube connected to an exhaust pump. On evacuation of the tube the usual colour change associated with deoxygenation of hæmocyanin was noted. The blood was then shaken in an atmosphere of hydrogen sulphide, left to stand, and again connected to the exhaust pump. I thought I could again detect the change from blue to colourless, but could not be certain due to the obscuring effect of precipitated sulphur. The tube was therefore connected to a Barcroft–Verzar respirometer immediately after allowing the air to rush in. The control tube contained an equal amount of gastropod Ringer of *p*H 7·8, which had been treated in the same way. The blood absorbed over six times as much oxygen as the control. On repeating the experiment with fresh hæmolymph the ratio was 7·5. Thus contact with sulphide does not appear to interfere permanently with the oxygen-carrying ability of *Bullia* hæmocyanin. On the other hand, I have not found any evidence to suggest that the animal is protected by the enzymatic oxidation of sulphide. When hydrogen sulphide is allowed to interact with *Bullia* hæmolymph, using the methods described by Patel and Spencer[1], a large amount of oxidation does, in fact, take place, but I find that this is not greater than occurs in controls consisting of gastropod Ringer of *p*H 7·8.

A final observation which may well be of some considerable ecological significance is that *Bullia* can detect quite small quantities of hydrogen sulphide and tends to avoid such pollution. When ten individuals of *B. digitalis* were placed in a tank, half of which had a substratum composed of sand which had had hydrogen sulphide bubbled through it while wet, all buried themselves in the other half of the tank, where the substratum had not been treated. This was repeated four times (with the same snails) with the identical result. If the entire substratum was treated or if hydrogen sulphide had been briefly bubbled through the water itself, the snails refused to burrow and eventually turned on to their backs and spread their feet, a position which, in the field, encourages transport by waves and water-currents[5]. Such behaviour might well account for the absence of *Bullia* on the beaches mentioned. The detection of hydrogen sulphide is not confined to the osphradium or some other specific chemoreceptory organ, for if a piece of cotton-wool is soaked in sea-water through which hydrogen sulphide has been bubbled, and then applied to any part of the head or foot of a snail which has been removed from the water, violent brushing-off movements are initiated and the proboscis is often everted and extended towards the affected region. The same reactions are used as a defence mechanism against would-be predators, but do not occur if cotton-wool soaked in unadulterated sea-water is applied to the foot.

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Adhesive Disk of *Echeneis naucrates*

Recently, Raghbir Singh*, director of fisheries, Madras, sent me a specimen of *Echeneis naucrates* for study. Having suggested[1,2] the presence of a pump at the hind end of the sucker, I dissected it under the binocular microscope and discovered two pairs of longitudinal pigmented canals one of which is broader and overlaps the basal plates on their ventral side towards their outer margins. The other is still further towards the outside, closely bordering the outer tips of the lamellar bars. Having no other specimens for study, I recently re-examined the two specimens sent to me by Dr. Mees, of the Western Australian Museum, Perth, and found that they had not been transferred to the preservative fluid after I had examined them last, so that they had become dried up. But on examining them now I noticed that grooves had formed on the disk in the position in which the outer pigmented canals are present internally.

Some time ago I gave a specimen to a local biological model maker to prepare a plaster of Paris impression of the sucker. He found that it adhered to the plaster and therefore tugged at the tail to get it off. While doing so the lamellar plates were partially opened, just as it happens when a live fish which is attached to a glass plate is pulled by the tail. I noticed no other change at the time in the specimen, which had dried up as the result of the heat produced in the plaster of Paris.

On further scrutiny of the Australian specimens the lateral grooves were intercepted at the hind-end by the presence of the pear-shaped pyriboss, but in the third the groove was continuous posteriorly without any interception. On trying to find out the cause of the difference I noticed that the pyriboss had shifted forwards and was fully accommodated in the posterior chamber exactly as I had pictured, ref. 2.

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Pigmentary System of *Diadema antillarum* Philippi

The echinoid *Diadema antillarum* Philippi shows both morphological and physiological (reversible) changes in colour[1,2]; the former is far more complex than previously supposed and the intimate mechanism of the latter may be, in part at least, of a type hitherto unsuspected.

Several kinds of pigment are involved, reddish or purplish hydroxynaphthaquinone[3] and pigment ranging in colour from golden yellow to black. The latter is chemically heterogeneous. Some shows the properties claimed to be characteristic of melanin[4–6], some appears to be chromolipoid, part is a newly discovered, iron-containing pigment of nuclear origin and part may be derived from the naphthaquinone[3].

Pigment accumulates, but not in a simple way. In the skin, the proportion of the various kinds alters: naphthaquinone becomes relatively more conspicuous. There is a turnover of melanin, some of which is discharged directly from effete pigment cells, some is excreted by amœbocytes[5] and much disappears in an unknown fashion. Such pigment is replaced, but not necessarily by the same kind; moreover, not all the melanin has the same origin. It appears in the young in finely granular form in the cytoplasm of epidermal chromatophores, but the melanin, which accumulates later, arises in association with characteristic spheroids, in the cytoplasm of amœbocytes containing phenolases[7], tyrosine[8] (the presumed substrate) as well as 3-indolyl compounds[6]. The existence of the last hints at the presence of ommachromes, but none was found.

Amœbocytes distribute the pigments widely. Much is egested, or left behind by their disintegration, as interstitial pigment in the connective tissue of the test, spines and viscera[3], as well as in the hæmal vessels of the axial organ[8].

Most of the skin pigment is contained in an extensive network of intercellular channels, with walls pervaded by a well-developed system of fibrils which appear elastic in living preparations. Such pigment is aggregated in chromatophores, apparently of the conventional type, which assume appearances varying from punctate to stellate, according to the prevailing lighting[1]. In reality the situation is very different. Only in the very young is cutaneous melanin wholly contained in cells (chromatocytes). They are located at the intersections (nodes) of the channels, along which their processes extend. Most of the chromatocytes soon degenerate, though they persist in some adults to varying extents, particularly in the vicinity of the pattern of iridophores[9]. Degeneration sometimes reveals a remarkable pattern of fibrils spanning the nodes formerly occupied by chromatocytes (Fig. 1).

The pigment in the shrivelled cytoplasm of degenerated chromatocytes becomes compacted to form large rounded masses of melanin constituting the primary pigment (Fig. 2), around which secondary pigment (*S*) accumulates progressively, due to the activities of amœbocytes. Such aggregations, cast in the mould of their cellular precursors, may be called 'chromatoglyphs' (γλυφίς). They embody all the varieties of pigment. Hydroxynaphthaquinone is often predominant, though it is discharged on fixation, and escapes when the channels in living skin are pierced by a micromanipulator.

As secondary pigment accumulates, the reversible colour change, so characteristic of the young, becomes obscured, though it may persist in adults in the vicinity of the iridophores[9].

In preparations of living skin, pigment undergoes continual movement so that parts of chromatoglyphs may become separated and later rejoin. Under the influence of light, pigment is dispersed from the nodes of the network until the tongues extending from adjoining nodal centres meet. When the pigment concentrates again in darkness, it may redistribute itself so as to flow into different nodal centres. Chromatophores may be cut across, and the separate pigment masses can continue to disperse and concentrate under appropriate lighting. The mechanism of movement is obscure. Since the chromatocytes lie free in the nodal centres of the channels, they could extend processes along them in amœboid fashion, but such a possibility would not explain the movement of the 'dead' chromatoglyphs, in which no active streaming movements could be seen during their change of shape. It is possible that the walls of the channels may produce the movements by exerting localized pressure on their contents.

Certain features of the observed movements recall those elegantly revealed by Yoshida in *Diadema setosum* (Leske)[10–12], in which the precise nature of the chromatophores is unknown, though they were assumed to be cellular. If, as in *D. antillarum*, there are chromatoglyphs forming part of an extensive network of channels, progress might be made in explaining some of the observations reported, which have proved difficult to interpret in conventional ways[11]. Thus, the effect of minute light spots on pigment-free areas of skin[10] might be due to motor effects in the channel walls. The apparent re-union of separated portions of chromatophores to form a functional unit[11], and the displacement over considerable distances of pigment *en masse*[11], are at least easier to envisage were there chromatoglyphs, rather than chromatocytes, in fixed locations.

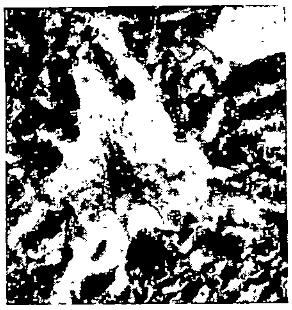

Fig. 1

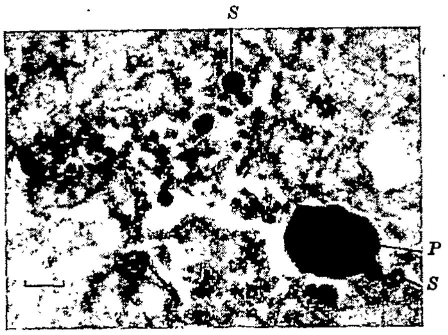

Fig. 2

Fig. 1. *D. antillarum*—a section of the skin in the aboral region of an interambulacrum, cut tangential to the surface, showing an early stage in the formation of a chromatoglyph. The central nucleus and surrounding fibrillar mass is left behind by the degeneration of a chromatocyte. Secondary pigment such as melanin has not yet invaded this region. Heidenhain's Susa fixative, Mallory's triple stain. Magnification as in Fig. 2

Fig. 2. *D. antillarum*—portion of a chromatoglyph as seen in a tangential section of skin, showing the compacted residual cytoplasm of a chromatocyte which forms the primary pigment (*P*) surrounded by secondary pigment (*S*). The pigment is selectively stained by Lillie's ferrous iron method for melanin, the tissue being counterstained in Van Gieson. Scale: 8·0μ

The investigation is being continued, and a full account will be published later. The early part of this work was undertaken in collaboration with Dr. Brenda Manly, whom I thank. I also thank Dr. M. Yoshida for comment and criticism.

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ENTOMOLOGY

Phyletic Significance of Some Insect Muscle Pherograms

The analysis of insect hæmolymph proteins has been shown to reflect relationships among species in several insect groups[1]. We have been unable to apply this technique to the bees because the quantities of hæmolymph in adult bees are too meagre to permit analysis. The knowledge that muscle extracts have been shown to be species specific in fishes[2] and are distinct between the lobster and earthworm[3] led us to examine the electrophoretic properties of muscle extracts from several species of bees. Since the thorax of bees is occupied predominantly by muscle, only this tissue was used.

Seven species, representing 5 genera of bees, were collected in the late summer and chilled in an ice chest to immobilize them until they could be quick frozen and stored at –18° C. The cockroach, *Periplaneta americana* L., was used as a standard because of its availability and was obtained from an inbred culture maintained by the department. The frozen bees were removed from the freezer and all of the body parts severed from the thorax. The thorax was opened, rinsed with distilled water and the muscle tissue immediately removed. The tissue was pre-minced with a scalpel and then macerated in a small-volume Potter–Elvehjem type tissue grinder with three times its volume of chilled 0·25 M sucrose for 10 min by hand. The homogenate was then centrifuged at 9,000*g* for 10 min. 15–18 μl. of the supernatant was removed and mixed with large pore sample gel which in turn was applied to the sample tube of a 'Disc Electrophoresis' apparatus (Canalco, Bethesda, Md.), as described by Ornstein and Davis[4]. The 'lower gel' concentration was 7·5 per cent. Electrophoresis of the samples was performed at *p*H 8·9 for 40 min at 2·5 m.amp/tube. The gels were stained for total protein for 3 or more hours in a 1 per cent amido-schwartz 10*B* dye dissolved in 7·5 per cent acetic acid and destained electrophoretically in 7·5 per cent acetic acid.

Representative scans of the protein extract patterns on a Model *E* Microdensitometer (Canalco, Bethesda, Md.) for each species are illustrated in Fig. 1; that for the cockroach is shown at the bottom for comparison.

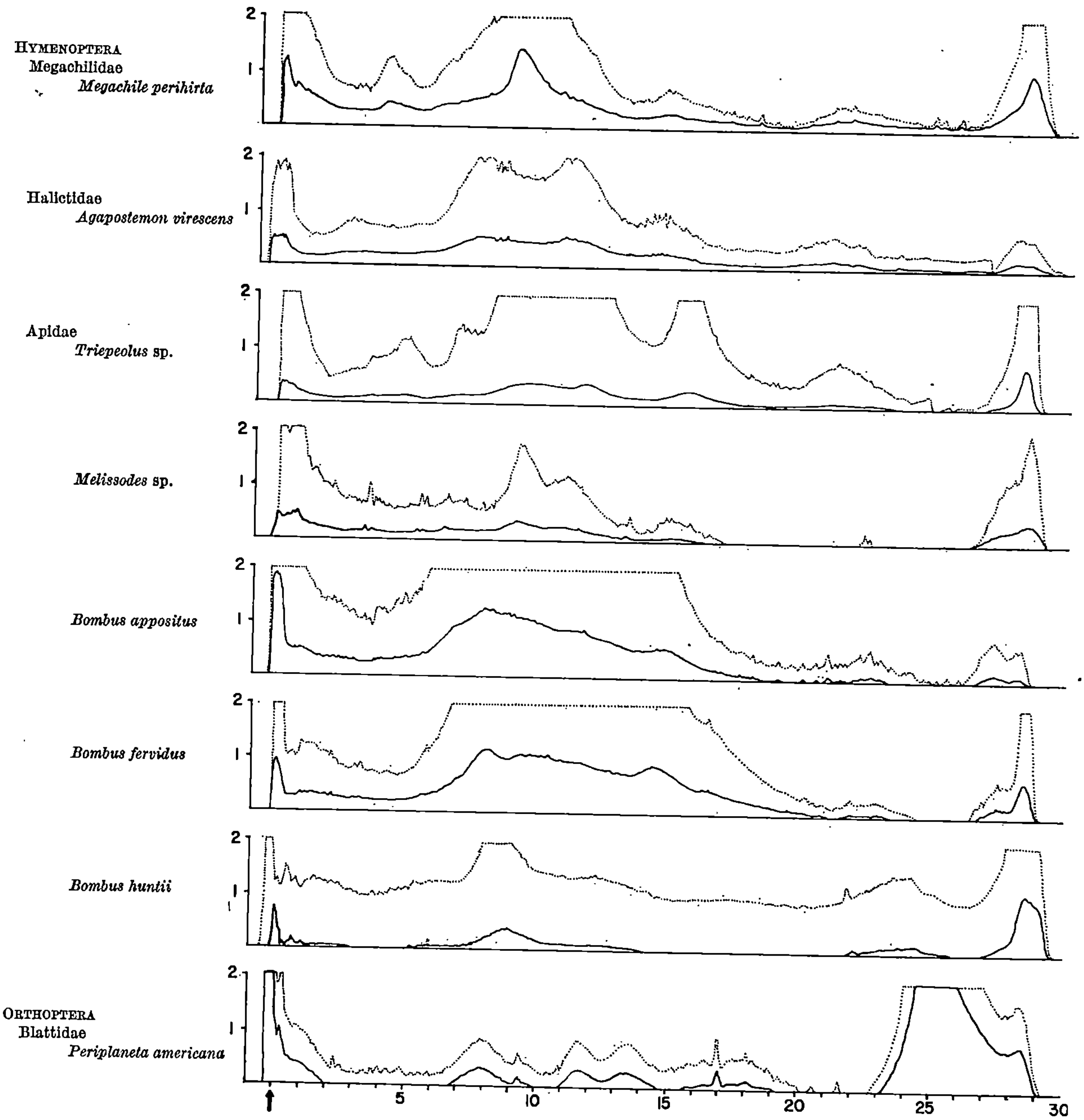

Fig. 1. Microdensitometric pherograms of sucrose extracted muscle proteins. The position at which samples entered the 'lower gel' is indicated by the arrow below the figure. The abscissa scale identifies fractions. The ordinate is optical density. Solid trace is at an optical density of 2 and the dotted trace at 0·2. The anodic area is toward the right

The patterns show a diversity in number and location of soluble muscle proteins from the different species. At this state qualitative differences only are considered.

A comparison of the pherograms of the Hymenoptera as a group with that of the cockroach (Orthoptera: Blattidae) illustrates a definite ordinal distinction. Quantitatively the bees have the major proteins intermediate in mobility while the two major fractions of the cockroach are its most rapidly migrating ones. Further, both groups possess six well-defined peaks of intermediate mobility, but only two or three of these appear to have similar mobilities.

The muscle protein patterns among the three species of *Bombus* resemble each other much more closely than they do any of the other genera, yet they exhibit species specificity. The scans of two of the subgenera, *B.* (*Subterraneobombus*) *appositus* and *B.* (*Fervidobombus*) *fervidus*, are more nearly alike than they are to the third *B.* (*Pyrobombus*) *huntii*. The amount of tissue in *B. huntii* is much less than that of the other two species of bumblebees, which accounts for the reduced concentrations of its fractions. These data suggest that the subgenera *Subterraneobombus* and *Fervidobombus* are more nearly related to each other than they are to *Pyrobombus*, a conclusion that agrees with the phyletic relationships as determined on morphological grounds. The major fractions of the four species of bees, representing the genera *Agapostemon*, *Triepeolus*, *Megachile* and *Melissodes*, closely resemble those of the species of *Bombus* in their mobility. The occurrence of several major fractions in the 6–17 range appears to be characteristic of the bees, contrasting with the trace amounts of these fractions and the excessive amounts of fractions 24–27 in the cockroach (Fig. 1).

There is no difficulty in distinguishing among the five bee genera on the basis of their pherograms, but comparison for the purpose of grouping the genera is premature at this time. The congeneric similarities of the *Bombus* pherograms, their resemblance to those of the other bee genera, and their marked dissimilarity to that of the cockroach suggest that the muscle myogens of insects may prove to be a valuable adjunct to the biochemical systematics of the Hymenoptera. The value of this technique as a tool in interpreting the interspecific, and more particularly the higher category, relationships among the bees and other Hymenoptera is at present being explored in this laboratory.

We thank W. S. Bigley for his assistance and F. W. Plapp, Entomological Research Division, U.S. Department of Agriculture, Corvallis, Oregon, for the use of the Model *E* Microdensitometer.

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A High Incidence of Dieldrin-resistance in *Anopheles gambiae* Giles from an Unsprayed Area in Northern Nigeria

IN the course of recent investigations in the northern Guinea savannah of Nigeria on the ecology[1] of *Anopheles gambiae* Giles and *A. funestus* Giles and on their behaviour in huts sprayed with insecticides[2], determinations were made of the susceptibility of both species to DDT and to dieldrin in a area near the town of Kaduna. Apart from very small-scale trials in which a total of 8 village huts were sprayed with DDT and a mixture of DDT and malathion, and apart from the fairly regular use of DDT (but not dieldrin or BHC) as a larvicide on streams in the township of Kaduna, the whole of the northern Guinea savannah can be considered an unsprayed area. The nearest area receiving regular insecticidal house-spraying is the Western Sokoto area, about 200 miles north-west, where DDT has been used since 1957. Prior to this both BHC and dieldrin had been used and dieldrin-resistance in *A. gambiae* shown to be prevalent from 1955[3]. Dieldrin-resistance was also found in *A. gambiae* in 1956[4] in the township of Kano, some 150 miles north-east of Kaduna, and was attributed to the use of BHC mainly for agricultural purposes.

One of us (M. W. S.) performed tests on blood-fed females of *A. gambiae* and *A. funestus* caught from village huts in Kangimi village, some 20 miles north-north-east of Kaduna, in September 1963, using the World Health Organization adult mosquito susceptibility test method. In late October, eggs obtained from gravid female *A. gambiae* from the same village were taken by air to the Ross Institute of Tropical Hygiene, London, where a colony was raised and further adult and larval susceptibility tests carried out by the other of us (G. D.)—adult tests between mid-November and the end of January 1964; larval tests at the end of February and the beginning of March 1964. The results of both field and laboratory testing of adult *A. gambiae* are given in Table 1. Those for *A. funestus* are presented elsewhere[5].

It has previously been shown with many different strains of *A. gambiae* that an exposure of adults to 0·4 per cent dieldrin in 'Risella' oil for 1 h kills all susceptibles but not heterozygous-resistant individuals, while an exposure to 4·0 per cent dieldrin for 1 h kills nearly all heterozygotes but no homozygous-resistant individuals, where the genetic factor involved is partially dominant in its expression[4]; the fact that the mortality at 4·0 per cent is considerably higher than at 0·4 per cent indicates partial dominance in this Kaduna strain. If, then, dieldrin-resistance in this Kaduna strain is the same as the partially dominant resistance previously recorded from other parts of Africa, including Kano and Western Sokoto, the field population consists approximately of 26 per cent susceptibles, 29 per cent heterozygotes and 45 per cent homozygous resistants, and the laboratory colony of 18 per cent susceptibles, 25 per cent heterozygotes and 57 per cent homozygous resistants. The similarity between field and laboratory results is close and the slight differences could be due to the greater survival rate in transit of eggs giving rise to resistant individuals. In addition, differences could be caused by differences in age composition of the two samples of mosquitoes used in the tests, and by differences in the physiological state at the time of testing (in the field only blood-fed females of unknown age were used; in the laboratory unfed females and males 1–3 days old were used).

Table 1. PERCENTAGE MORTALITIES OF WILD-CAUGHT AND LABORATORY-REARED ADULT *A. gambiae* FROM KADUNA, NIGERIA, EXPOSED TO VARIOUS DOSAGES OF DIELDRIN AND DDT IN 'RISELLA' OIL FOR 1 H

Insecticide and dosage (%)	Wild-caught, blood-fed females			Laboratory-reared (London) males and females (unfed), 1–3 days old		
	Total tested	No. dead	Mortality (%)	Total tested	No. dead	Mortality (%)
Dieldrin						
0·4	145	54	26*	62	11	18
0·8	149	69	36*			
1·6	182	100	47*			
4·0	191	118	55*	192	82	43
DDT						
4·0	103	103	100	182	165	91
Control	152	23	15	152	4	3

* Corrected mortalities.

Exposure of blood-fed females in the field to 4·0 per cent DDT in 'Rosella' oil for 1 h resulted in a complete mortality indicating susceptibility to this insecticide. In the laboratory some 10 per cent of unfed females and males escaped the action of this concentration and exposure time of DDT. This is not unusual in the laboratory with susceptible *A. gambiae* using the World Health Organization adult test.

Resistance to dieldrin and susceptibility to DDT were confirmed by the susceptibility testing of fourth-stage larvæ using the World Health Organization larval test method. The LC_{50} for DDT was less than 0·004 parts per million, while survivors occurred on all dosages of dieldrin from 0·0008 to 2·5 parts per million.

Thus, in an area never sprayed with dieldrin or BHC, and where other insecticides have been used only on a very limited scale, 74 per cent of the *A. gambiae* population is already resistant to dieldrin, 45 per cent being homozygous resistant. Such a high incidence indicates that the genetic factor for resistance is not only not a deleterious character but also that under the conditions prevailing in this particular area of northern Nigeria is of some advantage. Other investigations of the natural incidence of dieldrin-resistance in *A. gambiae* have also shown that this resistance is not uncommon in unsprayed areas[6], but nothing as high as the incidence here reported has been recorded before.

Crossings performed in London between the Kaduna colony and known mating-types A and B of the *A. gambiae* complex[7] show it to be an *A* form. Both the dieldrin-resistant strains from Western Sokoto and from Kano are *B* forms. This could indicate that the resistance in the Kaduna area is natural to that area and has not originated from areas further north, or that the resistance has originated from these more northern areas and spread through hybridization between *A* and *B* forms.

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Maturation and Egg-laying of the Rabbit Flea (*Spilopsyllus cuniculi* Dale) induced by the External Application of Hydrocortisone

In a previous communication[1] it was shown that maturation of the female rabbit flea could be induced by injecting castrated buck or virgin doe rabbits with a commercial preparation of the anterior lobe of the pituitary gland ('Ambinon *B*', Organon Laboratories, London). In Nature the rabbit flea can only mature its ovaries feeding on a doe during the last 10 days of pregnancy, or on new-born baby rabbits during the first 5 or 6 days of life.

Experiments have now been carried out to investigate which of the anterior lobe hormones controls maturation, and these strongly suggest that the principal factor is the adrenocorticotrophic hormone (ACTH), acting on the adrenal gland to produce corticosteroids above the usual blood-level. Injections into the host of two U.S.P.E. (1 ml.) daily for 14 days of the thyrotrophic hormone ('Actyron', Ferring *AB*, Malmo), and 0·5 mg/day in 3 daily injections of thyroxine ('Eltroxin', Glaxo Laboratories, England) for 14 days, also produce accelerated defæcation and limited ovarian development in the flea (but not full maturation and egg-laying)[2]. This is probably an indirect effect, due to the stimulation by thyroxine of the rabbit's adrenal cortex[3], since female fleas undergo normal maturation and egg-laying when feeding on pregnant and pseudo-pregnant thyroidectomized does. Massive injections daily of 2 mg, for 5–7 days, of œstradiol ('Oestroform', British Drug Houses, London) into the ovariectomized and thyroidectomized host also produce accelerated feeding of both sexes and full maturation and egg-laying of the female fleas in 5–7 days; but this again is possibly the result of a rise in the corticosteroid level in the rabbit's blood due to these abnormally large doses of hormone[4]. In Nature it is unlikely that maturation of the flea is directly influenced by œstrogens, since the ovaries of matured fleas regress rapidly if specimens are fed on does immediately after parturition[4,5], which is nevertheless a period when œstrogens must reach a high level in the rabbit's blood. Maturation is probably initiated and maintained only when the corticosteroids circulating in the blood reach a relatively high level. Thus, daily injections of the non-pregnant adult host (7 lb.), with 1·3 mg of cortisone or more, induced partial to complete maturation of the fleas, but 0·6 mg or less does not.

Negative results were obtained by injecting the host with purified extracts of luteinizing hormone and prolactin, and commercial preparations of progesterone, progesterone and thyroxine combined, oxytocin, testosterone and adrenaline. Full details of these experiments will be published elsewhere.

When it was determined that a daily injection (for 12 consecutive days) of six international units of a commercial preparation of the adrenocorticotrophic hormone ('Acthar', corticotrophin gelatin injection, Armour Pharmaceutical Co., London) or daily injections (for 4 consecutive days) of 1·3–5 mg of cortisone ('Cortisyl', Roussel Laboratories, London) into castrated buck rabbits, virgin doe and thyroidectomized doe rabbits produced uniform maturation of all the female fleas present on the host, an attempt was made to introduce the hormone directly into the parasite.

The fleas were removed from the host and placed in a glass jam-jar. One controlled dose, containing 0·2 mg of hydrocortisone from a new metered 'Hydrocortisyl' skin spray (Roussel Laboratories), was directed into the mouth of the container from a distance of 4 in. The fleas were then replaced on castrated buck and virgin doe rabbits. Maturation of all the female fleas occurred within 2–4 days, generally associated with exaggerated yolk formation; accelerated defæcation of both sexes was noted. The ovaries of these female fleas did not regress for 2–4 weeks, whereas those on hosts injected with 5 mg of cortisone daily, for 8 consecutive days, began to regress after 2–11 days after the injections were stopped. A similar experiment was performed in which the hydrocortisone was replaced by an equal amount of corticosterone. These results were also uniformly positive, although maturation was not so rapid nor yolk production so exaggerated as in the case of fleas sprayed with 'Hydrocortisyl'.

Sprays containing the excipient only, and progesterone instead of hydrocortisone, were used as controls. These sprays did not induce any sign of maturation or increased defæcating rates in the fleas.

The results of the experiments seem to show that hydrocortisone and corticosterone exert a direct influence on the male and female rabbit flea. It is possible, however, that the sprayed fleas could introduce sufficient hydrocortisone on their mouth-parts, while feeding, to produce an effect on the rabbit itself.

It should be stressed that there is a very close relationship between maturation and the feeding behaviour of the flea. In natural infestations on pregnant doe rabbits the defæcation rate of those fleas which are feeding while fixed to the ears (and counts are therefore practicable) is one fæcal pellet expelled every 15 min or longer. Frequently the interval may be more than 30 min. During the last 6–10 days of the rabbit's pregnancy the defæcation rate of both male and female fleas increases rapidly. By the last 2 days before parturition the defæcation rate of a fully matured female flea reaches one pellet or blood droplet (the fæcal matter at this stage is fluid or semi-fluid) expelled every 1–4 min. The same phenomenon can be observed in fleas matured on cortisone-treated hosts (injected with 5 mg) except that in this case the defæcation rate increases more rapidly. This probably reflects an increased feeding and digestion rate rather than merely a more rapid passage of ingested food through the gut. Mead-Briggs[6] has noted hypertrophy of the cells lining the mid-gut in maturing fleas.

When the host is injected with 5 mg of cortisone and massive doses of œstradiol the fleas quickly respond by becoming restless, and all but a few detach themselves from the ears and are found running about freely in the fur on the head or face of the rabbit. (They do not, however, desert the host for the nest containing young[4,5], as they do after parturition.) These fleas which are loose on the head mature less rapidly and less evenly than those which remain fixed to the ears. Feeding sites near the base of the ears, rather than the edges or the tips, produce the most rapid and even maturation.

Before the experiments with the Roussel sprays it was assumed that the fleas free on the head were not feeding so steadily and were consequently not ingesting such large amounts of corticosteroids as those which were fixed to the ears. Such an explanation will not suffice in the case of the sprayed fleas, which presumably absorb

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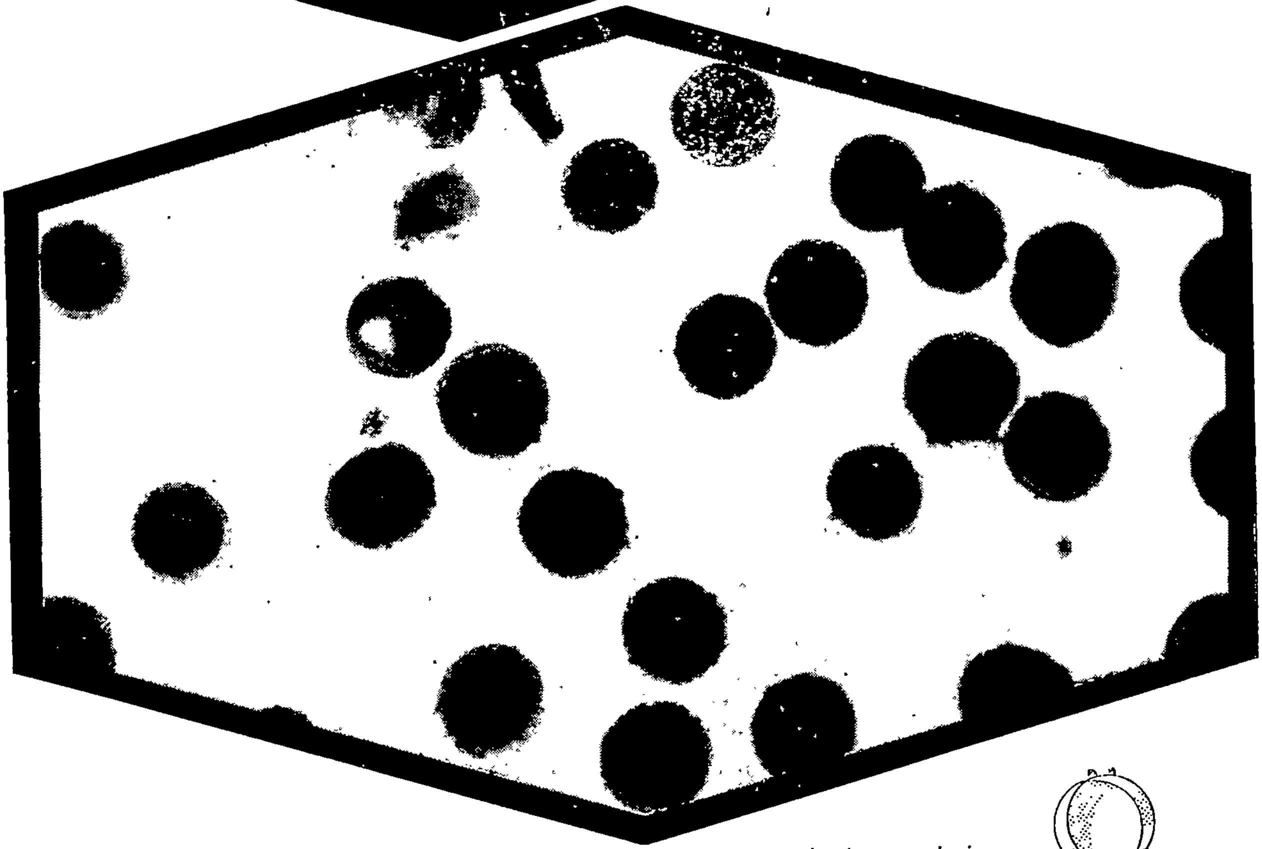

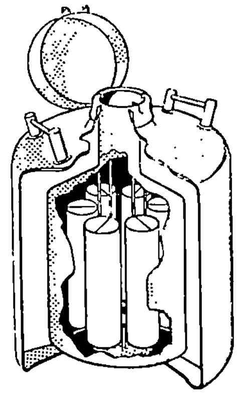

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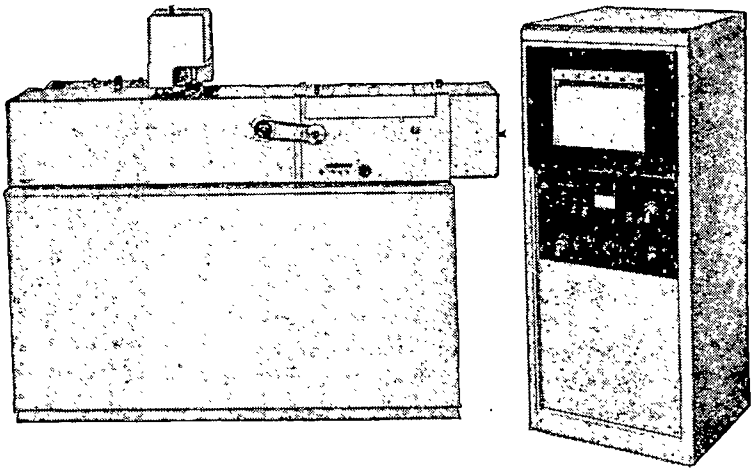

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the same amount of corticosteroids through their cuticle in either situation on the host. However, they probably ingest more blood when fixed, which may be significant. The relationship between the hormone, maturation, increased defæcation rate and feeding behaviour of the fleas requires further investigation.

It should be noted that on hosts injected with large doses of cortisone, maturation proceeds more rapidly and is maintained for a longer period than on hosts receiving smaller doses. In view of this fact the observations made on the course of maturation in Nature, when fleas mature in 8–10 days on the doe and in 3 days on baby rabbits[4], suggest that the corticosteroid-levels in the rabbit's blood follow a somewhat similar course to that said to be found in man[7]; a sharp rise possibly occurs during the last 10 days of pregnancy in the female rabbit, which gradually increases or at any rate is maintained until parturition, when it falls abruptly; the level in the blood of the new-born young would appear to be higher than in the doe immediately before parturition.

It was noticed that when the fleas were sprayed *in situ*, on the tip of an unshaved ear of the castrated buck rabbit, with 2 measured doses (0·4 mg of hydrocortisone) from the 'Hydrocortisyl' skin spray, their ovaries matured and had failed to regress after a period of 4 weeks on the host. New batches of fleas were afterwards introduced on to the head of the same rabbit and their ovaries matured although seven weeks had elapsed since the initial spraying of the host. It would thus appear that extremely small amounts of hydrocortisone, which presumably reached the skin via the covering of hairs on the rabbit's ears, remained active for considerably longer periods than the cortisone (5 mg) which was injected intramuscularly for periods of 10–11 days.

Experiments are now in progress with other groups of blood-sucking insects using the Roussel spray technique.

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MICROBIOLOGY

Host-range of Temperate *Serratia marcescens* Bacteriophages

It has been reported[1] that phages isolated from sewage against *Serratia marcescens* lyse a single strain each of *Escherichia coli* and *Shigella flexneri*. *S. marcescens* can receive[2,3] lac+ genes from *E. coli* and *Salmonella typhosa* via an episomic vector and may in turn transmit these genes to *S. typhimurium* and *Sh. dysenteriae*.

As part of an investigation to determine whether genes of *S. marcescens* can be transferred interspecifically via phage vectors the host-range of temperate *S. marcescens* phages was investigated. Sixteen N.C.T.C. strains, 1 American Type Culture Collection strain and 14 local strains of *S. marcescens* were examined for lysogeny by methods previously[4] used. Fifteen of the strains (including the American and 7 of the British) proved lysogenic for one or more of the 31 *S. marcescens* cultures. Twelve of the phages were obtained by ultra-violet induction and 3 were present in the supernatants of 10-day broth cultures. The 15 phages could be differentiated by means of their host-ranges.

The action of the 15 phages was tested on a series of *Proteus hauseri*, *P. morganii*, *P. rettgeri* and *Providence* cultures previously used[4] as well as on 12 *S. typhosa* Vi types, 28 other *Salmonella* serotypes, 48 strains of *E. coli* and 24 *Shigella* serotypes. The methods used have been described[4]. Although none of the *S. typhosa* cultures was affected 11 other *Salmonella* serotypes were productively lysed by one or more of all 15 phages. No abortive infections were encountered and the efficiency of plating was unity in all cases. The 11 sensitive *Salmonella* strains belong to the *B*, *C*$_1$, *F* and unclassified groups of the Kauffmann-White schema. No action was noted on strains of the other genera tested.

Many strains of *S. marcescens* are bacteriocinogenic and kill *E. coli* cultures[5,6]. This property of *S. marcescens* has been used[6] as an argument in favour of the classification of *S. marcescens* among the Enterobacteriaceae. The results reported here support this opinion.

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Antibacterial Interaction between Bromthymol Blue and Polymyxin *B*

Polymyxin *B* and bromthymol blue have been used in various selective and differential bacteriological media[1-3]. In the course of recent investigations of various experimental selective media for use in the enumeration and isolation of staphylococci from foods, it was noticed that *Staphylococcus aureus* and *Bacillus subtilis* grew on the surface of plates poured with polymyxin *B* media containing bromcresol purple and phenol red, but did not grow, or grew poorly, on plates of polymyxin *B* media containing bromthymol blue.

A number of experiments have since been carried out to enlarge on these observations because of the possibilities for useful employment of the polymyxin *B*–bromthymol blue interaction in selective media and for other purposes.

A basal-plating medium consisting of: 'Bacto'*-tryptone, 10 g; 'Bacto'-yeast extract, 5 g; *d*-glucose, c.p., 5 g; NaCl, c.p., 5 g; 'Bacto'-agar, 15 g; and distilled water, 1,000 ml., was utilized. Before autoclaving, the reaction of the basal medium was adjusted to *p*H 7·2 and 'Bacto'-bromthymol blue (BTB) was added in the form of a 1 per cent alcoholic solution[3]. The medium was sterilized at 121° C for 15 min and cooled slowly to 45°–50° C. Polymyxin *B* (PB) (obtained from Nutritional Biochemicals Inc., Cleveland, Ohio) was added aseptically as a Seitz-filtered 1 per cent solution. The final reaction of the medium was approxim-

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* Trade name for Difco Laboratories, Inc., Detroit, Michigan. The names of the manufacturers are given for materials solely for the purpose of identification and not as endorsement thereof by the Public Health Service.

Table 1. Effect of Increasing Concentrations of Polymyxin *B* (PB) on the Recovery of Three Bacterial Species

Organism	No. of colonies counted after incubation for 24 h at 35° C on basal medium containing polymyxin *B* at *p*H 7						
	PB concentration (μg/ml.)						
	0	40	60	80	100	120	140
B. cereus	1.6×10^7	1.3×10^7	1.5×10^7	1.6×10^7	1.6×10^7	1.6×10^7	1.4×10^7
P. vulgaris	1.3×10^9	7.6×10^8	5.4×10^8	5.8×10^8	5.9×10^8	5.2×10^8	5.6×10^8
Staph. aureus	7.7×10^8	8.0×10^8	7.8×10^8	5.4×10^8	5.5×10^8	4.7×10^8	5.2×10^8

Table 2. Effect of Increasing Concentrations of Bromthymol Blue (BTB) on the Recovery of Three Bacterial Species

Organism	No. of organisms counted after incubation for 24 h at 35° C on basal medium containing bromthymol blue at *p*H 7							
	BTB concentration (per cent)							
	0	0·001	0·002	0·003	0·004	0·005	0·006	0·007
B. cereus	1.6×10^7	1.7×10^7	1.9×10^7	1.5×10^7	8.7×10^6 *	7.5×10^6 *	4.7×10^6 *	2.1×10^6 *
P. vulgaris	1.3×10^9	1.3×10^9	1.3×10^9	1.2×10^9	1.3×10^9	1.2×10^9	1.2×10^9	1.2×10^9
Staph. aureus	7.7×10^8	7.0×10^8	5.9×10^8	6.9×10^8	5.3×10^8	5.2×10^8 *	4.5×10^8 *	3.1×10^8 *

* Colony size was reduced.

Table 3. Effect of *p*H on the Recovery of Five Bacterial Species on Media containing 0·003 per cent Bromthymol Blue (BTB), 80 μg/m Polymyxin *B* (PB) and the Combinations of these Two Agents

Organism	Plate counts after incubation for 24 h at 35° C											
	*p*H 6·2				*p*H 7·0				*p*H 7·6			
	Medium additives				Medium additives				Medium additives			
	None	PB	BTB	BTB + PB	None	PB	BTB	BTB + PB	None	PB	BTB	BTB + PB
B. cereus	1.3×10^7	9.3×10^6	$< 1.0 \times 10^4$ *	$< 1.0 \times 10^4$ *	9.3×10^6	1.2×10^7	6.8×10^6	$< 1.0 \times 10^4$ *	1.1×10^7	1.1×10^7	9.4×10^6	2.9×10^6
B. subtilis	6.1×10^7	6.1×10^7	$< 1.0 \times 10^5$ *	$< 1.0 \times 10^5$ *	8.1×10^7	7.1×10^7	3.6×10^7	$< 1.0 \times 10^5$ *	8.3×10^7	8.0×10^7	7.4×10^7	8.0×10^6
P. vulgaris	1.2×10^9	6.8×10^8	1.2×10^9	3.1×10^8	1.1×10^9	7.6×10^8	1.3×10^9	3.4×10^8	1.2×10^9	6.3×10^8	1.3×10^9	5.0×10^8
Staph. aureus	7.8×10^8	8.2×10^8	6.8×10^8	4.0×10^8	7.6×10^8	7.4×10^8	7.2×10^8	4.4×10^8	8.4×10^8	6.6×10^8	7.2×10^8	3.3×10^8
Strep. faecalis	1.4×10^9	1.3×10^9	1.1×10^9	$< 1.0 \times 10^7$ *	1.3×10^9	1.1×10^9	1.1×10^9	1.1×10^9	1.2×10^9	1.3×10^9	1.3×10^9	1.2×10^9

* No growth at the lowest dilution plated.

ately *p*H 7–7·2. The effect of *p*H on the interaction of BTB–PB was studied in three batches of basal medium prepared and adjusted to yield final reactions of *p*H 6·2, 7, and 7·6 after sterilization. Cooled media were poured into Petri dishes and allowed to dry overnight at room temperature before use.

Several PB-resistant test organisms representative of common Gram-positive, Gram-negative, and spore-forming types were employed. *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus* 196*E*, and *S. faecalis* were obtained from our collection. *Proteus vulgaris*, A.T.C.C. 4669, was obtained from Dr. C. K. Williamson, Miami University, Oxford, Ohio. Stock cultures were maintained by bi-weekly stab inoculations into tubes of low-nutrient, semi-solid medium[4]. Cultures were incubated at 35° C for 24 h and stored at room temperature. For each experiment, fresh transfers were made and used within three days as a source of inoculum. Transfers from these cultures were made into trypticase soy broth (Baltimore Biological Laboratory) and incubated without shaking for 24 h at 35° C. Serial dilutions of these broth cultures were made in phosphate buffered dilution water[5], *p*H 7, and 0·1 ml. of each dilution was spread over the surface of triplicate plates by means of a sterile, glass spreader bar. The seeded plates were incubated for 24 h at 35° C, and resulting colonies were counted on a Quebec colony counter. Each experiment was repeated at least three times.

Tables 1 and 2 summarize typical data on the effect of PB and BTB added singly to the basal medium. Recovery of *B. cereus* was not affected and *S. aureus* only slightly (above 60 μg/ml.) by PB alone. PB decreased the *P. vulgaris* count, but not in proportion to concentrations above 40 μg/ml. These findings compared with those of Pulaski *et al.*[6], who found the sensitivity of *Proteus* species to polymyxin *in vitro* to be greater than 100 μg/ml., and those of Finegold and Sweeney[1], who reported some *Bacillus* species to be resistant to 100 μg/ml. and *S. aureus* to withstand more than 100 μg/ml. The number and size of *B. cereus* colonies on BTB medium were significantly reduced only in concentrations of dye above 0·003 per cent. *P. vulgaris* was not affected by BTB. *S. aureus* colonies were reduced in size and/or numbers in the presence of 0·005 per cent or more BTB; a decrease in viable count of 50 per cent was noted in 0·007 per cent dye. For purposes of studying the BTB–PB combined effect, 0·003 per cent BTB best satisfied the criterion of a dye concentration below which, at *p*H 7, no visible influence was exerted on the most susceptible bacteria, but above which a biological effect was noted. PB at a level of 80 μg/ml. was somewhat arbitrarily chosen for the combined-effect study because it was: (*a*) within the range of slight effect on *P. vulgaris* and *S. aureus*; (*b*) no other clear-cut effect 'end point' was found (Table 1); (*c*) it was the concentration at which the first-chance observations were made.

Because BTB is an acid-base indicator, the effect on plate counts of BTB and PB was tested at *p*H 6·2, 7·0, and 7·6. Table 3 shows marked reductions in viable counts of *B. cereus* and *B. subtilis* on both BTB and BTB–PB-containing media at *p*H 6·2, which indicates that the effect of the combined agents was probably masked by the single effect of BTB at this *p*H level. The combined interaction of BTB and PB against the *Bacillus* sp. was quite apparent at *p*H 7·0 and still detectable at *p*H 7·6. BTB–PB inhibition of *P. vulgaris* was not so great as in the *Bacillus* sp. It occurred at *p*H 6·2 and 7 and was detectable, though less, at *p*H 7·6. The slight suppressive effect of PB alone on *P. vulgaris* noted in Table 1 was again apparent at all three *p*H levels. Viable staphylococcal counts were reduced by about 50 per cent at all three *p*H levels on BTB–PB media and *S. faecalis* counts were reduced at *p*H 6·2, but not at *p*H 7·0 or 7·6.

The results (Table 3) suggest that at *p*H 6·2 enhancement of the activity of BTB was primarily responsible for the observed inhibition by the indicator alone and in combination with PB, although other mechanisms, such as cellular changes that might have predisposed the organisms to the agent, are possible.

Since polymyxin is surface active[6], 'Tween 80' (80 µg/ml.) was substituted for the antibiotic singly and in combination with BTB in experiments similar to those presented in Table 3 to determine if a plain non-ionic surfactant would interact to inhibit bacteria. No significant inhibitory effect was noted at the 3 *p*H levels, except for a slight reduction (usually less than one-half log cycle) in the number of *S. aureus* colonies appearing on media containing both BTB and 'Tween 80' at *p*H 6·2.

Bactericidal action of polymyxin has been reported to be due to a combination with and disorganization of cellular structures that are responsible for maintenance of osmotic equilibrium[7]. Bromthymol blue (3,3′-dibromothymolsulphonphthalein) is an aryl sulphonate chemically similar to many synthetic anionic detergents. The effects of detergents on biological systems include inactivation of viruses, enzymes, toxins, and also bacteriostasis[8]. Like the anionic detergents, the efficacy of BTB in the present investigation was increased by acidity.

One indication of a possible mode of interaction between BTB and PB may be found in the studies of Newton[7] on the mode of action of polymyxin. When washed cells of *Ps. aeruginosa* were suspended by him in dilute solutions of the dye, *N*-tolyl-α-naphthylamine-8-sulphonic acid (which forms a fluorescent conjugate with proteins under ultra-violet light), no fluorescence was observed, indicating the absence of combining groups on the bacterial cell surfaces. Addition of polymyxin to such cell suspensions was followed by immediate fluorescence because of penetration of the dye into the protein-containing portions of the cells. The present experiments suggest that, although PB alone was not fully inhibitory to the resistant species studied, the antibiotic may have been able to exert sufficient disruptive influence on cellular structures to permit penetration of BTB, which exerted the main inhibitory effect. This and the possibility of formation of a PB–BTB antibacterial complex may be worthy of further examination.

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Survival of *Shigella* in Sea Water

THE survival of enteric bacteria in sea water has been extensively investigated[1–5]. However, there is a paucity of information on the survival of *Shigella* in sea water. We felt that an examination of the survival of *Shigella* species in sea water was important since it has been a common practice of communities situated on coastlines to utilize the adjacent oceans as a convenient receiver for all domestic wastes. There has been an increase in the use of ocean beaches and tidal areas for recreational activities and commercial shell-fishing; this, coupled with evidence that enteric infections are primarily water-borne, has necessitated certain precautionary measures. This communication records our work on the persistence of *Shigellae* in natural sea water, sea water treated in various ways, artificial sea water and saline solutions. The investigations were performed at various temperatures.

The *Shigella* strains used in these experiments were isolated from Eskimos in Alaska. The cultures were maintained in both nutrient broth and in a chemically defined medium[6]. The organisms were checked periodically by biochemical, morphological and serological methods for purity. Survival of the cells was studied by using a 24-h culture containing approximately 10^8 cells ml. *Shigella* cells were placed in test-tubes containing untreated sea water, filtered sea water, autoclaved sea water, and artificial sea water to determine longevity. In addition, survival was investigated in solutions of 5, 10, 15, 20 and 25 per cent sodium chloride.

Detection of viability was made by placing 0·25 ml. of the sea water into nutrient broth, incubating at 37° C for 24–48 h, and visually measuring growth due to the turbidity. Each experiment was performed in triplicate and the average values are reported. The artificial sea water used in these experiments was a modified formula of ZoBell[1].

The results on the survival of *Shigellae* in sea water are given in Table 1. In general, the organisms did not survive as long in natural, untreated sea water as compared with autoclaved or filtered sea water. There was a wide variation in the ability of different strains to survive in untreated sea water, that is, strain *B*-2501-3 survived more than 70 days whereas strain 12403 survived only 15 days. As a rule the *Shigellae* survived more than 69 days in autoclaved sea water and more than 70 days in filtered sea water. The survival of strain *B*-2547-3 in artificial sea water varied considerably depending on the temperature at which the cells were held (Table 2). Survival was longest at 15° C and 25° C and shortest at 8° C and 37° C.

The *Shigellae* were quite resistant to the osmotic effects of high concentrations of sodium chloride. However, survival depended a great deal on the holding temperature. For example, the cells survived for more than 25 days at −13° C and for only 4 days at 37° C at varying concentrations of sodium chloride. In some instances the *Shigella* were viable even when kept in 45 per cent sodium chloride for 25 days at 8° C.

Untreated, natural sea water has been reported to contain heat-labile toxic substances[7–10]. This could explain the reduction in survival times of *Shigella* in sea water compared with survival in autoclaved sea water. Some workers contend that antimicrobial substances are produced by the micro-organisms in the sea water[11–13]. Our results with investigations using bacteriologically

Table 1. SURVIVAL OF *Shigella* IN SEA WATER

Organism and strain no.	Survival period (days) at 15° C Untreated sea water	Autoclaved sea water	Filtered sea water
S. sonnei, 5819	33	> 69	> 70
S. sonnei, 7616	19	> 69	> 70
S. sonnei, 11439	38	> 69	> 70
S. sonnei, 12403	15	> 69	68
S. sonnei, 13036	71	> 69	68
S. sonnei, 13037	47	> 69	68
S. sonnei, 13038	51	> 69	> 70
S. sonnei, 13374	71	> 69	> 60
S. sonnei, 14785	19	> 69	55
S. sonnei, 16901	47	> 69	> 70
S. sonnei, 18317	68	> 69	> 70
S. sonnei, 18319	68	> 69	> 70
S. sonnei, 18564	68	> 69	> 70
S. sonnei, 18621	33	> 69	> 70
S. sonnei, *B*-2501-3	> 70	> 69	> 70
S. sonnei, *B*-2569-2	> 70	> 69	> 70
Alkalescens-dispar, 9775	> 71	> 69	> 70
S. flexneri 2*a*	72	> 69	> 70
S. flexneri 5*a*, 11434	> 72	44	> 70

Table 2. SURVIVAL OF *S. sonnei*, *B*-2547-3, IN ARTIFICIAL SEA WATER

Holding temperature (°C)	Survival period (days)
−13	43
8	23
15	> 106
25	> 106
37	23

Table 3. SURVIVAL OF *S. sonnei*, *B*-2547-3, IN VARYING CONCENTRATIONS OF SODIUM CHLORIDE

NaCl concentration (%)	Holding temperature (° C) −13	8	15	25	37
0	> 25	> 30	> 30	> 30	24
5	> 25	> 30	> 30	> 30	5
10	> 25	> 30	> 30	29	4
15	> 25	25	25	14	4
20	> 25	25	25	9	4
25	> 25	25	15	9	4

filtered sea water tend to corroborate this, since the removal of microbial life or some large particulate material by filtration allowed longer survival of *Shigella* in the water. The variations observed in the survival periods in untreated sea water suggest that different strains have different sensitivities to the agent(s) found in natural sea water. Carlucci and co-workers reported that *Escherichia coli* survived longer in heat- and filter-sterilized sea water than in untreated sea water[14].

In artificial sea water the *Shigellae* survived for only 23 days at 37° C compared to more than 106 days at 15° C and 25° C. One possible explanation for this is that the cells were undergoing steady metabolism and therefore death may have resulted from the exhaustion of metabolites. The frozen cells appeared to be preserved for longer periods of time than cells held at 8° C.

Shigella survived for 15 to more than 70 days in untreated sea water. Survival was longer in autoclaved and filtered sea water. The survival of the organisms in artificial sea water varied with the temperature of storage.

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PSYCHOLOGY

Units Sensitive to Direction of Movement in Goldfish Optic Tectum

THE retinæ of frog[1], rabbit[2], and pigeon[3] have recently been shown to contain a variety of ganglion cells which signal the direction in which stimuli cross the visual field. The response of such units to a small stationary spot of white light which is briefly flashed at some point within the receptive field has been generally found to be ON–OFF everywhere within the sensitive area[2–4]. Recently, certain units have been found in the retina of the goldfish, *Carassius auratus*, which give a similar pattern of response to stimulation with small stationary spots of white light[5]. However, in the goldfish, no attempt has been made to examine the response of these units to stimuli other than to brief flashes of stationary spots of light. The investigation recorded here examines the response to more complex visual stimuli, and especially the response to moving visual objects.

The fish was clamped between two foam-rubber pads and water containing urethane was circulated through the mouth and past the gills. A small opening was made in the skull to expose the optic tectum, and this was the only part of the fish which remained above water-level. The eye, which was below water, peered out through the transparent side of a 'Perspex' tank at a white screen. Small spots of white light, which subtended an angle of 0·5°, 1°, or 2° at the eye, were projected on to the screen; alternatively, small circular disks of black cardboard (2°, 4°, diam.), mounted on transparent glass rods, were moved in front of the screen by hand. A background illumination of the screen of 1·6 and 1·4 millilamberts was generally maintained during recording sessions to ensure that the retina was light adapted and to reduce the effects of scattered light. Woodsmetal microelectrodes[6] were used for recording action potentials from units in the superficial plexiform layer of the optic tectum. The characteristics of these units were similar to those recorded in the optic nerve and excised retina[5], and this suggests that the responses originated in the terminal arbors of optic nerve fibres.

As the microelectrode penetrates the dorsal surface of the tectum it passes through a region where initially no response is recorded to visual stimulation. The microelectrode then reaches a region where many of the units give responses of the ON–OFF variety to abrupt changes in illumination. Within this layer, many of the units have a concentric field organization which has previously been described for the goldfish by Wolbarsht *et al.*[7]. In units where the ON component of the ON–OFF response increases as one approaches the centre, movement of a small 1° spot of white light from the outside to the centre of the receptive field produces no response. However, the unit responds if the spot is moved radially outwards from the centre. In units where the OFF component of the ON–OFF response increases towards the centre, the unit responds to centripetal movement of the spot. The receptive field of these units subtends an angle of approximately 7°–10°.

Within the ON–OFF layer one also finds units that do not respond to changes in ambient light. Many of these are directionally selective in their response to movement and respond only if the spot of light or black cardboard disk crosses their field in a particular direction and display no response to movement in the reverse direction. Sensitivity to direction of movement is independent of whether the stimulus is lighter or darker than the background, and units respond well to both a small 1° white spot moving against a dark background or a small 2° black cardboard disk moving against a light background. The directional response is also largely unaffected by the level of background illumination (Fig. 1) and is independent of the part of the receptive field through which the stimulus is moved. The response to stimulation with a small stationary spot of white light is generally ON–OFF throughout the receptive field although the relative magnitude of the ON and OFF components at a particular locus may vary somewhat with successive stimulations. The receptive field of this type of unit subtends an angle of approximately 3°–5°. Table 1 presents the number of units which were directionally selective in their response to movement along the vertical and horizontal axes of the fish's visual field. Directional units which responded to horizontal

Table 1

	Direction of movement across visual field	No. of units
Horizontal axis	Temporo-nasal	32
	Naso-temporal	13
		45 Total
Vertical axis	Dorso-ventral	9
	Ventro-dorsal	14
		23 Total

movement were recorded almost twice as often as those which responded to vertical movement, and a χ^2 test shows the difference to be significant at the 1·0 per cent level. In view of the small number of directional units that were examined, any statistical inference drawn from these results must be tentative. However, the results suggest that the goldfish retina may be better equipped to detect horizontal movement within the visual field than it is to detect vertical movement. It is well known that fish possess a visually controlled reflex which allows them to compensate for drift and enables them to maintain a constant visual impression of the bank and bottom of the stream[8]. It therefore is possible that horizontal directional detectors in the goldfish retina participate in the control of this reflex. In addition, the data also suggest that the retina as a whole may be more sensitive to horizontal movement in a temporo-nasal direction within the visual field than it is to movement in the reverse direction (the difference is significant at the 0·5 per cent level). It is known that in a moving stream, a fish generally orients itself so that the head points upstream[8]. When this position is assumed, any tendency for the fish to drift with the current moves the image of the bank and bottom of the stream past the eye in a temporo-nasal direction thereby firing those directional units the sensitivity of which is to movement in this direction. If, indeed, directional units participate in the control of the 'rheotactic' reflex, then the fish's preference to orient itself with the head upstream might account for the fact that the retina is better equipped to detect movement in a temporo-nasal direction.

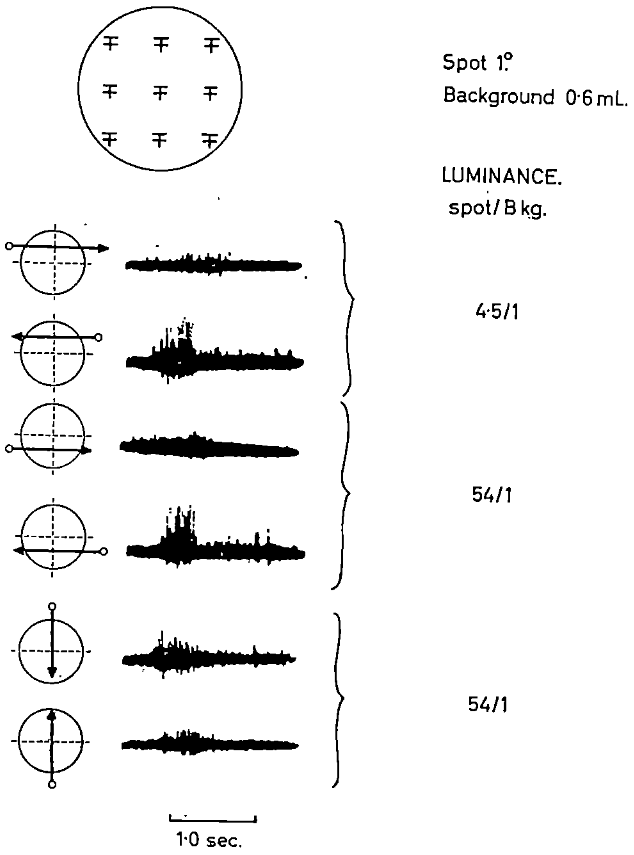

Fig. 1. Effect of intensity on directional response. The unit responds when the spot of light is moved through the field from anterior to posterior, but does not respond to movement of the stimulus in the reverse direction or to movement along the vertical axis of the visual field

As the microelectrode penetrates beyond the ON–OFF layer it enters a region where all recorded units give sustained discharges which signal the level of ambient light. These units are of two kinds, both of which possess characteristically large receptive fields of approximately 15°–18° from which responses of only one kind, either exclusively ON or exclusively OFF, can be elicited when the field is explored with a stationary spot. The sustained OFF units are similar to the dimming detectors described by Lettvin *et al.*[1] for the frog. These units signal a decrease in ambient light and continue to discharge for prolonged periods in the dark. The sustained ON units signal any increase in the level of general illumination and discharge rhythmically for a prolonged period in the presence of an illuminated field. The finding that ON fibres enter the tectum in the goldfish was a little unexpected since in the frog it is reported that ON fibres pass to the diencephalon and that the tectum contains only OFF and ON–OFF fibres[9].

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Visual Response to Gradients of Varying Colour and Equal Luminance

Are human visual responses to scenes of constant luminance paralleled by those to scenes of constant chromaticity? This question is of particular interest since MacNichol and Svaetichin have shown[1] that the responses to luminance and chromaticity are separated at the retinal level in the goldfish, and De Valois has shown that they are partly separated in the lateral geniculate body of the monkey[2].

In most phenomena luminance and chromaticity responses are parallel. Consider, for example, the phenomena of brightness constancy and colour constancy. However, Mach bands, which are present in the *Limulus*[3] as well as in man[4] in gradients of varying luminance, are believed to be absent in man in gradients of constant luminance and varying chromaticity. Thouless did not see bands in any of the pairs of colours which he tried, when they were of the same luminance[5]. Koffka and Harrower found bands with one combination of colours only, red on a grey background, and even in this case the bands were vague[6]. Mach himself does not appear to have investigated the question[4].

Mach bands can be seen in many situations, and are particularly evident in two, which will be described as

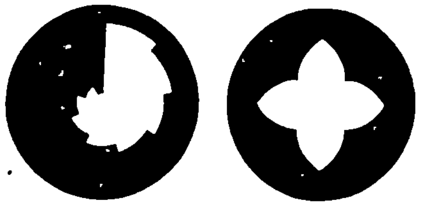

Fig. 1. Disks which, when rotated, show Mach bands. Left disk produces a step tablet, right disk produces a continuous gradient

the 'step tablet' and the 'continuous gradient'. A circular step tablet can be obtained by rotating a Masson disk made from black and white papers (see left half of Fig. 1) at a speed above flicker fusion frequency. This produces a series of steps, each of which is physically uniform, but most of which do not appear to be so. The centre step appears uniformly white, and the outermost step uniformly black, but each of the intermediate steps appears to be dark along the edge lying next to the lighter steps, grading into light along the edge lying next to the darker steps.

A continuous gradient can be obtained by rotating the shape shown in the right half of Fig. 1. In this case it is the centre and outermost areas which are physically uniform but do not appear to be so. The centre area appears to be grey, surrounded by a narrow white circle, and the outermost area to be dark grey, with a narrow black band on its inner edge. The annulus in between shades from white to black.

Gradients of colour are produced by replacing the black and white papers with coloured papers. If, in addition, the disk is illuminated by two projectors with filters over them and a rheostat attached to each of them, the colours of the filters corresponding to the colours of the papers, the observer can vary the relative luminance of the pair of papers continuously. Thouless, and Koffka and Harrower, did not have any means of varying the relative luminance of the papers which they used, in a continuous manner.

Previous investigations were confined to continuous gradients. Most of the following experiments were performed with step tablets, containing ten steps, each 9·5 mm wide. The luminance lay between 1 and 10 millilamberts, and the background was dark. Many combinations of papers and filters were tried: this report concerns six of them, each observed by five people. All observers had normal colour vision.

First the disk was set into rotation, and the observer set the rheostats so that all the steps appeared to him to be of equal brightness. Then he commented on whether or not he saw any Mach bands. These were seen as non-uniformities of the intermediate steps. In the case of a red to green step tablet, for example, the edge next to the greener steps appeared redder than the edge next to the redder step. The centres of the steps progressed from red in the innermost step to green in the outermost, through some unsaturated and neutral colours, rather than through yellow and orange. Next, the observer turned the rheostats slowly to change the relative luminance of the papers, and observed if there was any setting at which the steps appeared uniform, or disappeared altogether. He was asked to turn the rheostats until one paper was lighter than the other, then to turn them back until the second was lighter than the first. During this time his eyes were allowed to move as he wished. Finally, the rheostats were returned to the observer's original setting for equal luminance, and the wheel was stopped. The observer matched the colours of the two papers with samples in a Munsell book, which was illuminated by a Macbeth Illuminant *C* lamp in an isolated area beside the wheel. (In some cases the Munsell book did not contain a sample saturated enough to match the coloured paper on the disk, in which case an estimate was made. The Munsell specifications are valid only for papers of equal luminance. At other settings of the rheostats the Munsell lightness values change. The hue and saturation values change also, but only to a minor extent in the range used in this experiment, since none of the combinations of paper and filter overlapped by more than 15 per cent.)

The Munsell values of the six pairs of papers are given in Table 1. All observers saw Mach bands in all the combinations, regardless of the relative brightness of the colours, from a distance of 4 feet (angular subtend 27 min). The pairs of colours in these cases were, in common terminology, red and green, red and blue, green and blue, blue and yellow, red and orange, and red and greyish green. From a distance of 40 ft. (angular subtend of each step 2·7 min) all the step tablets appeared to be continuous gradients of colour. At distances between 4 ft. and 40 ft., the observer saw either a continuous gradient of colour, or a series of bands of alternating colours, two bands for each step. At no distance did any intermediate step appear uniform, unless the observer was so close that a single step covered a substantial portion of his field of view. All this parallels the results from step tablets of varying brightness, where the observer sees either a smooth gradient or a set of alternating light and dark bands, depending on his distance from the display.

Table 1. SPECIFICATION OF THE COLOURS IN MUNSELL VALUES, WHEN THE VARIACS ARE SET FOR EQUAL BRIGHTNESS

Values are the average for the five observers

Case	Inner paper	Outer paper
1	5 *GY* 7/9	10 *R* 7/9
2	7·5 *PB* 4/16	5 *R* 5/8
3	7·5 *PB* 5/14	10 *GY* 6/8
4	7·5 *PB* 4/16	10 *Y* 5/4
5	10 *YR* 7/10	10 *R* 6/14
6	10 *GY* 7/4	10 *R* 6/12

Six observers were also asked to look at a continuous gradient of constant luminance, using the two subjectively most different colours of Table 1 (case 1), and describe what they saw. Three reported Mach bands and three did not. All reported Mach bands when one projector was turned off, giving a gradient of varying luminance.

There is no conflict between the results of these experiments and those of Thouless, and of Koffka and Harrower, since the experiments described here deal primarily with step tablets, and previous experiments dealt solely with continuous gradients. The conflict lies in the implications of the results and the conclusions to be drawn from them. It seems reasonable to assume that the mechanism causing Mach bands in the step tablet is the same as that causing them in the continuous gradient. In the *Limulus* the mechanism has been shown to be one of inhibitory interaction between neighbouring ommatidia[3]: in higher animals which can distinguish colour, the nature of the mechanism has not yet been elucidated. My results suggest that the mechanism acts on changes of chromaticity in the same way that it acts on changes of brightness. This could be reconciled with the results of Thouless, and of Koffka and Harrower, by saying that the action of the mechanism is below threshold for continuous gradients of constant brightness.

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FORTHCOMING EVENTS

Monday, July 20

Zoological Society of London and the Anti-Locust Research Centre (at the Zoological Society of London, Regent's Park, London, N.W.1), at 10.30 a.m.—Symposium on "The Endocrine Control of Metabolism and Maturation in Insects".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer or Lecturer (with a special interest in one of the following fields: plant geography, cartography, human geography, or industrial geography) in the Department of Geography—The Secretary, University College, Gower Street, London, W.C.1 (July 15).

Research Students or Assistants (with degrees in physiology, biochemistry, chemistry, zoology or allied subjects) in the Department of Physiology and Biochemistry—The Secretary and Registrar (Room 9), The University, Southampton (July 15).

Assistant Cataloguer (graduate with good academic qualifications and experience or training in librarianship) in the University Medical Library—The Registrar, The University, Manchester, 13 (July 17).

D.S.I.R. Research Students in Physics for research on nuclear magnetic resonance under Prof. J. G. Powles—The Registrar, University of Kent at Canterbury, Westgate House, Canterbury, Kent (July 17).

Reader/Senior Lecturer, a Lecturer and an Assistant Lecturer (preferably with previous experience and research interest in one of the following fields: electron physics, wave optics, cryogenics, nuclear physics, solid state physics, or microwave physics) in the Department of Physics—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham, 4, quoting Ref. 376/6 (July 18).

Research Assistant (well qualified in sociology and interested in its application to education) to the Professor of Education—The Registrar, The University, Keele, Staffs (July 18).

Assistant Lecturer or Lecturer in Pure Mathematics—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (July 20).

D.S.I.R. Research Student (with a good honours degree in zoology) in the Zoology Section of the Department of Botany and Zoology, to join a group working on the ecology and physiology of protozoa with special reference to water pollution and purification—Dr. R. B. Purchon, Head of the Department of Botany and Zoology, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (July 20).

Laboratory Instructor (with a degree in chemistry and preferably some teaching experience, not necessarily in a university) in the Department of Physical Chemistry—The Registrar, The University, Leeds, 2 (July 20).

Biologist (Assistant Lecturer grade) to study growth characteristics of animal tumours by techniques which include autoradiography—The Director, B.E.C.C. Research Unit in Radiobiology, Mount Vernon Hospital, Northwood, Middlesex (July 21).

Senior Lecturer/Lecturer (dental, medical or science graduate with bacteriological experience) in Dental Microbiology—The Secretary, The London Hospital Medical College (University of London), Turner Street, London, E.1 (July 21).

University Librarian—The Registrar, The University, Manchester 13 (July 22).

Veterinary Officers (men and women aged at least 23, M.R.C.V.S. with at least one year's practical postgraduate experience) with the Ministry of Agriculture, Fisheries and Food, for posts in various parts of England—The Civil Service Commission, Savile Row, London, W.1, quoting Ref. 130/64 (July 24).

Lecturer in Clinical Psychology in the Department of Psychological Medicine—Secretary of the University Court, The University, Glasgow (July 25).

Post-Doctoral Research Fellow in Chemistry at Wollongong University College (an associated college of the University of New South Wales)—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (July 25).

Chair of Agriculture; and a Senior Lecturer-Reader in Agriculture at Makerere University College, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (July 30).

Lecturer in Agricultural Education; and a Lecturer in Agronomy (Oil Palm Research Division) at Njala University College, Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (July 30).

Assistant Lecturer/Lecturer in Cybernetics—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 14/G (July 31).

Lecturer or Assistant Lecturer in Physiology at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (July 31).

Lecturer (preferably with research interests in plant pathology) in Botany—The Registrar, The University, Manchester (July 31).

Lecturer (with an honours degree in pharmacy or equivalent qualifications, and preferably with a special knowledge and experience in pharmaceutical analysis and of the use of instrumentation methods) in Pharmaceutical Chemistry—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1 (July 31).

Research Fellow in the Department of Statistics of the Research School of Social Sciences, Institute of Advanced Studies, Australian National University, to work in the field of mathematical statistics and its application to other sciences—The Secretary, Association of Commonwealth Universities, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 31).

Senior Lecturer in Medicine—The Registrar, The University, Sheffield (July 31).

Senior Lecturer or Lecturer in Anatomy; and a Demonstrator (Assistant Lecturer) in Anatomy—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1 (July 31).

Senior Lecturer (with a good honours degree in geography and either a higher degree or actively engaged in research leading to a higher degree, and preferably some lecturing experience) in Geography at the University College of Townsville, University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, July 31).

Lecturer or Assistant Lecturer in the Computing Laboratory of the Department of Applied Mathematics—The Registrar, The University, Sheffield (August 1).

University Lecturer in Psychopathology—The Secretary of the Appointments Committee, Office of the General Board, The University, The Old Schools, Cambridge (August 1).

Lecturer/Senior Lecturer, and a Lecturer in Psychology at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 14).

Research Associate (normally with some post-doctoral experience) in the Physics Department, to assist in work in the area of solid state thin film physics, and will be encouraged to initiate new projects in this field—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (August 15).

Senior Lecturer or Lecturer in the Department of Bacteriology, University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (August 17).

Chair of Organic Chemistry at the University of Western Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 22).

Lecturer or Assistant Lecturer (with prospect of advancement to Lecturer) in Pharmacology in the Department of Pharmacology and Therapeutics, Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee (August 22).

Chair of Electrical Engineering at the University of Malaya, Kuala Lumpur—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, August 31).

Mycologist (with a good honours degree in botany or equivalent with considerable postgraduate experience in mycology and plant pathology, an authoritative knowledge of the taxonomy of the phycomycetes, practical experience of abstracting and editing an abstract journal, and a working knowledge of at least two European languages other than English) at the Commonwealth Mycological Institute—The Director, Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey (August 31).

Lecturer (preferably with field experience as a mine geologist or exploration geologist concerned with metalliferous deposits) in Geology—The Registrar, The University, Leicester (September 15).

Senior Lecturer and a Lecturer in the Department of Genetics—The Registrar, The University, Leicester (September 15).

Senior Tutor-Demonstrator in Biochemistry at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

Chair in Engineering Thermodynamics within the Department of Mechanical Engineering—The Registrar and Secretary, The University, Senate House, Bristol, 2.

Chemist or Biochemist (with a good honours degree) to work on the mutation of trace elements in man—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berkshire, quoting Ref. GEH/3.

Clinical Psychologist (Senior Grade) for duties which will include diagnostic and assessment work for the Psychiatric Departments at Salford Royal and Hope Hospitals—The Group Secretary, Salford Group Hospital Management, Committee, Fairhope Avenue, Eccles Old Road, Salford, 6.

Head (with high academic qualifications in physics with teaching and research or industrial experience) of the Department of Physics—The Principal, Huddersfield College of Technology, Huddersfield, Yorkshire.

Lecturer and an Assistant Lecturer in Pure Mathematics at the University of Uppsala, Sweden—Anthony Abrahams, 1 Garden Court, Temple, London, E.C.4.

Lecturers or Assistant Lecturers in Mathematics (two vacancies); and Lecturers or Assistant Lecturers in Applied Mathematics (two vacancies) in St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Scotland.

Post-Doctoral Fellow (spectroscopist, physicist or physical chemist) in the Department of Chemistry, to co-operate with Dr. A. J. Hyde on *the determination of molecular space-time correlation functions in liquids* using scattering from laser beams—Dr. Hyde, Department of Chemistry, The Royal College of Science and Technology, George Street, Glasgow, C.1.

Research Assistant (graduate in biology) in a newly formed Neurobiological Group (the work will be centred round cellular relations in peripheral nerves and spinal cord under normal and experimental conditions)—The Secretary, Institute of Neurology, The National Hospital, Queen Square, London, W.C.1.

Research Student (first- or second-class honours graduate in physics, chemistry or biochemistry) in Biophysics in the Department of Zoology, for biophysical research into the interaction of steroid hormones with micromolecules—Prof. I. Chester Jones, Department of Zoology, The University, Sheffield, 10.

Research Student (with a good honours degree in chemistry or physics or an equivalent qualification) in the Department of Physical Chemistry for work on the kinetics of capillary phenomena in collaboration with Prof. D. H. Everett and a post-doctoral fellow—The Administrative Officer (Mr. A. W. Honey), School of Chemistry, The University, Bristol 8.

Senior Electronics Technician in the Physics Department, to be concerned with the design and construction of electronic apparatus for research purposes—The Assistant Secretary, St. George's Hospital, London, S.W.1.

Senior Lecturer or Lecturer (with an honours degree in electrical engineering or physics and a sound knowledge of electronics, together with, if possible, experience of radar, guidance of missiles or telecommunications) in Electronics, to lecture to degree courses and specialized postgraduate staff courses and to supervise laboratory classes—The Registrar, Royal Military College of Science, Shrivenham, Swindon, Wiltshire.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Gateways: Guide to Courses of Further Education in Middlesex, 1964–65. Part 1: Vocational Courses. Pp. 53+x. Part 2: General Interest and Recreational Courses. Pp. 50+x. (London, S.W.1: Middlesex County Council, 1964.) [75

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TRAFFIC PROBLEMS AND INDUSTRIAL DEVELOPMENT IN BRITAIN

THE *South-East Study, 1961–81*, prepared by the Ministry of Housing and Local Government, was published at the end of March, together with a White Paper, *South-East England*, explaining how far the Government felt able to adopt the analysis of the problem and the proposal suggested in the *Study*. The commissioning of the *Study*, like the commissioning of the earlier studies of north-east England and central Scotland, and of the transport studies embodied in the Buchanan Report, is superficial evidence that the Government appreciates what is needed. Nevertheless, the successive White Papers and the debates on them in Parliament supply little concrete indication that the Government is prepared to take the necessary action, at least in time, and this impression is confirmed on every occasion when the Government is confronted with opportunities for immediate and effective action—as, for example, over the Morecambe Bay barrage and road scheme or the Liverpool Corporation's road scheme (see *Nature*, 203, 125; 1964).

To make sure that its traffic plans are adequate for the City's imaginative Shankland Plan, the City's Planning Department has commenced a traffic survey which will cost £75,000, and has shown that on its new road system alone the City of Liverpool must spend at least £100 million over the next twenty years, of which the City can provide £25 million. The rest must come from the Government; but for this the first opportunity of putting into practice the principles of the Buchanan Report, which the Government professes to support whole-heartedly, the Ministry of Transport at present intends to spend only £3 million over this twenty years on roads over the whole of Merseyside. Nor has the Ministry, while rejecting the regional proposals of the Buchanan Report, given any indication how the gap is to be filled, either at regional or at national level.

This weakness is outstandingly demonstrated in the way Mr. Marples rejected the Morecambe Bay barrage proposal of Mr. L. Leeming as an alternative route for the proposed extension northwards of the M6 motorway, but it is also illustrated in the White Paper, *South-East England*, and the debate on this Paper in the House of Commons on May 4. The *Study* assumes that between 1961 and 1981 the population of south-east England, which as defined in the *Study* lies south and east of a line between the Wash and Dorset, will increase by 3·5 million, of which 1·1 million will be due to net immigration. This will give Britain a population of at least 53 million in 1981, and, according to the White Paper, while not departing from its policy of channelling economic growth from the south-east to other parts of Britain, the Government accepts the basic objectives of the *Study*, including the need for new and expanded towns to accommodate up to 1·75 million people in ways providing effective relief for the pressures on London.

The Government also endorses the view expressed in the *Study* that high-quality agricultural land should not be taken for urban development wherever there is a practical alternative. In spite, however, of the attention directed to the heavy pressures on the green belt around London both in the *Study* and in a recent debate in the House of Lords, the White Paper will do little to remove misgivings already entertained in regard to that belt. It is clear that the Government is prepared to sanction certain inroads on the existing green belt, and the White Paper specifically states that the Minister of Housing and Local Government does not propose to approve proposals of local planning authorities for extending the green belt.

There is a reference in the White Paper to the possible effects on the region of the construction of a Channel Tunnel to which the Government has already agreed in principle. The plans for re-shaping the railway system to meet future demands are to include any proper through link between the Tunnel terminal and other parts of the country so that the benefits of improved communications with the Continent will be spread far beyond the south-east region itself. The White Paper also affirms the Government's intention, before reaching decisions on particular passenger closure proposals, to take full account of possible developments put forward in the *Study*. This at least is some advance on any earlier pronouncements relating to the Beeching Report, and, in particular, the Government now states that it will consider whether the possibilities of future development make it desirable that a line should remain available for future use if required.

It is recognized that the *Study* and its proposals raise many important issues on some of which public opinion outside the area must be consulted before decisions are taken. It is also clear that population pressures in the region are such as to demand planning now, even though the greater part of development in the expansion schemes will not take place until the 1970's. By determining the extent and location of public investment and industrial building, and by phasing the release of land for development in expansion schemes, it is claimed that the Government will ensure that the proper development of the south-east does not prejudice growth in other parts of the country. Its confidence in the organization for keeping under examination policies which have the most direct bearing on regional development is affirmed, as is its belief that regional planning can contribute greatly to the well-being and prosperity of Britain as a whole, and that this new programme and those already published and being implemented for Central Scotland and the north-east identify the special needs of the individual regions and contribute to the achievement and maintenance of a proper national balance (see *Nature*, 201, 458, 745; 1964).

In opening the debate on May 4, Mr. M. Stewart said that a national policy, such as this and other studies implied, called for a more stringent use of industrial development certificates, greater co-ordination in the activities of Departments, including the co-ordination of decisions on transport with general policy affecting the location of industry, and a thorough review of local government finance. Mr. Stewart stressed particularly the importance of finance and especially in regard to the acquisition of land, and in this connexion was the first of several speakers to refer to the siting of offices. This was admitted by Sir Keith Joseph, who followed, and he suggested that here automation might soon have an ameliorating effect. He also said that the Government was determined to move out of London as many as possible of the headquarters of civil servants: the decision to move the Post Office Savings Bank to Scotland was the latest large example. The Government was also determined to persuade firms that as much office work as possible should be moved out of London, and preferably out of the south-east region. The tough policy of the Board of Trade with regard to Industrial Development Certificates, which would continue, was a further factor towards the present disproportionate draw to the south-east.

For the rest, Sir Keith said that the Government recognized growth in the region was likely, and orderly provision for it must be made so as to improve life for all in the south-east, and in particular to ensure good housing, either in London or elsewhere, for all born in London or already living there. It accepted the need to relieve the pressure on London and to break the vicious circle of growth creating more growth. The strategy now was to provide attractive, vigorous big communities well outside London and well outside the green belt on a scale to cope with the foreseen overspill of more than one million. Moreover, these new communities would not consist of one section of the population but would comprise members of nearly every income group who could not find in London suitable accommodation at a price they could afford. He pointed out that more than 85 per cent of the acreage in the south-east was at present undeveloped, and by 1981, if all the proposals of the *Study* or their equivalent were accepted, more than 83 per cent would remain undeveloped. There would be careful consultation with all the planning authorities and local authorities concerned, but the Government hoped to reach a firm decision on the total to be provided by individual projects by the end of the year.

On office accommodation, he added that the first six months' working of the Location of Offices Bureau warranted the hope that in the next few months and over the short period of years ahead the Bureau might manage to offset by persuasion of individual offices a large part, if not all, of the increased office work in London presaged by planning permissions already given. No Government could allow the growth of office jobs to continue at its present rate. Finally, he admitted that possibly a new town type of corporation might be required to deal effectively with some of the new proposals and also that we needed an agency to acquire the land, make the plan, provide the services and then dispose of the land according to the plan, either by selling or letting to private or public enterprise. Re-zoning sufficient land for the entire period should suffice to give public and private authorities confidence that land would be available when required.

While there was some keen criticism of the adequacy of the Government's proposals in the debate, particularly in regard to the acquisition of land, the restriction of office buildings and co-ordination of policy, Sir Keith Joseph's statement embodied some definite assurances, and his speech was supported at some points by that of the Parliamentary Secretary to the Board of Trade, Mr. D. Price, who wound up for the Government. On immigration, Mr. Price observed that overseas immigrants, especially in the Greater London area, had been filling posts in the service industries which without them would have broken down. While it would be unrealistic to expect a substantial exodus of offices out of the south-east as a whole, he maintained a substantial move of offices from Central London to anywhere else in the south-east would be a great gain. He reiterated that the Government's proposals were consistent with its plans for the development of central Scotland and north-east England and elsewhere.

There were five factors safeguarding development outside the south-east. First, the developing regions where the needs were greatest would have a long start. Programmes were already being implemented for central Scotland and the north-east of England, whereas the major schemes in the south-east would not make their full demands for capital investment and labour until the 1970's. Further, the Industrial Development Certificate policy would continue to give first priority to the development districts, and, subject to this, would seek to get industry out of the main conurbations into new and expanded towns which are receiving their populations. Next, the north-east of England and central Scotland which, with 13 per cent of the population, had 18 per cent of the public investment programme, would continue to receive such priority for some years. Present inducements to attract industry to development districts would remain and the growth zones in both these regions had received assurances of a sustained and continued effort. Mr. Price saw a fifth safeguard in the extent to which the town expansions in the south-east would generate their own employment. Finally, he said that the study teams for Wales, the west midlands and the north-west had reached the stage of detailed local consultations. There would be comprehensive regional studies covering changes in population, industrial structure and employment, land use and urban renewal and communications, and a study for the East and West Ridings of Yorkshire was being considered by the Secretary of State for Industry and Trade. No decisions had yet been taken on the places suggested for expansion in the *South-East Study*; but while Mr. Price declined to give a flat undertaking that Industrial Development Certificates in these towns would not be given to firms from the north or west, he undertook that they should be limited essentially to firms from London.

However reasonable the Government's position may appear as presented in the White Paper and in this subsequent debate, confidence is badly shaken by its failure to take effective action on the Buchanan Report and the co-ordination of traffic policy with other national needs. The *South-East Study* stands at marked issue, for example, with many recommendations of the Beeching Report to which the Minister of Transport has repeatedly declared his support, while the Minister's short-term policy on road transport has been severely criticized by Sir Robert Matthew, professor of architecture in the University of Edinburgh. Mr. F. Medhurst, in a university extension lecture, has pointed out that a regional development authority set up by the Government could be expected to execute the proposed Morecambe Bay barrage scheme. The scheme involves the creation of a freshwater lagoon, a barrage able to carry a motorway and railway tracks, and a dozen square miles of salt marshes would be cleared of salt and made available for agriculture.

These factors would accordingly involve the River Board, the Ministries of Transport and Agriculture, if not the Ministry of Industry and Trade also, as well as British Railways, the Lancashire County Council and Manchester Corporation. The prescience of Mr. Medhurst's comment that each of these would be likely to examine the scheme in a particular aspect and against limited alternatives is demonstrated as clearly by Mr. Marples's subsequent brusque rejection of the Plan as by the inadequacy of the Government's whole approach to such problems. Mr. Marples declined to consider it as an alternative to the inland route for the motorway to Scotland and intends to proceed with the route running east of Shap through Tobay.

Mr. Marples did not dismiss entirely the coastal route over the barrage, but while he emphasized the importance of the time-factor, he was more than a little naïve in suggesting that the barrage route must be considered as a regional project. Mr. Medhurst's lecture makes nonsense of that idea and Mr. Marples's hasty refusal should stimulate the technical colleges and universities in the north-west to take up urgently Mr. Medhurst's suggestion and undertake some of the research needed within the scope of the scheme, presenting the findings to the Government with a request for combined regional action. Meanwhile, Mr. Marples's decision should be firmly challenged, and every effort made to expedite the surveys and enquiries which are necessary before technical decisions on the scheme can be reached. It is also essential that the full implications of the scheme, not only in respect of road transport between England and Scotland but also no less in respect of water supply for Lancashire, and the revival of industrial west Cumberland, not forgetting the marginal implications in respect of agriculture and land utilization, should be brought to public understanding. If such a scheme is to be rejected it should be in a rational perspective and not through departmental myopia.

A GREAT ENGLISH BOTANIST

Joseph Dalton Hooker

Botanist, Explorer, and Administrator. By Dr. W. B. Turrill. (British Men of Science.) Pp. xi+228+25 plates. (London: Thomas Nelson and Sons, Ltd., 1963.) 21*s.* net.

JOSEPH DALTON HOOKER was twenty-two at the start of his Antarctic voyages in 1839: in 1911 his botanical work came to an end only a few months before his death at the age of ninety-four. From that long life one can select four chapters for comment: Antarctic botany, Indian botany, the association with Charles Darwin, and the directorship of Kew. Any one of these would rightly give Hooker a place in the series, *British Men of Science*, and Dr. Turrill's achievement in condensing all this, and more, into 228 pages is considerable. Author, editor and publisher all earn our gratitude for presenting, at a reasonable price, this concise and interesting account of a great botanist.

The Antarctic and Indian phases are well summarized and numerous passages in Hooker's own words add authentic flavour to the story of the discomforts and the rewards of pioneer botanical work in the field. Hooker not only wrote vividly of his experiences, he was always busy with his sketch books, and selected pages from these provide the most attractive illustrations in this volume. In telling us of Hooker and his work, Turrill says enough about the items he selects for comment to make them botanically informative. For example, Hooker's account of the Kerguelen cabbage (*Pringlea antiscorbutica*) is brought up to date with more complete information on its distribution and vitamin C content. Throughout, there are frequent references to more recent evaluations of Hooker's data, so that the student who reads these pages is in no danger of coming away with a lot of out-of-date ideas. Not that Hooker's views on classification or plant distribution need much modernization; perhaps only his positioning of the gymnosperms (as on pp. 81–82) now seems grossly inaccurate.

One amusing point in the preparation of this review may be mentioned. I found (p. 60) what seemed to be a very surprising statement: that the upper limit of *Pinus longifolia* (= *P. roxburghii*, the *chir* pine) in Sikkim was at 2,500 ft. This seemed incredibly low. Surely there must have been a slip somewhere? But no; Hooker carefully records that it is restricted to this altitude in southern Sikkim because it is a plant of the dry hills: elsewhere it may ascend to 7,000 ft., but only where its habitat is found at that height. Southern Sikkim above 2,500 ft. is too wet for this species. Hooker was not known as an ecologist: the term was not yet in use.

The enigma of Hooker's life, as Turrill brings out quite clearly, lies in the nature of his scientific thinking between 1844, when he read Darwin's preliminary essay, and 1859, when the publication of *The Origin of Species* threw Darwin's views on evolution open for public discussion. Excerpts from Hooker's writings of this period are quoted, and these show that he was by no means fully satisfied with the idea that species were immutable, yet for practical purposes he did accept them as fixed entities. Did he feel obliged, out of loyalty to Darwin, to suppress his own thinking on this subject? Or was he, in fact, so fully occupied with prosecuting his practical classificatory work that the theoretical side did not greatly concern him? Hooker was Darwin's friend and his adviser on botanical matters and provided him with many taxonomic (and phytogeographical) data. They were in constant communication. One can scarcely avoid the conclusion that Hooker was content to stick to taxonomy and leave evolution to Darwin. When Turrill (p. 212) seeks reasons why Hooker did not, after his open acceptance of Darwin's views, "attempt to work out a classification of plants on evolutionary principles", he surely misses a simple point. Darwin had provided the evolutionary explanation, in acceptable terms, for Hooker's taxonomic facts. What need was there to alter the taxonomy?

Turrill's own association with Kew ran from 1909 until his death in 1962 and, although he met Hooker but once, he lived surrounded by the Hookerian tradition. Naturally, therefore, he gives due attention to Hooker's achievements as director of Kew. Yet here one is conscious of the biggest gap in this biography. Hooker's lasting influence on Kew was not in re-organizing the Gardens' water supply, not in the extension to the Herbarium nor in planting the Cedar Avenue. He did not build Kew with pipes and bricks, nor even with plants: but with men. Daniel Oliver, J. G. Baker, Sir William Thiselton-Dyer, W. Botting Hemsley, Otto Stapf—these were the men Hooker gathered around him at Kew, yet they scarcely figure in these pages. W. B. Turrill was in the direct line of succession; it is strange that he failed to recognize in them the final proof of Hooker's greatness.

B. L. BURTT

HAZARDS OF SMOKING

Smoking and Health

Report of the Advisory Committee to the Surgeon-General of the Public Health Service. (U.S. Department of Health, Education and Welfare: Public Health Service.) Pp. xviii+387. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1964.) 27*s.* 6*d.*

IN 1963 the Surgeon-General of the United States Public Health Service set up an advisory committee to undertake a comprehensive review of all data on smoking and health. This book represents the fruits of their labours. It is in fact the latest in a series of evaluations of the evidence relating smoking and health which have been published during recent years. It covers much the same ground as the report on the same theme published in Britain in 1962 by the Royal College of Physicians and reaches the same general conclusions: cigarette smoking is a hazard to health and, more particularly, is an important cause of lung cancer in men and of chronic bronchitis. It is also associated with coronary artery disease and with a number of other less common diseases, although it is not certain in these cases if the association is causal.

The book is in two parts. In the first part the methodology of the study is described and the main conclusions summarized. The evidence on which these conclusions are based is set out in detail with numerous references to the original literature in the second part. The large amount of published material available is dealt with critically from both a general point of view and on the basis of disease categories. It is not possible here to give more than a rough outline of the ground covered. Separate chapters are concerned with consumption of tobacco products in the United States, chemical and physical characterization of tobacco and tobacco smoke, the pharmacology and toxicology of nicotine, mortality of smokers, and the relation of smoking to cancer, other respiratory diseases, cardio-vascular disease and other conditions. Beneficial effects of tobacco, psycho-social aspects of smoking and the morphological constitution of smokers are also considered.

The great bulk of the report is taken up with consideration of the epidemiological investigation of the effect of smoking on health. In seven large prospective investigations it has been constantly found that death-rates in general for cigarette smokers are higher than for non-smokers, and are substantially higher for cancer of the lung and other forms of cancer of the upper respiratory tract, for bronchitis and for coronary artery disease.

Most general interest will probably attach to the findings on lung cancer. A careful review of the evidence leaves the committee in no doubt that cigarette smoking is an important element in the causation of lung cancer in men, and while it recognizes that it cannot be the only cause, it gives it as its opinion that the magnitude of the effect of cigarette smoking far outweighs all other factors. Twenty-nine retrospective and seven prospective investigations all agree in showing a higher incidence of lung cancer among smokers than non-smokers, and the incidence increases regularly with the amount smoked. This evidence is supported up to a point by pathological studies of the tracheobronchial tree of smokers and non-smokers, and by the evidence from several laboratories that tobacco smoke condensate is carcinogenic to the skin of several animal species. A number of carcinogenic hydrocarbons and heterocyclic compounds have also been detected in cigarette smoke although, contrary to the report, only one has actually been isolated. The committee clearly feels that the smoke exerts its effect, in the main, by a direct carcinogenic action on the lung tissue, possibly through the agency of the polycyclic hydrocarbon and heterocyclic carcinogens contained in it, but it recognizes that the experimental evidence for this view is not conclusive. Tumours have not yet been produced in the lungs of experimental animals by direct inhalation of cigarette smoke, although they have been obtained in a few instances by direct application of smoke condensate. In addition, the amounts of the hydrocarbon carcinogens so far detected in cigarette smoke appear to be insufficient by a large factor to account for the activity of the smoke condensate towards the skin of experimental animals. However, the likelihood that hydrocarbon carcinogens may be involved is increased by the recent demonstration by Shabad that epidermoid carcinoma can be produced in the lungs of rats by intubation of a hydrocarbon carcinogen absorbed on carbon particles. Cigarette smoke has also been found to contain substances with tumour-promoting properties, and these may enhance the effect of the small amounts of carcinogens which are present. The effect of damage to the defence mechanisms of the respiratory tract by tobacco smoke must also be taken into account.

The other main diseases with which cigarette smoking appears to be related are bronchitis and coronary artery disease. In the case of bronchitis, the association is so strong as to be considered one of direct cause and effect, and the report states categorically that cigarette smoking is the most important cause of chronic bronchitis in the United States. In this the American experience differs somewhat from that in Britain, where other factors besides cigarette smoke are considered in the report of the Royal College of Physicians to play a part in the development of chronic bronchitis. It has been established also, in a number of studies, that male cigarette smokers have a higher death-rate from coronary artery disease than non-smoking males, but it is uncertain whether there is a causal relationship. A number of other factors are associated with coronary artery disease and it is not easy to disentangle the effect of any one.

The book is well produced; there are very few misprints and the tables and diagrams are excellent. An index would have been useful. W. CARRUTHERS

ANIMAL SOURCES OF HUMAN DISEASE

Diseases Transmitted from Animals to Man

Compiled and Edited by Dr. Thomas G. Hull. Fifth edition. Pp. xxii + 967. (Springfield, Ill.: Charles C. Thomas, 1963.) 17.75 dollars.

THIS useful book, well known since its first publication in 1930, appears now, in its fifth edition, with the collaboration of 32 specialists who have contributed to it one or more chapters. Thus the book now contains authoritative articles on a wide variety of subjects, all of which are important to both medical men and veterinarians.

In Section 1, there are articles on the diseases of domesticated animals and birds and on some of the diseases of wild animals and rodents. Section 2 deals more specifically with the diseases of rodents and wild animals, but includes some diseases of domesticated animals. Section 3 is devoted to the relationships between diseases of man and those of other animals, this important subject being considered under the headings of: animal diseases of minor public health importance; human diseases that are spread by other animals; animals that are passive carriers of pathogenic organisms; and the part played by different animals and birds in relation to diseases of man.

It will be realized that a brief review cannot give an adequate idea of the large number of diseases considered. Most of the space is given to bacterial, viral and fungus diseases and only one chapter, about 10 pages long, is specifically given to diseases caused by parasitic animals (Protozoa and Helminths). This chapter, however, carries all the authority of its author, Prof. Faust, and parasitic animals inevitably have their place in the final chapter on the part played by different animals and birds in diseases transmitted to man.

It is, indeed, in this final chapter, written by Dr. Hull, that the theme of the book is epitomized; and the tables in it, which list the large number of diseases that may be transmitted to man from other animals, will be of value to medical men and veterinarians. The layman who keeps dogs, cats, birds and other pets should also be interested but he will not, one hopes, be unduly alarmed by the possibilities here outlined. Dr. Hull names 36 diseases that may, in various parts of the world, be transmitted to man, either directly or indirectly, from the horse, 57 from the cow, 47 from the hog, 48 from the sheep and goat, 65 from the dog, 39 from the house cat, 32 from rats and mice, and 27 from the fowl and other poultry. He also gives a list, 2½ pages long, of diseases that may possibly be transmitted to man from wild animals and birds. It should be realized, however, that many of these diseases are repeated in each of the lists, so that the total number of diseases is correspondingly reduced. A final list, 9 pages long, gives a useful summary of all the diseases, with their ætiological agents, their common animal hosts and the usual methods by which man may be infected.

One purpose of the book is to provide a common meeting ground for the physician, health official, veterinarian and research worker, and in this it should succeed. It will help, one hopes, to foster the movement, now gathering force, towards close co-operation between the medical man, the veterinarian and the biologist in the fascinating field of comparative medicine and surgery. Some readers may find that the space given to some of the diseases considered might have been extended and that given to others shortened, but opinions about this will vary according to the special interests of the reader and the importance of certain diseases in particular parts of the world. The scope of the book is, it should be remembered, world-wide, and the 32 contributors have been given considerable freedom in their treatment of the subjects allotted to them.

The book is well printed on thick, glossy paper and this makes it rather a heavy book to handle; but no doubt this paper helps the reproduction of the illustrations, most of which are photographs of lesions in man and other animals. Many of these will be valuable to practising medical men and veterinarians. The general production of the book maintains the high standard that we have learnt to expect from its publisher.

G. LAPAGE

ABSORPTION OF LIPIDS FROM THE INTESTINE

Biochemical Problems of Lipids

Edited by A. C. Frazer. (B.B.A. Library, Volume 1.) Pp. ix+474. (Amsterdam, London and New York: Elsevier Publishing Company, 1963.) 130*s*.

THIS volume contains the fifty-eight papers read at the seventh international conference on "Biochemical Problems of Lipids", held in Birmingham during June 24–27, 1962. The main theme was the mechanism of fat absorption from the intestine. Papers concerned with this subject were classified thus: (1) intraluminal aspects of fat absorption; (2) structural aspects of the intestinal cell and absorption studies; (3) enzymatic aspects of intestinal lipid metabolism; (4) chylomicrons and lipoproteins.

In Section 1 there is frequent reference to the use of thin-layer chromatography for detecting the products of enzymatic hydrolysis of triglycerides and identifying individual bile acids. The role of bile in the solubilization of synthetic hydrocarbons and dyestuffs has also been investigated, as have the effects of diversion of the bile on the recovery of lipids in the thoracic-duct lymph. In addition, there are papers on the amount and composition of the fæcal fats of several species, which have been examined with particular reference to the influence of the intestinal bacteria.

There are important papers in Section 2 dealing with the fine structure of intestinal epithelial cells and particularly with the brush border, as observed under the electron microscope. Results obtained by different groups have been presented in support of possible mechanisms of lipid absorption from the intestine. It appears that at present there is still a great deal of uncertainty regarding the nature of this process. Opinion seems to be rather evenly divided in favour of absorption of lipid droplets by pinocytosis on one hand, and of a highly dispersed molecular or micellar species on the other.

In Section 3, the main line of research concerns the re-synthesis of triglyceride in the intestinal mucosal cells. It seems to be generally accepted that the most important pathway in this tissue involves the condensation of monoglyceride with two molecules of long-chain acyl-coenzyme A. Thus intestinal mucosa differs from other tissues in which triglyceride synthesis begins with the acylation of L-α-glycerophosphate.

One of the papers in Section 4 deals with the composition of rat thoracic-duct lymph as a function of the nature of the dietary triglyceride. Lipids containing fatty acid radicals of short and intermediate chain length ($C_4 - C_{10}$) are not found in the lymph draining the intestine, following oral administration of the triglycerides of these fatty acids; on the other hand, the long-chain component fatty acids ($C_{12} - C_{18}$) do appear in the lymph under comparable experimental conditions.

Only about two-thirds of the papers presented deal with the main absorption theme. The rest are divided into two additional sections: (5) mitochondrial and other aspects of lipid metabolism, and (6) wider aspects of lipid metabolism. Some of the most interesting papers in the whole volume are to be found in these two sections. Thus in Section 5 there is an excellent account of the role of lipids in mitochondrial electron transfer and oxidative phosphorylation; also a very careful investigation of the cause of fatty livers in rats following injection of either ethionine or puromycin.

In Section 6 there are important investigations on the lipids of the erythrocyte membrane. The solubilization of these lipids has been examined following treatment with hypotonic saline and various hæmolytic agents. The lipid composition of the erythrocyte membranes of different species has been determined and incorporation of lysolecithin and linoleic acid into the lecithin component has been observed. Other papers of interest deal with the synthesis of unsaturated fatty acids by yeast, the effect of essential fatty acids in the diet on the lipid composition of rat heart and the metabolism of linoleic acid in rat liver.

Each paper is followed by a discussion of variable length, but only about half the papers contain a summary. A subject index has been provided, which seems to have been compiled exclusively from words appearing in the titles; similar topics are thus not necessarily brought together.

This volume does not attempt to give a connected account of the absorption of lipids from the intestine. Instead, it presents a series of investigations designed to solve some outstanding problems. For this reason, the book will probably appeal to lipid workers, whatever their specialized interest, but it is doubtful to what extent it will be read by others.

C. LONG

HIGH-PRESSURE PHYSICS AND CHEMISTRY

High Pressure Physics and Chemistry

Edited by R. S. Bradley. Vol. 1: Pp. xi+444. 100*s*. Vol. 2: Pp. xii+361. 80*s*. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1963.)

THE progress of the physical sciences may be likened to an incoming tide, the advancing waters presenting an uneven front but the general level rising at a fairly uniform rate; new techniques and discoveries in a particular sector invariably generate points of growth in neighbouring sectors and thus contribute to the overall advance.

The history of high-pressure technology affords an excellent example of this process. Improvements in experimental methods, the specific demands of industry and the need to confirm the predictions of theory have, in turn, stimulated activity in the field. In the latter half of the nineteenth century, the work of the great French physicists Amagat and Cailletet was associated with the problem of the liquefaction of the so-called 'permanent gases'. It was followed in the early years of this century by the pioneering work of Le Chatelier and Haber on the effect of pressure on chemical equilibria and, in particular, on the synthesis of ammonia. During the period 1912–60, P. W. Bridgman advanced the subject by greatly extending the range of pressure accessible for experimental observation and by measuring the physical properties of numerous liquids and solids up to pressures of several hundreds of thousands of atmospheres. The latest step forward has carried observations into the range of pressure found within the Earth and in astral bodies.

Since pressure is one of the thermodynamic functions which determine the state of a material system, its effects are not confined to any one branch of science. The literature is scattered and it is by no means easy to obtain a coherent picture of a subject which is advancing rapidly on many fronts. Workers in the field and students will, therefore, welcome a series of reviews, edited by R. S. Bradley, in which important aspects of recent work are summarized and accompanied by bibliographies. The scope of *High Pressure Physics and Chemistry* includes an account of the methods of generating and measuring high pressures, descriptions of apparatus and experimental techniques and the applications of pressure to the investigation of reaction rates, chemical equilibria, the physical properties of matter and the Earth sciences.

The discoveries which have aroused greatest interest in recent years are those resulting from the combined use of high temperatures and high static pressures, and those in which transient high dynamic pressures have been applied by shock-wave methods. In the former, a number of solid–solid transitions have been investigated, including boron nitride–borazon, silica–coesite and graphite–diamond, and also certain phase changes in metals which are presumed to involve an electron transition; this work is well summarized in two contributions by R. S. Bradley and W. Paul respectively. In the latter, electrical and metallurgical effects have been observed up to pressures as high as 10^6 atmospheres and new $P-V$ data have been obtained over a similar range.

The theory and application of shock-waves are dealt with in some detail by G. E. Duvall and G. R. Fowles, and the problem of an equation of state applicable to ultra-high pressures by L. Knopoff. The development of shock-wave methods for determining equations of state allows direct comparisons of pressure–density relations to be made and is affording important information on the composition of the Earth's interior. Thus it has been shown that the density of iron is somewhat larger than the densities in the Earth's core and that a mean atomic number of about 23 is consistent with core conditions.

In giving a survey of the applications of high pressures to the Earth sciences, P. J. Wyllie points out that we are now passing through a period of reconnaissance—"a reconnaissance of possible processes that could lead to the formation of a particular assemblage of minerals with specific textural relationships"—and that this must be followed by a period in which more precise data are sought, particularly in the higher pressure–temperature ranges. The possibility which now exists of reproducing a geological process within a pressure vessel is opening up new fields in experimental petrology.

It would be wrong to conclude this review without a reference to contributions by B. F. Dodge and S. D. Hamann on the effect of pressure on chemical equilibria in gaseous and condensed systems and on reaction rates. The aim of the authors is a very practical one, that is, to provide a basis for calculating the conversion at equilibrium of a reactant, either in a single reaction or in a set of simultaneous reactions as it is influenced by pressure, predicting whether or not a proposed new reaction is thermodynamically possible and under what conditio of temperature and pressure it could be expected to gi favourable yields, and determining the role of pressu on the rates of homogeneous liquid phase reactions.

Dr. Bradley has been fortunate in enlisting the c operation of so many distinguished workers in the hig pressure field in the preparation of his book, and he is to congratulated on the successful manner in which he h discharged the onerous duties of editor.

D. M. NEWITT

PLASMA PHYSICS

The Theory of Plasma Waves

By Prof. T. H. Stix. Pp. x+283. (McGraw-Hi Advanced Physics Monographs Series.) (New York an London: McGraw-Hill Book Company, Inc., 1962.) 76s

THE theory of waves in a plasma may be said to hav originated with the pioneering papers of Langmui and Tonks in the late 'twenties. Interest in the subjec was revived after the Second World War because of it importance in the problem of thermonuclear fusion— the 'philosopher's stone' of plasma physics. Since the a vast number of papers on the subject of plasma wave have appeared, but up to the present these lay scattere among the pages of many scientific journals. In thi beautifully produced book the author had collecte together as an organic whole the many complex topic connected with the behaviour of waves in plasmas contained in these papers. The book is a model of expositio and, in my opinion, a classic among the extensiv literature on the physics of plasmas.

The book deals with small-amplitude waves in a cold plasma, boundary value problems in plasma waves, and the theory of waves in a hot plasma—both in the absence and in the presence of a magnetic field. In the first chapter the topological genus of the wave-normal surface is discussed and in the succeeding chapter the small amplitude waves are analysed more fully in terms of the now well-known diagram due to Clemmow, Mullaby and Allis. Here will be found derived in a logical sequence the hydromagnetic waves of Alfvén and Aström, ion-cyclotron waves, the Appleton–Hartree dispersion relation and the so-called 'electrostatic waves'.

The flow of energy associated with the passage of plasma waves is discussed here and such concepts as group velocity and ray optics are admirably treated. The author also introduces the notion of 'accessibility' in a plasma of high density.

Problems of stability of equilibrium plasma configurations associated with the names of Kruskal and Schwarzschild are regarded as boundary value problems in plasma waves—though the classical methods of Kruskal and Schwarzschild are faithfully followed.

An interesting account is given of the plasma models with discrete structure and especially those due to Dawson and van Kempen, and also of the 'trapping' of charged particles in the potential wells of the electric field of the plasma waves. Mention is also made of the important work of Bunemann of two-stream instability using a charged-sheet model of a plasma.

The major portion of the book is concerned with a 'hot' plasma; there are clear expositions of the physical nature of Landau damping, that is, the phenomenon of the absorption of energy from a plasma by a collisionless process, and of the Nyquist criterion for instability. A chapter devoted to the theory of small amplitude waves in a hot plasma in the presence of a magnetic field includes such topics as cyclotron damping, magnetic trapped particle modes, concluding with a discussion o the solution of Boltzmann's equation for a hot plasma. This covers damped, stable and undamped waves. In the final chapter, the author considers the propagation of waves in the case where both the plasma density and magnetic field are inhomogeneous with the associated phenomenon of reflexion and absorption of these waves in certain critical cases.

An interesting feature of the book is the admirable collection of examples (of which there are nearly one hundred) at the end of each chapter. Some contain hints for the proof of theorems enunciated in the text, others serve as useful illustrations of points discussed therein. There is also a comprehensive bibliography. The author is to be congratulated on this excellent treatise, and every plasma physicist will feel indebted to him for this. It will surely find a place on the library shelves of every serious student of plasma physics.

V. C. A. FERRARO

SOLVING FIELD PROBLEMS

Analysis and Computation of Electric and Magnetic Field Problems

By K. J. Binns and P. J. Lawrenson. Pp. xiv+333. (London and New York: Pergamon Press, 1963.) 84*s*. net.

FREQUENTLY an engineer or physicist has to solve a field problem involving complex mathematical analysis. More often than not it is difficult to find someone who can understand the problem and advise on the best way of solving it. Although the situation is improving it is still a rare department that has a highly competent engineer–mathematician on its staff who can advise others on the best methods for tackling specific problems.

This book goes a long way towards providing many of the answers that are required. It is directed at practising engineers and physicists, as well as students seeking a better understanding of field problems. The standard of mathematics required to start reading the book is within the syllabus of a normal engineering degree; where more advanced mathematical topics are introduced, they are fully explained in the text. Throughout the book the emphasis is on the physical significance of the mathematics, and to this end practical examples are used wherever possible.

The book is divided into four parts. Part 1 serves as an introduction to the rest of the book; it deals with basic field theory and the methods of calculating force, inductance and capacitance from a knowledge of the field distribution. Part 2 is concerned with images and direct methods of solution, much along the lines of the book by Hague. However, in addition, attention is directed to the solution of the Laplace equation in Cartesian coordinates, and where the method of images is used the solutions are expressed in a convenient complex variable form.

The largest part of the book, Part 3, deals with transformation methods, and the authors claim that their treatment of the subject is the most comprehensive available. This certainly appears to be so, for in the five chapters in this part the authors present in a most clear and concise fashion a vast amount of transformation theory. The first chapter in this section presents the basic transformation theory leading up to the transformation equations. The second chapter is concerned with curved boundaries and the following chapter with polygonal boundaries. There follows a chapter on the application of elliptic functions and finally one on general considerations. Of particular interest in this third part are the treatments of line and doublet sources, the transformation of regions exterior to finite polygonal boundaries and a most useful technique for the direct numerical integration of functions of a complex variable, which permits solution of problems involving boundaries of greater complexity than is otherwise possible.

In Part 4, the powerful finite-difference methods are discussed. Again, the treatment is from first principles, and practical problems serve as examples for the application of the techniques evolved. The practical advice on the actual methods to be adopted is most useful. The section devoted to convergence and the optimum choice of parameters to provide the most rapid convergence possible for any given problem is probably the best so far available anywhere in the literature. Other topics include gradient boundary conditions and errors. In the conclusion to this part, the authors make the point that if a digital computer were used, it might be possible to reach the solution to a problem much more quickly by means of finite-difference methods than by analytical methods—that is, if an analytical solution exists anyway.

The appendixes include a useful list of transformations, many of which have not previously been published. These, for the most part, involve elliptic functions.

Every chapter ends with two lists of references. The first refers to specific points in the text, and the second indicates related literature which could be of interest. In addition, at the end of the book, an extensive bibliography is included.

Even if one knows a method for solving a specific problem one would be well advised to look within the covers of this book to ensure that no better method exists for the solution. This is an excellent volume, which is certain to prove most popular. The authors are to be congratulated.

E. M. FREEMAN

PHOTOCHEMISTRY

Advances in Photochemistry

Vol. 1. Edited by W. Albert Noyes, jun., George S. Hammond and J. N. Pitts, jun. Pp. ix+443. (New York and London: Interscience Publishers, a division of John Wiley and Sons, 1963.) 125*s*.

DURING the past few years, there has been a rapid rise in the number of *Progress in . . .*, *Advances in . . .*, *Aspects of . . .*, and other such series about various topics in chemistry. As a result, with any new series of this general type, the reviewer must not only consider the merits of the particular volume being discussed, but also whether the series itself fulfils any real need.

The growth of virtually all aspects of photochemistry has been particularly marked during the past fifteen years. This has undoubtedly been as much due to the development of new analytical techniques (for example, gas chromatography) as to developments in spectrometers and progress in spectroscopy generally. Considerable advances have been made in the elucidation of mechanisms of many photochemical reactions and progress has also been made in determining the nature of the intermediate excited states resulting after the absorptions of radiation. A surprising number of compounds obtainable only with extreme difficulty by conventional synthetic procedures can now be made simply by photochemical methods, and often on quite a reasonable scale. Indeed, the first really large-scale manufacture of materials using a photochemical reaction has recently been undertaken.

Few of these developments have yet appeared in chemical text-books, and surprisingly few works specifically devoted to photochemistry exist. While several reviews and articles on various photochemical topics have been published, they are very widely scattered. Thus we see that there is little doubt of the real need of a series of reviews on photochemistry.

Volume 1 of *Advances in Photochemistry* consists of nine chapters, and contains, in addition, author and subject indexes. The first chapter on "The 'Vocabulary' of Photochemistry", by J. N. Pitts, jun., F. Wilkinson and G. S. Hammond, deserves to be read not only by all practising and intending photochemists but also by all undergraduates specializing in chemistry. It presents a clear and remarkably concise account. In the space of some twenty pages the terms for symmetry, and state symbols for the description and presentation of spectra, are considered and discussed, as are selection rules and chemical processes. Even such topics as high-pressure, medium-pressure and resonance lamps are covered. These terms are, of course, obvious (or even trivial) to the expert, but often present very real difficulties for the novice and undergraduate.

This chapter is followed by "The Photochemistry of Aromatic Hydrocarbon Solutions" by E. J. Bowen; "Photochemical Gas Phase Reactions in the Hydrogen–Oxygen System" by D. H. Volman; "Photochemistry of Cyclic Ketones" by R. Srinivasan; "Addition of Atoms to Olefins in the Gas Phase" by R. J. Cvetanović; "A New Approach to Mechanistic Organic Photochemistry" by H. E. Zimmerman; "Isotopic Effects and the Mechanism of Energy Transfer in Mercury Photosensitization" by H. E. Gunning and O. P. Strausz; "Photochromism" by R. Dessauer and J. P. Paris; and "Photochemical Rearrangements of Organic Molecules" by O. L. Chapman. These chapters are of a high standard, as is to be expected, for their authors have all made important contributions to various aspects of photochemistry. The range of topics is very wide and it is unlikely that more than a very few will be equally interested in all chapters. This is by no means a disadvantage and could lead to much useful cross-fertilization.

Even though the range of subjects in Volume 1 is wide, there are still large areas of photochemistry not mentioned. We are promised that this series will cover topics ranging from the elucidation of spectra of polyatomic molecules to the photochemistry of biological systems in plants and animals. Volume 2, which will follow Volume 1 in quick succession, will doubtless include many of those areas which have not appeared in the first volume. Thereafter, about one volume should appear each yaer.

Advances in Photochemistry is well produced and seems to be remarkably free from misprints, etc. The structural formulæ, especially in the long final chapter, are very clear and beautifully reproduced (which makes a few formulæ in an earlier chapter, which appear to have been drawn by only a semi-skilled hand, look even more out of place). The book can be strongly recommended to all photochemists and will certainly find a place in all science libraries. It is a pity that its price will probably prevent its purchase by a wider audience. The editors, Profs. Noyes, Hammond and Pitts, are to be congratulated on their choice of authors and topics for this first volume, which augurs well for the future of this series.

H. M. Frey

THE SEA AS AN ENVIRONMENT

Marine Distributions

Edited by M. J. Dunbar. (The Royal Society of Canada Special Publications, No. 5.) Pp. viii + 110. (Toronto: University of Toronto Press; London: Oxford University Press, 1963.) 40*s*. net.

THE publication in book form of a series of papers, the substance of which has been, or probably will be, published in the usual journals, must have behind it a strong belief in the need to direct attention to the interrelationships of the individual subjects. It was the belief that emphasis should be placed on the interdisciplinary nature of oceanography that led to the organization of a symposium in June 1962 at which these six papers on aspects of marine distribution were read. The symposium was to serve, in particular, to introduce the *Serial Atlas of the Marine Environment*, which was sponsored jointly by the U.S. National Academy of Sciences and the Royal Society of Canada.

The object of the *Serial Atlas*, which is described in the first paper, is to provide a new type of journal in which distributional investigations from all disciplines will be published. By plotting these distributions on a uniform series of charts prepared by the American Geographical Society (the body responsible for the preparation and publication of the *Atlas*), it is hoped to build up, over the years, a volume of data in which the separate distributions can be compared. The problem of the comparability of methods, although mentioned, has yet to be resolved.

The second paper is concerned with the vertical distribution of temperature in the upper 100 m of the eastern Sub-arctic Pacific and describes the processes involved in the development and decline of the seasonal thermocline. This is followed by three papers which directly relate the distribution of physical and chemical properties to biological distribution; they deal with attached algae of the North-east Pacific and zooplankton species in the North Atlantic. One of these papers includes a plea for more taxonomic investigations. It is certainly a feature of the literature that whenever wide-scale distributional investigations are undertaken, many systematic problems have to be cleared up first.

Another paper shows how a knowledge of the life-history of a species increases its value as an indicator of water movements. It might well be added that to understand fully the distribution of a species, a knowledge of its life-history is essential. Such points emphasize the theme of *Marine Distributions*—that the distribution of plants and animals cannot be examined in isolation. The final paper is concerned with osmoregulation in some of the larger Crustacea, which the author groups according to their capacity to adjust to variations in salinity. This paper, based on experimental work, is refreshingly different from the three previous ones and demonstrates the use of physiological methods in distributional investigations.

The book ends with comments and a summary by Dr. L. A. Walford, without which it would lose a great deal of its value. He links the papers together and raises many of the problems involved in the investigation of the sea as an environment. Although the difficulties he lists are numerous the result is not wholly depressing, as he also suggests ways in which some of them may, at least partially, be overcome. He believes that one approach lies in the "... systematic mapping of all the many elements of the marine environment—currents, bathymetry, bottom sediments, salinity and temperature fields and distributions of all species of plants and animals", which is the plan of the *Serial Atlas*. Put this way, one may be excused, perhaps, for thinking that this is rather a tall order.

Some comment is necessary on the layout of the papers. Whether, in a publication of this type, abstracts at the heads of the papers are necessary or not, at least a consistent pattern should be followed. Four of these papers have neither abstract nor summary, one has an abstract only and one provides both an abstract and a summary. Some of the figures could be much clearer and it is unfortunate that, in the paper on temperature–salinity–plankton diagrams, Figs. 10*b* and 11*b* have been transposed in printing, particularly as it makes the necessary simultaneous comparison of two of the diagrams and their corresponding charts impossible.

Ultimately the success of this book depends on whether it directs attention to relationships between papers that would be overlooked if they were read separately, possibly over a long period. To those readers already familiar with the subjects discussed such relationships will probably already be apparent, but to those whose investigations at present appear to have less obvious connexions with these problems this book will be of considerable use.

A. de C. Baker

EDUCATION AND INDUSTRY*

By S. P. CHAMBERS, C.B., C.I.E.

Chairman, Imperial Chemical Industries, Ltd.

A SUBJECT such as I have chosen may conjure up the prospect of a disquisition on the balance which should be held between 'pure education' in the academic or philosophical sense, and vocational training, but this is not the case here. It is to quite a different aspect of education to which I wish to direct your attention. This aspect is concerned with neither the academic content of education nor with vocational training, but is concerned with the extent to which education in its broadest sense fits men and women for responsibility and control in industry. It may be that what goes wrong in the education which some people get is as wrong for vocations in other walks of life as for those in industry; but it is for other people to judge in these other fields.

In an organization as large and complicated as mine (Imperial Chemical Industries, Ltd.), there is need for men of many kinds with widely different training and different aptitudes, but, except for quite low-level routine workers, certain characteristics are needed in all of them. Briefly these characteristics are the capacity: (*a*) to weigh up factors and to come to firm decisions; (*b*) to control other men fairly and firmly and win their confidence; (*c*) to delegate responsibility and to tolerate errors of judgment in subordinates within reason and without inquests; (*d*) to take firm action, however unpleasant, where the standard of performance has fallen below the tolerable limit.

Even where the work does not involve the direct control of other men, there is usually the indirect and intangible control which is exercised when passing judgment on the work, character or capacity of other men.

These characteristics are needed right through from the charge-hand directly controlling the work of a few other men to the highest executives dealing with long-term policy. What is more important is that one or more of the essential characteristics can be absent from somebody who has been appointed—wrongly appointed—to quite a senior post and yet be present in a man who is doing a subordinate job really well but whose aptitude and training do not fit him for a more responsible post.

I believe that having these characteristics is basically a matter of education in its fullest sense and is neither an inherent quality nor a matter of vocational training; but there is no clear pattern from which we can determine the likely source of the 'haves' and the 'have-nots'. Some top-rank public schools and university colleges produce men of brilliant academic achievement who have poor judgment, no power of decision and no capacity to delegate work or to control men. These men can be the tragedies of industry because their deficiencies are not revealed in their academic record and are difficult to detect at a selection interview. They can get started on a promising career, but end in the wilderness of the unpromotable clever boys. On the other hand, the same schools and colleges can produce second-rate graduates who are first-rate men with all the characteristics I have listed.

The history of British industry during the nineteenth century and up to the outbreak of the Second World War teems with examples of men who have not had any formal education but who have had all the necessary characteristics and have become notable industrial leaders. With the growing complexities of modern large-scale industry with its scientific basis, the 'self-made man' with little or no formal education is vanishing. Yet some of the highest places in industry have been filled successfully by men whose education has been obtained the hard way. In these cases, the task of getting education and training the hard way has imposed personal disciplines which have probably led imperceptibly to the acquisition of those characteristics needed in industry. Sometimes, however, such a course produces an almost characterless 'swot'.

As I see it, the educational system of Great Britain produces from the point of view of industry good and bad candidates on a 'hit-or-miss' basis which is most unscientific. I have said that academic achievement is no guarantee that the other attributes of a good industrialist will also be present, but undoubtedly the ideal is a combination of academic brilliance and these other qualities.

From what I have said so far it will rightly be concluded that I am concerned here neither with academic education nor with vocational training, but with the development of character. I do not mean, and I want to emphasize this, the development of moral standards. All aspects of education are important—the acquisition of knowledge; the development of the power of thinking and reasoning; specialized vocational training, whether the vocation is to be that of a doctor, an engineer or an electrician; the inculcation of moral values; and, finally, the development of all those elements of character which enable a man to work effectively and harmoniously with his fellow workers. There are, of course, many other aspects of education such as the development of the power of appreciation in cultural matters so that in leisure time there can be full enjoyment in the fields of art, literature, music and so on; but I am not concerned with these here.

I have given a list of the characteristics which are needed for those in industry who are not engaged solely on routine work. I want now to explain the importance of these characteristics in a world in which large sections of industry are becoming more mechanized, more complex, and controlled in units which continue to grow in size.

First, a word about size. Economists in general like to see the maximum amount of competition as a spur to efficient and economic production, and I am sure that, in general, they are right. But the economic unit in many sections of industry grows with technological progress. For example, and I take an example from the chemical industry because I am on familiar ground, a plant to produce 20,000 tons a year of ammonia would have been regarded only a few years ago as large; to-day we are erecting two plants each to make 300,000 tons in a single stream of gas, and these, with one other plant, will supply the whole of the present requirements of the country. Moreover, the gases produced pass unseen through a series of plants from the raw material (naphtha) to a plant making concentrated mixed fertilizer which is fed straight into polythene bags for delivery to farmers and stockists. It is inevitable that all these plants, costing millions of pounds, should be under the same ownership. The economics of joint production are such that a whole range of other plants must be in the same ownership if there is to be economic production. Indeed, it is the spur of international competition which makes units in this type of production in Britain reach, for Britain, monopoly size. This is only one example of what is happening in many parts of industry, although, of course, there will always be some parts of industry in which production and ownership will be in quite small units.

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* Substance of the Chuter Ede Lecture delivered before the National Union of Teachers on June 2.

In any really large undertaking the decisions which have to be taken can be classified. There are some decisions of policy which are of wider application throughout the whole organization and also of long-term significance; these clearly must be made at the highest level. Others are important but are more limited either in their effect on the organization as a whole or in the area of application. Decisions at lower levels must, of course, conform to the general policy. Too often there is a lack of co-ordination because the general policy in an organization has not been adequately communicated, and this, in turn, may be due to a failure to define and decide such a policy.

At each level in Imperial Chemical Industries, Ltd., there has to be the exercise of judgment on the relative facts, decision, and the communication of decision to lower levels of management. This is true of the main board of directors, to the chairmen of divisions, factory managers, plant managers and foremen, with similar lines of communications horizontally to those engaged on research and commercial operations. Very difficult questions of organization arise, and if these are settled badly, friction, frustration and bad management will follow. For example, the co-ordination of the functions of production and marketing has generally to be done below that of a Division Board level. If they are co-ordinated at a higher level, which used to be the case, relatively low level executive decisions involving several different functions would have to be referred to unnecessarily high level committees, the members of which are almost certainly less competent to make the decisions than men at a lower level because their contact with the material factors is more remote. If you only have the co-ordination of these functions at a high level anything involving a number of functions has got to go to the highest level, therefore co-ordination must be at a lower level. This is important. The 'man on the spot', if in fact the organization is bad and decisions go to a high level, then tends to despise those who have authority over him and then to distrust their judgment not only in those matters which could and should have been settled locally but also in matters which are proper to these men at higher levels of management—matters in which their judgment is likely to be superior.

The need to keep decisions down to the level where they belong is essential to the proper organization of a large business. There must also be the recognition that human beings, to whom powers of decision have been delegated, are fallible and that mistakes will be made. When mistakes are made, there is a natural temptation to bring the relevant decisions up to a higher level or to issue detailed instructions which, in practice, has the same effect. This temptation has to be resisted. In a large industrial organization the power to decide quite important matters must be delegated, and when there is doubt about the exercise of this delegated power, a firm self-discipline is needed at the higher level to resist the temptation to withdraw the delegated power. Where the record over a period of years reveals incompetence, then changes in personnel may be needed, but this is a different matter.

The capacity to delegate and not to get flustered if things go wrong at a lower level depends on the recognition at a higher level that mistakes will be made and that there must be no recrimination or the withdrawal of the power to delegate. The system of delegated power must be adhered to firmly and must be understood throughout the organization.

Only in this way can a large organization function effectively in conditions which are changing and which require the exercise of judgment and discretion at different levels without seeking detailed instructions from above. Ideally there should be communication of policy and of higher decisions to lower levels of management and no instructions on how the lower levels of management should carry out the policy communicated to them. In practice some instructions are needed, and care has to be taken on occasion to see that a decision to be taken at a lower level does not conflict with an important decision of policy, but such instructions should be kept to a minimum.

The alternative to this system of delegation and reliance on the judgment and capability of other people is a vast bureaucratic machine in which there is the fiction that, assisted by expert advisers, the top management is omniscient and capable of issuing detailed instructions on everything that has to be done. All initiative is taken away from lower levels of management for whom deviation from detailed instructions is an offence, to be put on the record. Such a system is productive of a vast amount of paper, and is grossly inefficient, slow and inflexible. Some Government departments are suspiciously like this; but they need not be.

There are at least two major objections to detailed control from the top. The first is inflexibility and slowness to cope with changed or changing conditions. The one which comes to my mind (this is a little controversial but it is a simple example) is the case where planning approval is required to put up a building, and where the local authority, the county authority and a central Government Ministry all have powers entitling them to go into every detail and to exercise a power of veto. Our multi-layer system of Government is, I am sure, right as a matter of democratic principle, but extraordinarily inefficient in practice because no attempt seems to have been made to introduce into it any principle of management by delegation which can cope with changing conditions.

The second objection is that a bureaucratic system fails to take into account the great need of intelligent men to express themselves by the exercise of their individual judgment and in so doing to act as free men doing effective work instead of being the cogs in a machine driven only by the centralized force of decision at the top. For men of intelligence to know that they are the slaves of detailed decisions by men who have been put in authority over them, but who have neither the knowledge nor the time to make these decisions well, there is a sense of frustration and humiliation which communicates itself still further down the line.

In a simple and static society faults of organization or management are relatively unimportant; they get ironed out by experience; in a modern and progressive society they can lead not only to disorder and other difficulties of management, but they can lead ultimately to decadence. The need for men to be able to express themselves and to use their mental and moral powers in their work is as important as the need to maintain high material standards of living and the environment for intellectual enjoyment during the extended hours of leisure.

It is here that I come back to education. How often have I met men of unblemished character, of high academic achievement, but whose life, sheltered from the harsh need to make important decisions, has left them timid and irresolute, or stubborn because of the fear of making a mistake. Life at a university with its intellectual and inconclusive discussions at the postgraduate level is, on the whole, a bad training for the real world and only men of very strong character surmount this handicap. Training in the principles of management at a later stage will not convert a timid man into a man of decision. Who along the educational chain is watching this failure of character development in men who may be getting high praise for their academic achievements ?

In some men of high intellectual ability, there is a failure of another kind. Concentration and success in a narrow field, which for my subject is likely to be scientific, can lead to an arrogance which reveals itself in a contempt for the problems in other and broader fields. The pure scientist who is infected in this way, and only a few of them are, will regard applied work as needing only a lower standard of intelligence, but he will not say so. He will also regard the problems of management and of working harmoniously in a heterogeneous team as non-existent or just simple matters to be dealt with *ad hoc* by anybody with average intelligence. He will not understand that that combination of powers of intellect and judgment which is needed for applied scientific work by brilliant scientists struggling with a specific objective is something which he probably does not possess; nor will he understand how much harder is the task of working successfully in a team of men than the indulgence of intellectual skill on abstract theorems.

I have met men of this kind who are contemptuous of economists because they have been deceived by the day-to-day environment and data which are subjects of economic study; they have failed to observe that the tasks of economic analysis are in some ways more difficult and more baffling than, say, those of nuclear physics because so much of the material is both familiar and immeasurable. The fact that we have made less progress with the former than with the latter is perhaps some evidence of this.

One of the reasons for our failure to produce enough men of high academic standards who have also the developed characteristics to cope with the hard problems of real life may be that our university education is almost wholly in the hands of academic people who have not these characteristics themselves. I have too often attended meetings chaired by dons who have been pathetically indecisive and have allowed discussion to drag on and to wander in the most undisciplined manner into wholly irrelevant by-paths.

Perhaps the changes envisaged in our system of higher education may lead to improvement; but I feel that this aspect of character development requires more positive attention. There is no doubt that organized competitive sport, particularly where teams are involved, makes an important contribution to some aspects of character training; but sport is confined to the physical world and we need its equivalent in the intellectual world.

So far I have been dealing with higher education and have had in mind the filling of higher posts in industry. The problem exists also at the earlier stages of education and is no less important here.

I am treading on delicate and, for me, less familiar ground when I say that, for what seems to me to be a growing proportion of teenagers, there appears to be a loss of a sense of purpose. If the proportion is smaller than I fear, and others are better able to judge than I am, so much the better; but the evidence so far as I can see spreads across teenagers from many walks of life and is not confined to those from under-privileged families.

Nobody would wish to return to the harsh disciplines of the nineteenth century; but the decline of the influence of Christian churches seems to have left a vacuum, and has left too many people rudderless. Those who have reached university-levels of education have generally applied to themselves their own disciplines and have achieved their own sense of purpose, but others receive formal education at school but apparently little leadership or direction. Outside influences seem to me to be generally degrading rather than inspiring. We may all disagree with Karl Marx when he said that religion was the opiate of the people, but would he have given this same description to the great quantity of sensuous and sentimental rubbish poured by pop-singers into the ears of teenagers every day? Listening to this outpouring from the radio or television requires no mental effort and is a form of dope, which for its time-wasting alone must do damage at an age when there is the maximum amount of mental and physical energy available. Perhaps it is this dope, coupled with an absence of any impelling self-discipline, which produces a listlessness and a lack of enthusiasm for anything outside this world of sentimental fantasy; I do not know: I wish I did.

Work in industry which is done in a perfunctory manner by people with no enthusiasm and no sense of purpose is nearly always bad work; for those who are needed for leadership at the level of charge-hand, foreman, supervisor, or higher posts, it can have a bad and widespread influence. Nobody who lacks enthusiasm can inculcate it in others, and the great need in industry, particularly in large-scale industry, is enthusiasm at all levels.

It is in this matter of a sense of purpose that I have been struck and rather worried by what I have seen in the U.S.S.R., from which I have recently returned. My observations, both in Britain and the U.S.S.R., are too few and too accidental to be reliable, but there appears to be a great difference in this matter of inculcating a sense of purpose and enthusiasm. The difference goes right back to training at the pre-kindergarten stage in the crèches provided for the babies of mothers working in factories or offices. My mind rebels against what I saw, and I would hate to see its equivalent in Britain; but it revealed, in my judgment, a vacuum in our own system which I feel must somehow be filled.

After the crèche stage (four months to three years of age), and I visited these, there are in the Soviet Union kindergarten schools for children from three to six years of age whose parents are working and who cannot conveniently be looked after at home because of the absence of a grandmother. Many of these are boarding kindergartens which the parents visit only on Sundays. As most married women work, there are many such kindergartens. My wife visited one of these boarding kindergartens and listened to some good singing by three-year olds. The songs were all in praise of Lenin, of Communism, of the U.S.S.R., or of the cosmonauts of the U.S.S.R. At another school which I visited some time ago in another country in Eastern Europe I saw the same thing—the teaching to the very young of little songs in praise of Lenin and of the principles of Communism. From then onwards education and indoctrination go together at all levels. I went into classes at higher levels and found what, in my judgment, were good standards of teaching and a down-to-earth relationship of this teaching to the needs of everyday life. The teaching of chemistry as a general subject to all pupils was excellent and taken further than in British schools; it was related to the chemical industry and, as in other subjects, there was always the background of the needs and purposes of the community as a whole. Special facilities for children and teenagers to follow their own inclinations in music, art, literature, and so on, were provided in the 'Palaces of Pioneers', for which the only qualifications were good work and conduct at school.

In the U.S.S.R. I am sure that loyalty to Communism is firmly built into the minds of everybody less than forty-five years of age, and that this is attributable to a large degree to this indoctrination from the earliest years. A parallel can be seen in the teaching of Christianity in small children's hymns; and the indoctrination in the U.S.S.R. to-day seems comparable to that of the Christian church in Europe, particularly prior to the time of the Reformation when deviation in belief was heresy.

I am repelled by this use of the educational system for the purpose of indoctrination along a single path from which deviation in act, if not in thought, is a grievous sin. On the other hand, it is impossible to spend any considerable amount of time in the company of men, women and children in the U.S.S.R. without observing that, to all appearances, they have, and work with, a sense of purpose. Having thrown off one form of indoctrination through education, without accepting any other, I am left wondering whether, for certain age-groups, there is left in Britain any sufficient sense of purpose or of social responsibility and, in particular, whether there is that training of character which is needed not only in social or intellectual fields but also in the vital work of industry. Absence of a sense of purpose may not be so widespread as I fear; many people are in a better position to judge than I am, but if the problem is a growing one it will need deep study and that free public discussion which is possible in Britain to-day and which can alone lead to the development of solutions.

In another way, and one which is very relevant to the efficient operation of the economy, there has been a marked change in the U.S.S.R. Under Stalin the centralization not only of long-term plans but also of much detail was accompanied by ruthless action if the performance of individuals fell badly short of requirements, particularly if this might be attributed to disaffection or even disloyalty. There was no doubt a control by a form of terrorism. Fear of making decisions, even relatively unimportant ones if these involved risks of making mistakes, coupled with the belief that a gigantic central plan could control the whole economy in detail, led to decisions by committees at all levels. The result was delay and a good deal of muddle and inefficiency. Decisions which should have been taken at lower levels were taken at higher levels—sometimes at very high levels—with added strains on the occupants of higher posts.

Since Stalin's death there has been far less recrimination and the abandonment of terrorism as a method of enforcing conformity. Decentralization and delegation of authority are the order of the day, and individuals are encouraged to take responsibility. Stalinism has left a legacy which has not yet been wholly eradicated. The exercise of the power of decision by individuals at high levels is noticeable. I saw a good deal of it, but at lower levels there appears to me to be still far too much committee work, and too much passing down of detailed instructions. The paper work for quite simple matters seems altogether excessive. Moreover, when men have passed much of their earlier working lives working on committees on the basis of detailed instruction from above, it is not easy to change to a system which requires initiative, judgment and the power to take decisions. Indeed, it may be some time before all down the line there are sufficient men who can take responsibility on their own shoulders, and the need for committees and detailed instructions may remain for some considerable time yet. There is no doubt, however, that the policy and the trend are in this direction.

Perhaps it is a coincidence but there appears to be to-day less rigidity in the U.S.S.R. in matters of doctrine. Home ownership through the co-operative purchase of flats is encouraged, and is spreading. The desire to own a flat and to have furniture that is different is accompanied by a desire to have better and different clothes. All these seem to me to be indications of a desire for self-expression by individuals who are rediscovering the joys of individuality as well as those of community. They may have a long way to go. Perhaps we, in our educational system, also have some way to go to rediscover how to inculcate a sense of purpose in those who seem to lack it, and how to train men and women with the character needed to shoulder the burdens of a world of growing complexity.

INCIDENCE AND POSSIBLE SIGNIFICANCE OF INHALED OR INGESTED POLONIUM

By Sir ERNEST MARSDEN, C.M.G., C.B.E., F.R.S.

Formerly Member of New Zealand Council of Nuclear Science

RECENT papers by Hill, Osborne and Mayneord[1] of the Physics Laboratories of the Institute of Cancer Research, by Radford and Hunt[2] of the Harvard School of Public Health and by Marsden and Collins[3] of the Dominion Physical Laboratory, New Zealand, have indicated the extent and possible human importance of naturally occurring polonium-210. In this article I shall record some further experimental evidence of polonium and its possible effects in cancer initiation at various sites, including the lung, and also in relation to certain possible induced genetic changes.

Polonium-210 RaF is an α-particle-emitting nuclide of half-life 138 days. The amount present is normally controlled by its parent once removed, RaD or lead-210 of 22 years half-life. The intermediate product RaE has a half-life of 5 days and emits soft β-rays. It is considered that α-particles can initiate protein molecular changes to an extent far greater than the proportionate ionization they produce compared with β-rays and γ- or X-rays.

Polonium can enter the body either through the water consumed, or as a contaminant of the air inspired, or as a trace constituent of foodstuffs via radioactive soil or from natural fall-out as the result of disintegration of radon which has escaped into the atmosphere from soil. In the case of drinking-water, the amount does not appear to be relatively important except for certain artesian waters generally also highly charged with radon. Normal air is said to contain lead-210 to an average extent of 0·003 pc./kg with upwards of 0·09 of this quantity of polonium-210, according to season. This figure would correspond to some 0·007 pc. inhaled by an average person per day.

Two widespread probable sources of inspired polonium, however, are: (1) inhaled tobacco smoke, when certain tobaccos are used in the cigarette blend and in the absence of a suitable filter; (2) the exhaust of motor-car petrol engines when lead compounds are used for enhancement of octane rating. These two sources could assume varying relative importance in different conditions and regions, though too little is known regarding the magnitude of the latter. The average retention period in the lung of such inspired polonium is of the order of 70 days but will probably vary with particle size, the nuclide presumably being more readily removed before disintegration when not associated with certain types of particulate matter, for example, smoke condensate or car exhaust material under certain conditions or urban pollution.

Tobacco

The series of α-particle measurements reported in the paper by Marsden and Collins[3] and others have been extended with results confirming the wide variation, more than 30 : 1 in the activity of tobaccos grown on different soils, the highest about 15 pc./g raw leaf (wt. of leaf tobacco in one cigarette about 1 g). The tobaccos showing highest activity are those grown on soils of low *p*H such as are derived from radioactive older granites or granodiorites occurring in parts of Southern Rhodesia, North Queensland, and elsewhere. The α-activity of a large range of parent granite materials has been measured and will be reported separately. They vary in total α-activity from 150 pc./g to 5 pc./g, the granites with lowest activity being, in general, those with less potash or soda granites,

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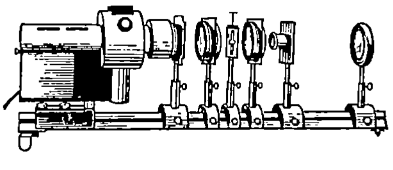

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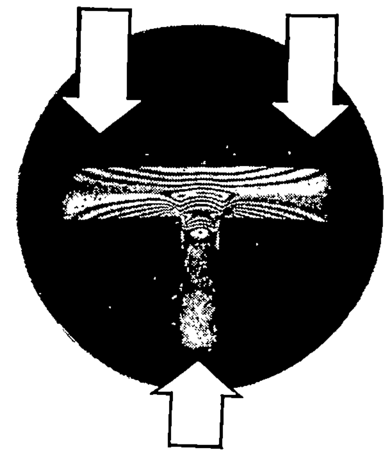

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and this extends to soils derived therefrom. Many granites derive most of their activity from thorium; but there are many in which uranium predominates as a source. This question has relevance to the proportion of α-particle contribution from polonium, which is one of the uranium series. In equilibrium, and in the absence of leaching effects, polonium would contribute one-eighth of the total α-particles from the uranium series.

Tobacco plants were grown in a greenhouse at the New Zealand Tobacco Research Station, Motueka, in large pots, using low-activity soil as control; but in other pots artificially enriched with finely ground different radioactive uranium ores and also monazite. Overseas field conditions regarding high radioactivity could be simulated, both in regard to soil and tobacco activity, and studies made, for example, of distribution of activity in leaves at various levels in the plant so-called lugs, cutters and tips. Although monazite itself (from Bryon Bay, Queensland) was not readily taken up by the plant, yet in the case of other ores in which uranium and thorium contributed, the uptake of uranium and thorium measured by the occurrence of 'doubles' (thorium and thorium *A*) was in many cases about proportionate, that is, there was little evidence that mesothorium (Ra 228) was the only thorium family parent picked up by the plant. Further observations are in progress.

A reconnaissance experiment was made to throw light on the question of the probable contribution of polonium from natural fall-out absorbed by the surface of the leaves. Dried commercial tobacco leaves were taken and equivalent parts of the leaf were placed with: (*a*) the upper surface against a phosphor; (*b*) the lower surface; while in some cases the leaf was dried until brittle and the powdered material placed against a third phosphor surface and scintillation counts made. All the tobaccos were Virginia cigarette type. The thickness of the leaf is several times greater than the range of α-particles. Typical results are given in Table 1.

Table 1. α-COUNTS/H FROM 50 CM²

Country of origin	Upper side of leaf	Lower side of leaf	Approx. percentage from thorium family
South Africa	12	5	30
New Zealand	9	8	35
S. Rhodesia	91	95	50
Thailand	21	20	50
U.S.A. (Eastern)	18	16	30

In general the lower side appeared to show a slightly higher proportion of thorium, but numbers are too small for statistical significance. It would be interesting to know in which locality the particular South African sample was grown.

The foregoing tests were carried out on board ship and weighing facilities were not available to give moisture content, which would affect α-particle absorption of the powdered sample, so the activity of the powdered leaf is not given, but it differed little from the mean values in Table 1. It is concluded that in spite of possible effect of surface waxes and small estimated amounts of soil dust on the surfaces of the leaf, there is probably some small effect of polonium fall-out; but in the case of higher activity tobaccos it is only a minor factor compared with activity derived from soil through the roots.

The foregoing figures are not to be taken as representing averages from the countries concerned as there is a large variation within each country, particularly Southern Rhodesia and Australia.

A large number of samples have been measured from tobacco leaf imported from various countries into New Zealand, and made available by co-operating commercial concerns, but if we are to attempt to give reliable representative figures, systematic sampling would be necessary, also selection of leaves from various soil types and positions on the plant from which the leaves are taken, also the soil moisture and climate conditions of the particular year of crop growth. For example, the New Zealand figure quoted is an average from soils derived mainly from 'Separation Point', low potassium granite, but with an admixture of so-called Motueka gravel soil of New Zealand greywacke origin. The purely granite soils over a larger part of the growing area gave an α-activity of 5 pc./g and the background γ-radiation was 0·0060 mrad/h, while only a mile away over the soils on which the sample referred to in Table 1 was grown the radiation was 0·010 mrad/h. The α-activity of soils and tobacco was found to show correspondence. There is also a probable influence of variety or genetic strain as in the case of wheat referred to in previous papers, although it is not so evident when using Virginia type cigarette tobacco only. There is apparently a preponderance of 'Hicks' variety grown in Southern Rhodesia, a proportion of 'Burley' in the United States and a type called 'Virginia Gold' in New Zealand, each bred and grown with regard to yield, quality and local disease resistance. Samples of the three varieties were grown on the same Motueka soil in New Zealand, and the specific activities were measured and found to show little difference.

Polonium in Motor-car Exhaust

It has long been known that lead processed from most of its ores contains considerable lead-210 and consequently polonium. Such lead was avoided for construction of electroscopes, etc., in the early days of radioactive measurements. It is natural, therefore, to suspect that lead in the anti-knock agent, of petrol lead tetraethyl, might contribute polonium to the combustion exhaust gases. Polonium salts are volatile, the chloride, for example, at 200° C only. A test was made of lead from lead tetraethyl solution as used commercially, and there was considerable α-activity when the material was treated with fuming nitric acid before precipitation to remove organic components. In a normal method of precipitation the polonium remained in the organic portion and the precipitated lead was at first inactive by α-particle measurement, but grew in activity in the following months, showing that polonium was involved.

Two sheets of lead of differing origin, of age possibly 10–15 years, were taken from the workshop and were given a fresh clean machined surface. The α-activity was in each case approximately 180 particles/100 cm²/h. A similar piece of lead, age known to be of the order of 120 years, supplied to me by Harry L. Dowsett of Dowsett Engineering Co., gave an activity of only 4 particles per 100 cm²/h. These figures correspond roughly with the decay of lead-210 of period 22 years.

By courtesy of Shell Co. of New Zealand, scrapings were obtained of the inside of the exhaust pipe of the C.F.R. engine used for testing leaded fuels. The pipe was an old one taken out of service four months earlier and had been surrounded by water-cooling pipes. The scrapings were measured for α-particles and displayed considerable activity, corresponding when corrected for lead content, roughly 16 per cent, to that of the lead tetraethyl used. Another test was made of the scrapings from the inside of the exhaust pipe adjacent to the engine of a new motor-car. The car had been in use for one month only using leaded petrol. The α-activity was pronounced and has grown roughly 10 per cent in the four weeks which have elapsed since the sample was taken, indicating that much of the original polonium may have escaped into the air, being more volatile than the lead which was more readily deposited. To test whether the anti-knock properties might be due to the initiating action of α-particle produced ionization or radicals, the Dominion Laboratory kindly prepared samples of lead tetraethyl from the two samples of lead referred to already. The lead tetraethyl produced had good anti-knock properties in both cases, some 80 per cent that of material supplied commercially but with the old lead possibly slightly less effective. However, these tests need repeating in a few months' time when polonium may have grown, but they did show that lead tetraethyl, prepared from old lead or from inactive ore, such as certain samples of galena which were also tested, can be used for octane enhancement without giving polonium to the car exhaust gases. It should be possible to design an exhaust pipe system which will condense out the lead itself should this be considered desirable for other reasons. The average amount used appears to be of the order of 2–3 g lead per gallon of petrol.

It may now be speculated generally and briefly on the possible significance of inhaled or ingested polonium neglecting the effects of other more fugitive α-radiating nuclides and also free radicals in the smoke condensate, as discussed in the paper by Marsden and Collins[3]. It is apparent that unlike radium and strontium-90, which accompany calcium to bone sites, polonium moves more to the soft tissue, and Osborne has reported quantities in the lung and reproductive organs. The destructive power of its α-particles is well illustrated in a photograph following the article by Radford and Hunt[2], already referred to. A man inhaling the smoke of 25 average British cigarettes a day probably deposits on his lungs and trachea some 8 pc./day of polonium, whereas the total dietary intake of radium is only about 2 pc./day excluding daughter products, and the latter is more subject to excretion or transfer to less hazardous body sites. In passing, however, mention may be made of the possible ingestion of polonium when a certain popular breakfast food is used. One sample of such gave an activity of 1·2 pc./g of which possibly 1/12 was polonium. If 2 oz. of this were consumed each day, the corresponding dietary intake would be 5 pc. Formerly the use of such active wheat was not considered a possible hazard because of the amount of dilution of its radium with the relatively large amount of inactive calcium in milk normally consumed with it, but when we consider polonium the position could be different.

To revert to cigarette smoking, as is well known there has been a striking increase in rates of diagnosed death from lung cancer during the past 50 years, that is, some 40 times in certain age groups in Great Britain. If we superimpose on the curve of increase among adult males since 1916 and, using a suitable scale factor, points representing the figures of imports of Southern Rhodesian tobacco, we obtain a startling correspondence, with a lag of 10 years in cancer figures corresponding to latent development period and delay in manufacture. However, such a correspondence may be largely fortuitous and diagnosis has improved, and possibly one could obtain much the same degree of correlation with figures relating to use of certain leaded petrols, or even, as a friend facetiously pointed out, of radio licence numbers, though not over the whole period to 1963.

The death rates of males from lung cancer for South Africa or New Zealand, Australia, United States and Great Britain are very roughly in the ratio of 22 : 30 : 39 : c. 60. These show a very general relationship to the amount of polonium activity inhaled from cigarettes consumed possibly if we include a factor in the United States and Great Britain for the possible effect of lead from petrol. The South African tobacco seems to be low in activity, though only two samples have been measured. As to Southern Rhodesia, I am informed that the male lung cancer death rate is the same as the figure quoted for South Africa and New Zealand, but am uncertain regarding age grouping. In New Zealand the deaths from lung cancer have followed the general world upward trend until 1956, when the curve abruptly levelled off significantly for 5 years. This may correspond to very considerably increased use of local tobacco, less radioactive than imported leaf or manufactured cigarettes. However, during 1957–61 considerable Rhodesian tobacco was imported, some 6 per cent of total, and it may be interesting to note that the male lung cancer incidence in 1962 and 1963 has again shown an increase, probably significant. The crude male deaths from lung cancer during the years 1957–63 were 357, 338, 363, 342, 395, 438 and 461 respectively. Australia has recently used some 25 per cent of its large consumption of cigarette tobacco from Rhodesia, plus that from North Queensland, both high activity tobaccos. The American figures are higher than Australian although tobacco activity is much the same, but considerably lower than those of Great Britain, as is the average activity of its cigarette tobacco correspondingly. Great Britain now imports some 30 per cent of its tobacco from Southern Rhodesia and Nyasaland and the activity of its cigarette blends was found, from examination of old cigarettes back to 1914, to have increased with the years and also the number of cigarettes smoked per head has increased in all countries. In studying correlations the difficulty is (*a*) latent period, (*b*) proportion of activity due to polonium. It may well be that the generally assumed latent period, that is, 20 years, is too high.

Mention may be made of the evidence derived by G. Dean[4] and D. F. Eastcott[5], who found higher lung cancer rates among migrants from Britain to New Zealand, South Africa and Australia compared with white local population. This may possibly be explained in part by the higher radioactivity of average British cigarettes. The correspondences quoted here, however, do not constitute proof of causation; but they arouse suspicion and are indicative of directions for further investigations and for remedial actions. It is probable that even if polonium is not absorbed directly by plant roots from the soil, since its compounds tend to have colloidal properties, yet lead-210 is freely transported. Thus within two years of gathering, which is less than the time normally allowed for fermentation, maturation, manufacture and retailing, polonium will have reached practical equilibrium, that is, within 3 per cent with such lead-210. It is not known whether lead is selectively taken up from soil compared with, say, radium. This may well be so under certain acid soil conditions. Some early results obtained by me point in this direction[3].

The possible case against lead anti-knock compounds, etc., needs much more investigation than that recorded in this article. The lead-210 in lead ores and compounds varies considerably with different origins and possible use of older reclaimed lead in processing. Some, probably secondary, ores have been examined which show little activity.

It is not improbable that some lead octane agents may contain polonium up to 10 pc./g. If 3 g per gallon are used this would correspond to 30 pc./gallon. Roughly converting this to content per litre of exhaust gases and assuming a man operates for 8 h/day in such exhaust, say, 100 times air diluted, one may deduce that such a person inhales of the order of 0·12 pc./day. This is small compared with that of the average cigarette smoke inhaler in Britain but may be more comparable in some other countries, as indeed might be inferred from the investigations of Poche *et al.*[6] in Germany. Tests of two samples of German tobacco leaf gave values 0·4 of the average used in the United Kingdom—a sample of Russian similarly—but considerations arise as to particle size and sites of deposition in the two cases. However, the figures, although an order of magnitude greater than under natural conditions, are too uncertain for further speculation, and experimental data are required relating not only to sources of lead components, but also of the actual content of the car exhaust gases under various conditions of car operation, that is, 'idling'. It would also be well to investigate the free radicals present and the lower aliphatics which might be the source of carcinogens in conditions of incomplete combustion.

Further mention must be made of possible effects of ingested polonium from diet. In a previous paper[7], the case of a group of Niueans was discussed and now further biological chromosomal tests are in progress, also an examination of food polonium-status. Apart from the very special case of certain Niueans, the Polynesians generally, including the Maoris, have a considerably greater intake of polonium-210 than Europeans, because of the large amount of sea shellfish they consume many times that of Europeans. The plankton food of such shellfish contains large amounts of polonium. I have made measurements in many ocean areas. The activity is very high and of the order of 12 pc./g plankton, dried at 120° C, and is predominantly polonium. It may possibly be significant that the Maoris have a large incidence of stomach cancers and also, to take one genetic impairment only, a 6-fold greater incidence of club foot[7,8]. This ratio, which has been recently investigated and confirmed by Dr. Peter W. Tapsell of Rotorua (private communication), does not appear to me to be wholly explainable by consanguinous marriages.

An examination of the figures of incidence of cancers of the digestive track in New Zealand in different regions lends support to the idea of a relation to soil and foodstuff radioactivity. Regional figures were supplied to me by N. H. Taylor (lately director of the Soil Bureau, and with whom I have discussed soil trace element questions during many years), and these are significant, but will be converted to a soil type basis. In particular an area in which the soil is of low activity, largely because of derivation from low-activity granite and rocks, gives an incidence of cancer of the digestive track only 40 per cent of that of a neighbouring area in which the soils are derived from highly active Karamea Granite and from greywacke. The relative figures indicate that investigation is worth while since remedial action is likely to be simple. There are, of course, other factors, such as rainfall.

In conclusion, it is appreciated that the various questions raised are perhaps somewhat hypothetical and naturally controversial. The importance of the subject justifies us in exploring such considerations as are advanced, since if causal connexions can be established the matter would assume wider social and political implications. The directions of these need not be enumerated at this stage.

I thank the director (W. H. Ward) and staff of the Dominion Physical Laboratory for facilities and advice, the Wellington Branch of the British Empire Cancer Campaign for a small grant, the New Zealand Tobacco Research Station, in particular Mr. D. S. C. Wright, for undertaking laborious experiments, New Zealand Tobacco Companies, Ltd., for assistance, and the Shell Co. of New Zealand and the United Kingdom, and Dominion Laboratory for ready help in work on leaded compounds used in petrol.

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Director

In order to provide increased facilities for research the Anti-Locust Research Centre, one of the scientific units of the Department of Technical Co-operation, has recently moved to new accommodation in College House, Wrights Lane, Kensington. The new Centre was officially opened on April 28 by the Right Hon. Robert Carr, Secretary for Technical Co-operation. The opening ceremony was attended by representatives of many of the international organizations with which the Centre collaborates, such as the Food and Agriculture Organization of the United Nations, the World Meteorological Organization and the international locust control organizations, by representatives of Embassies and High Commissions of countries which the Centre assists or with which it co-operates in locust research and control; and by scientists and others who are specially interested in the work of the Centre.

At College House all sections of the Centre except the Taxonomic Section, which for obvious reasons remains in the British Museum (Natural History), are together under one roof for the first time. This has meant bringing in the Insecticide Section, previously attached to the Chemical Defence Experimental Establishment at Porton Down, and the Behaviour Unit, previously working in the Hope Department of Entomology at Oxford. Despite these additions, it has been possible in the new building to extend the space and increase the facilities of all sections.

In the laboratory, there are specially designed constant-temperature rooms for locust breeding and experimental work, including a cold room; all rooms in the central section are air-conditioned. Special planning was necessary in the Insecticide Section to prevent the toxic chemicals used therein affecting biological work, and this section has a separate air-conditioning and filtering plant. There are biological, physiological, biochemical and behaviour laboratories, and special facilities include an interference microscope and counter-current, chromatographic, electrophoresis and electro-physiological apparatus. There are two wind tunnels, one for studying locust flight and the application of insecticides to flying locusts and the other a special insecticide spraying tunnel. Because of the allergy to which locusts give rise, and to which some people are extremely sensitive, the laboratories and locust breeding rooms can only be entered through changing-rooms in order to minimize the transference of the allergens to other parts of the building (Fig. 1). Cartographical and biogeographical research facilities include the provision of new map rooms, archive and data-storage rooms and modern map filing and display facilities. Accommodation is also now provided for the recently formed Field Research Section, which was established to meet added overseas requirements when the Centre became a special unit of the Department of Technical Co-operation in 1961. As such, its terms of reference now include the promotion of international technical co-operation in the field of locust research and control, and the provision of facilities for visiting scientists and students for research and training. Thus in addition to special rooms for visitors, there is a conference room with sound cinematograph projection room attached, which is used for seminars and training courses.

Current research at the Centre covers three main fields—biology, biogeography and control. Biological

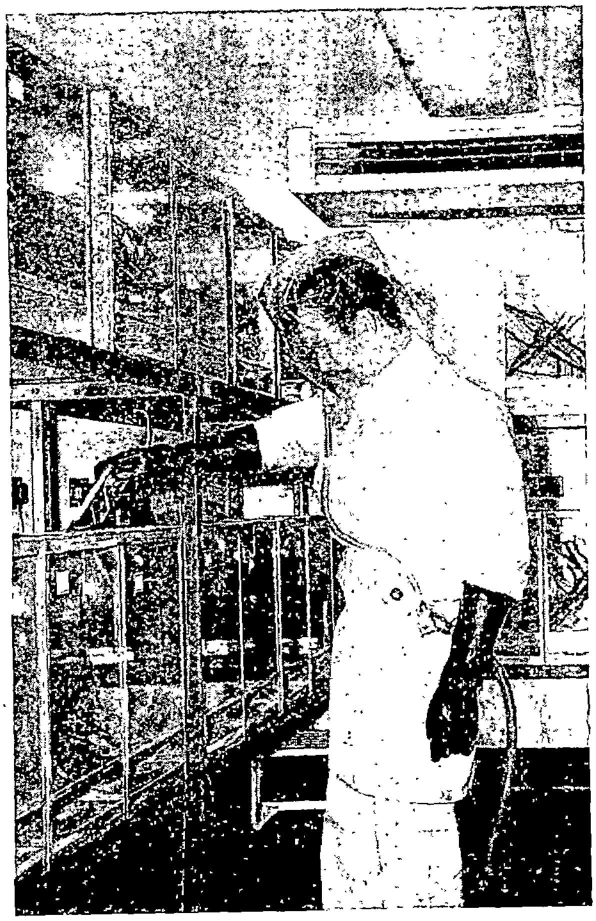

Fig. 1. A corner of one of the constant-temperature breeding rooms, showing a member of the staff wearing special clothing as a protection against locust allergens

research includes the taxonomy and identification of locusts and grasshoppers sent in by sources throughout the world; Dr. V. M. Dirsh is in charge of this work and, as already remarked, his section remains at the Entomological Department of the British Museum (Natural History), together with the Centre's extensive collection of specimens. The breeding facilities, in charge of Mr. Hunter-Jones, allow the rearing of a million or so locusts a year, and make it possible to investigate the life-histories of economically important locusts and grasshoppers. The experimental rooms enable the effects of environmental changes on the development, maturation and reproduction of locusts to be studied. A major effort is being directed to those aspects of locust behaviour and physiology which might ultimately lead to the development of new methods of control, such as the study of attractants and the hormone systems involved in locomotor and reproductive activity. This work is being conducted by Dr. P. T. Haskell, Dr. D. B. Carlisle and Dr. P. E. Ellis.

The examination of the biogeography of locusts has always been considered by the Centre as an essential basis for the strategy of their control. The Biogeographical Section, led by Miss Z. Waloff, is at present concerned with the analysis of historical data on the plagues and recessions of the desert locust, the initiation, development and decline of the last major plagues of the red and the African migratory locusts and the seasonal distribution and breeding of tree locusts in relation to rainfall and vegetation. A further project concerns the assessment of the economic importance of locusts, and involves the assembly and analysis of agricultural statistics and locust damage records.

Another section engaged on biogeographical work the Desert Locust Information Service, in charge Dr. R. C. Rainey. This section, which is also sponsor by the Food and Agriculture Organization of the Unit Nations, maintains the record of desert locust activi which has been built up in the Centre over the pa thirty-five years. Locust situation reports, sent regula to the Centre by countries affected by the desert locu are correlated with daily weather data supplied with t co-operation of the U.K. Meteorological Office and t World Meteorological Organization and form the ba for a monthly summary of the current desert loc situation and a forecast of expected developments wh is sent to countries in and around the invasion ar The section also carries out research on the influence climatic and meteorological factors on the desert locust

Research on locust control includes work undertak by the Field Research and Insecticide Sections. T former section, led by Mr. C. Ashall, is staffed entir by scientists with very long practical experience locust research and control in the field and it carries c field investigations of locust and grasshopper cont in close co-operation with overseas anti-locust organ ations and plant protection departments. Thus, officer from the section is now based in the Trucial Sta to carry out surveys of desert locust populations in t area and in other parts of the Arabian Peninsula. A visory and training visits are carried out in response increasing requests from overseas Governments; example, recently a preliminary survey of the red loc situation in Mauritius was begun, and in the past year t training courses have been given in the Sudan and th in Saudi Arabia.

The Insecticide Section, led by Mr. R. D. MacCu is concerned with testing new insecticides and inves ating the factors affecting the toxicity of insectici and their formulations to locusts. Recent laborat work has shown that the volatility of spray solvents c greatly influence their toxicity, and further experime are being undertaken to estimate the importance of t in control operations. A standard insecticide test kit for locusts has been prepared so as to enable susceptibility of locusts of the same species in differ regions of the world to be determined.

The section carries out field work concerned with testing and calibration of spray gear, the assessment control methods and the development of techniq for testing spray pick-up and residues, all of which is d overseas in co-operation with international locust org izations or plant protection departments.

The 'servicing sections' of the Centre include Statistical and Photographic Section, which advises the design of experiments, sampling techniques related subjects; the Training and Liaison Section, wh deals with home training courses, publications and pu relations; and the Library. The latter contains m than 13,000 separate publications on locusts and gr hoppers in more than twenty-five languages, aut catalogued with subject and species indexes. It looks a the photographic collection and the unique collectio taxonomic works on locusts and grasshoppers and publishes abstracts of current literature on locusts grasshoppers.

The Centre plays a leading part in the furtheranc international co-operation in locust control and rese and it has achieved world recognition as the pre-emi establishment for anti-locust investigations. The facilities at College House are worthy of this role. interesting to reflect, particularly at the present time w much attention is being directed to the problems of ai developing countries, that the Centre, paid for by British taxpayer, has for the past eighteen years pione international technical assistance in locust research control and exists solely for the benefit of ove territories.

SOUTHAMPTON MEETING OF THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

By R. N. M. ROBERTSON

Secretary and Registrar, University of Southampton

THE British Association first met in Southampton in 1846, sixteen years before the opening of the Hartley Institution, in which the University of Southampton originated. In 1882 when the Association next came to Southampton the programme included a *soirée* at the "Hartley Hall" and in 1925 at the following meeting various sectional meetings were reported as having been held at the Hartley University College. The Hartley Institution, later known as the Hartley College, was incorporated in 1902 as the Hartley University College by the Board of Education. The *Centenary History of the University*[1] refers to the support of the British Association for the establishment of full-time technical colleges in all major towns aided by government and local grants, and it seems likely that this strengthened the Institution's case for help for this aspect of its work. New buildings in Highfield for the University College were opened by Lord Haldane in June 1914, but the establishment of a military hospital soon afterwards prevented a move from the old buildings in the High Street until 1919.

The fourth visit of the Association to Southampton, to be held this year during August 26–September 2, comes twelve years after the dissolution of the Hartley University College on the constitution of the University of Southampton by Royal Charter in 1952. *The Centenary History* is largely devoted to financial difficulties, as a result of which the buildings available to the College, when the British Association meetings were held in 1882 and 1925, were ill-equipped and to some extent unsuited to their purpose. This year's activities will mainly centre on new buildings erected in a period of unprecedented growth in student numbers, which justifies all the struggles of the past. In the session 1963–64 the student population has been 2,100, and, according to the plan approved by the University Grants Committee to meet the needs outlined in the Robbins Report, the numbers will increase to 4,000 by session 1967–68. No decisions have been reached about later expansion, but the confident hope is that Southampton will take its place as one of the multi-faculty universities of 8,000–10,000 students envisaged by the Robbins Committee. By 1967–68 sixty per cent of the students will be in the Faculties of Science and Engineering and further evidence of the swing in this direction is perhaps to be seen in the appointment of Prof. Kenneth Mather, of the University of Birmingham, to succeed the present vice-chancellor, Dr. D. G. James, at the end of session 1964–65 (*Nature*, 203, 24; 1964).

Southampton, Britain's principal ocean passenger port, and a major cargo centre, occupies a central position on the south coast of England within eighty miles of London. A borough for almost eight centuries, its history stretches back through settlements established by the West Saxons and Danes to the Roman port of Clausentum on the River Itchen. Canute was chosen as King by the Saxon Council at a meeting in the town in 1017, and the story of his attempt to turn the tide is reputed to be connected with one of his subsequent visits. In medieval times the prosperity of the port, with its interesting double tides, was founded on the wine and wool trades, but a period of decline followed until the town was transformed into a fashionable spa in the eighteenth century. The coming of the railway led to the rise of the transatlantic passenger trade, culminating in the completion of the New Docks in 1934. More recently Southampton has become a centre of the chemical industry, largely as the result of the siting of the Esso refinery at Fawley on the south side of Southampton Water. The refinery, the largest in Great Britain, produces 11,000,000 tons of petroleum products per annum and supplies nearly a quarter of the country's requirements. With the introduction of economic methods of converting waste material from the refinery into useful chemicals the growth of Fawley has led to the erection in adjacent sites of large chemical plants by such firms as Monsanto,

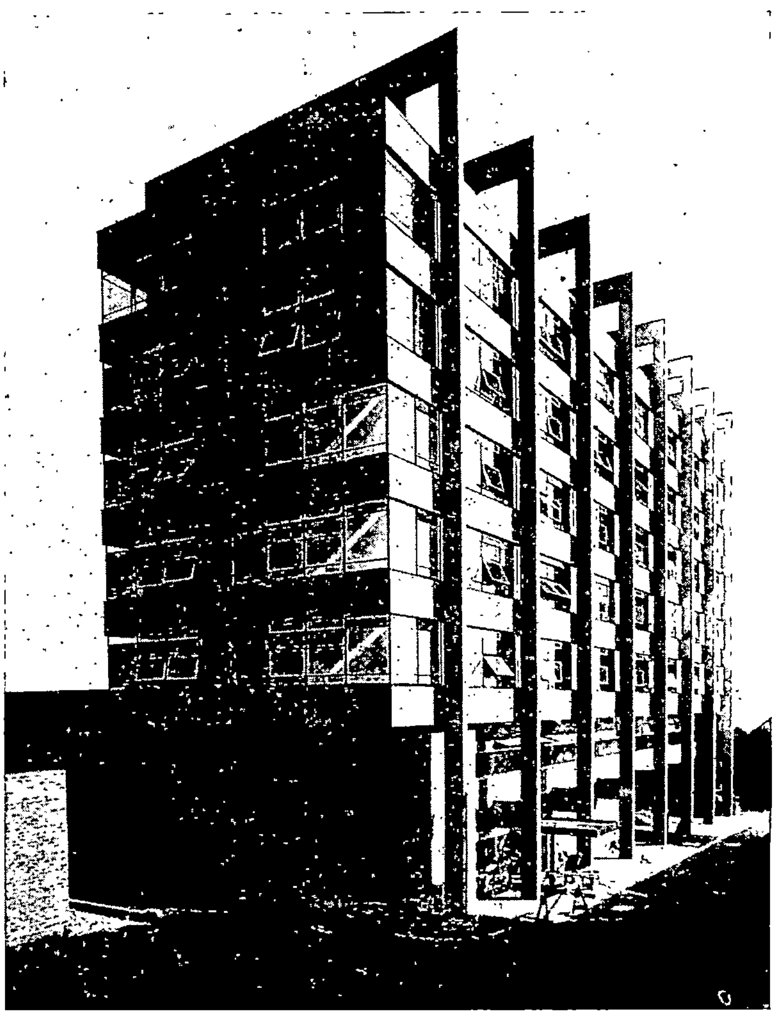

Fig. 1. New Chemistry Building, University of Southampton

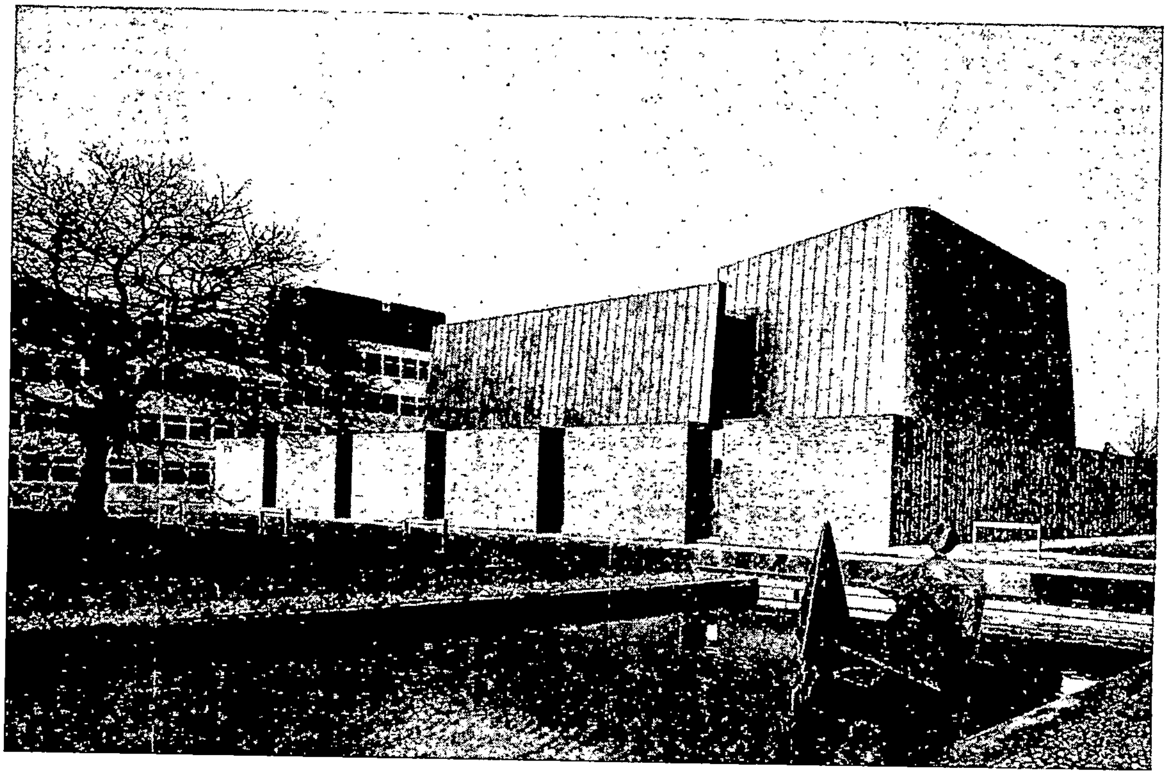

Fig. 2. Nuffield Theatre with Arts Building in the background

International Synthetic Rubber and Union Carbide. Local industry is not restricted to these activities and other firms with extensive plants in Southampton include AC-Delco, Standard Telephones and Cables, Mullards, Pirelli-General, British American Tobacco, Ford, Vickers-Armstrong, Hawker Siddeley and Thornycroft. In 1846, on the occasion of the first meeting, Southampton was described as "this beautiful and flourishing sea port" with considerable value to the naturalist and geologist. Although the description is still to some extent true, it must be emphasized that the area is now also one of great industrial significance.

These local interests of the city are well supplemented by the regional characteristics of Wessex, with its cathedral cities, country towns, forests, heaths and downs, the whole presenting a fitting setting for the 1964 meeting. The programme of excursions, general and sectional, is widely varied. There will be many opportunities to visit factories, industrial plants and other establishments not only in the city but also farther afield at the Royal Aircraft Establishment at Farnborough, the Road Research Laboratory at Crowthorne, Berks, the Atomic Energy Establishment at Winfrith and the National Institute of Research in Dairying at Reading. In addition to visits to Southampton Docks, including conducted tours of the R.M.S. *Queen Elizabeth*, there will be a specially organized day cruise of Southampton Water and the Solent. Historic buildings in Southampton feature in the programme as well as visits to the great cathedrals of Winchester, Salisbury and Chichester and to such notable country houses as Wilton, Longleat, Beaulieu, Breamore and Stratfield Saye. The Ordnance Survey office in Southampton will also be open to members. Natural and prehistoric features include the New Forest, Old Winchester Hill, Maiden Castle, the Furzebrook Nature Conservancy Reserves, the Isle of Wight and the Dorset Coast.

One of the first steps taken in planning the meeting was the appointment of Prof. F. J. Monkhouse, professor of geography in the University, as editor of *The Southampton Survey*, which has been compiled and published for the meeting. The first part of the Survey deals with the wider region of which the City of Southampton forms part, the whole of Hampshire, the Isle of Wight and the adjoining parts of Wiltshire, Dorset and West Sussex; the second part is concerned with the historical background both of the wider region and of the city; the third looks to present-day Southampton from economic, social and administrative points of view. A copy of the survey, which has been printed locally by the Camelot Press, will be presented to each member of the Association attending the meeting. This year copies may, if desired, be obtained by members in advance, and after the meeting they may be purchased on application to the University (35*s*. or 5 dollars, including postage).

The previous presidents at Southampton were Sir Roderick Murchison (1846), Dr. C. William Siemens (1882), and Prof. Horace Lamb (1925). The president of the 1964 meeting is Lord Brain (*Nature*, 199, 891; 1963), the distinguished neurologist and former president of the Royal College of Physicians, who will deliver an address on "Science and Behaviour". Before the address, the Chancellor of the University, Sir Keith Murray, former chairman of the University Grants Committee, will confer the honorary degree of doctor of science on Lord Brain and also on Sir Eric Ashby and Sir Cyril Hinshelwood.

The two evening discourses will be by Prof. W. J. G. Benyon on "The International Years of the Quiet Sun" and by Prof. A. S. Parkes on "Biological Aspects of the Population Explosion". There will also be a public lecture by Dr. A. T. J. Dollar on August 30, entitled "Island Volcano", which will cover the recent new volcanic island off the coast of Iceland. The presidential addresses in the sections will present the usual wide range of topics, many of particular interest to non-scientific members; but a new note will be sounded on August 31 with "Science in Science Fiction" by Prof. W. T. Williams of the University Department of Botany and some of his colleagues. This year, for the first time, the Council of the Association has made arrangements for live television transmissions of the morning sessions of a number of the sections.

By special arrangements with Southern Television, which covers an area from Bridport to Dover with an extensive hinterland, there will be about fifteen hours in all of transmissions from the University's Nuffield Theatre (Fig. 2). This new Theatre, which was built largely with the aid of a grant of £130,000 from the Nuffield Foundation, has a seating capacity of 500 and was opened earlier this year by Dame Sybil Thorndike. In addition to television presentations, scientific film shows and an Open Forum for young people will also be given there.

The exhibitions at this year's meeting include one staged by the Ordnance Survey in the entrance hall of the University Main Building, which will contain a comprehensive display of their work. Members of the staff of the Survey will be in attendance to give practical demonstrations of some of the techniques used in field surveying and cartography. The National Book League will stage an exhibition "Science for All" together with a supplementary exhibition on the Social Sciences. There will also be special demonstrations set up by the Departments of Zoology and Botany and by the Institute of Science Technology in the Department of Chemistry (Fig. 1), and the University Library will feature exhibitions of books on Botanical Illustrations and the local history of Southampton. The hydraulic model of Southampton Water, which is housed in a special building, will be demonstrated daily by the Civil Engineering Department, and in the Electronics Department a number of commercial and government organizations will sponsor an exhibition of some recent developments in the fields of microminiature circuits, electrical generation and communications equipment.

The young people's Science Fair will be held in the new Southampton College of Technology, where more than 130 projects from 61 secondary schools and three training colleges will occupy laboratories and demonstration rooms on six floors. There will also be illustrated lectures for young people in the Guildhall on four days of the meeting by Prof. R. V. Jones, Dr. N. Tinburger, Dr. H. A. Thomas and Dr. E. J. Ambrose. Prof. L. Dudley Stamp will be chairman of the Open Forum in the Nuffield Theatre and the panel will consist of Dr. J. D. Cartney, Dr. V. Milledge, Mr. D. E. Broadbent and Prof. W. T. Williams. The social activities of the week will include two evening receptions given by the City Council in the Guildhall. The University will give a Garden Party to all members in the gardens of South Stoneham House, an early eighteenth-century mansion now used as a University Hall of Residence, and a number of other functions will be offered by private organizations, including a particularly appropriate gesture of hospitality, lunch on board R.M.S. *Capetown Castle*.

The local organizers in Southampton feel that they are well prepared for the meeting and they anticipate that it will not only be the best of the Southampton meetings but also one of the outstanding meetings in the history of the Association.

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[1] Patterson, A. Temple, reader in regional history, Univ. Southampton (1962).

NEWS and VIEWS

The Royal Society: Research Appointments

The Royal Society recently agreed to co-operate with Wadham College, Oxford, in setting up a Royal Society research fellowship in the biological sciences at Wadham College. The establishment of this post marks the three hundred and fiftieth anniversary of the foundation of Wadham College and also the Royal Society's past historical associations with the College. The Research Fellow will be a full official Fellow of Wadham College and the appointment has been made for a period of five years from October 1. It has now been announced that Dr. D. C. Smith, university demonstrator in the Department of Agriculture at the University of Oxford, has been appointed to this post. Dr. Smith will carry out research in the physiology of lichens.

The Royal Society has also announced that the Foulerton Gift research fellowship, for two years in the first instance from October 1, has been awarded to Dr. J. J. B. Jack, at present house surgeon at the Radcliffe Infirmary, Oxford, to enable him to work on excitation and inhibition in the spinal cord at the University Laboratory of Physiology, Oxford.

Galileo Celebration

On the evening of July 9 the Royal Society celebrated the quater-centenary of the birth of Galileo Galilei (*Nature*, 201, 563; 1964) with a special Conversazione held in the Society's apartments at Burlington House. Among the guests were representatives of the Accademia Nazionale dei Lincei, of which Galileo was a member, the British–Italian Society, the British Society for the History of Science and the British National Committee for the History of Science. Among the exhibits was a rare collection, arranged by Prof. E. N. da C. Andrade, of books by and about Galileo, including a number of early English books in which mention of Galileo is made, including *Paradise Lost* and *Areopagitica* by John Milton. A few of the experiments which Galileo himself carried out relating to fundamental laws of mechanics were repeated by the staff of the Royal Institution. Other exhibits compared what Galileo saw through his telescope with modern achievements on the same subject. For example, his description of sunspots is contrasted with contemporary photographs obtained by the Royal Greenwich Observatory, and the Department of Astronomy, University of Manchester, likewise compared up-to-date knowledge of lunar topography with that observed by Galileo. The Nuffield Radio Astronomy Laboratories, Jodrell Bank, University of Manchester, described some recent radio astronomical work relating to Venus and to radio sources in the Milky Way, both Venus and the Milky Way being subjects of observation by Galileo. The National Physical Laboratory demonstrated a reversible pendulum for the absolute determination of gravity, and Mr. D. G. King-Hele, of the Space Department of the Royal Aircraft Establishment, in a display showed what could be learned about the upper atmosphere and its dependence on solar activity as determined from the orbits of artificial Earth satellites.

Research on Metal Extraction and Mineral Processing

In a written answer in the House of Commons on June 25, the Secretary of State for Education and Science, Mr. Q. Hogg, stated that the decision to transfer forty staff of all grades employed on work on the extraction of metals from the National Chemical Laboratory to the Warren Spring Laboratory followed the Government's acceptance of a recommendation of the Brundrett Report on Technical Assistance for Overseas Geology and Mining. Of these forty, three junior members resigned before the decision was announced and one more senior had resigned since; eight had asked for transfer elsewhere. The Director of the National Chemical Laboratory gave the Department no reason for his resignation, but it is understood that he disagreed with the decision to implement this recommendation of the Brundrett Committee. While Mr. Hogg greatly regretted Dr. Mitchell's decision, he was satisfied that the national interest would best be served by arranging, as the Brundrett Committee recommended, for the work on extraction of metals and that on mineral processing to be integrated under the same research direction.

The Council for National Academic Awards

In a written reply on July 2 to a question by Mr. Julian Critchley, Mr. Hogg announced the membership of the new Council for National Academic Awards under the chairmanship of Sir Harold Roxbee Cox (*Nature*, 201, 975; 1964): The Viscount Caldecote (deputy chairman, English Electric); Mr. W. F. Cartwright (managing director, Steel Co. of Wales); Mr. Michael Clapham (director of Imperial Chemical Industries); Sir James Cook (vice-chancellor, University of Exeter); Mr. B. Downs (head of Department of Mechanical Engineering, Loughborough College of Advanced Technology); Miss Helen L. Gardner (reader in English literature, University of Oxford); Dr. D. E. R. Godfrey (head of Mathematics Department, Woolwich Polytechnic); Mr. H. N. Henry (principal, Paisley College of Technology); Prof. J. Lamb (professor of electrical engineering, University of Glasgow); Mr. J. Russell Lang (chairman, G. and J. Weir, Ltd., Glasgow); Dr. Kathleen Ollerenshaw (Manchester City Councillor and member of Manchester Education Committee); Dr. O. G. Pickard (principal, Ealing Technical College); Sir Arnold Plant (professor of commerce, University of London); Mr. R. H. J. Rhodes (vice-principal, Leeds College of Commerce); Prof. G. D. Rochester (professor of physics, University of Durham); Sir Lionel Russell (chief education officer, Birmingham); Dr. J. Topping (principal, Brunel College of Advanced Technology); Mr. D. Vitkovitch (head of Electrical Engineering Department, Nottingham and District Technical College); Dr. G. E. Watts (principal, Brighton College of Technology); Mr. R. E. Wood (principal, Leicester College of Technology and Commerce); Dr. E. G. Woodroofe (joint vice-chairman, Unilever). The new Council will take over the responsibilities of the National Council for Technological Awards as soon as it is in a position to do so, but the latter will continue to administer the Diploma in Technology in the meantime.

The Commonwealth Development Corporation

The annual report of the Commonwealth Development Corporation for the year ended December 31, 1963, welcomes the Commonwealth Development Act, 1963, which became law in July 1963 (Pp. vi + 102. London: H.M.S.O., 1964. 6*s*. 6*d*. net). The Act restored the Corporation's full powers of operation in all those countries within the Commonwealth which had achieved independence since the Corporation was established in 1948. The Corporation's area of activity had been contracting year by year and, now that the area of investment has been restored to that of 1948, it should again be possible to expand the volume of investment. The useful future of increasing activity now foreseen should be beneficial in sustaining the essential morale of the staff, which in some territories overseas had faced a year of danger with a steadiness to which the report pays tribute. Ten new projects involving a total commitment of £6,585,000 were approved during the year; three large projects involving £3,400,000 are in independent Commonwealth countries. Plans for further investment in existing projects have been approved for a total of £3,860,000; however, these figures do not give full weight to new projects approved by local development companies in which the Corporation has an interest. The falling away of new project approvals in 1962 was reflected in a further contraction of new investment from £9·5 million in 1962 to £6·8 million in 1963, but the whole of this new investment was financed from sources other than additional drawings from the Treasury, to which there was a net repayment of £100,000, in addition to the £629,000 formula payment in reduction of frozen balances. The operating surplus of £4,751,622 was £391,000 greater than in 1962, but the present arrangements do not give sufficient margin against the commercial and other risks that the Corporation is bound to run; the operating margin continues to narrow. Besides loans for public utility (infra-structure) purposes and investments in large-scale agricultural, industrial and estate projects, the Corporation's investment is increasingly directed to projects involving association with and support of the peoples of the countries in which it operates. Recently, a particular aim has been to make available to small farmers some of the benefits of present-day scientific advances in growing, handling and processing tropical crops.

Qualifications in Biology

The Institute of Biology has agreed to join with the professional institutes for other sciences and the Ministry of Education in a scheme for an Ordinary National Certificate in the Sciences. This will replace the Ordinary Endorsed Certificate in Biology and Ordinary National Certificate in Chemistry and Physics. Those intending to proceed to a Higher National Certificate in Applied Biology will study biology to a higher level than mathematics, physics and chemistry. It is likely that, of the time available for scientific subjects, one-half will be available for biology, one-quarter for chemistry and one-quarter for mathematics and physics. The Institute is represented on the Joint Committee for the Ordinary National Certificate in the Sciences by Prof. C. G. C. Chesters and Dr. R. J. Terry. The Committee will later issue rules and notes for guidance with the view of courses commencing in September 1965. The Institute has also been considering means of admitting persons to membership of the Institute as a result of an examination set by the Institute. It is envisaged that the examination will be taken after three years part-time study by persons who enter the course with a Higher National Certificate in Applied Biology. There would be a Part 1 examination at the end of the first year, at which candidates would take papers in the principles of biology and in a special subject. The Part 2 examination would be on the special subject alone. The subjects at present being considered are biochemistry, entomology, microbiology, pharmacology and plant pathology. For these subjects, panels are preparing syllabuses. A meeting was recently held with representatives of technical colleges and of the major employers of biologists at which comments were received on the scheme. A preliminary enquiry shows that some twenty colleges hope to be able to offer courses with a total intake of some 150 students. Courses leading to the examination for membership of the Institute of Biology will commence in September 1965.

The Macaulay Institute for Soil Research

The thirty-third annual report of the Macaulay Institute for Soil Research for 1962–63 summarizes the work in progress and includes a list of seventy papers, published or submitted for publication (Pp. 56. Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1964). The primary aim of the research continues to be the addition to fundamental knowledge of soils and soil : plant relationships supported by investigations directed towards the maintenance or improvement of soil fertility. Of the wide range of investigations described, only a short selection is needed to indicate the nature of the activities in the various departments. Electron microscopy and diffraction have been added to the methods for soil mineralogy investigation; pollen analyses of peat deposits are intended to supplement scanty records of vegetational history, and the physical properties of peat to help in investigations of tree growth. The soil survey has completed a further 580 square miles on the 2·5-in. scale, and another memoir (*Country Round Aberdeen, Inverurie and Fraserburgh*) has been published. Emphasis is laid on the need for more information on the trace element contents of different species at different stages of growth and on the possible dangers of lead toxicity. The interactions between major and trace elements on ion transport and exchange in plant tissues continue to receive attention and a start has been made on the role of vacuolar osmotic potential in nutrient uptake. Selected soil groups are being used for evaluating the properties that govern soil productivity, and investigations of responses to fertilizers have included methods and times of application.

The Furniture Industry Research Association

The third annual report of the Furniture Industry Research Association shows that, during 1963, a significant increase in membership occurred, and that total membership now amounts to 712 (Pp. 16. Stevenage: The Furniture Industry Research Association, 1964). This includes 538 firms registered with the Furniture Development Council, which cover about 80 per cent by value of the turnover of the industry. Considerable progress is reported on all present research projects covering such topics as the determination of the loads in chair frames, causes of show-through in veneered chip-board, the checking of veneers, and the investigation of the properties of flexible polyether foams. Work has been completed on polyvinyl acetate adhesives and on the development of a glossing-up test for matt lacquers. The optimum conditions for spray-gun operation have been determined by relating the quality of the finish to the waste of lacquer through fog and bounce. About 20 per cent of the research staff investigators have been concerned with the investigation of *ad hoc* enquiries, such as the performance testing of prototype furniture, causes of warping of panels, comparative testing of various finishes, the critical examination of chairs from the point of view of comfort, the suitability of different timbers, the noisiness of table tops and various fault investigations. The Technical Information Service has been called on to meet an increasing demand. A total of 2,200 enquiries submitted by 542 organizations has been dealt with. A new publication series, known as *FIRA Notes*, has been introduced with the view of giving in easily digestible form concise and up-to-date information drawn from the Association's own sources and elsewhere.

Rugby School Natural History Society

The ninety-seventh issue of the report of the Rugby School Natural History Society contains details of the archæological excavations carried out at Caves Inn, near Shawell, in 1963 by the Rugby Archæological Society and members of Rugby School (Pp. 22+2 plates. Rugby: Rugby School Natural History Society, 1964). The hill by Caves Inn Farm is referred to as Tripontium and is mentioned in the sixth itinerary of Antonius. Historians, through the ages, have used this itinerary in conjunction with the name of the hill to try to locate it. While there is no firm evidence of its identification, its situation in Roman miles fits the information in the itinerary. The evidence found so far suggests that the site consisted of a 'Mansio', surrounded by a settlement. Another article in the report includes some observations of solar activity made at the Temple Observatory during the summer term, 1963, as well as an account of the work of the Conservation Corps at Scolt Head by I. R. Whitehead.

The Cole Library: University of Reading

The books on zoology and early medicine, collected by the late Prof. F. J. Cole, have now been moved from his home at Kingwood, Oxfordshire, to the new Library Building of the University of Reading at Whiteknights Park, where they occupy an air-conditioned room in the special collections section on the fifth floor (see also *Nature*, 188, 1148; 1960). The Cole Library comprises eight thousand volumes dating from 1472 to the present day, and it contains many rare items. Author, title and subject catalogues were prepared by Prof. Cole, and are a valuable aid to the reader. A chronological catalogue down to the end of the eighteenth century is now being made by Dr. N. B. Eales, and this will include biographies and translations.

Scottish Journal of Geology

The first part of a new geological journal, the *Scottish Journal of Geology*, is due to appear in the autumn of this year. The new journal is the result of an amalgamation of the *Transactions of the Geological Society of Edinburgh* and the *Transactions of the Geological Society of Glasgow*. The Councils of both Societies consider that the new journal will provide a better forum for the presentation of the scientific work of the two Societies and of other work on Scottish geology. They also intend to continue their policy of encouraging the publication of geological papers of general or specialist interest. The journal will be published three times a year. Subscription rates will be £4 per annum for institutional subscribers and 30*s*. per annum for Fellows of either Society. Enquiries about subscriptions or editorial matters should be addressed to the Editors, *Scottish Journal of Geology*, Tweeddale Court, 14 High Street, Edinburgh.

The Longest Road

The Trans-Canada Highway, the longest paved road in the world, which stretches for 5,000 miles (with two short ferry-breaks) from St. John's, Newfoundland, to Victoria, Vancouver Island, was originally opened in 1962, and has already transformed the conception of east-west direct communication by road in the Dominion. It is now practicable for Canadians to motor across this vast territory without leaving their own country. The first car-ferry is across the Gulf of St. Lawrence to Cape Breton Island. This is now artificially joined to Nova Scotia by a causeway constructed by literally filling in the one mile wide and 200-ft. deep Canso Strait between Cape Breton Island and the mainland with some 5 million cubic yards of rock, an engineering feat of no mean order, considering that bridging this gap, often subject to fierce spring tides and ice jams, was abandoned as being an impracticable task. From Nova Scotia the highway passes through New Brunswick to Quebec, thence via Montreal, across Ontario by Ottawa north of the Great Lakes by Sault Sainte Marie and Wawa to Fort William. In this stretch another great engineering triumph is recorded. Between Sault Sainte Marie and Fort William, a distance of some 400 miles, there occurs a wilderness area called by the Canadians 'The Gap'. Before the advent of this great highway, this feature had constituted a barrier which had virtually split the Dominion in two; its avoidance was only possible by following northerly loop-roads adding hundreds of miles to the distance to be traversed. In a vividly written and illustrated article in *The Lamp* (quarterly magazine of the Standard Oil Company of New Jersey, 46, No. 1; 1964), the hazards of this wilderness are well summed up: "The Gap itself was just 164 miles across, but it was split up by more than a hundred rivers, thousands of lakes, deep ravines, granite ledges—and muskeg. Muskeg is a Canadian highway engineer's peculiar nightmare. It is seemingly bottomless mud and silt, sometimes a mile deep, which freezes solid in winter but in summer becomes quicksand. Muskeg can swallow men and machinery—even roads. One section of the Trans-Canada, representing an entire day's work, disappeared without a trace before the next sun came up." From Fort William, the highway traverses Manitoba by Winnipeg, Saskatchewan via Regina, thence to Calgary, Alberta, into British Columbia, here through the most difficult section, Rogers Pass, 4,335 ft. O.D. with Rocky Mountain peaks rising 10,000 ft. on either side. At Vancouver a short sea ferry completes the journey along this amazing road to Victoria, Vancouver Island.

Clare Hall: a New Development of Clare College, Cambridge

CLARE COLLEGE has decided to promote within the College a new development to be known as Clare Hall. The intention is that Clare Hall shall make a contribution towards meeting present-day Cambridge needs by providing a new graduate society for resident and visiting scholars. Its fellowship is designed to bring together four categories: (1) scholars holding appointments in the University or in research institutions in Cambridge, whose subjects may not be in great demand for undergraduate teaching; (2) post-doctoral and other senior research workers; (3) visiting scholars in Cambridge on study-leave; (4) Research Fellows, elected from among more senior research students. When the new society has been established, a body of research students who are not Fellows will be added. The University has a petition before the Queen-in-Council, asking for a repeal of the University Statute which excludes women from membership of men's Colleges. Subject to approval being given to this petition, fellowships in Clare Hall will be open to men and women on equal terms. Clare Hall will be legally part of Clare College, governed by statutes of the College. The Society will consist of about forty Fellows of Clare Hall, who will also be bye-Fellows of Clare College. It will be governed by a Council composed of five Fellows of the Hall, five Fellows of the College, the Warden of the Hall, and the Master of the College as chairman. It is proposed that the buildings for Clare Hall, which will include flats, studios, a seminar room, and dining rooms, will be on a site within five minutes' walk of Clare College, where the Hall will be able to develop an identity of its own and to maintain, socially and intellectually, a close relationship with the College.

The Governing Body has recorded its intention to elect into fellowships of Clare Hall and bye-fellowships of Clare College the following: Dr. T. E. Armstrong, Mr. R. W. David, Mr. E. P. M. Dronke, Miss M. B. Hesse, and Dr. W. H. Taylor.

Clare College has received from the Old Dominion Foundation of New York an endowment of 200,000 dollars. The income from the endowment will be used to subsidize research fellowships and visiting fellowships at Clare Hall.

The Night Sky in August

NEW moon occurs on Aug. 7d 19h 17m U.T. and full moon on Aug. 23d 05h 26m. The following conjunctions with the Moon occur: Aug. 2d 03h, Jupiter 2° N.; Aug. 4d 16h, Venus 5° S.; Aug. 4d 23h, Mars 0·2° N.; Aug. 23d 07h, Saturn 3° N.; Aug. 29d 13h, Jupiter 2° N. Venus is in conjunction with Mars on Aug. 28d 11h, Venus being 4° S. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 1h 25m, 1h 5m and 1h 10m on August 1, 15 and 31, respectively. Its stellar magnitude is $-4·1$; its distance from the Earth increases during the month from 46 to 67 million miles and the visible portion of the apparent disk from 0·320 to 0·516. Mars rises at 1h 05m, 0h 50m and 0h 35m at the beginning, middle and end of the month, respectively. Its stellar magnitude is $+1·7$; conditions for observation are not yet favourable. Jupiter rises at 23h 05m, 22h 15m and 21h 15m on August 1, 15 and 31, respectively. It is near the boundary of Aries and Taurus, its stellar magnitude is $-2·0$ and its distance from the Earth on August 15 is 456 million miles. Saturn is above the horizon most of the night. It is in Aquarius and in opposition on August 24. Its stellar magnitude is $+0·6$ and its distance from the Earth on August 15 is 816 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Aug. 22d 00h 24·2m, φ Cap. (disappearance); Aug. 28d 23h 18·2m, 147 B. Ari. (reappearance). The Perseid meteors are active during July 27–August 17, with maximum on August 12. The radiant is near R.A. 3h 04m, Dec. $+58°$; conditions for observation are very favourable.

Announcements

PROF. PAUL PHILLIPS, of the University of Wisconsin, Madison, Wis., has been awarded a "medal of merit" by the Istituto Sperimentale "L. Spallanzani", Milan, in recognition of his contributions to the development of a practical method for artificially inseminating cows and for research on mineral elements in nutrition.

THE New Zealand Department of Scientific and Industrial Research has issued a list of printed publications, indicating those out of print and where those still in print can be obtained; more than 70 are publications of the Geological Survey and more than 150 Research Bulletins of the Department (*List of Publications*. Pp. 35. Wellington: Government Printer, 1964).

A CONFERENCE on "The Use of Film in Higher Education and Research" will be held at the College of Advanced Technology, Birmingham, during September 9–11. Further information can be obtained from P. D. Groves, Department of Chemistry, College of Advanced Technology, Gosta Green, Birmingham 4.

A SERIES of lectures on "Plasma Physics and its Applications in the Field of Thermo-nuclear Reactions and Electrical Power Generation" will be held at Sunderland Technical College during September 8–11. Further information can be obtained from the Physics Department, Sunderland Technical College, Sunderland.

A COURSE on "The Genetics and Physiology of Bacterial Viruses" will be held under the auspices of Unesco and the International Cell Research Organization, at the International Laboratory of Genetics and Biophysics, Naples, during September 11–October 9. Further information can be obtained from the International Laboratory of Genetics and Biophysics, Casella Postale 104, Naples.

THE fifth international symposium on "Gas Chromatography", organized by the Gas Chromatography Discussion Group of the Institute of Petroleum, will be held in Brighton during September 8–10. The programme will include sessions on: techniques of separation and identification; column design; exploitation of molecular interactions. Further information can be obtained from the Organizing Office, Fifth International Symposium on Gas Chromatography, 61 New Cavendish Street, London, W.1.

A SECTIONAL meeting of the World Power Conference on "The Struggle against Losses in the Field of Energy Economics" will be held in Lausanne during September 13–17. The general theme of the meeting will be divided into the following sections: general aspects; avoidance of losses in conversion; avoidance of losses in fuel transport and power transmission; avoidance of losses in utilization of energy. Further information can be obtained from the Swiss National Committee, World Power Conference, Plaza B, Petit Chêne 38, Lausanne.

THE fifteenth international Astronautical Congress will be held in Warsaw during September 7–12. The programme will include sessions on: flight programmes; propulsion techniques; navigation; lunar international laboratory; bioastronautics; power systems for space vehicles; celestial mechanics and astrodynamics; physical problems of re-entry into planetary atmosphere; meteorological satellite systems; communication satellite systems; geodetic satellite systems; ground installations; education in astronautics; space vehicles in an ionized medium. Further information can be obtained from the International Astronautical Federation, 250 rue Saint-Jacques, Paris 5.

WELLCOME HISTORICAL MUSEUM AND LIBRARY

DR. F. N. L. POYNTER, formerly chief librarian of the Wellcome Historical Medical Library has been appointed a director to take charge of both the Wellcome Historical Medical Museum and the Historical Medical Library. His appointment is part of a general reorganization following the retirement of Dr. E. Ashworth Underwood as director of the Wellcome Historical Medical Museum.

Dr. F. N. L. Poynter joined the staff of the library as assistant librarian in 1930 when Sir Henry Wellcome was himself director of the Museum and Library. He became sub-librarian in 1938, deputy librarian in 1946 and chief librarian in 1954. He received his training as a medical historian from the late Prof. Max Neuburger. In addition to his other duties he is secretary-general of the International Academy of the History of Medicine and honorary secretary of the Faculty of the History of Medicine of the Society of Apothecaries. He is the author of a number of books and more than a hundred papers on the history of medicine.

The Wellcome Historical Medical Museum and the Historical Medical Library are housed in the Wellcome Building in Euston Road, London. Both are based on collections initiated by the late Sir Henry Wellcome, who died in 1936. The Library contains approximately 250,000 printed books, pamphlets and journals, 10,000 manuscripts (of which half are in oriental languages) and 100,000 autograph letters of medical and scientific interest. The Museum contains what is probably the most comprehensive collection yet made of scientific instruments and antiquities of interest for the History of Medicine.

As part of the reorganization also, Dr. Edwin Clarke has been appointed medical historian to the Museum and Library and Mr. J. W. Barber-Lomax is to be administrative officer and veterinary historian. Dr. Clarke was formerly associate professor of the history of medicine in Yale University. Mr. Barber-Lomax, who is one of the leading veterinary historians in Great Britain, was formerly manager of the Professional Services Department, Veterinary Division of the Wellcome Foundation, Ltd.

Mr. E. Gaskell, formerly sub-librarian, has been elected librarian of the Historical Medical Library and Mr. E. Freeman sub-librarian.

Dr. Ashworth Underwood retired from the Wellcome Historical Medical Museum this year after nearly eighteen years as its director. Despite the frustrations of space restriction, resulting primarily from the War, Dr. Ashworth Underwood was able to keep a representative exhibition open for the public and was always striving for the proper deployment of the collection so that full use could be made of it. Important exhibitions of topical subjects were regularly arranged by him during his term of office and his great knowledge of the subject enabled him to give much help to many students of the history of medicine.

Before his appointment as director of the Historical Medical Museum Dr. Ashworth Underwood had been medical officer of health for West Ham and an honorary lecturer in the Department of the History of Science and Philosophy in University College, London. He has published many papers and books, among them the second edition of Singer's *Short History of Medicine*.

BALTHAZAR VAN DER POL GOLD MEDAL FOR SCIENTIFIC RADIO

DURING the general assembly of the International Scientific Radio Union (URSI) in Tokyo, September 1963, an evening was devoted to honouring the memory of the late Dr. Balth. van der Pol (1889–1959), who had devoted an important part of his numerous activities to the growth of URSI, in which he was so keenly interested. The occasion was the first presentation of the Gold Medal founded by the generosity of Mrs. van der Pol in memory of her late husband. In consultation with the Board of Officers of URSI, it was decided that the Medal was to be awarded to an outstanding radio scientist who, during the three-year period preceding the year of each general assembly, has made a valuable contribution in one of the fields of activity of the Union either by his research work, discoveries or achievements, or by any other activity.

In a volume recently published (June 1964) under the title *Van der Pol Memorial Lecture 1963*, by the Secretary-General of URSI (7 Place Emile Danco, Bruxelles, Belgium), the proceedings at this special meeting are recorded in full. After a short opening address by the president, Dr. R. L. Smith-Rose, a review of the life and scientific work of Balthazar van der Pol was given by Prof. Ch. Manneback, treasurer of URSI.

He recalled that, after graduating at the University of Utrecht in 1916, van der Pol went to England, where he worked with Prof. (Sir Ambrose) Fleming in the University of London, and with Prof. (Sir J. J.) Thomson in the Cavendish Laboratory, Cambridge. He returned to the Netherlands in 1919, and, after obtaining his doctor's degree at Utrecht, he joined the research laboratories of the Philips Company at Eindhoven in 1922. He remained there until 1949, when he was appointed the first director of the International Radio Consultative Committee (CCIR) in Geneva. After his retirement in 1956, he was visiting professor in the University of California, Berkeley, and, in 1958, in Cornell University, Ithaca.

Following this exposition at the ceremony in Tokyo, the first van der Pol Gold Medal was then presented to Prof. Martin Ryle by Mrs. van der Pol in recognition of his leadership of the radio astronomy group at the Mullard Observatory of the Cavendish Laboratory, Cambridge. Remarking on the appropriateness of the award, Mrs. van der Pol recalled the period in 1917 during which her late husband had worked at the Cavendish Laboratory, where he formed a life-long friendship with Dr. (Sir Edward) Appleton and other British scientists. The new group under Prof. Ryle has made outstanding contributions to astronomy, problems of cosmology, and to scientific radio. In particular, the Cambridge scientists have developed new aerial systems, especially suited to radio astronomical research. Following his acceptance of the Medal, Prof. Ryle gave a lecture on "Giant Radio Telescopes", which is reproduced with diagrams and photographs in the volume just published. In the course of his lecture, Prof. Ryle outlined the two main problems of radio astronomy: the limited resolving power obtainable compared with optical astronomy; and the extreme weakness of the signals to be received. A brief description was given of the design and performance of the three telescopes operating on the principle of 'aperture synthesis', which was developed at Cambridge. Some details were also given of a fourth and considerably more powerful instrument under construction.

After the president had thanked Prof. Ryle for his lecture, Mrs. van der Pol presented a second Gold Medal to Colonel Ernest Herbays, secretary-general of URSI, in recognition of his continuous and devoted service to the Union since 1928. In the volume now published recording these proceedings, Colonel Herbays very appropriately provided a concluding section outlining the history of the Union since the first meeting in 1912 of a few scientists interested in the investigation of the phenomena associated with 'electric waves'.

The production of this little volume is excellent in every way; and it forms a worthy memorial to the achievements of URSI and the part played in them by the late Dr. B. van der Pol. R. L. SMITH-ROSE

CANADIAN SOCIETY OF PLANT PHYSIOLOGISTS

THE 1964 annual meeting of the Canadian Society of Plant Physiologists—la Société Canadienne de Physiologie Végétale—was recently held in Kingston, Ontario. The host institute was Queen's University.

The proceedings opened, on June 4, with a symposium on respiration under the chairmanship of Prof. Dorothy F. Forward (University of Toronto). Dr. D. F. Parsons (Department of Medical Biophysics, University of Toronto) discussed his recent investigations of the structure and junction of mitochondrial membranes, using a negative staining technique for electron microscopy. Two types of projecting sub-units were found, one on the outer membrane, consisting of 60 Å hollow cylinders, and the other on the cristæ, consisting of 90 Å mushroom-shaped structures. The latter structures have been found in a wide variety of plant and animal tissues. These sub-units are protein, but do not contain cytochromes. There is evidence for the presence of a flavoprotein and an ATPase. The sub-units may be associated with oxidative phosphorylation.

Dr. W. D. Bonner, jun. (Johnson Research Foundation, University of Pennsylvania), presented a paper on electron transport in plant mitochondria. He discussed the large number of cytochromes which have been described, and the difficulties in their characterization. Two *c*-type, three *b*-type and three *a*-type cytochromes are present in the mitochondria of all plant species, regardless of the respiratory characteristics. At normal concentrations of oxygen, when ADP is limiting (state-4 conditions), oxidation by plant mitochondria is not inhibited by hydrocyanic acid (HCN) or carbon monoxide (CO). However, when ADP is in excess (state-3 conditions), HCN + CO reduces the rate to that of state-4. State-4 rate corresponds to the 'ground respiration' of plant physiological literature. This is completely inhibited at low concentrations of oxygen by HCN and CO; the same is true in mitochondria which show HCN and CO insensitive respiration (skunk cabbage). Kinetic analysis suggests that oxygen reacts with two separate oxidases. This is supported by optical demonstration of two CO (O_2) binding pigments. The presence of a second CO-binding pigment provides an explanation for both 'ground respiration' and 'cyanide insensitive respiration'.

The symposium was completed by a paper by Prof. G. Krotkov (Biology Department, Queen's University) on the effect of light on respiration, thus continuing the development of the symposium subject from the sub-cellular structural level to that of the physiology of the whole organism. The question of whether respiration is affected by light has in the past been answered by indirect means. The techniques used in Dr. Krotkov's laboratory made it possible to show that light and dark respiration are two different processes. 'Photorespiration', or evolution of carbon dioxide in light, involves different pathways of metabolism, perhaps utilizing glycollic acid metabolism. Corn, a plant of exceptionally high productive ability, has no photorespiration, and appears to lack co-factors for glycollic acid oxidation. Corn does not 'waste' products of photosynthesis in respiration while it is illuminated, and is thus more efficient than other plants.

Six half-day sessions (two sessions always running concurrently) were devoted to contributed papers. A closely knit group of papers on translocation originated from the laboratories of the Division of Biosciences, National Research Council, Ottawa, under the direction of Dr. P. R. Gorham and Dr. D. C. Mortimer, and from the laboratory of Dr. G. Krotkov and Dr. C. D. Nelson at Queen's University, the host institute. Dr. Mortimer's results showed that downward translocation of photosynthate occurred at greatly varying rates and intensities in different vascular bundles of a leaf, which thus had to be analysed separately. The Queen's University group showed that translocation of photosynthate is under hormonal control, and that there is selective and preferential translocation of certain sugars.

The session on nitrogen included two papers from the laboratory of Dr. W. G. Boll (McGill University, Montreal) on the ability of ethanolamine to replace vitamin B_6 in the nutrition of excised tomato roots, and on the biochemical mechanisms associated with this ability. An interesting paper from the laboratory of Dr. C. L. Mer (Imperial College of Science and Technology, London (now visiting at Harvard University)) provided a nutritive hypothesis for growth responses in oat seedlings which precludes the necessity to postulate changes in auxin metabolism to account for various growth responses to changed environment.

A session on regulators was of general interest, and included papers on light quality and periodicity, on the regulation of enzyme synthesis, photosynthesis and nuclear activity by hormones and on the mode of action of herbicides.

Two sessions were held on metabolism, covering a wide range of research interests. A growing interest in the phenolic substances in plants was evidenced by a group of papers from the Halifax, Nova Scotia, laboratories of the National Research Council under the direction of Dr. A. C. Neish and Dr. G. H. N. Towers. These included very interesting work on the pathways of lignin biosynthesis and the interrelations of soluble and insoluble derivatives of the many C_6–C_1 and C_6–C_3 phenolic acids found in plants. Other papers included biosynthetic investigations on indoles, carbohydrates, phosphatides, alkaloids and chlorophylls. A number of papers were presented on various problems in plant metabolism, including organic acid metabolism, respiration, frost hardiness, and the influence of seasonal or environmental factors on plant metabolism.

A session was held on cell biology, which included cytological and cyto-physiological investigations on tissue cultures of Jerusalem artichoke and oat coleoptiles, originating from the Carleton University, Ottawa, laboratories of Dr. G. Setterfield and Dr. F. Wightman. It was shown that a number of synthetic auxins which promote cell expansion also promote cell division. Papers were presented on control mechanisms of amino-acid synthesis, on chloroplast bleaching in *Euglena*, and on histochemical tests. Dr. Nelson's group from Queen's University presented work on the effect of auxin in controlling cellular permeability, and on the electro-osmotic transport of sugars labelled with carbon-14 in *Nitella* cells.

The non-scientific event of outstanding significance at the meetings was the holding of a banquet in honour of Prof. D. L. Bailey (Botany Department, University of Toronto). Dr. Bailey is retiring from the editorship of the *Canadian Journal of Botany*, the official journal of the Society, after a number of years of outstanding service in which he has maintained the *Journal* at the highest possible standard. Special guests at the banquet included

Dr. Leo Marion editor-in-chief of the *Canadian Research Journals*, and Dr. Pauline Snure, manager of the editorial office.

At the business meeting it was decided that next year's annual meeting will be held in early June at the University of New Brunswick, Fredericton, New Brunswick. Dr. G. Setterfield (Carleton University, Ottawa) was elected president of the Society, Dr. A. R. A. Taylor (University of New Brunswick) was elected vice-president, and Dr. R. G. S. Bidwell (University of Toronto) was re-elected secretary–treasurer. Other officers of the Society are Dr. M. Shaw (University of Saskatchewan) (past president), Dr. M. Cailloux (Université de Montréal) (eastern director) and Dr. Mary Spencer (University of Alberta) (western director).

R. G. S. BIDWELL

UNIVERSITY LIBRARY SERVICES

A MEMORANDUM outlining the responsibilities and functions of university libraries has been submitted by the librarians of Britain's new universities to the Committee on Libraries of the University Grants Committee. It sets forth, first, the function of the university library; next, the level and scope of acquisitions; the need for undergraduate provision; and then discusses problems of building and accommodation and of library co-operation.

The memorandum begins by insisting that the first function of a university library is to support the teaching and research of the institution it serves, but that it also has a duty to the community of learning at large and access to its resources should be available to all serious students who need to consult them. As research grows and a larger proportion of the population receives a higher education, such extra-mural activities are likely to grow in importance, The memorandum insists that librarians must play a much more positive part in exploiting the books in their libraries, both by information work and by giving instruction and guidance to students and research workers. Dealing with the level and scope of acquisitions, it indicates the magnitude and urgency of the situation confronting the new university librarians, and the acquisition of books and periodicals is seen as still the most important task which the university library must perform.

The library provision varies widely among the existing universities in Britain, and the inequality has increased rather than diminished since 1945. Only Cambridge, Oxford and London provide really comprehensive collections for research in Britain, although in the United States several university libraries have doubled or more than doubled their size since 1945. The rise in income of British university libraries during the past 15 years has been almost totally absorbed by the increase in publishing and the steady rise in prices. The memorandum suggests that the new universities should aim at collecting 250,000 volumes apiece during their first ten years, excluding multiple copies of undergraduate texts. Substantial capital grants will be required. The minimum cost of provision of periodicals in mathematical, physical, chemical and biological sciences may approach some £10,000 a year, although two-thirds of Britain's present universities spend less than this figure on periodicals and one-third spend less than £5,000.

The need for the student to have the use of a comprehensive library is emphasized by the memorandum, and it is pointed out that multiple provision of text-books makes possible a better lending service while provision of a reference copy ensures that both students and other users of the library can usually obtain access to certain books. The setting-up of separate undergraduate libraries in new universities is not favoured, but it is suggested that duplication of minimum book needs for each undergraduate course should be on a scale depending on the number of students involved. Funds available to university libraries, at least outside Oxford and Cambridge, are quite inadequate to provide multiple copies on the scale necessary, and students in some subjects cannot reasonably be expected to provide themselves with more than a small number of the many books they will need to consult in the course of three years. Grants are therefore needed for multiple book provision.

On buildings and accommodation, the memorandum points out that the new universities are in a position to take immediate advantage of the developments in university planning, particularly in the United States, since the Second World War and of recent developments in fast building techniques. University teaching methods will influence planning and the typical new library is likely to be designed on a flexible plan, with large open floors in which readers are placed close to blocks of bookshelves grouped according to subject. More generous space allowances are required and variety of provision for readers is essential. Some experiments in extended hours of opening are already being made. On co-operative storage and technical developments, the memorandum affirms the intention of the new universities to build up their libraries on the principle that the university library must possess within its own walls copies of almost everything required by its members for the promotion of their studies and the advancement of learning. However, it is believed that a proper policy for the withdrawal of obsolescent material should also be admitted. A national co-operative scheme now ensures that a copy of every recent worth-while British book is available for loan somewhere, but the universities still need to buy their own copies to cover their own requirements, and the possible advantages of a co-operative approach are regarded as worthy of investigation. Some advantage might accrue in the acquisition of periodicals from the co-operative coverage of all new titles of academic interest. The reductions available when a number of libraries purchase specially reproduced copies of out-of-print books are large enough to justify an attempt to set up a co-operative non-profit-making organization scheme. Any scheme for co-operative storage must provide for bibliographical care and maintenance so that material is available quickly when needed. The most immediate help in co-operative work that could be given is the provision of more funds for the important comprehensive catalogues, in particular, the national catalogues in the National Central Library. It is submitted in the memorandum that there is a strong case for amending the copyright regulations in the interests of efficiency and economy in learned institutions so as to facilitate the reproduction of copies from telefacsimiles and the request of photocopies by 'Telex'. There are difficulties in acquisition and in the use of microforms which militate against any substantial savings for libraries. However, the amount of bulky and little-used material kept in microform is likely to increase and to offer some economy in storage costs. More information is needed on many aspects of library organization, and none of the existing organizations is capable of undertaking this work. The memorandum suggests that there is scope for a small library development agency, the staff of which might both collect information and assist the co-operation of academic libraries.

Copies of the memorandum can be obtained from Mr. P. E. Tucker, librarian, the University of Warwick, Coventry, Warwicks.

RESEARCH IN TEXTILES, COLOUR CHEMISTRY AND DYEING

THE eighty-ninth report to the Worshipful Company of Clothworkers of the City of London of the Advisory Committee on the Departments of Textile Industries and Colour Chemistry and Dyeing in the University of Leeds* covers the session 1962–63. During the session there were 400 students in the Department of Textile Industries, of whom 96 were graduates reading for higher degrees or diplomas, compared with 12·5 per cent such students for the University as a whole. Twenty-eight students were awarded Bachelor degrees and 27 gained diplomas.

In textile physics, a method was devised for determining the contribution of the relevant molecular configuration to the overall crystallinity from the dimensional change of the microfibrils measured in electron micrographs of the transverse sections of wool fibres: the results confirmed the view that an intermediate molecular phase was involved in the α–β transformation. Work in textile chemistry during the session was concerned with attempts to devise methods for the continuous curling of worsted and mohair yarn, for lustring all-wool fabrics, and for imparting permanent creases and pleats to all-wool fabrics under simpler conditions. Work was also continued on the prevention of heat discoloration of wool textiles, and the properties of the by-products of the fleece were further examined. The fundamental investigation of traveller burning was extended and a complete analysis made of the position of the traveller during flight. A novel method of cleaning wool which might eliminate the card and produce wool of much more even fibre length is being examined. An extensive programme of work to explain the mechanical behaviour of cloths in terms of yarn properties and cloth geometry yielded some useful results. In textile technology the effect of the arc of contact in a carding machine on the efficiency of the carding process was investigated, and, in worsted spinning, attention was directed to the drawing and spinning of coloured tops. An investigation was completed in the production and properties of nylon and 'Terylene', false-twist mixture yarns. Work in textile finishing covered the chemistry and practice of textile processing with particular attention to the flame-proofing of textiles and the reactivity and properties of nylon- and wool-setting operations.

In the Department of Colour Chemistry and Dyeing, there were 42 undergraduates during the eighty-fourth session and 14 graduates working for research degrees.

In research, a preliminary study of the mechanism and formation of dioxazine dyes was completed and the reactions of 9,10-benzo*meso*-benzanthrone with nucleophilic reagents were examined, as well as the acetylation of 1,1-dinaphthyl and perylene.

Research continued on the measurement of the vapour pressure of disperse dye crystals, and investigations were made of the behaviour of dispersed dye particles in the dye bath, of the ring dyeing of circular filaments of nylon, 'Terylene' and 'Courtelle', and of the important technical process of rosination in the manufacture of azo pigments. Investigations of the treatment of wool in various media on its load-extension and setting characteristics were completed, as well as others on the solvent-assisted dyeing of nylon 66. An investigation into factors influencing the strength of the dye fibre bond formed by reacting halogeno triazinyl dyes and cellulose was commenced.

Lists of departmental publications during the year are appended to both departmental reports.

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* Report to the Worshipful Company of Clothworkers of the City of London of the Advisory Committee on the Departments of Textile Industries and Colour Chemistry and Dyeing in the University of Leeds for the Session 1962–63. Pp. 54. (Leeds: The University, 1964.)

THE ZOOLOGICAL SOCIETY OF LONDON

THE 135th annual report of the Council of the Zoological Society of London* shows that, at the end of 1963, there were 3,526 Fellows and 1,448 Associates. Although public attendances at the Society's Zoological Gardens improved a little in the year, the 1963 figure was still some 65,000 below the average of the previous seven years. This recovery did not extend to Whipsnade, where attendances, at 616,000, were below even the 1962 level. Since the Society must rely on substantial annual surpluses, and since the bulk of income must still come from the public, the Council has commissioned a firm of consultants to report on the Society's revenue-earning activities and to suggest ways by which they can be improved.

To further its policy that zoos should, wherever possible, take positive action to ensure the survival of endangered species, the Council sent the female Arabian oryx, which has been in the collection since 1958, on loan to the Phoenix Zoo, Arizona, where a breeding herd of captive oryxes was being established. This herd consists of two adult male and three adult female oryxes, two males and one female having been captured by the expedition sent to the Aden Protectorate by the Fauna Preservation Society and another female presented by H. E. Sheikh Jabir Abdullah Al Sabah of Kuwait. The first offspring, a male, was born at Phoenix Zoo in October.

There have been several rare births in the Gardens during the year. From the point of view of conservation, probably the most important birth was that of a second orang-utan to the breeding pair at Regent's Park, 'Toli' and 'Charlie'. This young male is being successfully reared by its mother, while its elder sister (born in 1961) continues to thrive with a chimpanzee as a companion. During 1963, the upper section of the stables in the Children's Zoo at Regent's Park was completely remodelled as a small pilot experiment for the nocturnal section of the new Small Mammal House. Here small nocturnal mammals are being kept under various lighting conditions in an attempt to find out the best way of reversing their daily rhythm. The tests have already proved successful and, when they are completed, the building will be opened as a temporary display until the completion of the new Small Mammal House. Animals housed there include bush-babies, lorises, pottos, douroucoulis, mouse lemurs and giant flying squirrels.

The report contains some outstanding photographs recording the births of various animals at Regent's Park and Whipsnade.

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*Annual Report of the Zoological Society of London, 1963. Pp. 51+ 11 plates. (London: The Zoological Society of London, 1964.)

EXCITATION OF CEREBELLAR PURKINJE CELLS BY THE CLIMBING FIBRES

By Sir JOHN ECCLES, F.R.S., R. LLINAS and K. SASAKI

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IN 1888 Cajal (ref. 1) discovered a most remarkable synaptic structure in which single afferent fibres were uniquely related to single Purkinje cells by synaptic contacts of extraordinary extent and intimacy. As illustrated in Fig. 1*A*, each so-called climbing fibre (*CF*) traverses the granular layer of the cerebellar cortex up to the soma of a Purkinje cell (*P*) and enters into close contact with the trunk of the dendritic tree at its origin from the soma, thereafter branching profusely so that the primary and secondary dendritic branches are entwined by climbing fibrils. There are descriptions of climbing fibres that branch to entwine dendrites of two or even more Purkinje cells[2]; nevertheless in the great majority there is the one-to-one relationship described by Cajal[1]. With the electron microscope[3] it can be seen that, as the climbing fibre twines round the first and second order dendrites of a Purkinje cell, it makes numerous synaptic contacts that have the features of Gray type 1. These contacts are with the dendritic shafts and not with dendritic spines, which invariably are the sites for the synapses (also Gray type 1) made by the parallel fibres[3-7].

The origin of the climbing fibres was controversial until careful degenerative investigations[8] showed that the great majority arose from the inferior olivary nucleus in the medulla, which is unique in that it does not contribute at all to the only other type of cerebellar afferent fibre, the so-called mossy fibres (Fig. 1*A*, *MF*). Experimentally, therefore, it should be possible to stimulate climbing fibres selectively by means of an electrode inserted into the inferior olive. Dow[9] and Jansen[10] found that stimulation of the inferior olive produced a complex potential change (often positive-negative) in most areas of the cerebellar cortex, especially contralaterally, and the sensitivity to anoxia indicated the synaptic generation of this potential. It will here be shown that the potentials synaptically generated in Purkinje cells by stimulation of the inferior olive have precisely the characteristics that would be expected for the synaptic action of impulses in climbing fibres.

Fig. 1*A* illustrates diagrammatically the experimental arrangements which in general resemble those already described[11]. In a small segment of the anterior lobe of the cerebellar vermis there are indicated several somata of Purkinje cells, only one (*P*) being shown with dendrites and the associated climbing fibre (*CF*). Antidromic stimulation of the Purkinje cell was effected by means of a concentric needle electrode (*ANT. STIM.*) in the white matter adjacent to the fastigial nucleus, but, as indicated, such stimulation would also be likely to excite climbing fibres (*CF*) and mossy fibres (*MF*) as well as Purkinje cell axons (*PA*). By contrast the other concentric electrode (*I.O. STIM.*) in the inferior olivary nucleus should excite only climbing fibres. A surface bipolar electrode (*LOC. STIM.*) was used for local stimulation of the parallel fibres, which are the axons of granule cells (*G*). The insertion of the potassium citrate-filled microelectrode (about 10 MΩ) is indicated. The animals (cats) were anæsthetized by pentobarbital sodium and paralysed by 'Flaxedil'. Conventional procedures were used in order to control the movements of the cerebellum: pneumothorax with artificial ventilation, and coverage of the exposed cerebellar cortex by an agar gel.

In Fig. 1*B* the very early diphasic spike potential (positive-negative with 0·6 msec latency) evoked by stimulation through the *ANT.* electrode shows that the microelectrode was in very close proximity to a Purkinje cell responding antidromically to this stimulus. The second spike (about 3 msec latency) would be evoked synaptically, presumably by the following pathway: the mossy fibres stimulated as they pass by the *ANT.* electrode, thence to the granule cells and their parallel fibres, and so to the Purkinje cells. Correspondingly, in the initial response of Fig. 1*G*, direct stimulation of the parallel fibres (*LOC. STIM.* in Fig. 1*A*) evoked a spike potential with a latency of almost 4 msec. In *C* the stimulus to the inferior olive (*I.O.*) was just at threshold for evoking a response of this same Purkinje cell, there being three superimposed traces, one below threshold and two above that each evoked 4 or 5 spike potentials, the traces of the first two spikes being superimposed. The details of this complex spike response are better seen in the single trace of *D*. The latency of the first spike was about 11 msec and there was a considerable diminution in the size of the later spikes, which presumably is attributable to the maintained depolarization of the cell. Further increase in the *I.O.* stimulation did not cause any addition to the response in *C* and *D*, which was thus strictly all-or-nothing. Since stimulation of parallel fibres produces a very large inhibitory postsynaptic potential (*IPSP*) via basket cells[11], it would be expected to inhibit the response evoked by the *I.O.* stimulation, as is illustrated in Fig. 1*E*–*G*. However, the first spike could not be suppressed even at the optimal interval of about 10 msec (*E*) after the

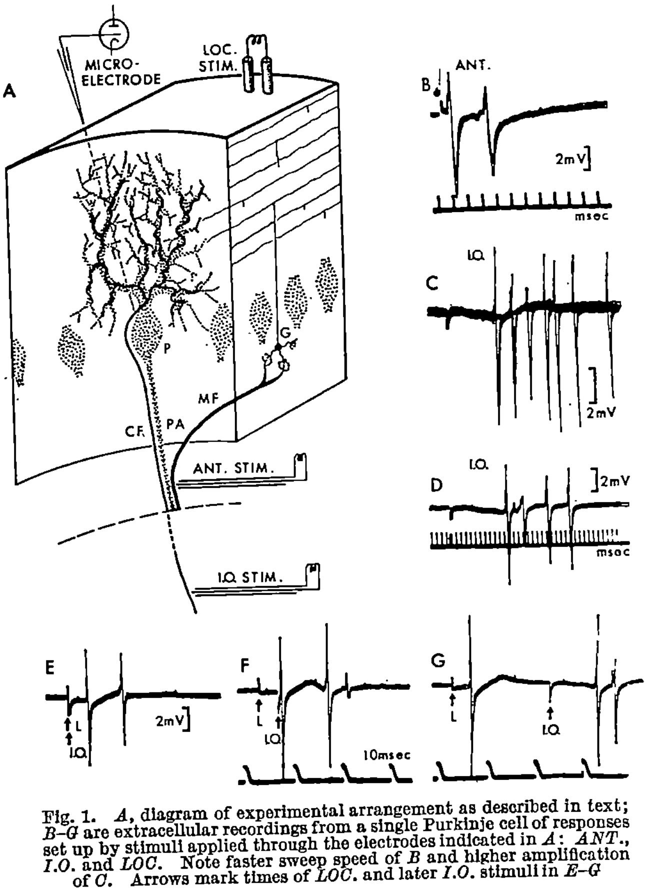

Fig. 1. *A*, diagram of experimental arrangement as described in text; *B*–*G* are extracellular recordings from a single Purkinje cell of responses set up by stimuli applied through the electrodes indicated in *A*: *ANT.*, *I.O.* and *LOC.* Note faster sweep speed of *B* and higher amplification of *C*. Arrows mark times of *LOC.* and later *I.O.* stimuli in *E*–*G*

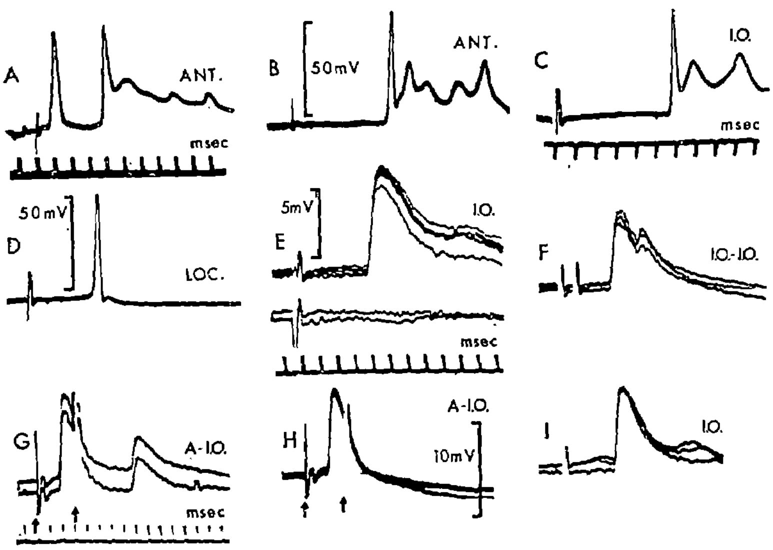

Fig. 2. Intracellular recordings from three Purkinje cells. In two, *A* and *B–D*, the membrane potential was −50 mV and the spike potentials 50–60 mV. *A* and *B* are responses to *ANT*. stimulation, *C* to *I.O.* and *D* to *LOC.* stimulation. All are at the same amplification, and *B–D* at same sweep speed. The other cell (*E–I*) had such a low membrane potential, declining from −40 mV, that there were only *EPSP*'s uncomplicated by spike potentials. *E* was at higher amplification and faster sweep than *F–I*, and illustrates the all-or-nothing character of the *EPSP*, the two lower traces with zero response being evoked by a slightly weaker stimulus. *F* is the double *EPSP* evoked by double *I.O.* stimulation at the shortest effective interval. In *G* similarly there is the double *EPSP* response at the shortest *ANT.-I.O.* stimulus interval, there being failure of the second stimulus at a very slightly shorter interval (*H*). *I* shows control *EPSP* to *I.O.* stimulation alone

local (*L*) stimulation, which shows how powerful is the excitatory action from the inferior olive. Progressive recovery is seen at longer intervals (*F*, *G*), but full recovery did not occur until at least 100 msec after the *L* stimulus, which corresponds to the duration of the *IPSP* recorded intracellularly[11]. It can be concluded that in Fig. 1*C–G* the stimulation of a single fibre in the inferior olive resulted in an extremely powerful synaptic excitation of that Purkinje cell. Similar observations have been made on the large number of Purkinje cells that were activated by an *I.O.* stimulation.

Intracellular recording (Fig. 2) gives further information on the extraordinary power of synaptic depolarization that is always exerted by a single impulse from the inferior olive. In Fig. 2*D* stimulation of the parallel fibres (*L* stimulus) typically evoked a single spike discharge (latency 3·2 msec) followed by the prolonged hyperpolarization of the *IPSP*. With *I.O.* stimulation (*C*) there was an initial spike (latency 5·8 msec) followed by two large slow depolarizing waves, a response that was almost exactly reproduced in four successive tests, and was also observed as a spontaneous response. Fig. 2*B* also shows that a similar response was produced by stimulation through the *ANT*. electrode, but with a briefer latency (4·7 msec). Evidently this electrode was unfavourably placed for stimulating the axon of the impaled Purkinje cell, which was affected solely by the excitation of the same fibre that was excited by the *I.O.* stimulus. The *ANT*. electrode was more favourably placed in another experiment (Fig. 2*A*), where the initial antidromic spike potential (latency 0·7 msec) was followed 3·1 msec later by a spike potential and the subsequent complex depolarizing potential resembling that of Fig. 2*B*.

That the *ANT*. stimulus can synaptically excite a Purkinje cell by setting up an impulse in a nerve fibre from the inferior olive is shown in the series of Fig. 2*F–G* from another Purkinje cell that was so depolarized by the microelectrode impalement that no spike potentials could be generated. In *E* the *I.O.* stimulation is seen to generate in an all-or-nothing manner a depolarization (*EPSP*) in the Purkinje cell with a latency of 4·1 msec. A similar all-or-nothing *EPSP*, but with a latency of 1·9 msec, was generated by the *ANT*. stimulus. In *G–H* the *ANT*. stimulus preceded the *I.O* stimulus and the least stimulus interval a which the latter stimulus evoked an *EPSI* was 3·2 msec (*G*), whereas with two *I.O* stimuli the least stimulus interval was 1· msec (*F*). It can be concluded that botl stimuli were exciting the same nerve fibr and that the propagation time to the inferio olive of the impulse evoked by the *ANT* stimulus was 2·1 msec (3·2–1·1), which is i good agreement with the propagation tim in the reverse direction, 2·2 msec (4·1–1·9) that is given by the latency differential fo the *I.O.*- and *ANT*.-evoked *EPSP*'s. Simila experimental tests have been applied t many other cases where the *ANT*. and *I.C* stimul evoked the same responses of Purkinje cell, and always there were com parable results. From the conduction time so measured for the impulse propagatior mean conduction velocities of approxim ately 10–15 m/sec have been calculatec which is in good agreement with the dia meter of rather less than 3μ that is observe for the majority of olivo-cerebellar fibres[1]

It can be concluded that fibres excite in the inferior olive have a very powerfu synaptic excitatory action on single Pur kinje cells, exactly as would be expecte for the massive synaptic relationship ob served histologically for the climbin fibre, which is in special agreement wit Szentágothai and Rajkovits[8]. Furthermore, this actio has the expected uniqueness in that we have so far neve observed evidence of convergence of two such fibres on t a Purkinje cell. The extracellular records of Fig. 1*C–* show the intense repetitive discharge that an impuls in a climbing fibre evokes from a normal Purkinje cel so much so that it cannot be completely suppressed eve by the powerful inhibition produced by basket cells[1] In Fig. 2*A–C* the depolarizing waves after the first spik probably are caused in part by electrotonic spread fro spikes generated in the Purkinje cell dendrites th failed to propagate into the soma, presumably because its excessive depolarization, but impulses generated the initial segment (*IS*) of the axon would also contribut For example, the second spike potential in Fig. 1 probably is an *IS* spike.

Responses resembling those of Fig. 2*A*, *B* were observe by Granit and Phillips[13] also in response to juxt fastigial stimulation and were called by them 'inactivati responses' because of the suppression of spike generati by the depolarization, just as in Fig. 2*A*, *B*. Spontaneo inactivation responses were also observed by them. W would attribute all these responses to the very powerf synaptic excitatory actions of impulses in the climbi fibres because they can be duplicated by stimuli appli to the inferior olive. It can be predicted that this ve specific and powerful activation of Purkinje cells has most significant function in the co-ordination muscles.

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RECOVERY FOLLOWING X-IRRADIATION OF SYNCHRONIZED CHINESE HAMSTER CELLS

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AN implication of the shape of threshold-type radiation survival curves (called "Type C" by Gunter and Kohn[1]) is that damage must be accumulated before the cell is killed. Elkind and Sutton[2] used fractionated doses of X-radiation to investigate the heritability of this accumulated damage among surviving cells. They found their results to be consistent with repair of sub-lethal damage before the first division after exposure. Similar observations have been reported in other mammalian cell lines[3,4], in *in vivo* mammalian investigations[5,6] and also in plant cells[7]. The phenomenon is, therefore, rather general and may, in fact, always exist whenever thresholds or shoulders occur in survival curves.

The interpretation of the kinetics of the recovery process is complicated when asynchronous populations of cells are used. It has recently been shown by several investigators that the X-radiation sensitivity of mammalian cells varies with age of the cell[8], that is, with time in the cell cycle from one division to the next[9–12]; consequently, after an asynchronous population is exposed to X-rays, the age-density distribution[8] of survivors is different from that of an unirradiated exponentially growing population. Subsequent progression of surviving cells through different sensitivity states leads to changes in the response to a second or test dose which may obscure the effects of a repair process, or possibly even give the appearance of such a repair process when none exists. Kallman[13] has described a model in which variations in sensitivity with cell age are responsible for oscillations in the form of the response of asynchronous cells to two separated X-ray doses and in which no intracellular repair process is invoked. Although various arguments and some recent experimental evidence by Elkind and others[14,15] and by Berry and Oliver[16] support the hypothesis of intracellular repair, the fact is that under normal conditions, only when the exponential age-density distribution is restored can the recovery or repair process be investigated free of the effects of cell progression through different sensitivity states. The interpretation of the response of surviving cells in the transient period following a first exposure is further complicated by our own observation[17,18] that, in Chinese hamster cells, variations in X-ray sensitivity with cell age are mainly due to alterations in the threshold region of the survival curve, that is, that part of the dose-effect curve intimately concerned with sub-lethal damage. We have attempted to clarify the process of repair of sub-lethal damage by studying the responses of synchronized populations of Chinese hamster cells to two doses separated by various time intervals.

Chinese hamster cells derived from the *V*79 clone and grown *in vitro* have been synchronized by a selection procedure involving a brief cold shock, re-incubation at 37° C for a period and then controlled agitation under gentle trypsinization[11]. Cells at or near mitosis are obtained. On subsequent recultivation, the cells form 1 or 2 cell 'colonies' during the first cycle of synchronous growth. By various tests, which include mitotic index, cell-size distributions, growth and pulse labelling with tritiated thymidine, these populations have been shown to be quite well synchronized[18].

The response of these cells after a single X-ray dose has been discussed in earlier reports[11,18] and was obtained by exposing synchronized cells incubated for different lengths of time at 37° C to a given dose of X-rays from a General Electric Maxitron machine 250 kVp, 0·9 mm copper half-value layer, at an exposure rate of 110 r./min (absorbed dose = 0·945 rad/r.). The fractionated exposures were conducted by removing an entire set of plates at a given time after re-incubation (during the first cycle) and exposing to the first dose. The plates were then restored to the incubator and removed at intervals thereafter to receive the second dose. Final incubation for 10–12 days results in visible colonies, each representing a survivor. For each exposure, the plates were out of the incubator (that is, at ~ 25° C) for about 15–30 min. This length of time at room temperature may have a small effect on survival, but the degree of synchrony, as judged by plates pulse-labelled with tritiated thymidine after exposure (Fig. 2), is substantially unaffected.

Recovery of S Phase Cells. Fig. 1 shows the survival following a single dose of 660 rads at various times (curve 1*A*), 1,320 rads at these times (curve 1*B*) and a two-dose response for 660 rads delivered at 10 h (when most of the cells are in the *S* phase) followed by a further 660 rads at intervals thereafter (curve 1*C*). The survival after two separated doses of 660 rads (Fig. 1*C*) quickly rises above that for the same total single dose, 1,320 rads (Fig. 1*B*), as the interval between the two doses increases. (The first point on Fig. 1*C* should lie exactly on Fig. 1*B*; it is slightly below only because the two sets of plates were not handled exactly alike.) Since cells of age 10 h in this population (Fig. 1*A* and 1*B*) are near their maximum resistance and moving toward a more sensitive state, the first increase in Fig. 1*C* can only be the result of recovery from the first dose. Beyond the first maximum, the survival after the fractionated dose (Fig. 1*C*) follows a very similar pattern to that after the same single total dose (Fig. 1*B*) but displaced toward later times (by about 2·5–4 h) and higher survival levels. The higher survival level indicates that the recovery is permanent and not transient in character.

Degree of Recovery in S Phase Cells. In the same experiment, cells were pulse-labelled with tritiated thymidine (15 min, 0·4 μc./ml., 3 c./mM). Fig. 2*A* shows that at 10 h 80 per cent of the cells were still synthesizing DNA. Since *S* cells are quite resistant (Fig. 1*A* and 1*B*)[11,18] after the first dose (of a two-dose pair) given at 10 h, most of the survivors will be *S* phase cells. Thus the response to two doses shown in Fig. 1*C* is for primarily *S* phase cells. Fig. 2*B* shows the percentage of cells labelled versus time for a population given 660 rads at 10 h, but since only 20 per cent of single cells would survive this dose (Fig. 1*A* and equation (1)) this curve refers mainly to non-colony forming cells. However, Fig. 2*B* indicates that the irradiated population retains most of its synchrony. The principal effect of the exposure was a delay of about 2·5–4 h, depending on where the two curves were compared. This is consistent with the delay observed in the survival data of Fig. 1 (compare curves 1*B* and 1*C*) which is to be expected if the rate of progression of survivors and non-survivors is the same[19].

The increase in survival after two separated doses (Fig. 1*C*) may be evaluated quantitatively by comparing the response to the second dose of first-dose survivors, with the response of single, unirradiated cells. Such a comparison is possible, if, as we have seen, cells after a

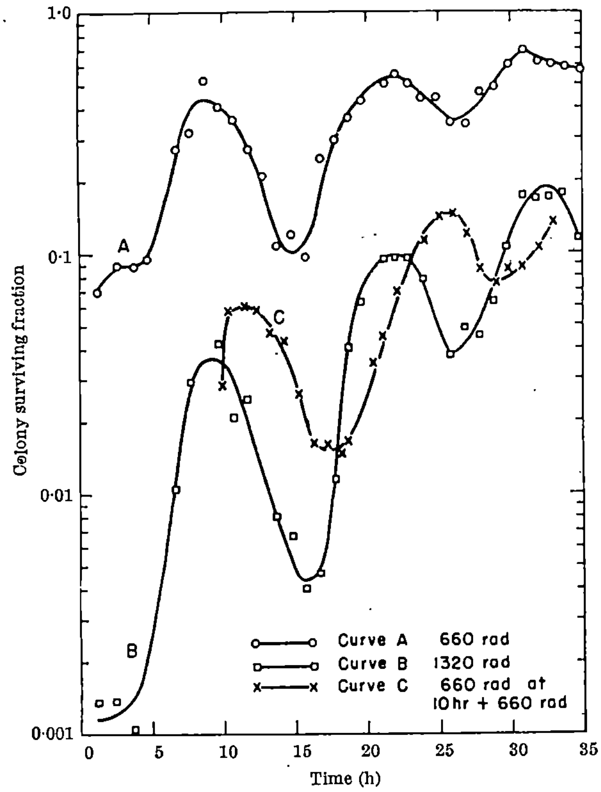

Fig. 1. Colony survival of synchronized hamster cells after 660 rads at intervals of time after incubation (curve *A*), 1,320 rads at intervals (curve *B*), and 660 rads at 10 h followed by 660 rads at further intervals (curve *C*). The first generation cycle ends at about 15 h and the second at about 26 h

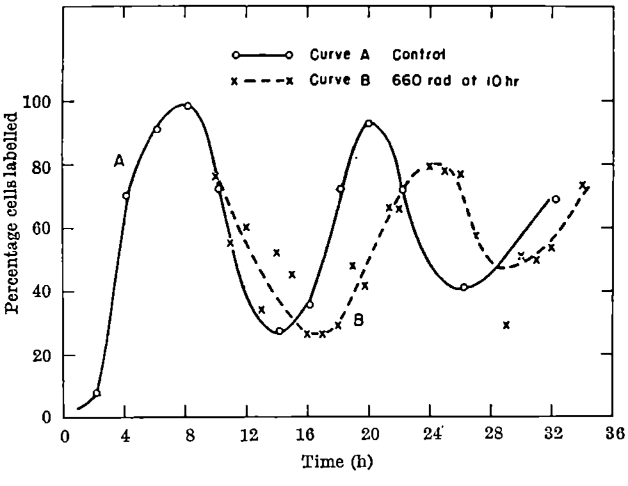

Fig. 2. Percentage of cells labelled with a pulse of tritiated thymidine (0·4 μc./ml. for 15 min) against time after incubation of the synchronized population. Unirradiated cells (curve *A*); cells after 660 rads at 10 h (curve *B*)

first dose are still quite well synchronized and if the initial distribution of cells per colony is known. In Fig. 3*A*, the average number of cells per colony for this experiment, $\bar{N}(t)$, is shown as a function of time. The single cell survival, $S(t)$, after 660 rads, shown in Fig. 4*A*, is then found from the colony survival after 660 rads, $f(t)$, (Fig. 1*A*) by means of the following equation[18]:

$$S(t) = 1 - [1 - f(t)]^{1/\bar{N}(t)} \qquad (1)$$

Values of $f(t)$ were taken from Fig. 1*A*.

Strictly speaking, $\bar{N}(t)$ should be that for viable cells only, and because the plating efficiency of these cells is not 100 per cent, $\bar{N}(t)$ shown in Fig. 3*A* includes both viable and inviable cells. The error in using the observed values for $\bar{N}(t)$ is not large, however, and has been discussed elsewhere[18]. The effect of a second 660 rads on survivors of a first dose of 660 rads at 10 h was found as follows. The observed colony survival after two doses, Fig. 1*C*, was divided by the fraction of colonies surviving a single dose of 660 rads at 10 h ($\sim$ 0·40 in this case, Fig. 1*A*). This gave the fractional survival to the second dose as a function of the time-interval between doses. Unfortunately, the survivors were not quite single cells, even initially, but the average multiplicity $\bar{N}_s$ of these survivors was calculated using equation (2) for $\bar{N}_s$ at 10 h:

$$\bar{N}_s\,(10\text{ h}) = \frac{S\,(10\text{ h})\,.\,\bar{N}\,(10\text{ h})\,.\,N_0}{f\,(10\text{ h})\,.\,N_0} \qquad (2)$$

where N_0 = total colonies that would form on an unirradiated plate. N_0 is constant, but in any event it cancels. $\bar{N}_s(t)$ at any later time t may then be plotted as in Fig. 3*B* assuming that $\bar{N}_s(t)$ follows a pattern just like that of $\bar{N}(t)$ but displaced from it by 2·8 h (Figs. 1 and 2). Equation 1 with $\bar{N}_s(t)$ substituted for $\bar{N}(t)$ was then used to calculate the single-cell survival after the second 660 rads (Fig. 4*B*). To summarize, Fig. 4*A* represents the single-cell survival after 660 rads as a function of cell age throughout 2·5 division cycles. Fig. 4*B* represents the survival after a second dose of 660 rads, for single-cell survivors given a first dose of 660 rads at 10 h. It may be seen from Fig. 4 that: (1) desynchronization of the population with time is evident in both curves; (2) the initial increase in the response to a second dose with time (Figs. 1*C* and 4*B*) is consistent with repair of sub-lethal damage; (3) concurrent with this repair, the survivors after 660 rads progress

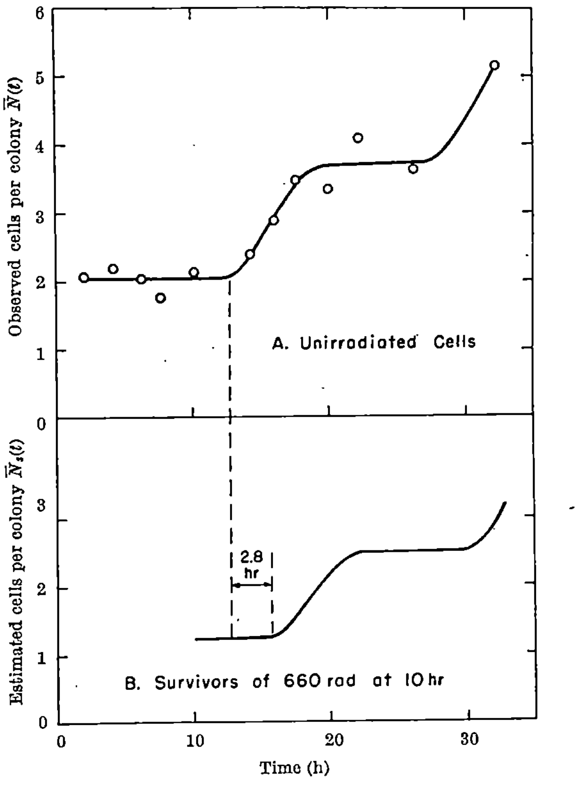

Fig. 3. Cells per colony observed by counting stained unirradiated cells (curve *A*). Cells per colony estimated for the survivors of 660 rads at 10 h (curve *B*) (see text)

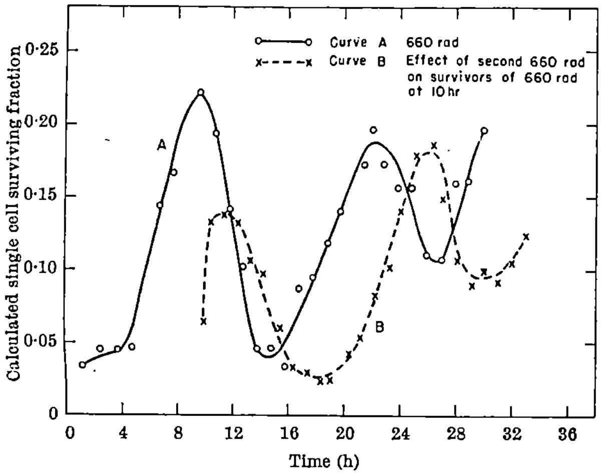

Fig. 4. Calculated age-response of single synchronized hamster cells to 660 rads. Curve *A*, previously unirradiated cells; curve *B*, cells which survived a preliminary 660 rads at 10 h

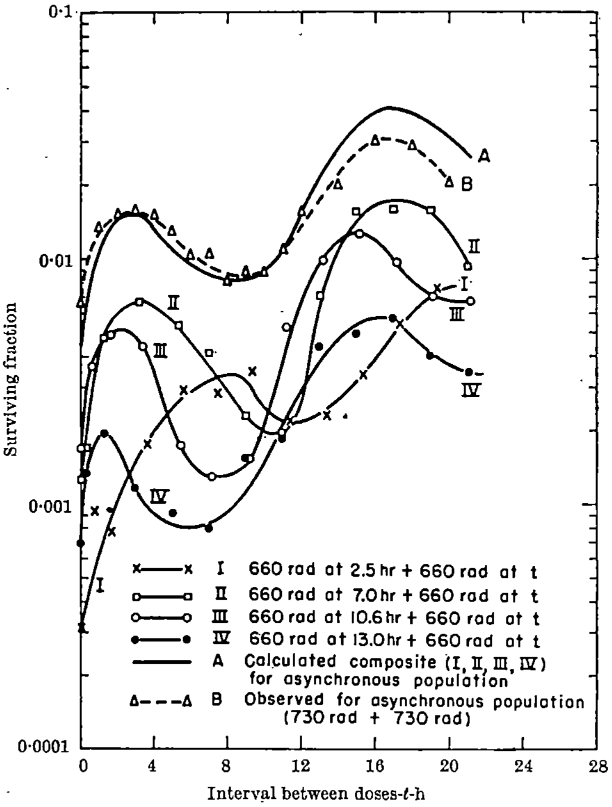

Fig. 5. The response of an asynchronous population of single cells to two doses of 660 rads separated by different time intervals. Curve *A*, estimated from the sum of four components I, II, III and IV receiving first doses at different cell ages, 2·5 h, 7·0 h, 10·6 h and 13·0 h, respectively. Curve *B*, experimental data for an asynchronous population exposed to 730 rads + 730 rads. Curve *B* had the same survival-level as curve *A* at the peak response, that is, at an interval of about 2·5 h between doses

through the cycle with a gradually increasing delay (that is, a decrease in rate); (4) apart from delay, Fig. 4*B* exhibits a pattern so similar to Fig. 4*A* after the first division that we conclude repair is complete, that is, the response of survivors to the first dose appears eventually to be just like that of unirradiated cells, at least at these dose-levels.

Recovery of Cells not in the S Phase. The foregoing results indicate that cells in the *S* phase are capable of complete sub-lethal repair, in so far as colony formation is concerned, within a relatively short time. It is possible, however, that the recovery process may be age dependent, that is, cells in the pre-DNA synthetic period G_1 or post-DNA synthetic period G_2 may not be able to repair sub-lethal damage as effectively or perhaps not at all. There are several reasons why these phases are much more difficult to investigate. First, if the amount of sub-lethal damage is indicated by the magnitude of the shoulder, the effect one might hope to see is smaller because G_1 and G_2 cells have survival curves with much smaller shoulders than do *S* cells[17,18]. Secondly, our synchronized cell population is never entirely free of some *S* cells at any cell age. For example, at 1–2 h in G_1 we may have about 5–10 per cent (Fig. 2) of the cells synthesizing DNA (perhaps because some cells have an extremely short G_1 or perhaps because the labelled cells represent a small fraction of non-synchronized interphase cells). Since cells in *S* are more resistant, their percentage is increased in the survivors of the first dose and recovery among these *S* cells can obscure the smaller recovery to be expected in the remaining G_1 cells. This situation is accentuated in G_2 where the fraction of cells still in *S* is higher. Thirdly, G_1 cells are progressing toward a state of greater resistance and thus a rise in survival due to recovery is less easy to distinguish. Cell progression operates more in our favour for G_2 cells, however. For these reasons, two-dose studies in Chinese hamster cells of the type described here yield information primarily about the most resistant component (that is, cells in *S*).

Response of an Asynchronous Population. In spite of these limitations concerning the specificity of recovery as a function of cell phase (G_1, G_2 or *S*), the two-dose response at different times in the synchronous population may represent an appropriate average for the purpose of analysing the response of asynchronous cells. Fractionation experiments at various cell ages in the synchronized population may, therefore, provide useful information concerning the form of the response to be expected for asynchronous cell populations.

A two-dose experiment was conducted in which first doses of 660 rads were delivered at 2·5 h, 7·0 h, 10·6 h, and 13·0 h followed by second doses of 660 rads at intervals of up to 20 h after the first dose. Other samples were exposed to single doses of 660 rads and 1,320 rads, respectively. An initial rise in the two-dose response (similar to that shown in Fig. 1*C*) occurred at all four cell ages but was greatest (as expected) for 7·0 h and 10·6 h cells. The data obtained were used to construct the response to be expected from an asynchronous population. The calculation, in outline, was as follows:

After an asynchronous log-phase population is exposed to a first dose of 660 rads, the age distribution of survivors was found by multiplying the age-response of single cells after 660 rads (for example, Fig. 4*A*) by the appropriate age-density distribution[20]. The curve obtained for the first-generation cycle was divided into four regions (I, 0–5 h; II, 5–9 h; III, 9–12·5 h; IV, 12·5–16 h) and the area under each multiplied by the effect of a second dose of 660 rads for first doses at 2·5 h, 7·0 h, 10·6 h, and 13·0 h, respectively. This procedure yielded the contribution of each of the four age regions to the overall two-dose response of asynchronous, log-phase cells as shown by curves I, II, III, and IV in Fig. 5. The survival response to be expected for the entire asynchronous population was then obtained by the addition of these four curves (Fig. 5*A*). For comparison purposes, the results of an actual experiment on an asynchronous population are included (Fig. 5*B*). Although slightly different dose fractions were used, this experiment yielded about the same survival at the maximum (that is, at ~2·5 h) and the form of the response is not strongly dose dependent[8]. In spite of minor differences, the general features of Fig. 5*A* and Fig. 5*B* are the same.

The following conclusions may be drawn from these investigations.

(1) Chinese hamster cells in the *S* period which survive a first dose of X-radiation repair fully the sub-lethal damage incurred, as defined by their ability to form colonies when challenged with a second dose. For a more comprehensive study of these effects at different dose-levels, full survival curves need to be obtained. (2) The repair process is rapid and may be complete in about 2–3 h. It certainly appears to be complete soon after the first division at these dose levels. (3) During the recovery process, the cells continue to progress through the cycle, although more slowly than unirradiated cells. (4) The kinetic pattern of recovery for asynchronous cells exposed to fractionated doses is due to the combined effects of partial synchronization of survivors, rapid recovery from sub-lethal damage, and subsequent cell progression through the cycle.

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EFFECTS OF INOSITOL-DEFICIENCY ON YEAST WITH PARTICULAR REFERENCE TO CHEMICAL COMPOSITION OF THE CELL AND OF THE CELL WALL

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INTERESTING changes in the morphological and physiological characters of *Saccharomyces cerevisiae* were observed when this brewer's yeast was grown in media lacking inositol[1]. Thus, in such media growth is restricted, the yeast cells clump together to form aggregates, the fat content of the cell is greatly increased and the cell wall is considerably weakened[1,2]. This article describes other effects of inositol-deficiency and reports on its influence on the chemical composition of both the cell and cell wall.

The yeast was grown at 30° in a glucose, ammonium salts medium containing growth factors including inositol, 1 mg/100 ml. (complete medium), and in the same medium lacking inositol (deficient medium) in 300 ml. volumes in culture bottles (Glaxo pattern, 2·5 l. capacity)[2]. After incubation for 4–5 days the yeast was gathered on the centrifuge, washed and freeze-dried. The yields (dry weight) were 600 mg and 300 mg and the yeast is named 'normal' and 'deficient', respectively.

Preliminary observations of the ribonucleic acid content of the yeast growing in the complete and that in the deficient media were made at five different stages of growth. No appreciable differences were observed between the RNA contents of cells of the 'two yeasts' at comparable physiological ages. This finding is similar to that recorded by other workers[3] for the related yeast *Saccharomyces carlsbergensis*.

Manometric measurements were made on the cells and on particle-free cell-extracts of each of the two yeasts. Deficient cells respired glucose at a normal rate but possessed impaired fermentative power both aerobically and anaerobically. Deficient particle-free extracts evolved carbon dioxide much more slowly than normal extracts from glucose and from each of four glycolysis intermediates.

The remainder of this article deals mainly with analyses of the normal and deficient cells and of their walls. Cells were ruptured by shaking in the Mickle disintegrator and the walls isolated from cell debris by differential centrifugation. The walls were washed very thoroughly (fifty times) with water, freeze-dried, weighed and were found to constitute 10·5 and 10·3 per cent of the dry weight of the deficient and normal cells respectively. The similarity of these results is perhaps surprising in view of the marked differences observed in chemical composition and in other properties. Analytical results are expressed as percentage dry weight of the whole cells or walls respectively unless otherwise stated. Lipid analyses are given in Table 1.

Table 1. Lipids in the Normal and Deficient Cells and Cell Walls

	Cells		Walls	
	Deficient %	Normal %	Deficient %	Normal %
'Purified' lipid	48	19	6·7	6·7
Lipid-inositol*	0·32	0·92	0·2	0·4
Lipid-phosphorus*	0·37	0·89	0·5	1·1

* Expressed as percentage of the lipid present in the cell or in the wall

The lipids present in whole cells were extracted after rupturing in the Mickle cell disintegrator. Extraction of the broken-down cells or of the walls was carried out at room temperature under nitrogen with neutral chloroform–methanol mixtures (2 : 1 v/v) followed by extraction with acidic (1 per cent hydrochloric acid) chloroform–methanol mixtures. 'Purification' of the combined crude extracts was effected by the washing procedure of Folch, Lees and Sloane Stanley[4]. Increase in lipid content resulting from inositol-deficiency, previously reported[1,2] (for lipid material extracted with light petroleum), was again observed. Thus the 'purified' lipid content (48 per cent) of the deficient yeast was approximately two and a half times as great as that (19 per cent) of the normal yeast. In contrast, the 'purified' lipid contents of the cell walls of each of the two yeasts were the same—6·7 per cent of the dry weight of the wall.

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Inositol present in these lipids was estimated by microbiological assay using *Schizosaccharomyces pombe*[5]. The deficient cell-lipids contained a lower percentage of inositol than the normal cell-lipids—0·32 versus 0·92 per cent—but when the *amounts* of total lipid-inositol present are considered (that is, after allowing for the higher lipid content of the deficient cells) the difference was small. In contrast, the deficient wall-lipids contained much lower amounts of inositol than the normal wall-lipids—0·2 versus 0·4 per cent—these latter percentages are proportional to the total amounts of inositol present in the wall-lipids, since each wall contains the same percentage (6·7 per cent) of lipid.

The total inositol contents of the cell and of the wall were also determined. Rather less inositol was found in the deficient cell—0·32 versus 0·44 per cent—whereas the deficient wall contained much less—0·02 versus 0·05 per cent—than the corresponding normal wall.

From the results of the two foregoing paragraphs it would appear: (1) that the yeast, when grown under conditions of inositol-deficiency, does have a lowered content of inositol in the cell and especially in its wall; (2) that the amount of inositol made available to cell-lipids is only slightly lowered, whereas that available to the wall-lipids is much reduced. The cell-lipids appear to have priority, as it were.

Phosphorus in the 'purified' lipids was estimated by the micro-method of Dryer, Tammes and Routh[6]. The percentage of phosphorus in the deficient cell-lipids was much reduced, 0·37 versus 0·89 per cent, but, after allowing for the much greater proportion of lipid present in the deficient yeast, the amounts of lipid phosphorus in the two yeasts were essentially the same. If the tentative assumption were made that the percentage of lipid-phosphorus gives some indication of the content of phospholipids the foregoing results would suggest that the two yeasts contained approximately the same amounts of this type of lipid; the increased amount of lipid observed in the deficient yeast could then be explained as due to an increase in the non-phosphorus-containing lipids.

In contrast to the foregoing results for cell-lipids the deficient wall-lipids contained much less phosphorus than the normal wall-lipids—0·5 versus 1·1 per cent. When these results are considered in conjunction with the lowered content of inositol, it seems possible that the deficient wall-lipids contain a reduced content of phosphoinositides. Such a finding may help to explain the fragility[1,2] of the wall of the deficient cell.

The fatty acids present in the wall-lipids were examined by gas-liquid chromatography and the relative proportions of the eleven fatty acids detected in each of the wall-lipids were essentially the same. A large proportion (76 per cent) of the fatty acids was unsaturated.

The cell walls were further examined and their contents of carbohydrate and protein were studied.

Total carbohydrate was estimated by the anthrone method. The polysaccharide glucan was estimated, after hydrolysis with acid, by glucose-oxidase; mannan was then determined by difference. Glucosamine was estimated after acid hydrolysis (hydrochloric acid) by the method of Cessi and Piliego[7].

Total carbohydrate constituted approximately 70 per cent of the normal wall and consisted mainly of the polysaccharides glucan (37·5 per cent) and mannan (31·6 per cent); a small amount of glucosamine (0·7 per cent) was also present. These findings are in general agreement with those of other workers for this species of yeast[8]. The foregoing three constituents were present in quite different proportions in the deficient wall—glucan was greatly increased (47·9 per cent), mannan greatly decreased (15·4 per cent) and six times as much glucosamine (4·2 per cent) was present.

Nitrogen was estimated by micro-Kjeldahl and protein calculated in the normal way after allowing for the nitrogen present as glucosamine. The normal wall contained much more protein than the deficient wall (15·9 versus 11·1 per cent).

The amino-acids present in the protein were detected by two-dimensional paper chromatography (butanol/acetic/water, 12 : 3 : 5 vol. and phenol, ammonia) after spraying with ninhydrin; estimation was carried out by means of an amino-acid auto-analyser (Technicon, Ltd.). Most of the naturally occurring amino-acids were present—seventeen in the normal wall and sixteen in the deficient wall; threonine was the most abundant and serine, glutamic and aspartic acids were also present in relatively large amounts in each of the walls. The dicarboxylic amino-acids formed a greater, and the basic amino-acids a much smaller, proportion of the total amino-acid content of the deficient wall. Only small amounts of the sulphur-containing amino-acids cystine and methionine were present in the normal wall; the deficient wall contained a smaller percentage of cystine and no detectable methionine.

The changes in composition of the cell-wall described above are of a major character. Compared with the normal the deficient wall contains materially different amounts of the two polysaccharides glucan and mannan, much more glucosamine, less protein (no methionine constituent), the same proportion of lipids which, however, have a lower phosphorus content and much less inositol both in the wall itself and in the wall-lipids. The decreased amounts of mannan and protein may indicate incomplete formation of the inner membrane (which, according to Northcote and Horne[9], is made up in part of these two substances) and this might account for the observed fragility of the inositol-deficient wall.

The results outlined in this article show that inositol-deficiency has a profound influence on the chemical composition of both the cell and its wall. These changes occur in yeast grown in the presence of oxygen in media containing no added inositol. Some of these changes have also been shown to occur in yeast grown in the presence of sub-optimal amounts of inositol.

Preliminary observations on yeast grown anaerobically in media containing oleic acid and ergosterol but lacking inositol have been carried out. The anaerobically grown deficient yeast cell contained essentially the same proportion (19 per cent) of lipid as the corresponding normal yeast, that is, increased formation of lipid in the cell did not occur. This very interesting result poses the question: Are the other effects of inositol-deficiency, as described here, also absent in anaerobically grown yeast? This question cannot be answered fully at present. Differences between the chemical composition of the walls of the anaerobically grown deficient and normal cells have in fact been observed, but these differences cannot yet be described more precisely, as preliminary results only are available.

We venture to suggest that investigations such as these on the weakened wall of the cell grown under conditions of inositol-deficiency may help in the understanding of the build-up of the wall of the normal healthy cell and, perhaps eventually, of the functions it performs.

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DEOXYCORTICOSTERONE ACETATE AND EXPERIMENTAL ATHEROSCLEROSIS IN CHOLESTEROL-FED RABBITS

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MOST recent work on the pathogenesis of atherosclerosis shows that probably the most important factors involved are: (*a*) changes in lipidic metabolism; (*b*) changes in the arterial wall, which could induce the deposition of plasmatic lipids into some particular vascular regions[1]. Among the endogenous factors regulating lipid metabolism, hormones seem very important, and for this reason their part in the genesis of atherosclerosis needs to be elucidated[2]. In this respect, cortisone, hydrocortisone, prednisone and corticotropin have been found capable of: (*a*) increasing the serum-levels of cholesterol and of low-density lipoproteins; (*b*) inhibiting atherogenesis in cholesterol-fed rabbits[2]. Similar results have also been obtained by other authors[3-7].

On the other hand, it has been previously shown[8-10] that hyaluronidase induces hypocholesterolæmia and more severe atheromasic lesions in cholesterol-fed rabbits, presumably because of an increased vascular permeability due to hyaluronidase. Adlersberg *et al.*[11], confirming the former and the latter results, have suggested that the effect of cortisone and its derivatives could be due to an antihyaluronidase action, already demonstrated by Benditt *et al.*[12] under different experimental conditions.

On the contrary, few investigations have been carried out on the relationships between deoxycorticosterone acetate (DCA) and both serum cholesterol-levels and atherosclerosis in cholesterol-fed animals.

However, it has already been shown that DCA, like hyaluronidase: (*a*) reduces the viscosity of the ground substance of connective tissue[13]; (*b*) depolymerizes the mucoproteic complexes of the ground substance[14,15]; (*c*) enhances the effect of hyaluronidase in stimulating osmosis[16,17]; (*d*) changes the vascular permeability when in high doses[18]. Furthermore, the results available on the relationships between DCA and atherosclerosis are often contradictory. In fact, according to refs. 19–22, DCA induces hypocholesterolæmia and decreases serum level of low-density lipoproteins and phospholipids in cholesterol-fed rabbits, dogs and chickens. This effect has been shown to be quite similar to that shown by hyaluronidase under the same experimental conditions[23]. On the other hand, a slight increase in cholesterolæmia has been demonstrated in dogs treated with low doses of DCA[24], while no changes of the serum levels of cholesterol have been found in rabbits treated with DCA[25]. The corresponding morphological findings concerning the arterial changes are also contradictory.

Preliminary investigations have been carried out by Cali and one of us (G. B. R.)[26] on the action of DCA on cholesterol-fed rabbits, with regard to the aorta only. Much more severe changes have been observed in aortæ of the cholesterol + DCA-fed rabbits than in those of the controls.

On the basis of the foregoing considerations and results, 87 female rabbits (average weight, 2·500 kg) were fed a high cholesterol diet (0·2 g/kg a day *per os*), in order to ascertain the possible effect of DCA on the cholesterolæmias and atherosclerosis of the hyperlipæmic rabbits. Three successive experiments were carried out, the first and the second with 25 animals each, fed with cholesterol for 60 days, and the third with 37 rabbits, fed with cholesterol for 67 days. In the first experiment, 13 out of the 25 rabbits were treated with 1 per cent DCA suspension in 10 per cent 'Tween 80' water solution at a dose of 12 mg/kg/day (300 mg, total), during the last 10 days of feeding with cholesterol, subcutaneously. In the second experiment, during the last 10 days of treatment, six animals received subcutaneously the foregoing dose of DCA (300 mg, total) and six were given the same DCA suspension at the dose of 18 mg/kg/day (450 mg, total) subcutaneously. The 37 animals of the third experiment were divided for the last 10 days of the treatment with cholesterol, in the following way: 9 animals were treated with cholesterol only (Chol), 11 rabbits received cholesterol + the foregoing suspension of DCA at a dose of 12 mg/kg/day (300 mg, total) (Chol + DCA + Tw), 8 animals were given cholesterol + 3 ml. of 10 per cent 'Tween 80' water solution a day (Chol + Tw), and finally, 9 animals were treated with the same dose of DCA, but given in a 2·5 'Vaseline' oil solution, besides cholesterol (Chol + DCA). DCA and 'Tween 80' were administered by subcutaneous way. Blood was drawn from the marginal vein of the ear of rabbits, after a fasting period of 12 h, before, during and after the administration of DCA and 'Tween 80'. Serum total cholesterol-levels were dosed according to the methods of Bloor and Knudson[27] in the first and second experiments, and of Pearson *et al.*[28] in the third experiment. Each value is the average of two determinations.

Four cross-sections of the hearts of the 37 animals of the third experiment, previously fixed in 10 per cent neutral formalin, were cut with a freezer microtome and the slices stained with sudan III and hæmatoxylin. At least 40 frozen slices were examined for each heart.

The relationships between the averages of the cholesterol values of the different groups of animals are shown in Figs. 1, 2 and 3. The statistical evaluation of data, carried out by means of calculation of Student's '*t*' and variance analysis, is illustrated in Table 1.

It has been pointed out that: (*a*) DCA induces a decrease of serum cholesterol-levels. This decrease is the more evident the higher were the serum cholesterol-levels

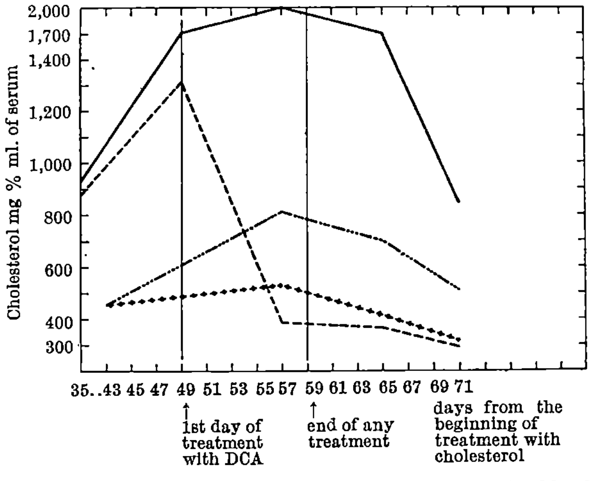

Fig. 1. First experiment. The averages of the serum cholesterol-levels of rabbits treated with DCA + cholesterol, and with cholesterol only, are reported. The experiment has been divided in two parts, because of the great difference between the values of some cholesterolæmias. Average of: ——, controls part No. 2; – – –, DCA treated animals part No. 2; + + + +, DCA treated animals part No. 1; —··—, controls part No. 1

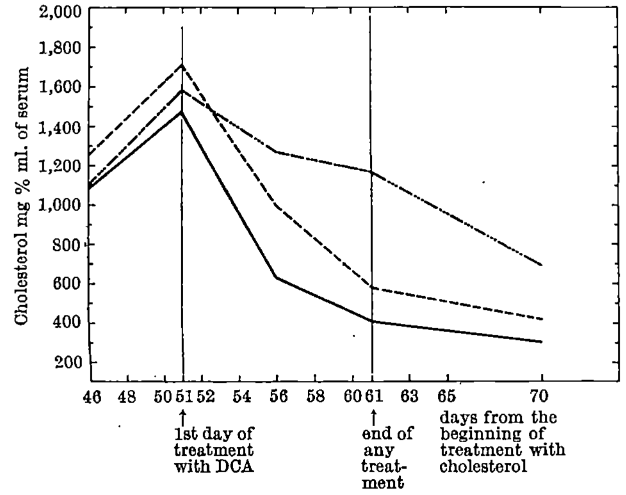

Fig. 2. Second experiment. The averages of the cholesterolæmias of the two experimental groups (rabbits treated with 300 mg and with 450 mg of DCA) and of control group are reported. ——, Average of values of rabbits treated with 300 mg DCA; – – –, average of values of rabbits treated with 450 mg DCA; —··—, average of controls

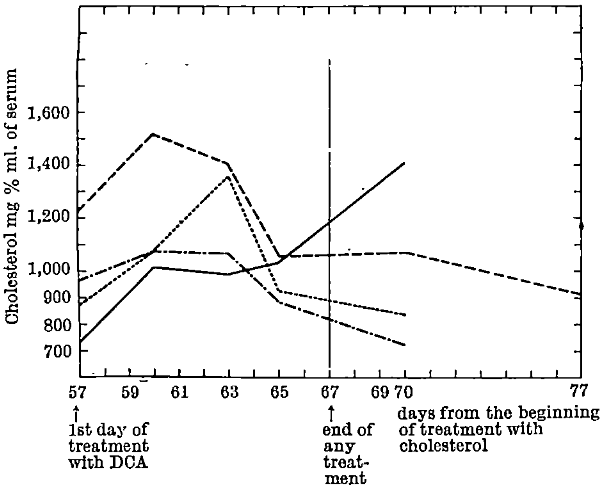

Fig. 3. Third experiment. The averages of the cholesterolæmias of the two experimental groups (Chol+DCA+Tw, – – – –; Chol+DCA, - - - -) and of the two control groups (Chol, ——; Chol+Tw, —·—·) are reported

Table 1. Statistical Evaluation of the Differences between the Averages of Cholesterolæmias of the Rabbits' of Experimental and Control Groups

(The standard deviation has been calculated for each average value)

1st Exp. (Student's '*t*' factor has been calculated):

(*a*) At the 43rd day $P > 0{\cdot}05$ for 18 *d.f.*
(*b*) At the 49th day $P > 0{\cdot}05$ for 3 *d.f.*
(*c*) At the 57th day $P < 0{\cdot}02$ for 23 *d.f.*
(*d*) At the 65th day $P < 0{\cdot}02$ for 23 *d.f.*
(*e*) At the 71st day $P < 0{\cdot}01$ for 21 *d.f.*

2nd Exp. (1) Variance analysis between the averages of the three groups (the factor '*F*' has been calculated):

(*a*) At the 51st day $P > 0{\cdot}05$ for 24 *d.f.*
(*b*) At the 56th day $P < 0{\cdot}02$ for 24 *d.f.*
(*c*) At the 63rd day $P < 0{\cdot}01$ for 24 *d.f.*
(*d*) At the 70th day $P < 0{\cdot}01$ for 23 *d.f.*

(2) Student's *t* factor has been calculated between the averages of cholesterolæmias of rabbits treated with 300 mg of DCA and of those treated with 450 mg of DCA:

(*a*) At the 51st day $P > 0{\cdot}05$ for 11 *d.f.*
(*b*) At the 56th day $P < 0{\cdot}05$ for 11 *d.f.*
(*c*) At the 63rd day $P > 0{\cdot}05$ for 11 *d.f.*
(*d*) At the 70th day $P > 0{\cdot}05$ for 10 *d.f.*

3rd Exp. (1) Variance analysis between the averages of the four groups (the factor '*F*' has been calculated):

(*a*) At the 65th day $P > 0{\cdot}05$ for 30 *d.f.*
(*b*) At the 70th day $P > 0{\cdot}05$ for 26 *d.f.*

(2) Student's '*t*' factor has been calculated between the averages of Chol+DCA+Tw group and of Chol+DCA group:

(*a*) At the 70th day $P > 0{\cdot}05$ for 13 *d.f.*

at the moment of DCA injection. The differences between the averages of cholesterolæmias of experimental and control rabbits are highly significant in the first and second experiment. An examination of Fig. 3 seems to indicate a decreasing effect of DCA in both the experimental groups (Chol + DCA + Tw and Chol + DCA), but the difference between the values of the experimental and control groups is not statistically significant. This could be due, in our opinion, to the small number of rabbits treated for each group and to an occasionally high biological variability. (*b*) The induced decrease of cholesterolæmia is not proportional to the injected dose of DCA. In fact, the difference between the averages of cholesterolæmias of the rabbits treated with 300 mg and, respectively, with 450 mg of DCA, is not significant. (*c*) 'Tween 80' does not interfere with the foregoing effect of DCA. In fact, no significant difference has been shown between the cholesterolæmia decrease induced by the DCA suspension in 'Tween 80' solution and that induced by DCA 'Vaseline' oil solution. Moreover, much higher doses of 'Tween 80' have been found capable of increasing and not decreasing, serum cholesterol-levels of cholesterol-fed rabbits[29,30].

So far as the morphological pictures are concerned, it has been observed that: (*a*) The coronary arteries of the animals of the Chol + DCA + Tw group show much more severe atheromatous changes (lipidic infiltration into the entire vascular wall) (Fig. 4) than those of the rabbits of the Chol group. Furthermore, the atheromasic lesions seem to be, as for the former group, very frequent in the majority of the small coronary vessels and also spread over great portions of the wall of each vessel (Fig. 5). (*b*) The coronary changes found in the animals of the Chol + Tw group are not so evident as those found in the rabbits of the Chol group (Fig. 6); this could be due to the small dose of cholesterol given in this experiment. (*c*) Finally, the vascular lesions of the animals of the Chol + DCA group are quite similar to those of the Chol + DCA + Tw group, both for their severity and for their frequency and diffusion. These results seem to indicate that: (*a*) DCA induces more severe and diffuse atheromatous lesions in DCA + cholesterol-treated animals than those observed in the controls. (*b*) 'Tween 80' does not interfere with the fore-

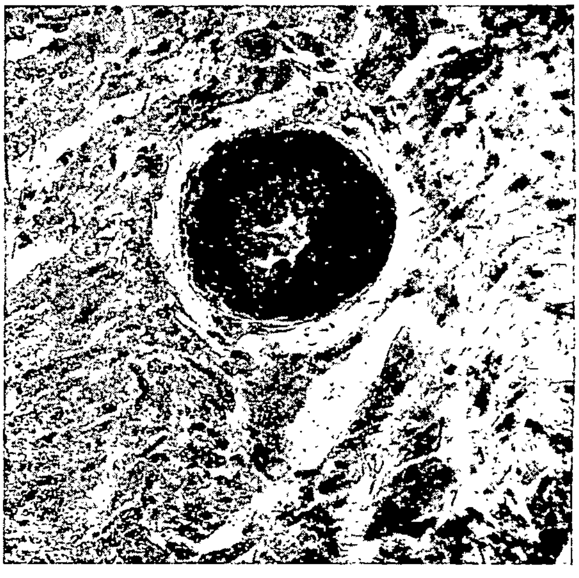

Fig. 4. Cross-section of a small coronary vessel, showing a massive deposition of sudanophilic material into the entire vascular wall (second group) (sudan III and hæmatoxylin, × 900)

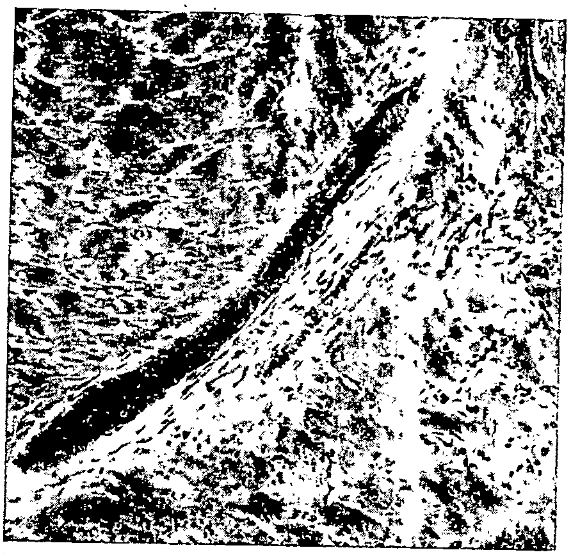

Fig. 5. A tangential section of a coronary vessel, in which the lipidic infiltration is spread over a great portion of the vascular wall (second group) (sudan III and hæmatoxylin, × 360)

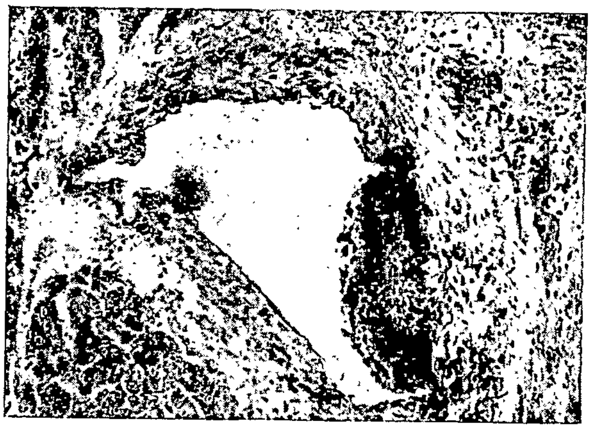

Fig. 6. A cross-section of a coronary vessel, with a small plaque limited in size and in extension (control group) (sudan III and hæmatoxylin, × c. 270)

going effect of DCA, which agrees with the observations that have been made of the serum cholesterol-levels. Moreover, much higher doses of 'Tween 80' have been found capable of slightly inhibiting, and not enhancing, the experimental atherosclerosis[29,30]. Similar results have been obtained on aortic specimens of animals treated in the same way as in this experiment[26].

From the foregoing considerations, DCA seems to play an important part in the pathogenesis of the plasmatic and tissue changes of experimental atherosclerosis due to hypercholesterolic diet.

On the basis of the suggested resemblance between the mechanism of DCA and hyaluronidase action, it has been suggested[26] that DCA probably interferes with the hyaluronic acid–hyaluronidase system of the chromotrope ground substance of the sub-intimal connective tissue. The depolymerization of the mucopolysaccharides of the ground substance could favour and increase the deposition of plasmatic lipids into the vascular wall, inducing both a decrease of the serum cholesterol-level and more severe atheromasic changes of the arterial wall.

An interference of DCA with the metabolism of thyroid gland is also possible. In fact, it has been demonstrated that thyroid hormones are capable of lowering serum cholesterol-levels and inhibiting atherosclerosis in cholesterol-fed rabbits[31] and chickens[32], whereas hypophysectomy[33], and hypothyroidism due to anti-thyroid drugs[34–38], enhance more and more hypercholesterolæmias and atheromatous lesions in cholesterol-fed rabbits[33], and succeed in inducing very high cholesterolæmias and atheromatous changes even in less-susceptible animals (dogs[34], rats[35–37] and hamsters[38]), fed with hypercholesterolic diet. Furthermore, it has been shown that the administration of cholesterol induces thyroid hypoplasia in rabbits and guinea pigs (species susceptible to develop high cholesterolæmias and experimental atherosclerosis) and thyroid hyperplasia in rats and hamsters (species less susceptible to show hypercholesterolæmias and experimental atherosclerosis)[39,40].

In this respect, DCA could inhibit the thyroid gland by means of the known 'adrenal cortex → hypophysis → thyroid gland' mechanism. If this were the case, it would be expected that DCA could, even slightly, increase thyroid hypoplasia due to the cholesterol feeding, inducing higher cholesterolæmias and, in this way, more severe arterial changes than in the controls. But the experimental results concerning the serum cholesterol-levels are quite opposite, and this seems to strengthen the aforementioned hypothesis of a direct action of DCA on the genesis of the deposition of plasmatic lipids into the arterial wall.

For all these considerations, even though a possible slight thyroid inhibition by DCA cannot be excluded, we suggest that the aforementioned interference of DCA in the hyaluronic acid–hyaluronidase system could be at least one of the most important mechanisms of DCA action on the atherogenesis of cholesterol-fed rabbits.

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URINARY TRYPSIN INHIBITOR (MINGIN): TRANSFORMATION INTO A NEW TRYPSIN INHIBITOR BY ACID HYDROLYSIS OR BY SIALIDASE

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THE trypsin inhibitor in human urine, purified by Shulman[1] and by Astrup *et al.*[2], is an interesting compound. In various physiological and pathological states it is excreted in increased amounts, apparently influenced by the pituitary-adrenal system[3]. Thus the diurnal excretion is augmented after administration of ACTH or cortisone, during fever, in the latter part of pregnancy, and in general after stress reactions. The compound is also an inhibitor of testicular hyaluronidase[4]. A parallelism between increase in level of serum trypsin inhibitor and the increase in diurnal excretion of the urine inhibitor has been reported[5]. Presence of ε-aminocaproic acid greatly potentiates its inhibition of urokinase-induced fibrinolysis[6].

Astrup *et al.*[2] prepared the inhibitor from human pregnancy urine and also suggested the term 'mingin' (from Latin: mingo) for this particular compound. Later, the compound was purified until it was homogeneous over a wide range of *p*H by paper electrophoresis, and it appeared that no further increase in trypsin-binding capacity could be obtained. A final capacity of 1·2 mg/mg crystalline trypsin ('Trypure', Novo Laboratories, Copenhagen: 24·4 Anson units/mg) was regularly reached. Assuming a molecular weight of about 25,000 for trypsin, this would, on a mole-to-mole basis, give a molecular weight for the inhibitor of about 30,000. Mingin is an extremely acid compound. Its isoelectric point is at *p*H 2·1. After complete acid hydrolysis, paper chromatography showed the presence of common amino-acids (including lysine and arginine) and of hexosamine. There was 4–5 per cent carbohydrate expressed as glucose[7].

We have now found that mingin can be transformed into a new trypsin inhibitor without loss of activity. This article describes experiments demonstrating the formation and some properties of the new inhibitor which differs from mingin in many respects. All experiments were performed with preparations of mingin having a trypsin-binding capacity of 1·2–1·3 mg/mg trypsin.

First an explanation was sought for the extraordinary low isoelectric point which could not be accounted for by the amino-acid composition. Analyses for sulphate and phosphorus were negative so that sulphuric acid or phosphoric acid groups could not be present. Our attention was then directed to the possible presence of sialic acid since our previous data had suggested a similarity with acid glyco-proteins[7].

When the 'direct Ehrlich' reaction[8] was applied to a solution of mingin (10 mg/ml.) no immediate colour reaction was obtained, demonstrating the absence of free reactive compounds. After heating the reaction mixture in boiling water for 30 min, 6 mg mingin produced a colour corresponding to 0·24 mg neuraminic acid, equal to 4 per cent (Beckman *DB* spectrophotometer. Linear dilution curves. Assayed against a sample of N-acetyl neuraminic acid, Nutritional Biochemicals Corp., Sialic acid concentrate; Lot No. 5487; Declaration of assay: 20 per cent).

The thiobarbituric acid method[8,9], applied to a solution containing 0·5 mg mingin/ml., yielded, after hydrolysis for 1 h at *p*H 2 and 80°, 2·3 per cent neuraminic acid (same reference sample as above).

Though the results of the two methods do not agree, they demonstrate that sialic acid reactive material is present in mingin in significant amounts. So far the reason for the discrepancy is unknown. However, differences between assays performed by the 'direct Ehrlich' reaction and by the thiobarbituric acid method have been reported by various authors and several explanations have been offered[10]. It could be of significance that the proportion between the two assays performed on mingin is approximately 58 per cent since this is the difference reported when the two methods are applied to *N*-glycolloyl neuraminic acid[10]. Using a pure compound, this could suggest the presence of this acid in mingin.

The release of large quantities of sialic acid during mild acid hydrolysis was a surprise, since mingin appears to be extremely stable in acid aqueous solutions. Thus little trypsin-binding capacity is lost by heating for 30 min at 100° C between *p*H 2 and 5. Above *p*H 5 there is rapid destruction[11]. It therefore appears that sialic acid can be removed without loss of trypsin-binding capacity, so that combination with sialic acid is not required for the inhibitor activity of mingin. After release of sialic acid, the remaining molecule would be more neutral than the original mingin, and it should be possible to trace the formation of the new compound by paper electrophoresis.

Hence, solutions of mingin were hydrolysed as in the thiobarbituric acid assay. Paper electrophoresis was performed in 0·1 M glycine-hydrochloric acid buffer at *p*H 2·3 where mingin would show little migration. Two components were observed. One of these migrated as untreated mingin. The other showed a much higher mobility at the *p*H value of the test, and therefore represented a compound considerably less acid than the original mingin. This assumption was supported by the observation that the new compound stained with bromphenol blue like a normal protein, while mingin is only weakly stained. This fact prevented a quantitative evaluation of the amounts separated on the electrophoresis strip. After elution, trypsin-binding capacity was found in both components. Hence, the formation of a new trypsin inhibitor, less acid than the original mingin, and derived from this by release of sialic acid, had been demonstrated.

The conditions for formation of the new inhibitor were then investigated, by varying the *p*H, temperature, and period of heating. Inhibitor activity was assayed on casein by ultra-violet spectrophotometry[12], using a Beckmann *DB* spectrophotometer with hydrogen lamp. Paper electrophoresis of the treated solutions was performed at *p*H 2·3 and 4·5. At the first *p*H mingin showed little migration, and at the latter the new compound migrated slowly. Conditions were established, at which little of the original mingin would remain as indicated on the electrophoresis strip, while most of the inhibitor activity was retained. Numerous experiments led to the final choice of 50 mg mingin/ml. in 0·1 M glycine-hydrochloric acid buffer at *p*H 2·3 and 4 h at 100°. About 75 per cent of the inhibitor activity was retained while less than 20 per cent of the original mingin remained. In general, the loss in activity was lower in these experiments than in the thermostability investigations previously reported. Most probably this is due to a stabilizing effect of the high concentration used in the preparative heat treatment, approximately 2,000 times higher than before. At a still lower *p*H the activity was rapidly destroyed. Thus at *p*H 0·8 after 1·5 h at 100° only 11 per cent inhibitor activity remained.

The next step was to see whether sialic acid could be removed by enzyme action with retainment of inhibitor activity. Such a possibility seemed likely in view of the recent finding that sialic acid can be released from human serum cholinesterase with sialidase with no loss of activity[13]. Supposedly an enzyme inhibitor would be even more resistant than an active enzyme.

Hence, a solution (50 mg/ml.) of mingin in 0·1 M sodium acetate buffer, *p*H 5·2, was prepared and incubated at 37° with 1 mg/ml. of a sialidase preparation (Sigma Chemical Co., St. Louis, neuraminidase, purified from '*Cl. perfringens*', Lot No. 338–691). The inhibitor activity of the mixture remained constant over a prolonged period. After 2 weeks less than 10 per cent had been lost. After 5 days no trace of the original mingin could be seen by paper electrophoresis performed as described here, and a new spot had formed, resembling, in migration and stainability, that obtained after acid hydrolysis. After elution this new component was found to possess inhibitor activity.

The electrophoretic mobilities of mingin have been determined before[7] using a method of paper electrophoresis by which corrections for evaporation, electro-osmosis, adsorption, etc., could be introduced, and the values for free mobilities could be calculated[11]. In order to compare the acid-hydrolysed mingin with the sialidase-treated product, and also with the genuine mingin, solutions of these compounds were prepared as described above and studied by this method. The following buffers were used, all at ionic strength 0·1; glycine–hydrochloric acid, sodium acetate–acetic acid, *tris*, glycine–sodium hydroxide, carbonate–bicarbonate. The data obtained, calculated as described, are presented in Fig. 1. Each value is the average of 3–12 estimations.

The electrophoretic mobilities of mingin correspond to those previously published[7], but have been extended over a wider range of *p*H. The mobilities recorded for acid hydrolysed mingin and for sialidase-treated mingin produced identical curves. Hence, these two compounds were electrophoretically identical. The new curve was approximately parallel to the original mingin curve with a difference over the whole *p*H range of about $+5 \times 10^{-5}$ mobility units. A highly acid component of the molecule had been lost such as had been anticipated. As a result, the isoelectric point had moved from *p*H 2·1 to about *p*H 4·5, the difference being approximately 2·4 *p*H units. In both curves little change in electrical charge occurred between *p*H 5 and 8. In this respect the new compound behaves differently from human serum albumin though the isoelectric points are nearly identical. It is interesting to compare these results with those reported for the sialidase-treated cholinesterase[15]. There the difference in mobility was 2–3 $\times 10^{-5}$ units, while the isoelectric point changed from *p*H 3 to about *p*H 7, a difference of about 4 *p*H units. Apparently the difference in number of ionogenic groups per molecule was less than when sialic acid was released from mingin, while the influence on the zero net charge was higher. Both cholinesterase compounds were less acid than the corresponding mingin compounds.

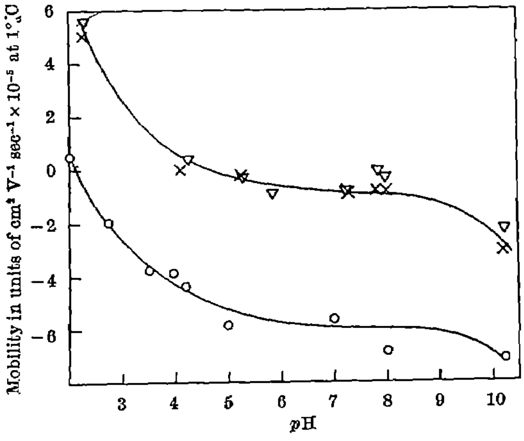

Fig. 1. Electrophoretic mobility of mingin (○), acid-hydrolysed mingin (▽), and sialidase-treated mingin (×)

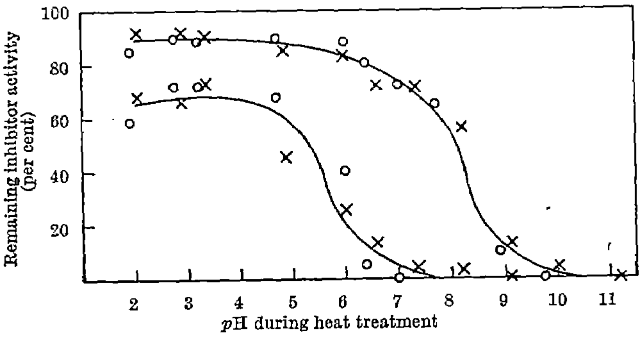

Fig. 2. Heat stability at 70° and 100° of mingin before (○) and after (×) treatment with sialidase

Since the thermostability curve is characteristic for mingin the thermostability of the new compound was investigated. Solutions of mingin and of a sialidase-treated compound, prepared as described, were heated for 1 h at 70° and 100° and at various *p*H values (using 0·05 M buffers as in the preliminary studies). The results are given in Fig. 2.

No difference is observed between the heat stability of the native mingin and of the sialidase-produced new compound. Apparently the heat stability, as the inhibitor activity, is a property of the protein moiety of the original mingin molecule. Loss of sialic acid does not change the heat stability of the protein part.

Separation and isolation of the new inhibitor were confirmed by column chromatography on a sulphonic acid resin (Fischer, 'Rexyn 101 (Na)'). After pretreatment of the column with 0·05 M acetic acid the acid- or sialidase-treated mingin solution, brought to *p*H 4, was passed through the column followed by 0·05 M acetate buffer, *p*H 4·25; by 0·1 M phosphate buffer, *p*H 7·6; and finally by 0·2 M sodium carbonate (*p*H ~ 10·6). The eluates were collected and brought to neutral reaction with N hydrochloric acid or solid sodium bicarbonate. Inhibitor activity was tested against trypsin by the plate method using unheated fibrin[16]. This method is less accurate for the assay of mingin for reasons explained before[11], but it requires little material and is easily performed on a large scale. Since no quantitative data were required for a separation of the active eluates from the inactive, this method was convenient. In the acid-treated solution two components were found. The first of these was eluted when the column was brought from *p*H 4·25 to neutral reaction. Native mingin behaved in the same manner on the column and this fraction therefore consisted of the unconverted mingin. The second component appeared when the *p*H was raised with carbonate. Sialidase-treated mingin produced only one component appearing in the second place.

It might be added that, in addition to sialic acid, other components of low molecular weight are released by acid hydrolysis or by sialidase treatment. They could be separated from the active compound by dialysis and gave a positive ninhydrin reaction, but so far have not been investigated.

This article is, to our knowledge, the first to describe a selective conversion without loss of activity of a natural protease inhibitor into a new inhibitor. We are at present engaged in a further characterization of the new inhibitor for which we would like to suggest the name 'minginin'. Its interaction with various proteases and fibrinolytic agents is being investigated. Several physiological questions are presenting themselves: What relation is there between inhibitors in blood and the inhibitor excreted in urine ? Does minginin exist as such in the organism ? Is the sialic acid-containing compound (mingin) derived from the sialic acid-free compound (minginin) or is the opposite true ? Does a coupling with sialic acid represent a new physiological mechanism of detoxication ? Or is it required for rapid excretion in the kidneys? It might be added for comparison that sialidase and a cholinesterase compound of low mobility have been observed in spinal fluid[13].

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UPTAKE AND BINDING OF CHLORAMPHENICOL BY SENSITIVE AND RESISTANT ORGANISMS

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D-THREO-CHLORAMPHENICOL (CAP) inhibits bacterial growth by blocking protein synthesis at some stage in the transfer of amino-acids from transfer RNA to protein[1]. However, CAP does not inhibit growth of yeasts, protozoa (with the unique reported exception of *Tetrahymena pyriformis*[2]), plants and animals.

It has been previously reported that D-threo-chloramphenicol (methylene-^{14}C) (^{14}C-CAP) is taken up by *Staphylococcus aureus* and *Bacillus megaterium*. After breakage and centrifugal fractionation of the bacteria all the radioactivity is associated with the ribosomal and soluble fractions. ^{14}C-CAP is also bound by ribosomes from these bacterial species in cell-free systems; the binding is immediate, not dependent on energy, unaffected by the incubation temperature and very easily reversible[3]. ^{14}C-CAP binds to 70 *S* ribosomes and to the 50 *S* sub-unit obtained from them. In both cases K^{+} ions are required for the binding of ^{14}C-CAP (ref. 4).

In more recent investigations the binding of ^{14}C-CAP to bacterial ribosomes has been shown to be prevented by pre-incubating the ribosomes in the presence of CAP, streptogramin *A* (synonyms *PA* 114 *A*–1, ostreogrycin *A*, staphylomycin M_1), ostreogrycin *G*, erythromycin, spiramycin, angolamycin, lankamycin, methymycin, carbomycin and oleandomycin, but was unaffected by streptogramin *B* (synonyms *PA* 114 *B*–1, ostreogrycin *B*), staphylomycin *S*, echinomycin, aureomycin, terramycin, puromycin, streptomycin and viridogrisein (synonym etamycin). A close relationship between the binding site and the primary point of action of an antibiotic seems probable. Thus antibiotics which reduce ^{14}C-CAP binding may inhibit the same reaction whereas others, although inhibitors of protein synthesis, may act at a different locus. The binding of CAP to ribosomes appears to be very specific and is unaffected by pre-incubation of the ribosomes with the CAP isomers L-erythro-, L-threo-, and D-erythro-chloramphenicol.

The uptake of ^{14}C-CAP by sensitive and resistant organisms and the binding of the antibiotic to their ribosomes have been investigated in an attempt to elucidate: (*a*) the relationship between the uptake and binding of ^{14}C-CAP and the antibiotic sensitivity of the organisms; (*b*) the possible mechanisms of resistance to CAP.

The uptake of ^{14}C-CAP by different organisms has been investigated and the results are shown in Table 1. There is a close relationship between the uptake of ^{14}C-CAP by the organisms and their antibiotic sensitivity. The uptake of ^{14}C-CAP by *E. coli B* 150 was lower than the uptake by *E. coli B* and this could possibly explain the increased resistance of *E. coli B* 150 to CAP. Spheroplasts of *E. coli B* and *E. coli B* 150 were prepared by the technique described by Kohn[5] and uptake of ^{14}C-CAP into the spheroplasts was investigated. The uptake of ^{14}C-CAP was almost negligible for spheroplasts from the resistant mutant (Table 2). It is possible that the low uptake of ^{14}C-CAP by intact bacteria of the resistant

Table 1. UPTAKE OF ^{14}C-CAP BY DIFFERENT ORGANISMS

Organisms	Growth inhibited (μg CAP/ml.)	Incubation conditions: Medium	Incubation time	Incubation temperature	mμg ^{14}C-CAP taken up/mg organisms
Staphylococcus aureus Duncan	10	*A* (ref. 6)	20 min	37°	9·8
Bacillus megaterium KM	10	*CG* (ref. 7)	20 min	30°	7·5
Escherichia coli B	5	*CG*	20 min	37°	19·6
**Escherichia coli B* 150	> 300	*CG*	20 min	37°	5·6
Pseudomonas striata	10	*A*	20 min	25°	26·0
Saccharomyces fragilis Jörgenson	> 300	Yeast extract (ref. 8)	20 min	30°	< 0·5
Crithidia (*Strigomonas*) *oncopelti*	> 300	Peptone glucose (ref. 9)	8 h	25°	0·5
Tetrahymena pyriformis W	150	Proteose peptone (ref. 2)	8 h	25°	2·5

**E. coli B* 150 derived from *E. coli B*

The organisms were collected during the exponential phase of growth, resuspended at a concentration of 3 mg dry wt./ml. (*C. oncopelti* and *T. pyriformis* 0·1 mg dry wt./ml.) and incubated in suitable growth media at their optimal growth temperature in the presence of 2 μg ^{14}C-CAP/ml. At the end of the incubation, the organisms were cooled to 4°, centrifuged, rinsed twice with growth media, resuspended in water and the radioactivity determined. Corrections were made for ^{14}C-CAP present in the intercellular fluid.

Table 2. UPTAKE OF ^{14}C-CAP BY *E. coli B* AND *E. coli B* 150

	mμg ^{14}C-CAP taken up/mg bacteria Whole bacteria	Spheroplasts
E. coli B	15·6	12·1
E. coli B 150	4·9	0·6

Bacteria were incubated in *CG* medium supplemented with 25 per cent sucrose. Spheroplasts obtained by the method of Kohn (ref. 5) were also incubated in *CG* medium supplemented with 25 per cent sucrose. Uptake of ^{14}C-CAP was determined as described in Table 1.

strain (Table 2) was due to a penetration of the antibiotic into cellular components outside the plasma membrane and that the resistance of *E. coli B* 150 is due to the impermeability of the membrane to CAP.

Although a permeability barrier could explain the CAP resistance of some organisms, it does not explain the failure of CAP to inhibit protein synthesis in ribosomal systems from yeasts, mammalian tissues, pea seedlings and *T. pyriformis*. The binding of ^{14}C-CAP to ribosomes from CAP sensitive and resistant organisms has been investigated and the results are shown in Table 3. The degree of binding of ^{14}C-CAP to ribosomes from different bacterial species was very similar and only *S. aureus* ribosomes bound ^{14}C-CAP to a significantly smaller extent. This was possibly due to the fact that the duration of breakage of *S. aureus* in the ultrasonic disintegrator was larger than for the other bacteria[10]. If the time of breakage of *B. megaterium* was prolonged to 13 min the amount of ^{14}C-CAP bound by the ribosomes was reduced by 50 per cent. On the other hand, the amounts of ^{14}C-CAP bound by ribosomes from *E. coli B* and *E. coli B* 150 were very similar, suggesting that the resistance of *E. coli B* 150 is due to a decreased permeability of the bacteria to CAP; this result is in agreement with the finding reported by Okamoto and Minuzo[13] that protein synthesis by cell-free systems from a strain of *E. coli* resistant to CAP was sensitive to the antibiotic.

^{14}C-CAP was not bound to ribosomes from *S. fragilis*, *C. oncopelti*, *T. pyriformis*, rat liver and pea seedlings either in 10^{-2} M *tris*-hydrochloric acid buffer containing 10^{-2} M Mg^{++} and 0·2 M K^{+} or in the buffer systems used to study protein synthesis in cell-free systems of these organisms[2,11,12,14].

The results obtained in this work suggest that all bacterial ribosomes (sedimentation constant 70 *S*) are able to bind ^{14}C-CAP. The resistance to the antibiotic shown by *E. coli B* 150 is due to the impermeability of the bacterial membrane; ribosomes from this strain bind ^{14}C-CAP to the same extent as those from the parent sensitive strain.

Ribosomes (sedimentation constant 80 *S*) from other organisms were unable to bind ^{14}C-CAP and this finding could explain the resistance of yeasts, protozoa, mammalian and plant systems to CAP. *T. pyriformis*, although a protozoan, is sensitive to high concentrations of CAP, but it has been reported that synthesis of protein by a ribosome system from *T. pyriformis W* is not affected by the antibiotic[2].

Table 3. BINDING OF ^{14}C-CAP BY RIBOSOMES

Organisms and tissues	Method of breakage	Ribosomal preparation	mμg CAP bound/mg ribosomes
S. aureus, Duncan	Ultrasonic, 13 min	70 *S*	18
B. megaterium KM	(1) Osmotic shock	70 *S*	30
	(2) Ultrasonic, 2 min	70 *S*	29
E. coli B	Ultrasonic, 1 min	70 *S*	29
E. coli B 150	Ultrasonic, 1 min	70 *S*	30
P. striata	Ultrasonic, 45 sec	70 *S*	33
S. fragilis Jörgenson	(1) Hughes press	80 *S*	Less than 1
	(2) Osmotic shock	80 *S*	
	(3) Ultrasonic, 2 min	80 *S*	
C. oncopelti	High-speed blending with glass beads	80 *S*	
T. pyriformis W	High-speed blending with glass beads	80 *S*	
Rat liver	See reference 11	80 *S* Polysomes	
Pea seedlings	See reference 12	80 *S*	

^{14}C-CAP (1 μg/ml.) was added to a preparation of ribosomes in 10^{-2} M *tris*-hydrochloric acid buffer *p*H 7·4 containing 10^{-2} M Mg^{++} and 0·2 M K^{+}. After 2 min at 4° the suspension was centrifuged at 150,000*g* for 60 min and the radioactivity in the pellet determined. In control experiments non-radioactive chloramphenicol (100 μg/ml.) was present during the incubation period and ^{14}C-CAP was added at the end. Volume of incubation mixture was 2 ml. containing 4 mg of ribosomes.

The lack of CAP inhibition of protein synthesis by ribosomal systems from yeasts, protozoa, mammals and plants may therefore be due to the inability of their ribosomes to bind the antibiotic.

The variation in CAP binding by ribosomes is in agreement with the ability of the antibiotic to inhibit protein synthesis in cell-free systems. If CAP inhibits only as a result of binding to the ribosomes, there is no need to postulate the existence of an additional CAP sensitive step in bacterial protein synthesis which is absent from mammalian systems as has been proposed by von Ehrenstein and Lipmann[15].

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CHLORAMPHENICOL AS AN INHIBITOR OF ENERGY-LINKED PROCESSES IN MAIZE MITOCHONDRIA

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IN the course of investigating the accumulation of calcium by maize mitochondria, it was observed that chloramphenicol in the concentrations used to inhibit ion accumulation by plants acted as an uncoupling agent[1]. Superficially, the compound seemed to act in the same fashion as the classic uncoupling agent, DNP (2,4-dinitrophenol). It appeared that the uncoupling properties of chloramphenicol could be responsible for the inhibition of ion accumulation.

We have now made additional observations on the action of chloramphenicol as part of present-day investigations of mitochondrial swelling and contraction, calcium uptake and ATPase. These observations indicate that chloramphenicol has a site of action distinct from DNP or oligomycin, and that it may act on one of the high-energy intermediates postulated for ATP formation and other energy-linked processes[2-10].

The experiments were conducted with mitochondria isolated from the shoots of ætiolated maize seedlings[1]. The properties of maize mitochondria with respect to reversible swelling, ion accumulation, and ATPase activity are not very different from those described for animal mitochondria. Complete descriptions will be published separately. We here abstract from the major work sufficient detail to compare the action of chloramphenicol with that of DNP and oligomycin.

Maize mitochondria swell spontaneously when placed in 0·2 M potassium chloride + 0·02 M *tris* (*p*H 7·5), and will contract on addition of ATP or succinate (Fig. 1). The curves are like those recently reported by Lyons and Pratt[11] for cauliflower mitochondria, except that these authors did not report substrate-induced contraction. In the experiments reported here the swelling and contraction were followed by changes in optical density at 520 mμ, but we have verified that volume changes are involved by measuring wet weight, and by electron microscopy. Pyruvate-malate or NADH will serve as well as succinate in powering contraction. Cyanide inhibits succinate-powered contraction, but not ATP-powered. Phosphate and magnesium are not required for succinate-induced contraction; on the contrary, phosphate is inhibitory (Table 1). Oxidizable substrate produces a more rapid contraction than ATP (Fig. 1).

Table 1. INHIBITION OF CONTRACTION BY UNCOUPLING AGENTS AND PHOSPHATE

Additive	% inhibition of contraction	
	Succinate	ATP
Dinitrophenol 5×10^{-4} M	56·9	64·0
Oligomycin 2 μg/ml.	−4·4*	79·5
Chloramphenicol 5×10^{-3} M	2·5	56·5
Phosphate 10^{-3} M	45·6	—
Magnesium 10^{-3} M	−5·4*	—
Phosphate and magnesium	46·7	—

Experiments with DNP, oligomycin, and chloramphenicol were performed as in Fig. 1, with the inhibitor added initially. Phosphate and Mg were added with the succinate. Inhibition was calculated from Δ optical density just before and 2 min after adding succinate or ATP.

* Promotion; these values and that with chloramphenicol (succinate-powered) are within errors of measurement.

Dinitrophenol accelerates the swelling-rate, but there is no change in final swollen volume (Fig. 1). Both succinate- and ATP-powered contraction are inhibited by DNP. Similar experiments have been done with the more effective uncoupler, *m*-Cl-CCP (carbonyl cyanide *m*-chlorophenylhydrazone). Except for the fact that lower concentrations are needed (about 10^{-5} M), the results are the same as with DNP. Neither DNP nor *m*-Cl-CCP is efficient in inhibiting contraction unless concentrations are used which virtually eliminate oxidative phosphorylation.

Table 1 compares the inhibitions of contraction found with DNP with those given by oligomycin, chloramphenicol and phosphate. Neither oligomycin nor chloramphenicol affects succinate-powered contraction, but they are effective with the ATP-powered system. Low concentrations of phosphate inhibit succinate-powered contraction.

Oligomycin blocks ATP-powered uptake of calcium but has little effect on substrate-powered uptake (Table 2; see also Brierley *et al.*[6] and Lehninger *et al.*[8]). Chloramphenicol, however, inhibits both the substrate-powered uptake[1] and the ATP-powered uptake (Table 3). Hence, although one cannot distinguish between oligomycin and chloramphenicol in the contraction system, they behave differently in the calcium-uptake system. Another difference which we have observed is that chloramphenicol accelerates the rate of initial swelling, while oligomycin does not.

Table 2. INHIBITION OF CALCIUM UPTAKE BY DINITROPHENOL, ADP AND OLIGOMYCIN

Energy source		Inhibitor	Ca uptake (mμmoles/ mg N/10 min)
Substrate	ATP		
−	−	—	74
+	−	—	281
+	−	$1{\cdot}2\times10^{-4}$ M DNP	76
+	−	$2{\cdot}6\times10^{-3}$ M ADP	104
−	−	$2{\cdot}6\times10^{-3}$ M ADP	65
−	+	—	265
−	+	$1{\cdot}2\times10^{-4}$ M DNP	106
+	+	—	595
−	−	—	252
+	−	—	1,960
+	−	2 μg/ml. oligomycin	1,630
−	+	—	1,640
−	+	2 μg/ml. oligomycin	364

Mitochondria isolated and experiment conducted as previously described (ref. 1). Media for the upper experiment contained 625 μmoles sucrose, 12 μmoles $MgCl_2$, 0·25 μmoles $CaCl_2 + {}^{45}Ca$ and 17 μmoles phosphoric acid in a volume of 2·5 ml. with *p*H adjusted to 7·5 with *tris*. Energy source was 8 μmoles succinate and/or 6·5 μmoles ATP. Media for the lower experiment had in addition 0·6 μmoles NAD, 0·4 μmoles thiamine-pyrophosphate and 0·1 μmoles coenzyme *A*. Substrate was 40 μmoles pyruvate + 5 μmoles succinate.

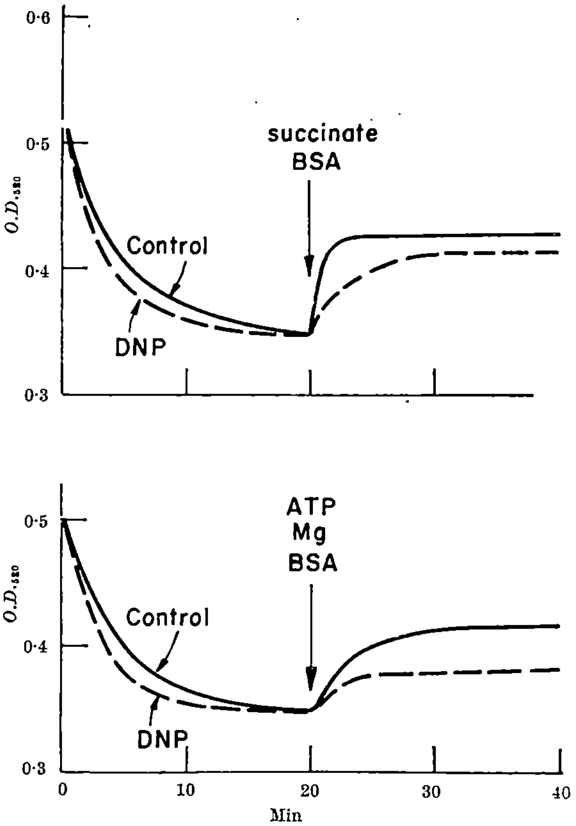

Fig. 1. Swelling and contraction of corn shoot mitochondria. Reaction started by adding 0·06 ml. of washed mitochondria in 0·4 M sucrose to 5 ml. of 0·2 M KCl + 0·02 M *tris* (*p*H 7·5) with and without 5×10^{-4} M DNP. Optical density changes at 520 mμ were followed in a Coleman model 11 spectrophotometer. Final concentrations of additives: 8 mM succinate; 5 mM ATP; 1 mM $MgCl_2$; 1 mg/ml. BSA (bovine serum albumin)

Substrate and ATP-powered uptake of calcium are inhibited by DNP (Table 2). Succinate-powered calcium uptake is inhibited by ADP, a result reported by Brierley *et al.*[6] for phosphate uptake. (Phosphate uptake accompanies uptake of calcium in these experiments.)

Both dinitrophenol and *m*-Cl-CCP enhance the ATPase activity of maize mitochondria (Heytler[12]); chloramphenicol does not (Fig. 2). At concentrations where all three agents begin to depress oxygen consumption (in parallel Warburg experiments), there is a depression of ATPase. With further increases in DNP or *m*-Cl-CCP concentration, there is a secondary rise in ATPase. Although the results are not detailed here, 1 μg/ml. oligomycin gives about 80 per cent inhibition of ATPase activity, which is comparable to the inhibition with animal mitochondria[13].

Oxidative phosphorylation by maize mitochondria is inhibited 50 per cent by approximately 2×10^{-4} M DNP,

Table 3. INHIBITION OF ATP-POWERED CALCIUM UPTAKE BY CHLORAMPHENICOL

ATP	Chloramphenicol	Ca content (mμmoles/ mg N)	ATP-powered Ca uptake (mμmoles/mg N)
−	—	108	—
+	—	415	307
+	2×10^{-3} M	276	168
+	4×10^{-3} M	184	76

Corn shoot mitochondria were isolated and experiment conducted as previously described (ref. 1), except that incubation time was 5 min. Medium contained 625 μmoles sucrose, 12 μmoles $MgCl_2$, 0·25 μmoles $CaCl_2 + {}^{45}Ca$, 17 μmoles phosphoric acid neutralized with *tris* (*p*H 7·5), 0·1 ml. ethanol, 6·5 μmoles ATP where indicated, and 0·456 mg mitochondrial nitrogen in volume of 2·5 ml.

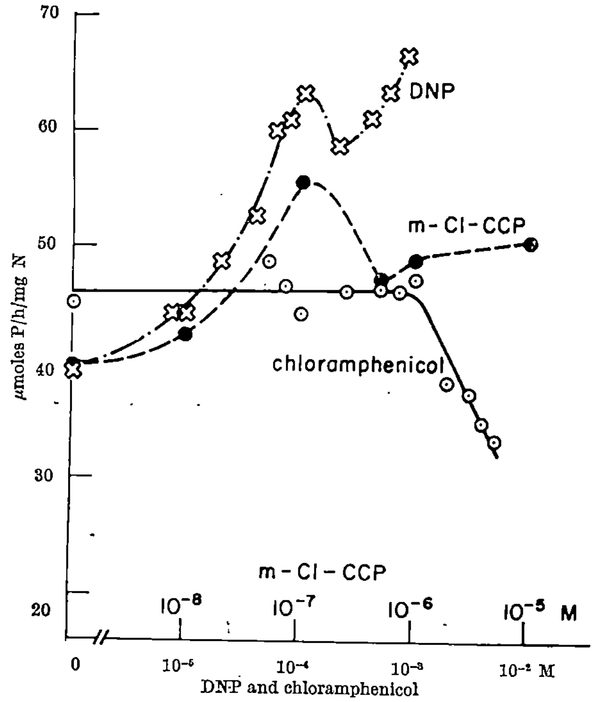

Fig. 2. ATPase activity of corn shoot mitochondria. Reaction at 28° C started by adding 0·1 ml. mitochondria (0·16–0·18 mg N) to 5 ml. of 0·2 M KCl+0·02 M *tris* (*p*H 7·5), 0·005 M ATP, 0·001 M $MgCl_2$ and uncoupler as indicated. Reaction terminated at 30 min with 5 per cent trichloroacetic acid, and change in inorganic phosphate from initial level determined. The ethanol added with the uncouplers was not sufficient to affect ATPase activity

0·08 µg/ml. oligomycin and 2 × 10^{-3} M chloramphenicol, using pyruvate-malate or pyruvate-succinate as substrate. These same concentrations suppress oxygen consumption by about 20, 40 and 20 per cent, respectively.

In all, we have examined four mitochondrial functions with three inhibitors; the results are summarized in Table 4. It is clear that chloramphenicol acts more like oligomycin than like DNP, the exception being that chloramphenicol inhibits substrate-powered uptake of calcium, while oligomycin does not. There is another distinction not indicated in Table 4 (cf. Tables 2 and 3); on a concentration basis, oligomycin is a much more effective inhibitor. Indeed, chloramphenicol is of interest here largely because of questions about its mode of action in stopping ion accumulation.

Table 4. SUMMARY OF EFFECTS OF INHIBITORS ON ENERGY-LINKED MITOCHONDRIAL PROCESSES

Inhibitor	Oxidative phosphorylation	ATPase	Contraction		Ca uptake	
			ATP	Succinate	ATP	Succinate
DNP	–	+	–	–	–	–
Oligomycin	–	–	–	0	–	0
Chloramphenicol	–	–	–	0	–	–

0, no effect; +, promotion; –, inhibition.

We have attempted an analysis of the action of chloramphenicol as compared with DNP and oligomycin by use of a modified 'Type *I*' scheme of high-energy intermediates (Fig. 3). The discussion of Boyer[7] proved very useful for this purpose, and the concept of reactive '*X*' and '*I*' groups on a common molecule has been adopted. There is no attempt to depict how the primary high-energy intermediate is formed, nor is any identification of the intermediate implied. The scheme simply provides a vehicle for examining the action of the inhibitors which is consonant with the observations summarized in Table 4.

The moiety shown as $\overline{X\,I}$ is assumed essential to the passage of electrons through 'coupling' sites. The first high-energy intermediate is $\overline{X \sim I}$. This state is considered to power directly contraction of maize mitochondria, for contraction requires neither phosphate nor magnesium, and indeed is inhibited by phosphate (Table 1).

The phosphate inhibition of contraction is considered to be due to depletion of $\overline{X \sim I}$ in a reversible reaction yielding $\overline{X\,I} \sim P$. The high-energy phosphate can be utilized in ATP formation or in phosphate (plus calcium) uptake (see Rossi and Lehninger[14] for comments on which ion is actively transported). The preferred pathway must be to ATP formation, for uptake of calcium is inhibited by ADP (Table 2).

Alternatively, ion accumulation may utilize the non-phosphorylated intermediate by a separate pathway (see the scheme of Slater *et al.*[9]). However, phosphate is essential to uptake of calcium in maize mitochondria; arsenate, chloride, and sulphate are ineffective; similar results are reported for uptake of magnesium by animal mitochondria[6]. Thus, any alternative pathway would also involve phosphorylation. For the sake of simplicity, a single phosphorylated intermediate is depicted.

Oligomycin is indicated to block the reversible transfer of phosphate between $\overline{X\,I} \sim P$ and ATP, much as in the scheme of Brierley *et al.*[6], and for the same reasons (Table 2).

The uncoupling action of DNP (and *m*-Cl-CCP) is deduced to be in the breakdown of the non-phosphorylated intermediate. Eisenhart and Rosenthal[15] recently concluded that DNP does not hydrolyse high-energy intermediates, but their pre-incubations included phosphate and presumably would have produced some phosphorylated intermediate which could phosphorylate ADP in the presence of DNP. Vignais[16], who preincubated in the absence of phosphate, concluded that DNP stimulates preferentially the hydrolysis of non-phosphorylated intermediates. With the present state of knowledge on high-energy intermediates, there seems to be no alternative which will explain the action of DNP in increasing the ATPase and the rate of swelling, and in inhibiting ATP-powered contraction and uptake of calcium. DNP clearly operates at the electron transfer side, yet it also functions to 'waste' energy channelled through from ATP. Some intermediate along the way must be affected in a fashion which will reduce the general level of intermediates available for osmotic work. Cleavage of the $\overline{X \sim I}$ bond would do this.

Within this frame of reference, the action of chloramphenicol can be examined. Chloramphenicol is not an uncoupler of the DNP type, for it does not inhibit substrate-powered contraction. It must therefore act beyond $\overline{X \sim I}$. It inhibits rather than promotes ATP hydrolysis, and blocks ATP-powered contraction, suggesting that it prevents $\overline{X \sim I}$ formation from ATP. Unlike oligomycin,

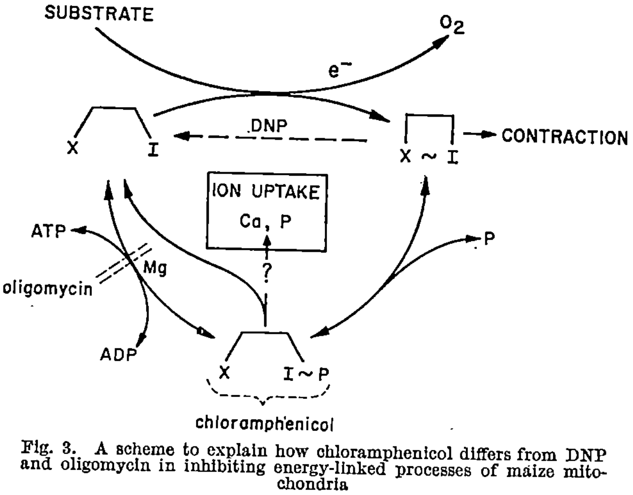

Fig. 3. A scheme to explain how chloramphenicol differs from DNP and oligomycin in inhibiting energy-linked processes of maize mitochondria

it will prevent substrate-powered uptake of calcium. In all, only one site is left to be involved; chloramphenicol in some fashion must block the formation or utilization of the phosphorylated intermediate.

This deduction, of course, is only as sound as the scheme, and it would be surprising if the scheme were correct in detail. However, until such time as the high-energy intermediates are known and their functions are described, little else can be done to localize sites of inhibitor action.

These experiments with chloramphenicol are with the high concentrations essential to inhibition of salt uptake. The inhibition of protein synthesis with lower concentrations is not necessarily related to the effect on mitochondria.

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Note added in proof. In order to simplify Fig. 3, we did not depict the action of the 'phosphate-collecting' enzyme which is thought to transfer phosphate from the intermediate to ADP[3].

$$\overline{XI} \sim P + E \rightleftharpoons \overline{XI} + E \sim P$$
$$E \sim P + \mathrm{ADP} \rightleftharpoons E + \mathrm{ATP}$$

We now observe that this omission gives the impression that oligomycin would block the ADP–ATP exchange, which is not always true (Huijing, F., and Slater, E. C., *J. Biochem.* (Tokyo), 49, 493 (1961); Wadkins, C. L., *Biochem. Biophys. Res. Comm.*, **7**, 70 (1962)). Oligomycin would presumably block the first reaction above, leaving the second to carry out the ADP–ATP exchange.

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ENERGETICS OF THE ELECTRIC ORGAN

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It was pointed out in 1906 by Bernstein and Tschermak[1] that if the immediate source of energy for the discharge of the electric organ was a movement of ions down their concentration gradients, then by analogy with the adiabatic expansion of a gas the electric organ would be expected to cool down during its discharge by an amount equivalent to the electrical energy dissipated externally. They performed experiments on the electric organ of *Torpedo*, which they regarded as verifying their hypothesis, though the sensitivity of their thermocouple and air thermometer (used as a joulemeter) was not really great enough to furnish very convincing evidence. After this hopeful beginning, no further examination of the thermodynamics of the electric organ was made until Abbott, Aubert and Fessard reopened the question in 1958. Using thermistors to measure the temperature changes in pieces of electric tissue from *Torpedo* they showed that there was indeed a phase of cooling during or soon after the electric discharge, but this was preceded and followed by warming phases which made it difficult to decide exactly how the magnitude of the cooling varied as a function of external resistive load (R_e).

Over the past five years we have made a fairly detailed investigation of the temperature changes in the electric organs of *Electrophorus*, *Malapterurus* and *Torpedo*, using somewhat smaller thermistor beads (diam. 0·25 mm) than in the earlier work[2], mounted on 20-mm lengths of twin glass capillary tubing so that they could be inserted between the electroplates with a minimum of damage to the tissue. The beads were insulated electrically by embedding them in 'Araldite' resin or (better) lead glass. The thermistors were used in pairs, connected in the opposite arms of a d.c. Wheatstone bridge, and in the case of *Malapterurus* each thermistor lay in the centre of a sandwich of electric tissue made by cutting the organ into pieces about 13 mm × 30 mm and putting two pieces together as shown in Fig. 1*A* with the same orientation of their electroplates and the skin outermost. The main reason for working differentially with two sandwiches or slices of tissue was to provide compensation for resting temperature drift, which all the same prevented the making of any useful measurements until the temperature of the preparation had been allowed to settle down for at least an hour inside a thermally insulated box. An additional advantage of this arrangement was that the observations could readily be duplicated (as, for example, in Fig. 3) by connecting the circuits for electrical stimulation and recording alternately to the two preparations. Unbalance of the bridge was detected by means of a Kipp Stylo galvanometer connected to a photoelectric feed-back

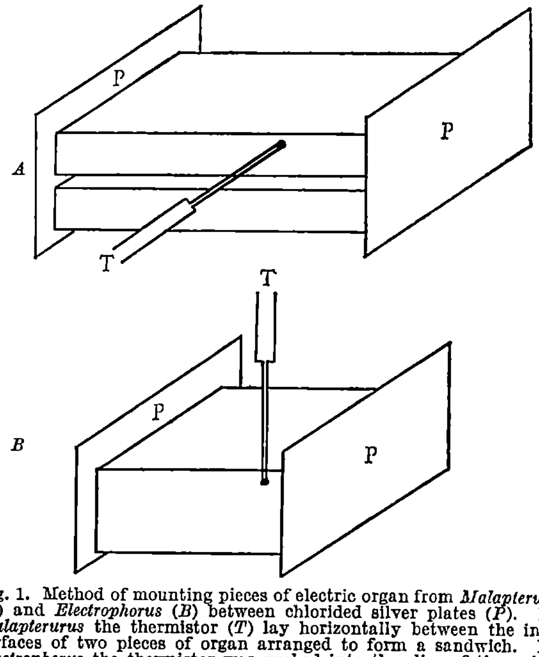

Fig. 1. Method of mounting pieces of electric organ from *Malapterurus* (*A*) and *Electrophorus* (*B*) between chlorided silver plates (*P*). For *Malapterurus* the thermistor (*T*) lay horizontally between the inner surfaces of two pieces of organ arranged to form a sandwich. For *Electrophorus* the thermistor was pushed into the slice of tissue in a direction parallel with the columns of electroplates through a nick in the the outer surface; the electroplates on either side of the thermistor were apt to be damaged in the process of insertion. When the electric discharge was generated, current flowed through the tissue between the silver plates, its size being governed by the value of the external resistive load connected across them

amplifier system as described by Hill[3], the output of which was displayed alongside the electric discharge on a Tektronix Model 555 double-beam oscilloscope and also on a high-speed (full-scale deflexion nominally 0·25 sec) Honeywell potentiometric recorder. The galvanometer response was speeded up by applying feed-back, and the overall time-constant for the thermistors and the galvanometer, tested with the thermistors inserted into uniform slices of agar jelly which were then abruptly warmed by a short pulse of current, was about 80 msec. The temperature coefficient of the resistance of each thermistor had been determined over a wide range of temperatures measured with a standard thermometer, and the records were calibrated by recording the deflexion corresponding to a known change in a standard decade resistance connected in parallel with a fixed resistance in one arm of the bridge.

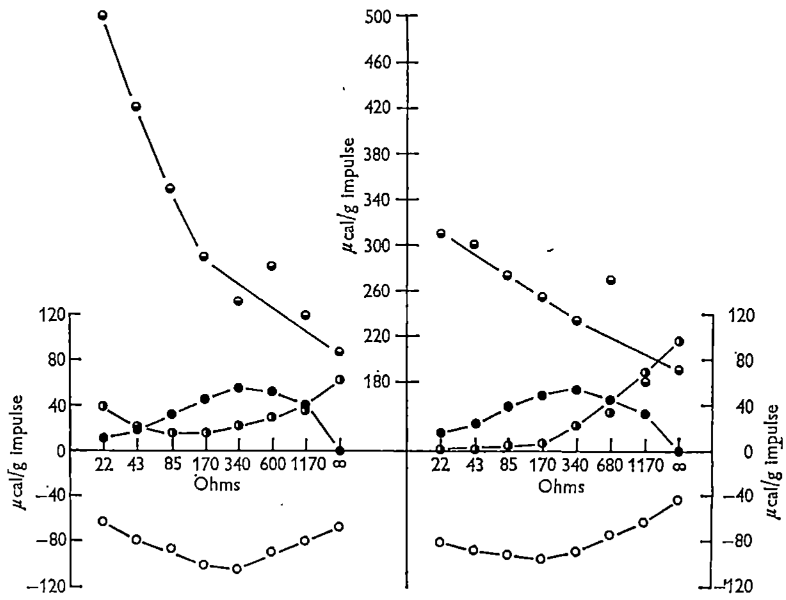

Fig. 3. Variation in the size of the thermal responses with external resistive load for two sandwiches of electric organ from a specimen of *Malapterurus*. ●, external electric work, measured with the microjoulemeter; ○, cooling phase Q_2; ◐, initial warming phase Q_1; ◒, recovery heat Q_3. Outer scales refer to work, Q_1 and Q_2; central scale is for Q_3. The measurements were made by reducing R_e in steps from infinity to 22 ohms, and then repeating the series in reverse order so as to be able to allow for fatigue by averaging the two values for each load. Recordings were made with 5 pulses at 10-msec intervals as for Fig. 2

Each sandwich or slice of electric tissue lay between two chlorided silver plates which were used for electrical stimulation (for *Electrophorus* and *Malapterurus*) and for application of the external resistive load. Trains of shocks of predetermined length (usually 5 pulses) and pulse interval (usually 10 msec) were generated by a programming device and applied to the tissue via a transistor switching circuit which was capable of delivering currents up to 1 amp without overloading during the shock, but the impedance of which as seen by the tissue at other times was very much higher than the selected loads. Corrections for the warming caused by the stimulus were sometimes necessary, but usually they were small; when working with *Torpedo* they could be avoided altogether by stimulating the nerve trunks rather than the tissue itself. Another important component of the apparatus was the joulemeter, which permitted direct measurements to be made of the external electric work performed during the discharge. This consisted of a resistance forming all or part of the load, which was wound round a small bundle of thermocouples connected to a galvanometer which registered their warming ballistically. It was calibrated by charging standard capacitances to known voltages and discharging them through the joulemeter. The joulemeter resistance was wound in sections switched so that its value could be changed by factors of 2 or 4; further variation of the load was achieved by connecting the joulemeter in series or in parallel with standard resistances.

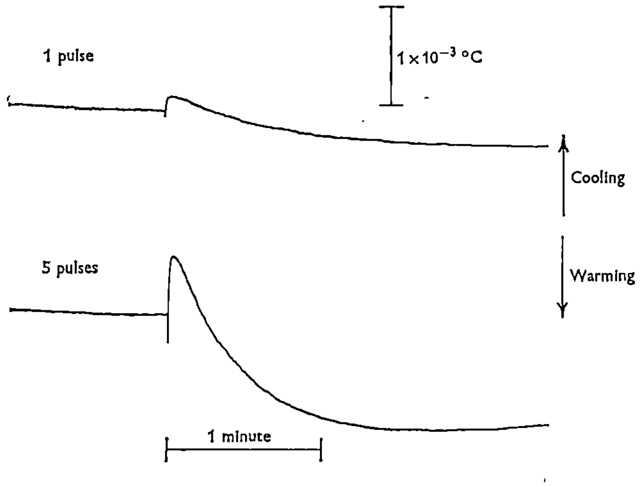

Fig. 2. Temperature changes in a sandwich of electric organ from *Malapterurus* after the application of 1 and 5 shocks. Shock strength was 16 V, duration 0·1 msec; even with 5 shocks, the warming due to the stimulus current alone was barely visible at this sensitivity. Temperature 19° C

Fig. 2 shows records of the temperature changes in a sandwich of tissue from *Malapterurus* in response to a single shock and to a train of five similar shocks spaced at 10-msec intervals. There was typically a very brief phase of warming which will be referred to as Q_1, then a more prolonged phase of cooling (Q_2), and finally a still more prolonged phase of warming (Q_3). For trains of up to 20 pulses there was a linear relationship between the peak amplitudes of Q_2 and Q_3 and the number of pulses, but since Q_3 was always the larger the summated value of Q_2 became relatively smaller for longer trains. Since each thermistor was placed in the thin layer of fluid and connective tissue separating the two pieces of electric organ which formed the sandwich, its most immediate surrounding was in effect a passive resistive element connected in parallel with the active elements (see Fig. 1*A*). The joule heat dissipated in such an element would be expected to be largest with the sandwich on open circuit ($R_e = \infty$), when the output voltage would be greatest. As may be seen in Fig. 3, the variation in size of Q_1 with R_e roughly fitted with what would be expected for a passive parallel element, and it hence seemed reasonable to interpret Q_1 as an artefact arising from the fact that the material nearest to the thermistor did constitute such an element. This interpretation was supported by the observation that in *Electrophorus*, where any damage to the electroplates while pushing the thermistors between them would give rise to a passive series element (see Fig. 1*B*), Q_1 was either absent (if such damage was slight) or only became conspicuous with low values of R_e. In *Torpedo*, where the electroplates are packed very closely together, it was difficult even with the smallest thermistors to avoid damaging the tissue so much that Q_1 became disconcertingly large; but in several of the most recent experiments records were obtained in which Q_1 was absent on open circuit.

The most interesting thermal event occurring at roughly the same time as the electric discharge was therefore the cooling phase Q_2. In the records shown in Fig. 2 it reached its peak with a time constant of about 250 msec; since the instrumental time-constant was only about 80 msec this might suggest that Q_2 actually took place slightly later than the electric discharge. However, the extra 170 msec might be accounted for by the presence of a passive layer of the order of 200µ thick on either side of the thermistor. Certainly there is a non-responding layer close to the thermistor, although this seems a rather high estimate for its depth. It is safest to conclude that Q_2 is either perfectly synchronous with the discharge or is not delayed after it by more than a fraction of a second. The average size of Q_2 on open circuit in *Malapterurus* at 20° C was 65 µ° C/impulse, and on loading the tissue electrically Q_2 increased to a maximum value of about 110 µ° C/impulse. The corresponding figures for *Electrophorus* at room temperature were 59 and 83 µ° C/impulse[4]. In *Torpedo* the open circuit and maximum values at 5° C were rather higher, 187 and 227 µ° C/impulse, but experiments on *Electrophorus* showed that Q_2 increased on cooling, so that all three species would have quantitatively similar values of Q_2 at room temperature.

When the size of Q_2 is plotted against R_e as in Fig. 3 and compared with the value of the external work performed at the same time, it is seen that in one respect Bernstein's prediction is fulfilled, since both Q_2 and the work attain a maximum at about the same value of R_e; in *Electrophorus* the maxima seemed to coincide less well, the value of R_e for greatest Q_2 being definitely smaller than for greatest work. The figures for the work fit very closely on to the theoretical curve for a constant-voltage generator with a fixed internal series resistance R_i, the condition for maximum power output being, of course, that $R_e = R_i$. However, it is clearly impossible to explain the results solely in terms of an adiabatic mixing process, since not only is there a substantial quantitative discrepancy between the maximum value of the cooling and the maximum external work when they are expressed in the same units (taking the specific heat of electric tissue as 0·87 cal/g), but also it is necessary to account for the appreciable cooling that takes place on open circuit, when no current flows in external circuits and the external work is zero. Our results seem best fitted by supposing that Q_2 is the sum of two components, one of which is fixed in size and is independent of load, and the other of which is the cooling predicted by Bernstein, equal and opposite to the external work. A possibility which can quickly be dismissed is that the open circuit cooling arises because there is always a leakage resistance round the edges of the slab of tissue which prevents true open circuit operation. It can readily be shown that such a resistance would have to be appreciably lower than the observed resting resistance of the whole tissue slice, and also very much smaller than the probable resistance of the thin inert layer of fluid outside the slice.

The Bernstein cooling arises, in effect, from the entropy change when work is done at the expense of the ionic concentration gradients. To the extent that the thermodynamic behaviour of the system is affected by changes in hydration of the ions with their concentration, a further heating or cooling of mixing would be anticipated, although this effect would be a second-order one. Unfortunately, the heat of mixing of Na^+ and K^+ is small and positive[5], and the only obvious way in which an extra cooling of mixing could be produced would be if the current flowing through the electroplates were carried by Na^+ ions entering the cells through one face (in *Electrophorus* the innervated posterior face[6], in *Malapterurus* the anterior face[7]) and by Cl^- ions entering through the opposite face rather than by K^+ ions leaving. The heat of dilution of sodium chloride, which is relatively large and is negative, might then be elicited in addition to the Bernstein cooling. However, the meagre electrophysiological evidence does not unequivocally support the idea that Cl^- ions may carry a large fraction of the current, and, in any event, such a mechanism would not explain the occurrence of open circuit cooling, when the only ionic movements are those necessary to alter the potential across the active face of the electroplate, which are almost certainly appreciably smaller than those that take place when large currents are drawn.

Another possible source of cooling would be an absorption of heat at the faces of the electroplates analogous with the Peltier effect. There seem to be two good reasons for rejecting this suggestion. In the first place, Peltier cooling during current flow only takes place at every other junction in a sequence of dissimilar conductors, there being an equal production of heat at alternate junctions, so that apart from joule heat the average temperature change over an even number of junctions is zero. Since the thermistors were large compared to the spacing of the electroplates, the temperature changes observed were certainly the average of those occurring at the opposite faces of each electroplate, and any Peltier cooling at one face would have been balanced by the corresponding warming at the other. Even if it were argued that in the electric organ the balance may not be exact because the current is carried by different ions through alternate faces, the Peltier analogy has to meet the further objection that any extra cooling attributed to it would be least on open circuit and would increase in proportion to the current drawn, instead of being independent of external load. The same objection applies to any explanation for the extra cooling which would make it vary with the total current flow, such as a hypothetical coupling of the ion transfer through the cell membranes to an endothermic chemical reaction. Thus, although some contribution from an effect similar to Peltier cooling is not excluded, the analogy seems unable to provide a complete explanation for the excess cooling.

A plausible further source of cooling which might meet the requirement of not varying greatly with external load would be the occurrence as an integral part of the permeability change in the active membranes of a process involving the melting or splitting of a membrane component. It would also be necessary to suppose that the restoration of the quiescent condition of the membrane at the end of the action potential did not simply take place through a direct reversal of the melting process, since, if it did, the net temperature change after each complete functional cycle of the permeability mechanism would merely be zero. This further condition means that the electroplate would have to have a reservoir of the component to be melted or split which was not instantaneously replenished after each impulse. The idea that the cell or the membrane contains a supply of a substance which is obligatorily consumed during electrical activity is, of course, contrary to recent evidence on the perfused squid axon[8], which is capable of conducting hundreds of thousands of impulses after replacement of virtually all its cytoplasm by a potassium sulphate solution, and in which the permeability mechanism seems likely to involve a completely reversible reorientation of charged dipoles within the membrane rather than the kind of process envisaged above for the electric organ. But the permeability mechanisms need not be assumed to be identical in these very different tissues. It may also be objected that the operation of the permeability mechanisms would be unlikely to have the same thermal consequences in all three types of electric fish considered here, since the active membrane in *Torpedo* is apparently strictly cholinergic and so exclusively chemically excitable[9], whereas *Malapterurus* is almost as exclusively electrically excitable[7], with *Electrophorus* in an intermediate position. Nevertheless, the open circuit cooling is roughly the same in each case. However, in the absence of any evidence as to the precise difference at the molecular level between chemical and electrical excitability, this argument is still not a decisive one. The suggestion that there may be some form of depolymerization in the membrane during the action potential has the further attraction that this might lead to osmotic effects which may be related to the changes in light scattering observed by Aubert, Chance and Keynes[10]. It is hoped that a more detailed comparison of the time-courses of the early thermal and optical effects will throw more light on this question.

Finally, the origin of the terminal phase of warming, Q_3, may be considered briefly. In *Electrophorus*[10] its time course is similar, both at room temperature and at 7° C, to that of the easily observed reduction and subsequent restoration of the size of the action potential which has been attributed to changes in the ionic concentrations within the electroplates, and its extent is roughly consistent with quantitative analyses of the breakdown of creatine phosphate[10,11]. Although similar evidence has not yet been obtained for *Malapterurus* and *Torpedo*, it is reasonable to assume that Q_3 arises from the operation of an ionic pump, driven by phosphate-bond energy, which restores the ionic concentration gradients the running down of which constitutes the immediate source of energy for the electric discharge. A still later recovery process, the activation of glycogenolysis to restore the phosphate potential, has been investigated in *Electrophorus* by optical[10,12] and chemical methods[11,13]. It takes place too slowly for any accompanying temperature changes to be readily separable from the dissipation of heat into the apparatus which inevitably follows its release as Q_3. As might be expected, the size of Q_3 increases steadily as R_e is reduced (Fig. 3) and as the downhill movement of ions in each impulse is correspondingly increased; this effect is more marked in *Malapterurus* than it sometimes is in *Electrophorus*.

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RECIPROCAL ACTIVATION AND INHIBITION OF CORTICAL NEURONES AND VOLUNTARY MOVEMENTS IN MAN: CORTICAL CELL ACTIVITY AND MUSCLE MOVEMENT

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IN 1906, Sherrington[1] introduced a theory of reciprocal innervation on the basis that simultaneous excitation and inhibition of the agonistic and antagonistic musculatures are required for execution of smooth phasic movements. Particularly in view of recent observations on reciprocal excitation and inhibition of unit discharges recorded with microelectrodes from the retina[2,3] and receptive fields of the cerebral cortex[4-6], this theory may also be applied to the integrative action of the sensory system.

The reciprocal behaviour of units in the receptive fields—the visual cortex and somatosensory cortex—has been extensively examined, but there are relatively few reports of similar investigations on the motor cortex. It is known that, under certain experimental conditions, inhibition of neuronal activity in the motor cortex occurs[7,8]; as yet, this phenomenon has not been investigated in relation to phasic movements.

Cordeau *et al.*[9], in Jasper's laboratory, produced 'postural' tremors in monkeys. By recording with microelectrodes from the sensorimotor cortex, these authors found that some neurones discharged synchronously with the rhythmic tremor movements. Since these movements were involuntary and comparable to passive movements, questions were raised. Could the cortical unit discharges be the result of afferent stimulation? Did they represent the central pace-maker for tremor movements? It has been shown that passive movements of a contralateral limb evoke spike discharges from the somatosensory cortex[5] and that stimulation of a contralateral peripheral sensory nerve can elicit a response from cortical neurones which also respond to stimulation of the pyramid in the medulla[8]. These findings were obtained in experiments on animals and support the argument that the alternating cortical discharges recorded by Cordeau from tremoring monkeys may have represented responses to stimulation of peripheral sense organs.

This article concerns cortical unit activity related to voluntary contraction and relaxation in the fingers of patients operated on for epilepsy or Parkinson's disease. The selection of patients and the method of recording are being reported elsewhere[10]. In brief, the micromanipulator, microelectrode carrier, and pre-amplifier were mounted on a ring which was, in turn, attached to the skull over a trephine opening. The assembled equipment weighed 30 g and was 1·5 cm in diameter and 13·5 cm in height. Before use, it was sterilized at 140° F for 4 h in a gas chamber. The recording was carried out under closed-skull conditions which not only preserved the physiological state of the cortex but also prevented respiratory and pulsatory movements of the brain[11]. This made it possible to maintain the recording microelectrode in one position in reference to the cortical cell for a long period of time. During recording, the patient was under local anæsthesia and was able to converse with the attending physician.

When the microelectrode was placed in the precentral cortex and the unit discharges were recorded, the discharge-frequency was often altered by contraction and relaxation of the patient's contralateral fingers. It soon became apparent that when the patient closed his hand, an increase in activity of some cortical units with a simultaneous decrease in activity of other units occurred. At the same time, the discharge pattern of some other units remained unchanged. This was best illustrated in the case described here.

The patient was a sixty-year-old housewife suffering from severe hypokinesia, rigidity and fine tremors, all of

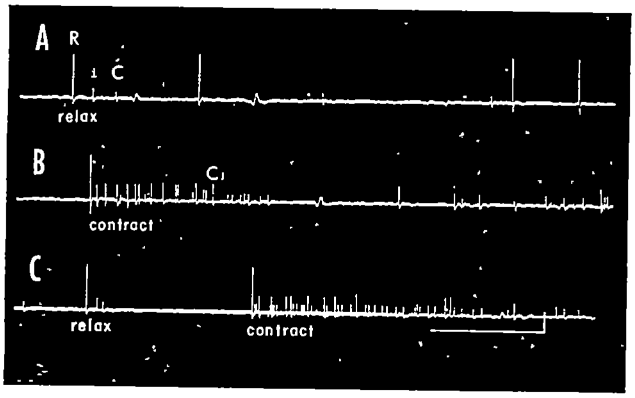

Fig. 1. Microelectrode recording from left precentral cortex of a conscious patient who intermittently relaxed and closed her right hand. The extracellular electrode recorded three units (*R*, *i* and *C*) in *A* and another (C_1) in *B* and *C*. *A*, *B* and *C* are continuous records. Upward deflexion represents positivity. Voltage calibration, 2 mV; time, 0·5 sec

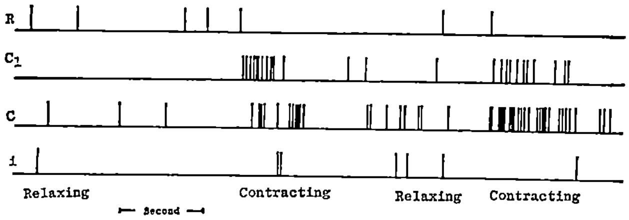

Fig. 2. The time relationship of the spike discharges from units *R*, *C*, C_1 and *i* during relaxation and contraction of the right fingers

which were most marked in the right upper extremity. Thalamotomy was carried out under local anæsthesia. Immediately following insertion of an electrode, presumably into the ventrolateral nucleus of the left thalamus, the tremor movements in the right hand ceased, However, there was still some rigidity in the arm. Microelectrode recordings from the left precentral gyrus showed that when the hand was closed, a burst of spike discharges occurred. When the right hand opened and closed in rapid succession, the recording shown in Fig. 1 was obtained.

Relaxation of the hand gave the tracing in *A* of Fig. 1. The amplitude of extracellular unit spike potentials varies with the distance between the recording electrode and the unit[11]. In this case, the extracellular microelectrode recorded spontaneous spike potentials of three different amplitudes; *R*, *i* and *C* were 3·1 mV, 1·0 mV and 0·66 mV, respectively. Thus, the extracellular electrode was recording spike activity from three units. It can be seen that during relaxation of the hand, the *R*-unit fired with an interspike interval ranging from 0·57 to 1·35 sec. The interspike interval of the *C*-unit potentials ranged from 0·7 to 0·9 sec. When the hand was closed (Fig. 1*B*), the *R*-unit stopped firing. Simultaneously, the *C*-unit discharged more rapidly, and the discharges occurred in bursts. In addition, a train of spike potentials was recorded from another unit (C_1-unit) which had been inactive during relaxation of the hand. When the hand was again relaxed, the previous activity pattern of the *C*-unit gradually resumed, the bursting discharges of the C_1-unit rapidly subsided and spike potentials of the *R*-unit reappeared. During this entire period, no significant change in the discharge activity of the *i*-unit was recorded. This unit was indifferent to movement of the fingers.

The discharge patterns of these four units (*R*, *C*, C_1 and *i*) are traced in Fig. 2. Since the number of cells under investigation was relatively small as compared with the cell population of the precentral cortex, no statistical treatment of the results in this investigation is possible. Nevertheless, the results clearly demonstrated that different cells in the precentral cortex behaved differently in connexion with voluntary movements. These movements were consciously motivated and were not involuntary. Therefore, it is proposed that reciprocal activation and inhibition of certain neurones in the motor cortex are necessary for contraction and relaxation of muscles in the execution of phasic movements. This is in keeping with Sherrington's theory of reciprocal innervation of agonistic and antagonistic musculatures.

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EFFECT OF ŒSTRADIOL ON BONE MARROW TRANSPLANTATION IN LETHALLY IRRADIATED MICE

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SEVERAL investigations have confirmed the observation by Treadwell *et al.*[1] that œstrogenic hormones afford some degree of radio-protection to sub-lethally irradiated mice. There is general agreement that either a single large injection or a 10-day course of œstrogen pretreatment offers maximal benefit. Mirand *et al.*[2] report some protection when œstrogen is instituted after X-irradiation, although other investigations[3,4] have demonstrated an increased mortality when hormone is administered during this period. It is well known that pharmacological amounts of œstrogens usually induce a progressive hæmatopoietic depression in dogs[5], but their influence on intact mice is much less striking[6,7]. Despite considerable examination, the mechanism of this hormonal phenomenon as well as that of œstrogen action on cellular elements of the hæmatopoietic and lymphoid organs remains unresolved[8,9].

Lethal irradiation followed by inoculation of bone marrow cells provide a means for investigating, in semi-quantitative circumstances, the influence of œstrogenic hormones on the proliferation and function of both hæmatopoietic and lymphoid cells. The purpose of this article is to describe the effects of pharmacological

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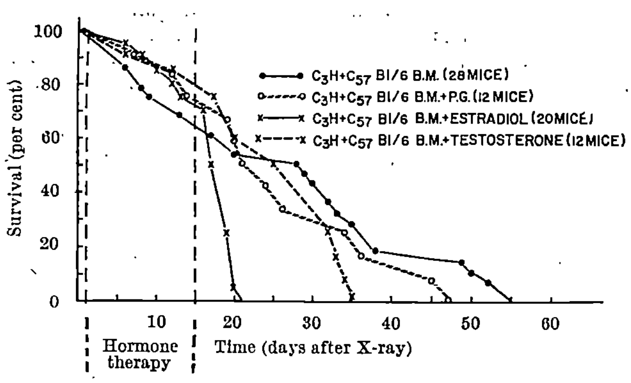

Fig. 1. The effect of œstradiol on the survival of lethally irradiated *C3H* mice injected with allogeneic *C57Bl*/6 bone marrow (*B.M.*)

amounts of œstradiol, administered to lethally irradiated mice receiving both syngeneic and allogeneic bone marrow and, in a separate set of experiments, administered to adult mice which served as bone marrow donors to lethally irradiated allogeneic hosts.

Twelve to sixteen week old *C3H/Anf* and *C57Bl*/6 female mice were used both as donor and host where indicated throughout the investigation. These mice were purchased from the Cumberland View Farms, Oak Ridge, Tennessee, and maintained on the 'Rockland Mouse Diet' and water *ad libitum*. The methods of irradiation, housing and feeding of animals were identical to those followed in previous investigations[10]. Irradiated mice received 900 r., in air X-irradiation, by means of a G. E. Maxitron-250 X-ray machine. Within 4 h of lethal irradiation 10 × 10^6 bone marrow cells suspended in Tyrode's solution were injected intravenously. Œstradiol and testosterone were dissolved in 10 per cent NNDMA (*N*,*N*-dimethylacetamide; Eastman Organic Chemicals, Eastman Kodak Corporation, Rochester, New York)[5] propylene glycol. A 0·1-ml. volume of solution containing 0·5 mg of hormone or the control vehicle was injected subcutaneously three times a week during the indicated treatment schedules. In those experiments in which the host was treated following lethal irradiation, the steroid and control injections were begun on the day after irradiation and bone marrow transplantation. These were continued for 2 weeks, during which time a total of seven injections was given. In the experiments in which bone marrow donor mice were treated, either three injections were given in 1 week or twelve injections were administered in a 4-week period. A daily record of animal death was maintained and expressed as percentage survival on any given day following radiation.

Fig. 1 indicates that œstradiol shortens the survival of lethally irradiated *C3H/Anf* mice given allogeneic *C57Bl*/6 bone marrow. Whereas this combination normally leads to 100 per cent mortality in 55 days because of a severe, progressive secondary disease syndrome, œstradiol treatment resulted in 100 per cent mortality by the twenty-first day after irradiation. Testosterone and control vehicle solutions appeared to have no effect on survival comparable to that seen with œstradiol.

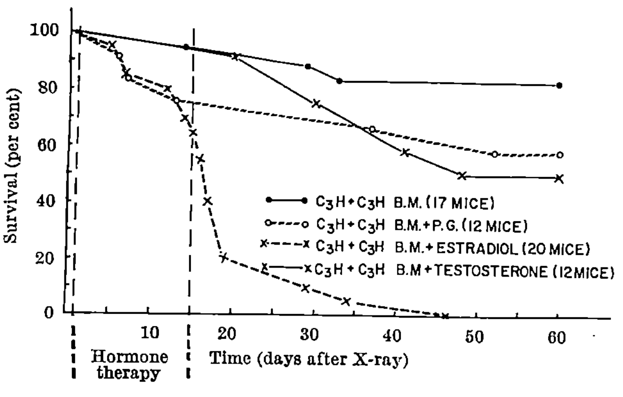

Fig. 2. The effect of sex hormones on the survival of lethally irradiated *C3H* mice injected with syngeneic *C3H* bone marrow (*B.M.*)

Fig. 2 reveals this pattern of accelerated death even more clearly in *C3H/Anf* mice given syngeneic *C3H/Anf* bone marrow. Within the 60-day period of observation only three of seventeen control animals which received neither hormone nor control solution injections died. Of those animals treated with œstradiol, 80 per cent had died by the nineteenth day and 100 per cent were dead by the forty-fifth day after irradiation and bone marrow transplantation. Testosterone and the control solution injections may have induced a modest reduction in survival, but the effect was not comparable to that of œstradiol.

Fig. 3 demonstrates the accelerated mortality of lethally irradiated *C3H/Anf* mice when donor bone marrow was obtained from *C57Bl*/6 mice which had been treated with œstradiol for 1 or 4 weeks. In this experiment, less than 50 per cent of the transplanted hosts were living 10 days after lethal irradiation and all were dead by 28 days in both groups.

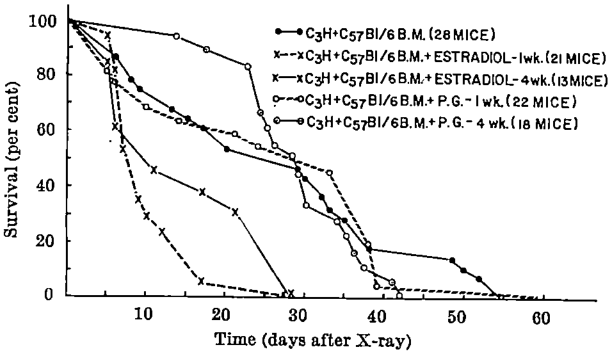

Fig. 3. The survival of lethally irradiated *C3H* mice transplanted with œstradiol-treated allogeneic *C57Bl*/6 bone marrow (*B.M.*); 1 and 4 weeks were time intervals of donor treatment with œstradiol or propylene glycol (*P.G.*)

Œstrogens regularly induce thymic involution, but their effect on other lymphoid organs is variable[9]. Although œstrogens may augment circulating antibody response to certain antigens[11], the results of Lurie[12] and more recently of Kappas *et al.*[13] indicate that these hormones also have a depressant effect on such manifestations of delayed hypersensitivity as cutaneous reactions to thyroglobulin and tuberculoprotein and the incidence of adjuvant arthritis in rats, a disorder shown by Waksman *et al.* to be of the delayed type[14]. These observations led us to consider the possibility that œstrogenic hormones might differentially affect various compartments of the lymphoid system.

Secondary or homologous disease, the wasting syndrome of delayed death following successful transplantation of genetically foreign bone marrow into lethally irradiated hosts[5], is thought to be an expression of immunologically competent cells. If œstrogens depressed the number or function of these cells, the survival of the mice injected with allogeneic bone marrow might be improved. The fact that œstradiol had a distinctly harmful effect when administered to lethally irradiated *C3H/Anf* mice given *C57Bl*/6 bone marrow and that the bone marrow transplant from œstradiol-treated allogeneic donors resulted in accelerated death of irradiated hosts tentatively discounts the selective suppression of these immunologically competent cells.

These experiments revealed an early pattern of death compatible with a primary failure of the transplanted cells to establish a successful graft when exposed to œstradiol before or after transplantation. Hæmatocrit and total leucocyte counts obtained 10, 15, 24 and 34 days after irradiation indicate that those mice treated with œstradiol remain leucopenic until death, whereas mice treated with testosterone or control injections showed a substantial rise of leucocytes by the fifteenth day. Since anæmia did not develop in any group, the observations suggest that under these experimental conditions œstradiol destroys leucocytes or their precursors and/or impairs the proliferation of leucocytic stem cells. Our results do not indicate whether this is a direct action on these cells or is mediated by an effect on other organ systems[9].

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AMINO-ACID SEQUENCE AROUND THE REACTIVE THIOL GROUPS OF ADENOSINE TRIPHOSPHATE–CREATINE PHOSPHOTRANSFERASE

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THE phosphotransferases are a very important group of enzymes found in most, if not all, living cells. They catalyse the reversible transfer of 'high-energy' phosphoryl groups from a nucleoside triphosphate, usually ATP, to a substrate and have an absolute requirement for divalent metal ions[1], usually Mg^{2+} or Mn^{2+}. In addition, many of these enzymes require unmodified thiol groups for activity[2–5]. It is becoming increasingly likely that their reaction mechanisms are similar and that generally applicable principles are likely to emerge from an examination of individual enzymes of this group[6].

So far, very little is known of their chemical structure. This communication describes investigations into the chemical environment of the essential thiol groups of one of these enzymes, ATP–creatine phosphotransferase (CPT).

Creatine phosphotransferase catalyses the reaction:

$$\text{creatine} + \text{ATP}^{4-} \overset{\text{Mg}^{++}}{\rightleftharpoons} \text{creatine phosphate}^{2-} + \text{ADP}^{3-} + \text{H}^{+}.$$

The enzyme has been highly purified and crystallized[7], and has a molecular weight of 81,000 (ref. 8). It contains 6–7 free thiol groups/mole[9–11], two of which react rapidly and irreversibly with alkylating agents such as iodoacetate (and iodoacetamide)[2,3]. Loss of enzymatic activity can be exactly correlated with alkylation of these two thiol groups[2,3], both of which react at precisely the same rate[3]. The remaining thiol groups react only after denaturation. The enzyme is protected against iodoacetic acid-inhibition by its substrate equilibrium mixture[3,12]. These and other data strongly suggest that the reactive thiol groups are directly involved in catalysis, and although not involved in binding of substrates are close to the substrate binding sites[3,12–14].

In the present investigation we have used ^{14}C-iodoacetate to label the reactive thiol groups of CPT, thus enabling the peptides derived from these parts of the enzyme to be characterized (see also refs. 15 and 16). The presence of the radioactive label proved invaluable in the subsequent isolation of the peptides and also in the determination of their structure.

Labelling of enzyme. CPT was purified to the pre-crystallization stage by method *B* of Kuby *et al.*, and had a specific activity of 70–80 units/mg[7]. The absence of contaminating peptides was shown by treatment with FDNB (fluorodinitrobenzene)[17]. The enzyme contained 6–7 free thiol groups/mole, as determined both by conversion to *S*-carboxymethyl-cysteine (CMCys)[18] and by reaction with DTNB (dithio*bis*(nitrobenzoic acid))[31]. In the absence of urea, 1·5–2·0 thiol groups reacted with iodoacetic acid under the conditions later used for labelling. Labelling of the enzyme with 2-^{14}C-iodoacetic acid was carried out as follows[19]: CPT ~ 50 mg/ml.; 2-^{14}C-iodoacetic acid, 2 moles/mole CPT; *p*H 9·0 (0·1 M glycine): temperature, 30° C. After 40 min, when negligible enzymatic activity remained, solid urea was added to bring the solution to 8 M, and the enzyme was further alkylated with inactive iodoacetate (4 times molar excess over thiol groups). This avoided oxidation of any remaining thiol groups, and prevented possible disulphide exchange reactions during purification. After a further 3 h at 30° C, the reaction was terminated with mercaptoethanol, and low molecular weight compounds were removed by exhaustive dialysis.

Purification of tryptic peptides. The fully alkylated CPT was then digested with trypsin (2 per cent w/w), in a *p*H-stat at 30° C for 1 h. (The trypsin had been predigested in N/16 hydrochloric acid to minimize chymotryptic activity[20].)

The tryptic peptides were purified by ion-exchange chromatography successively on columns of 'Dowex-50–X2' and 'Dowex-1–X2' at 37° C. The gradients and conditions were essentially those of Konigsberg and Hill[21] for 'Dowex-50', and of Schroeder *et al.*[22] for 'Dowex-1'. The column eluates were monitored continuously for radioactivity with a scintillation plastic flow cell (Nuclear Enterprises, Edinburgh), and the collected fractions with ninhydrin using a modified auto-analyser technique. Typical chromatograms are given in Figs. 1 and 2; the latter shows the purification on 'Dowex-1' of the major radioactive peptide from the 'Dowex-50' run. Buffer salts were then removed by passage through a column of 'Sephadex *G*-25'. In different preparations *T*5 accounted for 45–50 per cent of the protein-bound radioactivity, *T*1 for ~ 10 per cent and *T*3 for ~ 9 per cent. (See Fig. 1 for numbering of peptides.)

Structure of T5. Hydrolysis (105° C, 6·5 N hydrochloric acid 18 h) and quantitative amino analysis gave the following composition for *T*5 (the assumed residues/mole are given in parentheses):

S-Carboxymethyl-cysteine	0·71 (1)	Proline	1·75 (2)	Leucine	3·80 (4)
Aspartic acid	2·14 (2)	Glycine	4·27 (4)	Tyrosine	1·00 (1)
Threonine	1·86 (2)	Alanine	1·19 (1)	Phenylalanine	1·06 (1)
Serine	1·03 (1)	Valine	0·88 (1)	Histidine	2·16 (2)
Glutamic acid	1·14 (1)	Methionine	0·97 (1)	Arginine	1·05 (1)

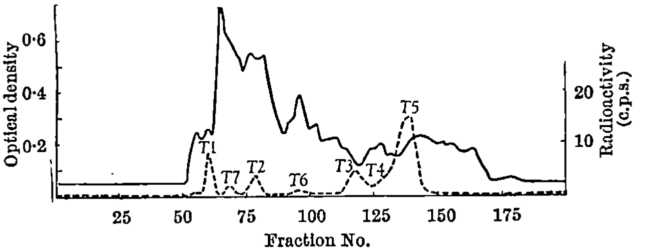


Fig. 1. Chromatogram of a tryptic digest of carboxymethylated-CPT on a column of 'Dowex-50-*X*2' (100 cm × 2 cm) with pyridine-formate and -acetate buffers. ——, Ninhydrin colour; - - -, radioactivity

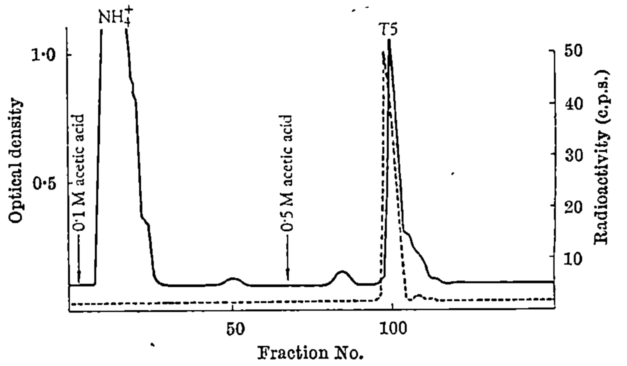


Fig. 2. Purification of *T*5 (Fig. 1) on a column of 'Dowex-1-*X*2' (150 cm × 0·9 cm) with α-picoline-acetate buffers. ——, Ninhydrin colour; - - -, radioactivity

End-group analysis by the Edman technique[21] indicated that the peptide contained only minor contaminants and showed alanine to be N-terminal. Also, virtually all the radioactivity was present as *S*-carboxymethyl-cysteine. To obtain smaller peptides more amenable to sequence analysis, *T*5 was digested with chymotrypsin (0·001 per cent, 1 h, 25° C) and the resulting peptides were separated on 'Dowex-50-*X*2' (50 × 1 cm) (Fig. 3). Peptide *C*1, which contained virtually all the radioactivity of *T*5, was then digested with pepsin (0·1 per cent, 16 h, 25° C) and separation of peptides was again carried out on 'Dowex-50-*X*2' (Fig. 4). Where necessary, further purification of peptides was carried out by electrophoresis at *p*H 2·0, 60 V/cm, 1 h.

The structures of the various peptides thus isolated were determined mainly by quantitative subtractive Edman degradation[21] and extensive use was also made of digestion with carboxypeptidase *A* and leucine aminopeptidase. The Edman technique was modified in that the cleaved peptides were purified, usually after every second step, by electrophoresis at *p*H 2·0, instead of by the column chromatographic method of Konigsberg and Hill[21]. Serine, glutamine and asparagine were distinguished by semi-quantitative electrophoresis at *p*H 2·0 since these amino-acids occupy the same position on the amino-acid analyser.

The data on which the structure of *T*5 is based are given in Table 1. It will be noted that the sequence of *C*4 has not yet been completed. Of the peptic peptides only *P*6 (yield ~ 15 per cent) is not consistent with the structure of *C*1, since on hydrolysis with acid it yields 1 mole of valine and 2 of leucine, and is not a mixture of valyl-leucine + leucine. Nevertheless, digestion of *C*1, from which it is derived, with leucine amino-peptidase yields 1 mole each of valine, leucine and threonine/mole *C*1. These points are still under investigation. (It should be noted that peak *C*2 (Fig. 3) consisted mainly of ammonium salts, together with a small amount of oxidized *C*1.)

The tryptic peptides *T*1 and *T*3 (Fig. 1) were also purified on 'Dowex-1-*X*2' as for *T*5. Amino-acid analysis of hydrolysates of these peptides, and end-group assays, indicated that *T*1 was probably *C*1 and *T*3 was probably *C*4 + *C*1, that is, they were derived by chromotryptic splitting of *T*5. This is not surprising in view of the susceptibility of the phenylalanine–methionine, and tyrosine–valine bonds to chymotrypsin already noted (Table 1).

The yield of peptides *T*5 (~ 47 per cent) together with its derivatives *T*1 (~ 10 per cent) and *T*3 (~ 9 per cent), a total of ~ 66 per cent of the protein-bound carbon-14 (actually ~ 80 per cent of the carbon-14 recovered as peptides), indicates that the chemical environment of the two essential thiols in CPT is identical. This strongly supports the view, based on kinetic evidence[3] and on substrate-binding experiments[23], that the enzyme contains two identical catalytic sites/mole each with one thiol group.

The enzyme contains eight half-cystine residues/mole and between 6 and 7 free thiols/mole[10,11]. Is the molecule then a dimer joined by a disulphide bond ? The available evidence suggests that it is not, since:

(1) Reduction followed by alkylation with iodoacetate yields 6·7–7 moles *S*-carboxymethyl-cysteine/mole CPT[10,11]. It cannot be excluded, however, that CPT contains a disulphide bond peculiarly resistant to reduction.

(2) No difference in molecular weight between native and reduced, fully alkylated CPT is detectable on 'Sephadex *G*-75' columns[11].

(3) Treatment with carboxypeptidase *A* indicates only one polypeptide chain in CPT[24]. (Digestion with carboxypeptidase *B* has yet to be carried out.)

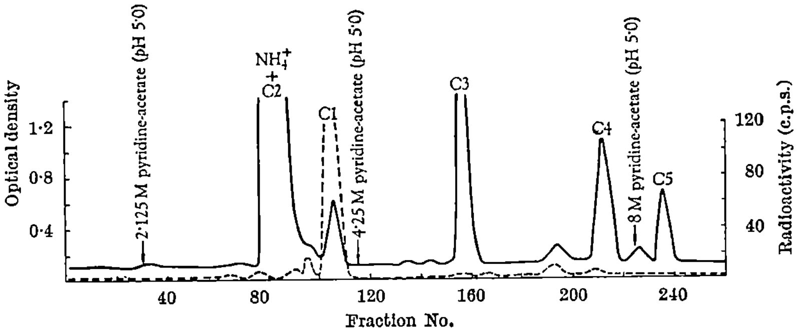


Fig. 3. Chromatogram of a chymotryptic digest of *T*5 on 'Dowex-50-*X*2' (150 cm × 1 cm) with pyridine-acetate buffers. ——, Ninhydrin colour; - - -, radioactivity

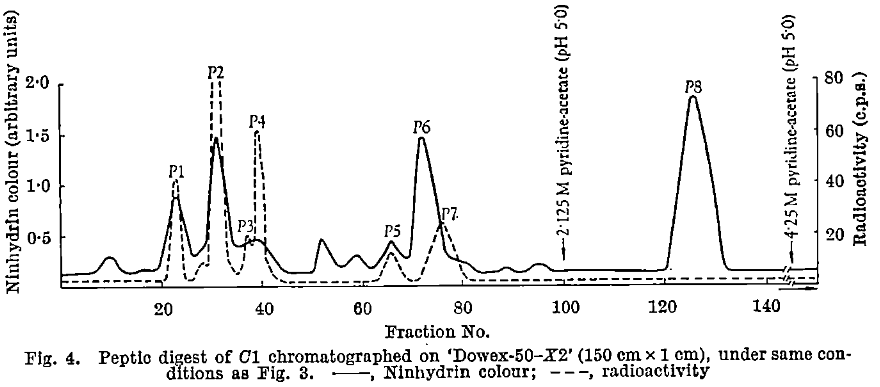
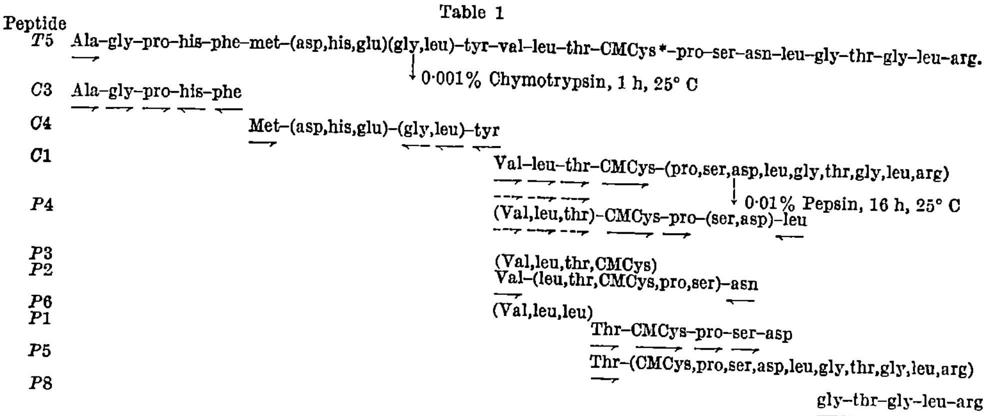


Fig. 4. Peptic digest of *C*1 chromatographed on 'Dowex-50-*X*2' (150 cm × 1 cm), under same conditions as Fig. 3. ——, Ninhydrin colour; - - -, radioactivity

Table 1


Peptide
T5 Ala–gly–pro–his–phe–met–(asp,his,glu)(gly,leu)–tyr–val–leu–thr–CMCys*–pro–ser–asn–leu–gly–thr–gly–leu–arg.
0·001% Chymotrypsin, 1 h, 25° C
C3 Ala–gly–pro–his–phe
C4 Met–(asp,his,glu)–(gly,leu)–tyr
C1 Val–leu–thr–CMCys–(pro,ser,asp,leu,gly,thr,gly,leu,arg)
0·01% Pepsin, 16 h, 25° C
P4 (Val,leu,thr)–CMCys–pro–(ser,asp)–leu
P3 (Val,leu,thr,CMCys)
P2 Val–(leu,thr,CMCys,pro,ser)–asn
P6 (Val,leu,leu)
P1 Thr–CMCys–pro–ser–asp
P5 Thr–(CMCys,pro,ser,asp,leu,gly,thr,gly,leu,arg)
P8 gly–thr–gly–leu–arg

*CMCys, *S*-carboxymethyl-cysteine; ⇀, Edman degradation; --⇀, Leucine amino-peptidase; ↽, Carboxypeptidase *A*.

On the other hand, peptide mapping of trypsin hydrolysates of the enzyme shows only about half the expected number of ninhydrin-positive spots. It seems highly probable, therefore, that CPT contains two identical active sites per molecule each with one thiol group. It is unlikely, however, on the present evidence, to consist of two polypeptide chains linked by a disulphide bridge, although the molecule appears to be in the form of two parts of essentially similar composition. Possible explanations of the data are that CPT is a cyclic molecule with a protruding C-terminal chain (for example, ovalbumin[25]), or alternatively is a dimer linked by a peptide or other bond. Further work is obviously required to resolve this point.

The most notable feature of *T*5, the peptide containing the essential thiol groups of CPT, is the presence of proline C-terminal to these thiols. This is the first report of such a sequence in a 'sulphydryl' enzyme and it seems reasonable to assume that, since this amino-acid is a 'misfit' in the protein α-helix, its occurrence in this position is of considerable significance. Whether its role goes beyond that of conferring a particular configuration at this point in the polypeptide chain cannot be decided at this stage.

One of the characteristics of CPT and several other 'sulphydryl' enzymes (for example, arginine kinase[5], yeast alcohol dehydrogenase[26] and phosphoglucomutase[27]) is the unexpected finding that the reactivity of their essential thiol groups to iodoacetate does not vary between *p*H 6 and 10. This has led to the suggestion that these thiol groups are hydrogen bonded to basic groups on the protein, probably imidazole (cf. ficin[28]), and, on this basis, Rabin and co-workers have proposed reaction mechanisms for CPT[12] and yeast alcohol dehydrogenase[26]. An obligatory condition for this mechanism to operate is the presence of histidine adjacent to the reactive thiol group at least in the three-dimensional structure of the protein. Evidence in support of this mechanism has come from the recent isolation by Harris of a peptide containing the 'active centre' thiol groups of yeast alcohol dehydrogenase[29]. The fact that this peptide contains the sequence –val–cys–his– is therefore very significant, and the dissimilarity between this sequence and that of the corresponding one in glyceraldehyde-3-phosphate dehydrogenase (GPDH)[16], namely:

–ser–cys–thr–thr–asn (asparagine)–

which might be expected to be similar (cf. proteinase and esterases[30]) is of great interest. The effect of *p*H on the reactivity of the thiols in GPDH to iodoacetate has not yet been investigated.

In *T*5, histidine is not adjacent to the reactive thiol groups. The absence of such an obvious association in *T*5 does not, however, preclude its existence in CPT, since folding of the polypeptide chain of the enzyme (which contains 33 histidines) might well lead to the formation of such a thiol–imidazole pair.

Whether there is any evolutionary significance in the similarity between the sequences round the reactive thiols in GPDH (see foregoing) and in CPT:

–thr–cys–pro–ser–asn–

both obtained from rabbit muscle, cannot yet be decided.

In view of the similarities in the properties of the phosphotransferases, comparative investigations are being carried out on other enzymes of this group, together with more detailed investigations on creatine phosphotransferase.

Full details of the work briefly described here will be published elsewhere.

We thank Mr. S. G. Milsom, Mrs. E. W. Tripp and Mr. J. F. Laws for their assistance.

Note added in proof. With the use of carboxypeptidases *B* and *A*, it has been confirmed that the C-terminal sequences in *T*5, *T*1 and *T*3 are identical, namely, -gly-leu-arg.

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SEGREGATION OF RADIATION-INDUCED MUTATIONS IN *Escherichia coli*

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IT is now accepted[1] that the genetic material of bacteria is composed of two main sub-units or strands each of which is conserved during replication. Some years ago Witkin[2] reported that with rare exceptions a single nucleus of *Escherichia coli* contained a single unit of the *lac* gene mutable by ultra-violet light, that is, all progeny of a mutated nucleus carried the mutant gene. In a small proportion of cases, however, segregation of both mutant and non-mutant progeny from a single nucleus was observed. This could not at the time be satisfactorily explained. Recent advances in our knowledge of the structure and replication of the bacterial chromosome[1] provide two possible explanations. The wave of replication might have already passed the *lac* gene at the time of irradiation in those nuclei or alternatively mutations might have been induced in a single strand of the chromosome. It is conceivable that all the mutations yielding pure mutant progeny were in fact induced on only one strand of the chromosome. If, for example, the lethal and mutagenic action of ultra-violet light occurred independently in single strands of genetic material, then with only $\sim 0·1$ per cent of cells surviving, the probability that a mutated strand would be accompanied by a viable sister strand would also be $\sim$ 0·1 per cent. Thus in most cases colonies of purely mutant progeny should have appeared, and this Witkin found.

In some recent experiments with ultra-violet light[3], phosphorus-32 (ref. 4), and nitrous acid[5], massive doses of mutagen have also been used with attendant high lethality and consequent ambiguity in interpretation. In our experiments with the tryptophan requiring (try$^-$) strain *WP*2 of *Escherichia coli** we have been able to follow the segregation of prototrophic revertants induced by relatively low doses of X- or γ-rays which resulted in more than 80 per cent of the bacteria surviving. Two factors which made these experiments feasible were the ease of detection of mutants and the low spontaneous reversion rate in this strain, approximately 10^{-10} per generation time[6]. Preliminary results with this system at 22° have been reported elsewhere[7]. The experiments recorded here indicate that the number of mutable units per nucleus, derived from the average numbers of mutable units and of nuclei per bacterium, depends on the temperature.

Nuclear-stained preparations of bacteria at the time of irradiation were prepared by the method of Robinow[8] modified to the extent that air-dried films were used and acid hydrolysis was continued for only 5 min at 55°–60°. Preparations were examined visually using a times 90 objective and a blue minus filter. Our chief problem was whether our staining procedure enabled us to detect a nucleus as having divided at the precise time that division actually occurred. If, for example, nuclei did not appear to have divided until some while after that event, then the observed mean nuclear number for the population would be an underestimate of the true number. To investigate this possibility we allowed non-replicating, mainly uninucleate cells[9] to replicate their nuclei synchronously at 37° on addition of fresh nutrients, and followed the sequence of events with concurrent nuclear stain preparations and DNA determinations[10]. It was observed that most nuclei divided synchronously at a time close to that at which the DNA in the culture had doubled. It therefore seems that our staining procedure gives a value for the mean nuclear number which is unlikely to be affected by any significant systematic error.

Segregation of mutable units. The determination of the number of mutable units per bacterium involved a comparison of the frequency of induced revertants in a growing population of bacteria immediately after a short irradiation, that is, before any segregation of mutated mutable units had occurred, with the frequency a few generation times later when mutant segregation was complete. When the number of mutable units per bacterium is 1, 2, 2^2, 2^3.... it may be seen from Table 1 that the initial frequency is greater than the final frequency by a factor equal to the number of mutable units per bacterium and this is generally true for any such number *p*.

Table 1

Time after induction	0		τ		2τ		3τ		After segregation
Total No. cells	N		2N		4N		8N		
Mutant cells / Mutable units per cell	No.	freq.	No.	freq.	No.	freq.	No.	freq.	freq.
1	m	m/N	$2m$	m/N	$4m$	m/N	$8m$	m/N	m/N
2	$2m$	$2m/N$	$2m$	m/N	$4m$	m/N	$8m$	m/N	m/N
4	$4m$	$4m/N$	$4m$	$2m/N$	$4m$	m/N	$8m$	m/N	m/N
.	.	.							.
.	.	.							.
.	.	.							.
p	pm	pm/N							m/N

The theoretical segregation pattern for induced mutations in cells containing 1, 2, 4 .... *p* mutable units. The mutant frequency in a very large population *N* is m/N per mutable unit at zero time. The population, numbers of mutants and their frequency at intervals of one generation time (τ) are shown and also the final mutant frequencies.

The segregation of mutable units in *Escherichia coli* *WP*2 was followed after short X-ray exposures (less than 5 min) of cultures in the process of exponential growth under full aeration. A turbidostatic system automatically controlled the addition of fresh glucose-salts minimal medium[6] plus 6 µg/ml. tryptophan, so that the optical density of the culture was kept constant to within ± 2 per cent. The usual volume of the culture was 300 ml. and the population density approximately 10^8/ml. Samples were removed from the flask or the overflow at intervals and assayed for numbers of mutants, viable bacteria, and total bacteria using a Hawksley Thoma counting chamber. Prototrophs (on 'Millipore' filters[6]) and auxotrophs were scored on minimal agar plates with 0·75 µg/ml. of tryptophan supplement. These plates allowed full expression of mutations, the number of colonies being independent of whether the mutant character was inherited by all daughter cells or only appeared in those arising from one nucleus or one mutable unit. Fig. 1 shows the results of a typical segregation experiment at 16°. Results from a number of such experiments at different temperatures are summarized in Table 2.

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* The synthesis of tryptophan is blocked at an early stage in this strain and the *try* gene referred to does not correspond to that determining tryptophan synthetase production.

Table 2. MUTATION RATES WITH STANDARD ERRORS AT DIFFERENT TEMPERATURES

Temperature	Mutation rate per rad: Per nucleus (× 10⁻¹¹)	Mutation rate per rad: Per mutable unit (× 10⁻¹¹)	Mutable units per nucleus: From ratio of mutation rates	Mutable units per nucleus: From segregation experiments	Mutable units per nucleus: Weighted mean
37°	6·55 ± 0·45	3·89 ± 0·18	1·68 ± 0·15	1·28 ± 0·20	1·5 ± 0·2
22°	4·1 ± 0·37	2·42 ± 0·20	1·70 ± 0·22	1·68 ± 0·38	1·69 ± 0·22
16°	2·33 ± 0·10	1·13 ± 0·17	2·06 ± 0·35	2·23 ± 0·51	2·13 ± 0·31

Only a small transitory inhibition of growth occurred after irradiation with the doses of 1–2·5 krads used in these experiments[6]. Slight temporary increases in cell-length and nuclear number were also observed presumably due to a temporary inhibition of cell division.

An alternative approach to the problem of estimating the number of mutable units per nucleus is to measure in different exponontially growing cultures the induced mutation rate per nucleus before segregation occurs and in other cultures the mutation rate per mutable unit after segregation is complete. The mutation rate per nucleus was derived from observations of mutant frequency in suspensions of log phase *E. coli WP*2 irradiated under aeration in minimal medium without glucose or tryptophan. Suspensions containing ~ 6 × 10⁹ bacteria/ml. were exposed to a range of X-ray doses and 0·1 ml. aliquots plated at once on the usual minimal agar with 0·75 μg/ml. tryptophan supplement. Stained preparations for nuclear counts were also made at the time of irradiation. When the observed frequency of induced revertants divided by the average number of nuclei per bacterium and by the radiation dose was plotted against dose, the graphs in Fig. 2 were obtained. These curves, each derived from at least three independent experiments, can be regarded as survival curves for those organisms which ultimately give rise to mutant colonies, provided the induction of mutants is proportional to the radiation dose, a condition apparently satisfied over the range studied here although not under other experimental conditions where lethality is great[11]. The zero dose extrapolate of the line, which appears straight when plotted semi-logarithmically, is the induced mutation rate per nucleus per rad. Values of these mutation rates are given in Table 2. The temperature-dependence which is evident from these results is partly associated with the temperature immediately after irradiation. Further experiments are in progress and will be reported elsewhere.

The mutation rate per mutable unit was determined in continuous cultures which were controlled turbidostatically and grown under continuous gamma irradiation[6]. The irradiations lasted many generation times and the mutant frequency increased at a constant rate except during the first one or two generation times. The constant rate of increase was a measure of the mutation rate after full segregation, that is, of the mutation rate per mutable unit[6] (Table 2). Knowing the dose rate, the mutation rate per mutable unit per rad was readily obtained. The results at the three temperatures are included in Table 2.

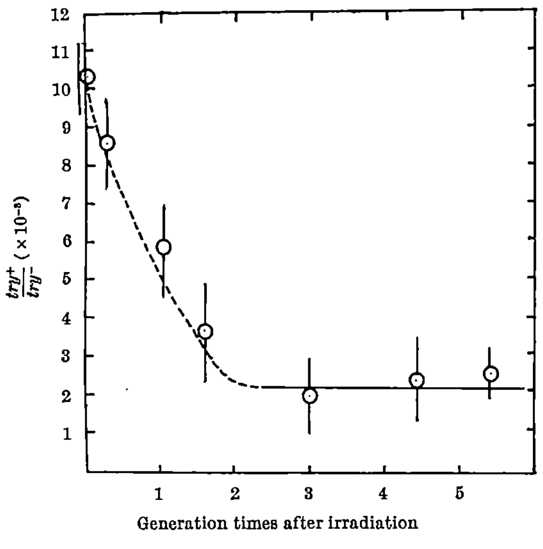

Fig. 1. Fall due to segregation in proportion of newly induced prototrophs in a population (mean nuclear number = 2·1) of *E. coli WP*2 growing exponentially at 16°. Vertical lines represent standard errors

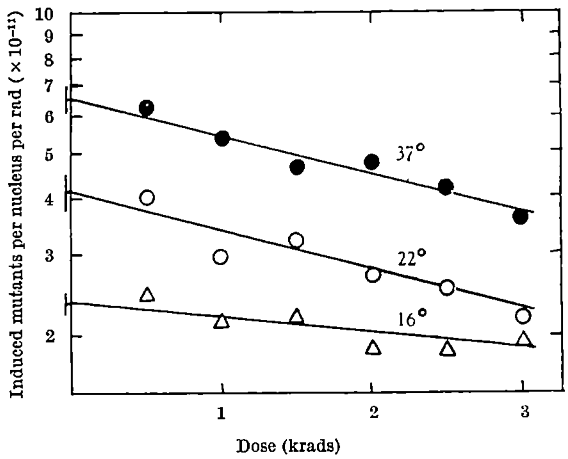

Fig. 2. Induced mutation rate per nucleus versus dose of 250-kV X-rays at different temperatures. Temperatures maintained throughout at values shown until 24 h after irradiation when plates at 16° and 22° were transferred to 37°. Vertical lines show standard errors of zero dose extrapolates

The estimates of numbers of mutable units per nucleus obtained by the two methods described here are in satisfactory agreement at 16° and 22°. At 37° there is a barely significant difference between the two estimates, but the trend to lower values with increasing temperature appears to be real.

Nature of the mutation event. Although it would be very premature to attempt to interpret the results of these experiments in terms of a general hypothesis relating to the primary events in mutation induction by radiation, some support for hypotheses already advanced by others is now apparent. The work of Deering[12], Kada, Brun and Marcovich[13] and Munson and Jeffery[6] has shown that with small X-ray doses the frequency of induced revertants of *E. coli WP*2 is proportional to the dose. Also experiments carried out by Deering[12] with particles of different LET showed a fall in mutagenic efficiency only at an LET greater than 190 MeV cm²/g. These observations suggest that the majority of mutants arise as the result of the passage of a single electron through or very close to that part of the DNA which corresponds to the mutable unit for this mutation. An analysis of Deering's LET data by the track segment method of Howard-Flanders[14], assuming a mutation rate of 4 × 10⁻¹¹/rad/mutable unit, shows that the sensitive region within which the production of one ion cluster induces a mutation with a probability of approximately unity is ~ 5 × 10⁻⁸ cm across. This would represent a rather small part of a nucleotide. It is interesting that Demerec and Sams[15] reported comparable X-ray reversion rates for three other auxotropic strains of *E. coli*. The hypothesis that a single ionization or cluster must be produced within one of a very few nucleotide pairs to cause a reversion does not appear on this evidence to be implausible.

The average number of genes of a particular locus to be expected in the nuclei of exponentially growing cells would depend on the mode of replication of the bacterial chromosome, and this is still subject to some uncertainty. If replication proceeded in a fixed linear order from a fixed point on the chromosome, the average number of *try* genes per nucleus would depend on the position of the *try* gene. An average of one gene per nucleus would imply a position at the end of the order and two genes per nucleus a position at the beginning. For all genes the average would be 1/ln 2, that is 1·44. Other replication models, for example, a fixed linear order but commencing at one of several points on a circular chromosome, or a fixed point of origin but proceeding in either direction round a circular chromosome, would give an average of approximately 1·4–1·5 genes per nucleus for all loci. Such models are, however, inconsistent with a strict interpretation of the results of Meselson and Stahl[16]. This difficulty could be overcome if it were postulated either that the probability of replication in one cycle beginning at a different point from that in the previous cycle is small but finite or that replication from a fixed point occasionally reverses its direction. Such small departures from strictly semi-conservative replication might not be detected in short-term experiments such as those of Meselson and Stahl, but would nevertheless have a sufficiently large randomizing influence over many generation times in a population to bring the average number per nucleus of every gene close to 1·44 and account for Nagata's[17] findings with an F^- strain of *Escherichia coli.*

Owing to our ignorance of the replication order in *Escherichia coli WP*2 the results in Table 2 can only be interpreted tentatively. The numbers of mutable units per nucleus at 37° and 22° do not differ significantly from 1·44, so if the replication order were randomized in the population, these results would be consistent with one mutable unit per basic (non-replicating) chromosome. This would imply that both strands are involved in the primary radiation damage or that damage to single strands is afterwards either 'lost' or extended to involve the complementary strand. If, on the other hand, the replication order were fixed, our observations would be consistent with entirely double-stranded mutational events if the *try* gene were 50 ± 20 per cent of the way along the replication order. If the gene were nearer the beginning of the order a proportion of events might be confined to one strand only, but it could not exceed 50 ± 20 per cent of the total. One other possibility cannot be ruled out, namely, that only one of the two DNA strands carries transmissible information, both new strands being coded directly or indirectly from this.

There appear to be two plausible explanations of the difference between the numbers of mutable units per nucleus at 16° and at the higher temperatures. In the first place there may be repair mechanisms which are more effective at 16° than at 37° and which are more efficient for the elimination of damage from double-strand events than from single-strand ones. In the second place, DNA synthesis may occupy only part of the mitotic cycle at low temperatures[18] instead of virtually the whole cycle as at 37° (ref. 19), the remainder being a rest period corresponding to that termed G_2 in higher organisms. One can readily derive expressions for the expected number of mutable units per nucleus in terms of the time γ spent in G_2, the generation time τ and the growth rate μ ($= \ln 2/\tau$). If one assumes that a completed chromosome pair is only microscopically resolvable into two nuclei at the commencement of the next round of replication, then with a randomized replication order among the population, the average number of mutable units per nucleus is $2\,\tau(1-\exp\overline{\mu\gamma-\tau})/(\tau-\gamma)\ln 2$ for mutations which involve both strands and twice this quantity for single-stranded mutations. When the replication order is fixed and the gene involved is replicated at time t after the beginning of the cycle the corresponding expression is $2\exp(-\mu t)$. It can be shown that our results are consistent with a random replication order, almost entirely two-stranded mutational events and γ/τ (that is, relative duration of G_2) increasing from almost zero at 37° to at least 0·5 at 16°. If, on the other hand, one assumes a fixed replication order, less precision in interpretation is possible. One can only conclude that between 37° and 16° there is a substantial increase in the proportion of single-stranded events or an appreciable decrease in t/τ reflecting a rise in γ/τ.

The observed segregation patterns at the different temperatures have led us to the following broad conclusions. At 37° one basic (non-replicating) nucleus contains one unit mutable by X- or γ-rays. The larger average number of mutable units per nucleus at 16° implies that approximately half the mutational events are confined to one strand only or alternatively that there is a G_2 non-DNA-synthesizing period in the cell-cycle lasting about half a generation time.

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DIFFERENCES IN THE CHARACTER OF C_6 TO C_9 HYDROCARBONS FROM GASEOUS METHANE IN LOW-FREQUENCY ELECTRIC DISCHARGES

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IN the study of the chemistry of primitive earth and Jovian atmospheres[1,2], there is considerable interest in the more complex products that may be formed from methane by electric discharges. There is an extensive literature on the reaction kinetics of methane in electric discharges[3,4]. A great amount of work has centred around such factors as power yields, pressure, cell design and presence of hydrogen. Few statements can be found linking these phenomena with possible pathways in primordial chemistry.

In the work recorded here the actions of several arc and semi-corona discharges on gaseous methane were studied.

Analysis by gas chromatography and mass spectrometry revealed a significant difference in the type of compounds formed in the two kinds of discharge. An attempt was made to find an explanation for the observed behaviour.

For a source of energy we used a Gardner luminous tube transformer (60 c/s, 120 V input; 15 kV/18 m.amp output; Cat. No. 1518). The two open ends of the secondary were connected to the discharge cell system, and the voltage was regulated by connecting the primary to a Variac autotransformer (General Radio Co. Type *W5LMT3*). Output currents were monitored by a 0–25-m.amp Simpson meter in one branch of the secondary. Voltages were measured with an Electrostatic kilovoltmeter (model *ESD*, Sensitive Research Instrument Corp., New Rochelle, New York). In cases where the 6-kV range would have been exceeded, the voltage was determined as the sum of voltages across three 22 megohm resistors in series. The current through the discharge cell was measured by the voltage drop across a resistor in series with the cell, and this resistor also functioned as a load to limit the current as desired, by using a value between 0·1 and 10 megohms. In two of the experiments described here, we used a pulsed arc which was automatically fired for 3 out of every 30 sec with the help of a Dual-Trol timer (Industrial Timer Corp., Parsippany, New Jersey). The discharge cell in each case was a 1,000-ml. round flask to which the respective cell heads with their electrode assemblies were attached by wide taper joints.

The semi-corona cell head consisted of a 'Pyrex' tube 100 mm in length, of internal diameter 30 mm with walls 2 mm thick. The inside electrode was made of 6-mm stainless steel tubing extended concentrically throughout the whole length of the 'Pyrex' cylinder. The glass tube was wrapped on the outside with a metal foil which covered 60 mm length in the middle section.

The arc assembly was a short cylinder into which two gold wires, 1 mm in diameter, were inserted. The electrodes were mounted through taper joints. Carbon deposited on the electrodes during the experiment could thus be shaken off by rotating the electrodes. The arc gap was maintained at approximately 10 mm.

The starting material was C.P. methane (99 per cent). At the end of each experiment, we isolated that portion of the cell contents which has more than 1 mm of pressure at −160°, and analysed it for the hydrogen–methane ratio using a G.E.C. 21-103 *C* mass spectrometer. After reading the pressure of the remainder, we transferred all those materials which could be distilled and condensed at 40μ mercury and −78° into a capillary cold finger. The volumes of these products were measured by the microsyringe used to transfer them to an Aerograph *A* 90-*P* gas chromatograph. A 0·25-in. column, 12 ft. long, packed with 10 per cent 'Octoil-*S*' on 60–80 mesh 'Chromosorb-*W*' was used. With helium at 40 ml./min, the absolute retention times were: benzene, 6 min; and toluene, 10 min. This column is similar to 'Apiezon' types, separating hydrocarbons by their boiling points. Fractions collected at −78° were examined in the mass spectrometer.

The data from seven experiments are shown in Table 1. Since the pulsed arc was operated only 10 per cent of the time, the numbers in line 3 give the net hours of current flow. The next line is intended to give a rough guide to the amount of energy consumed in the whole experiment. The apparent electrode voltage and V-amp-h reported for the semi-corona are probably too high, since the voltage has to be divided between the drop across the glass wall and that over the gas space, respectively.

Voltages and currents did not change by more than 30 per cent throughout any of the experiments, and we are listing the averages.

We have tabulated under "% loss of CH_4/h" (Table 1) the percentage of starting material consumed, divided by the number of hours. The volume of distillate reported as microlitres is intended as an illustration of the amount of material actually recovered in each experiment. In all cases the percentage composition of products was independent of the duration of the experiment.

Table 1

	Arc high continuous	Arc high pulsed		Arc low continuous		Semi-corona continuous	
Electrode voltage	2,150	850	1,450	1,275	2,260	8,900	9,425
Cell current (m.amp)	4·5	8	3·5	0·56	0·51	0·4	0·31
Total hours of current flow	5·5	3·8	2·0	21	41	94	41
V-amp-h	53	25·8	10·2	14·5	47·3	335	120
Initial CH_4 (mm Hg)	513	245	504	249	502	255	502
Final press. (mm Hg) CH_4	202	115	385	139	308	39	236
H_2	401	177	143	123	192	215	233
Others	48	28	19	19	38	8	30
Total	651	320	547	281	538	262	499
% loss of CH_4/h	11	14	11·8	2·1	0·9	0·9	1·0
Initial mg CH_4	328	157	323	160	321	163	321
Distillate							
μl.	16	3	2	2	10	4	26
% by vol. C_6H_6	59	53	35	<5	4	<1	0
% by vol. C_7H_8	7	22	24	<5	2	<1	0
% by vol. C_8H_{10}	2	7	7				
% by vol. C_8H_8	7	10	22				
% by vol. others	25	8	12	90	90	95	100
$C_{(s)}$	†	*	*	*	*	—	—
Non-volatiles	†	—	*	*	*	†	*

Note: $C_{(s)}$ and non-volatiles: *, deposit; †, considerable deposit.

In the high-intensity arc the distillate (at 40μ and −78° C) was a clear-yellow fluid which gave chromatograms of well-spaced peaks. On the basis of the peak height, benzene was most abundant. Next in order of magnitude was toluene. A third compound the mass spectrum of which shows a sharp termination at about 106 (C_8H_{10}) corresponds to a boiling point of about 135°, and the fourth suggests a parent mass of about 104 (C_8H_8), and an approximate boiling point of 155°. The mass spectra of both these materials have been obtained, but we have not been able to characterize them using published mass spectral data.

The semi-corona cell yielded a colourless distillate the gas chromatogram of which was poorly resolved. In Fig. 1 this chromatogram (low) has been superimposed on that from the high-intensity arc (high). A significant feature of this trace was the virtual absence of any peaks where benzene and toluene should have appeared. We have obtained a somewhat better resolution at lower column temperature and flow-rate and identified some prominent peaks by mass spectrometry as follows: 2,2-dimethylbutane; 2-methylpentane; 3-methylpentane; 2,4-dimethylhexane; 3,4-dimethylhexane.

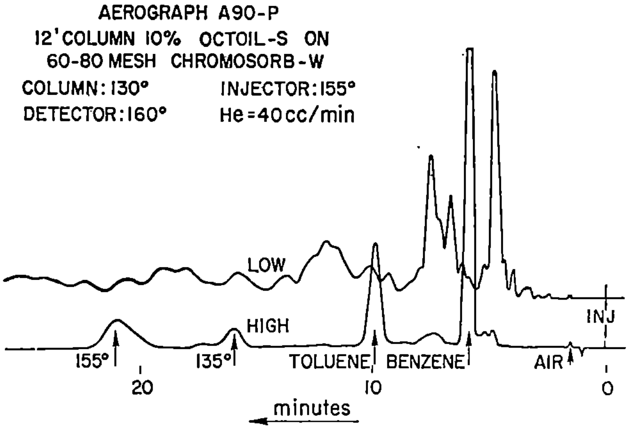

Fig. 1. Gas chromatograms of products from low intensity (semi-corona) and high-intensity (spark-discharge) cells

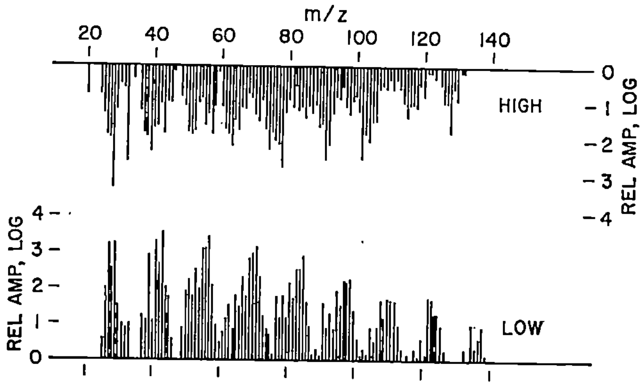

Fig. 2. Portions of composite mass spectra of products from low-intensity (semi-corona) and high-intensity (spark-discharge) cells. The ordinates represent logarithms of relative amplitudes

The low-intensity arc produced a mixture, the chromatogram of which was similar to that of the semi-corona. But, in this case, benzene and toluene were found present in small quantities (Table 1). In Fig. 2, the composite mass spectra of the high arc and of semi-corona products are contrasted to illustrate the differences in the products from the two types of discharge.

The results presented here show that the character of compounds in the range of interest appears to be determined by the type of discharge more than by any other factor. Variations in the duration of the current flow or in the cell dead volume did not at all affect the qualitative results. There is some indication that, at higher initial pressure, the relative yields of C_6 to C_9 material will tend to be higher because these materials have a better chance to condense away from the warm electrode region.

Neither discharge cell showed any gross rise in temperature under air cooling. Further, pulsing the arc brought no departure from the exclusive production of aromatics, while reducing the current did. Metal catalysis due to the gold wires or the stainless steel centre electrode is improbable, because all electrodes were soon covered with carbon deposits. In either case the possible catalytic action of carbon is not excluded.

In an attempt to explain our observations, we may consider that the arc has two primary features which distinguish it from the glow or corona discharge. One is the higher electron temperature, and the other the higher current density of the arc[5]. By inference, we also assume that the local gas temperature in the arc is higher, and that this might result in a depletion of target species in the arc zone. Since a reduction in arc current does not change the current density[6], the results of the low-intensity arc experiments suggest that here the gas temperature is lower than in the case of the high arc. Although there is a tendency toward current localization in the semi-corona after it has been operated for a brief time in this instance, the gas temperature gradient is probably far more moderate.

As it has been suggested that both the spark and the corona-type discharges could have occurred under primitive Earth conditions[7], these findings throw some light on the pathways of formation of aromatic and saturated carbon compounds under abiogenic conditions. In applying our results to the planet Jupiter, it is also conceivable that there is a correlation between the type of hydrocarbon molecule or radical appearing in its atmosphere and the form of the observed energy outbursts[8].

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A TECHNIQUE FOR MINIMIZING THE EFFECTS OF ATMOSPHERIC DISTURBANCE ON PHOTOGRAPHIC TELESCOPES

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IT is well known that images in astronomical telescopes are shifted and degenerated by atmospheric disturbance. This becomes extremely important with large apertures and high magnifications: the disturbances prevent the detailed photographs which would be expected from the theoretical resolving power of large instruments. The disturbances take several forms: (1) The image may be shifted as a whole, in any direction, with varying frequency and amplitude. (2) Parts of the image may move in different directions simultaneously. (3) The image may be degenerated, especially with large aperture instruments, when the effective wave-length of the disturbance is less than the diameter of the objective. This produces a 'milkiness' of the image. This is quite different from the effect of shift when seen visually, though it may appear similar in a long-exposure photograph when the shifts produce blurring of contours and loss of fine detail.

It seems that visual observation can be preferable to photographic recording in lunar and planetary work, because the effects of the shifts of images can to some extent be avoided by visually sampling those moments when the agitation of the images is least marked. The purpose of the technique described here is to enable photographic telescopes to select moments of quiescence to build up a correctly exposed photograph.

It is clear from the kinds of disturbances encountered that any attempt to compensate the disturbances by introducing equal and opposite movements of the image on the plate will be unsatisfactory, when movements can occur in several directions in different parts of the image. Further, to get a servo-system to perform this task it would be necessary to feed it with information of the direction and velocity of the shifts, which is extremely difficult, while the servo itself would be subject to some over-shoot and tremor.

The technique under investigation is to take, first of all, a long-exposure photograph of the (atmospherically disturbed) image. The resulting photograph is statistically correct, in the sense that the major features will fall near the centre of intensity gradients produced by the random disturbance of the image. But, although the position of the contours will be nearly correct, fine detail is lost, hence the problem. This long-exposure photograph is processed, and the resulting negative is placed in its original position in the optical system, so that the

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STEROID ANALYSIS BY GAS LIQUID CHROMATOGRAPHY

By

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and

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Albany, New York

Describes the detection and separation of steroids both in clinically obtained biological fluids and tissues and in certain experimental situations.

Selected methods which give reproducible results with semi-purified extracts and which permit the separation and identification of one or more steroids in biological fluids are presented. *The methods have been chosen for rapid clinical screening.*

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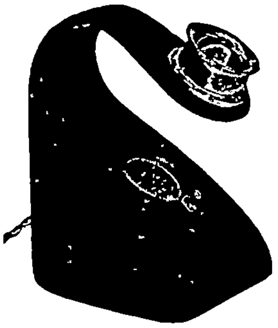

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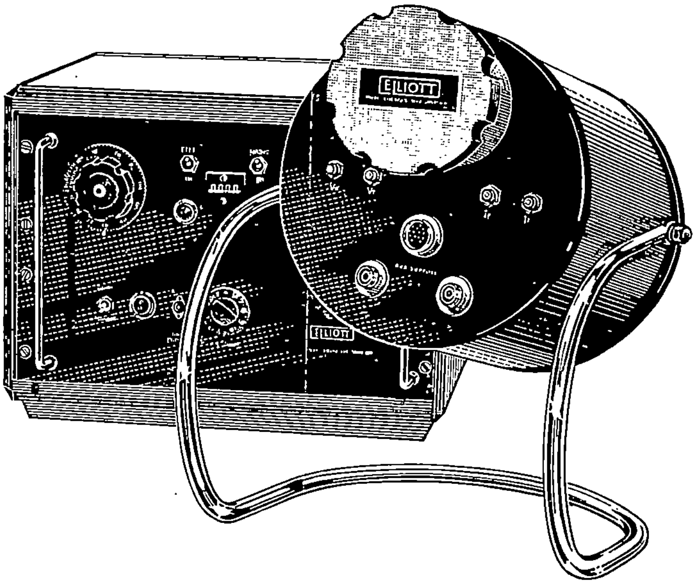

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fluctuating image now lies on the transparent negative. The image is now almost entirely cancelled by its negative. It is, however, most completely cancelled when the image most nearly corresponds to the negative. As the image is displaced, by the atmospheric or other disturbances, there is an increase in intensity. This is detected by a single photoelectric cell which covers the entire image plane, and so receives plenty of light. We can thus detect the presence of any shift of the image—though not the direction of shift—from increase in the output of the cell. The output rises with any discrepancy of the image from the statistically correct 'master' negative—not only shifts but also loss of focus and the 'milkiness' produced by regions of different refractive index smaller than the aperture of the instrument. (This last point I have established by means of a ripple tank.)

Having attained a signal indicating disturbance from the statistically correct image, it is a simple matter to use the signal to produce a second photograph free of disturbance. This may be done by using a second camera which shares the image with the first, by means of a beam splitter. This second camera is fitted with an electrically operated shutter which opens only when the output from the photo-cell is near its minimum value, corresponding to a close fit of the fluctuating image with the master negative.

In this way we separate informational integration from the integration of energy needed to expose the final picture, which is built up from many short exposures occurring whenever the image is close to the 'master'.

The preliminary experiments are limited to simulation of atmospheric disturbance, by placing an oscillating 'Perspex' sheet between the object and the optical system, and a ripple tank.

The beam splitter is a half-silvered mirror placed at 45°, so that the second camera (an 'Exacta' 35-mm single-lens reflex) is provided with the same image as the large camera carrying the master negative and the photo-cell. The second camera is fitted with a specially made shutter, consisting of a pair of electromagnetic vibrator units (Advance type VI) which drive a pair of metal vanes shaped to form a square opening, increasing in size as the vanes are withdrawn by the opposed vibrator units. The circuit consists of an oscillator (400 c/s) allowing a.c. amplification from a bridge which is unbalanced by the photo-cell changing in resistance with increasing light, when the image loses register with the master. The amplified output is rectified, and used to energize the vibrator units to close the shutter.

An example of how a shifting image is improved is shown in Figs. 1*a* and *b*. Fig. 1*a* was obtained from a long exposure of the Moon model while the 'Perspex' plate was oscillating about a vertical axis, to produce horizontal disturbance. The degeneration along the horizontal axis is very apparent. Fig. 1*b* shows the improvement obtained—the optical conditions being identical—when the shutter system is switched on. Some degeneration on the horizontal axis can still be seen: this may be further reduced by increasing the gain of the amplifier.

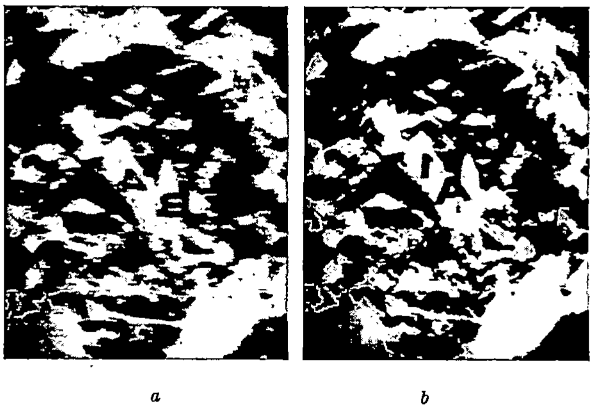

Fig. 1. *a*, Photograph of Moon model taken by the second camera through horizontal disturbance produced by oscillation of a 'Perspex' plate (1 in. thickness: rotated through 20°). This is the same degree of blurring as in the 'master' negative which gives Fig. 1*b*. *b*, The same Moon model, also taken by the second camera, but only when the electrically operated shutter is open for periods when the disturbed image nearly corresponded with the blurred 'master' negative. The difference gives an idea of the improvement to be expected for simple shift of images with high magnification telescopes. (Relative shift can be produced with a ripple tank, and this also gives signals which should produce improved photographs)

The improvement shown is given by a shutter open/closed ratio of about 6 : 1. This may be increased by increasing the gain in the present arrangement, or by introducing a gate, working the shutter as an all-or-none device. It is important to note that the improvement is from a blurred master negative identical with Fig. 1*a* since it was taken through the disturbance.

We are now simulating atmospheric disturbance with a layer of water agitated by an electromagnetic vibrator, driven from a low-frequency noise source. The resulting disturbances appear very similar to those experienced with an astronomical telescope. It remains to discover the efficiency of the technique under these more realistic conditions. It might then be directed to the Moon and the planets.

I thank Mr. Stephen Salter and Mr. William Matthews for their help in setting up the preliminary experiments. I also acknowledge receipt of grant *Af-EOARDC* 63–93 monitored by the European Office, Office of Aerospace Research, for work on visual perception.

AGE OF CALEDONIAN OROGENY AND METAMORPHISM IN BRITAIN

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As part of a continuing programme of research into the chronology of British Caledonian rocks, we have recently completed a critical review and reappraisal of the geological evidence and isotopic age data from Britain. Excellent summaries of the geological evidence, with very full bibliographies, can be found in *The British Caledonides*[1], also in Read[2], McKerrow[3], and Watson[4]. Published isotopic data are available in refs. 5–20. From

Table 1. New Potassium–Argon Age Determinations

Sample	Ref. and method	K_2O (%)	Atmo. contam. (%)	Vol. radiogenic ^{40}Ar (mm^{3}NTP) / wt. sample (g)	Apparent age and error (m.y.)
Epidiorite, Co. Mayo. Basic intrusion into Wenlock Beds: metamorphosed during Caledonian orogeny	612 K/Ar whole rock	1·77 ± 0·03	2·1	0·02737	417 ± 11 (that is, apparent age of Caledonian metamorphism)
Cataclastic chlorite-muscovite schist, Porth Trecastel, Anglesey. (*MR SH* 333707.) Gwna Beds (Pre-Cambrian) metamorphosed in Monian orogeny. Age discrepantly low as a result of Caledonian cataclasis	*FM* 125 467 K/Ar muscovite	3·06 ± 0·01	7·3	0·07300	611 ± 16 (that is, a minimum age for the Monian orogeny)
'Caledonian' chlorite, separated from sheared migmatic soda-granite, the Gader Inlier, Mona Complex, Anglesey. (*MR SH* 293923)	*FM* 199 591 K/Ar chlorite	1·52 ± 0·038	1·6	0·02304	410 ± 16 (that is, apparent age of Caledonian orogeny)

$\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr^{-1}. $\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr^{-1}.

the considerable volume of unpublished isotopic results at hand, certain relevant new age determinations are quoted here (Table 1).

The geochronological work of Giletti *et al.*[7] in 1961 suggested that the age of metamorphism of the Dalradian rocks of Scotland and Ireland was significantly different at 475 ± 15 m.y. from that of the last metamorphism to affect the Moine Series at 415 ± 15 m.y. This tentative conclusion, although derived from very scanty data, has become an unquestioned dogma, and the idea of a difference in age of metamorphism between the Moine and Dalradian rocks has persisted in both geological and geochronological thought. As a result of our reappraisal of the evidence, we have come to the conclusion that this supposed difference does not exist. We are convinced that the age patterns obtained from both the Moine and Dalradian rocks can best be explained as resulting from a combination of partial and complete 'overprinting' of older metamorphic rocks by a (420–410) ± 10 m.y. main Caledonian orogenic/magmatic/metamorphic event which was contemporaneous throughout Britain. Full discussion is reserved for future publication; but it was thought that this important re-interpretation should be communicated now.

Only in especially favourable circumstances can isotopic age determinations reveal the true or 'initial' age of crystallization of a rock. The various causes of discrepancy have been discussed fully in Fitch and Miller[18], Miller and Fitch[20] and in the discussion to Lambert[21]. Briefly, we do not accept the view that metamorphic complexes or igneous intrusions can, in general, remain above some 'threshold' temperature for daughter element retention over extremely long periods amounting to tens of millions of years. In the discussions already quoted here, we provide evidence which suggests that the cooling period is normally far less than that involved in the experimental error of an age determination, and is, therefore, not a factor of geological significance in this context. We do believe, however, that the principal cause of discrepancy in rocks of suitable retentive mineralogy and fabric is 'overprinting' by later geological events. Partial and complete overprinting of older rocks leads to recognizable patterns of age results from which it is possible to select the geologically significant dates. It must be emphasized here that to take an average of a series of partially overprinted ages leads to a completely meaningless figure. It is the statistical pattern of ages which is important. Results from Morar[7,12,16] and from the Glen Dessary Syenite[17] leave no doubts regarding the Moine Series. They are a late Pre-Cambrian sedimentary group (deposited after 1,600 m.y., most probably between 1,200 and 800 m.y.) involved in Pre-Cambrian metamorphism and magmatism (> 720–765 m.y.) and overprinted by the Caledonian event around 420–410 m.y. Unquestionable evidence of the Pre-Cambrian event can only be found in the western part of Inverness-shire and southern Ross; elsewhere the ages obtained from the Moines are almost entirely overprinted to become Caledonian. A pattern of ages is obtained which falls rapidly from rare Pre-Cambrian ages to a very prominent maximum between 435 and 405 m.y., with its peak between 420 and 410 m.y., then falls off again to a few scattered lower ages.

Taking into consideration the smaller sample available, the age pattern of Caledonian overprinting obtained from the Dalradians is identical to that obtained from the Moines. This conclusion was reinforced by an examination of the age patterns of Argyll and Perthshire in which ages from the Central Highlands granulites and Dalradian rocks were considered together. Unequivocal evidence of a Pre-Cambrian age of early metamorphism for the Dalradians is lacking, however, unless it be present at Ben Vuroch. The only certainty to be found in the Dalradian pattern is that the 420–410 m.y. Caledonian metamorphism is overprinted on rocks first metamorphosed at some earlier date, for no date above 470 m.y. has yet been obtained in the Cambridge laboratory from a Dalradian meta-sedimentary rock. The older whole-rock rubidium–strontium ages of Giletti *et al.*[7] from Ben Vuroch (590 ± 130 m.y. and 670 ± 160 m.y.) and from the intimately associated Central Highlands granulites of Perthshire (565 ± 160 m.y.) are suggestive of a similar Pre-Cambrian age for the early metamorphism of both the Moine and Dalradian rocks, but are not in themselves conclusive.

In 1961, a 475-m.y. first metamorphism of the Dalradian rocks was acceptable because it agreed with current geological hypothesis. Kennedy[22] in 1955 put forward a strong case for mid-Ordovician orogeny in the Scottish Highlands, and the earlier discovery of mid-Cambrian trilobites in the Highland Border Series by Pringle[23] was regarded as suggesting that much of the Dalradian succession was Cambrian in age. The complete absence of evidence suggesting 475 m.y. metamorphism in the Moine areas of the Highlands remained, however, an inexplicable puzzle. New geological evidence from Ireland (see, for example, refs. 24–28) has made it quite clear that the Connemara Dalradian was folded and first metamorphosed previous to the Arenig, and acted as a tectonically distinct basement during the Caledonian folding and metamorphism of the Ordovician and Silurian rocks of Murrisk. The available isotopic data from north-west Ireland are summarized in Table 2. It will be seen that there is clear evidence of the 'overprinting' of an earlier metamorphic complex by the Caledonian event at 420–410 m.y. (as defined by the age of 417 ± 11 m.y. obtained from a schistose epidiorite intrusive into Wenlock strata (Tables 1 and 2)). The overprinted metamorphic event can be defined as having occurred some time before 490 ± 20 m.y. (the oldest age quoted in Table 2).

It may be concluded with some confidence that the earlier Dalradian orogeny and metamorphism of Britain was pre-Arenig. On the 1959 time-scale of Holmes[29] this would be at least 500 ± 15 m.y. There is no reason at present to supppose Holmes's estimate to be drastically wrong[18]. If the mid-Cambrian rocks of the Highland

Table 2. PERTINENT AGE RESULTS FROM IRELAND

Apparent age + error (m.y.)	Method and mineral used	Details of rock	Ref.
365 ± 10	Rb/Sr biotite	Galway granite S.E. of Carna, Co. Galway	7
381 ± 16	K/Ar biotite	Garnet-mica schist, Loch Corrib.	16
386 ± 17	K/Ar biotite	Garnet-mica schist, Loch Corrib.	16
414 ± 19	K/Ar biotite	Biotite-schist, Cushenden	16
415 ± 19	K/Ar muscovite	Muscovite-schist, N. shore Kylemore, L. Connemara	16
417 ± 11	K/Ar whole rock	Epidiorite, South Mayo	See Table 1
417 ± 19	K/Ar biotite	Biotite-schist, Cushenden	16
424 ± 19	K/Ar muscovite	Muscovite-schist, N. shore Kylemore, L. Connemara	16
(465 ± 13)	Rb/Sr muscovite	Pegmatite in Connemara schist, near Clifden	7
455 ± 13	Rb/Sr muscovite	Re-run of above by	12
460 ± 12	Rb/Sr biotite	Connemara schist, Clifden, Co. Galway	7
460 ± 11	Rb/Sr muscovite	Pegmatite in Connemara schist, near Clifden	7
(495 ± 22)	Rb/Sr muscovite	Connemara schist Clifden, Co. Galway	7
485 ± 22	Rb/Sr muscovite	Re-run of above by	12
490 ± 20	Rb/Sr muscovite	Connemara schist, Clifden, Co. Galway	7

Border Series are truly part of a continuous Dalradian succession, then this orogenic episode must have been late-Cambrian or earliest Ordovician in age. There are many difficulties inherent in the acceptance of this correlation, for nowhere in the British Isles can definite isotopic or geological evidence be found which indicates major orogeny at this time. Studies of ancient rocks throughout Britain have so far failed to reveal any significant concentrations of partially or completely overprinted ages that might be referred to such an event. Neither will structural or stratigraphical considerations support such a conclusion. Evidence of post-Tremadocian/pre-Arenig parorogeny (the terms evorogeny and parorogeny are used here as defined by Simpson[30]) and unconformity is clear in North Wales[31]; but before this minor disturbance, Tremadocian sedimentation in Wales was a record of relatively quiescent conditions. The Upper Cambrian/Earliest Ordovician sedimentation of Skye and the north-west Highlands again suggests regional quiescence. Throughout Britain there is evidence of important Arenig transgressions, but we maintain that the time interval and style of this transgression (for example, as indicated by the analysis of the Arenig rocks of Murrisk by Dewey[26]) are not compatible with it representing the major (F_1/F_2) polyphase orogenic–metamorphic event of the Grampian Highlands. That such an event, accompanied by recumbent folding on the grand scale, could have been closely restricted to the Dalradian belt and have left the rest of Britain virtually unaffected is to us unacceptable. Recent structural investigations in the Caledonian belt south of the Highlands[28,32–37] clearly demonstrate the presence of a single unified Caledonian evorogenic structural complex involving Arvonian, Cambrian, Ordovician and Silurian rocks up to and including Ludlovian. In Anglesey the metamorphic basement of this evorogeny is the Pre-Cambrian Mona Complex (orogenic metamorphism > 611 ± 16 m.y. (see Table 1)); in Ireland and Scotland it is the Dalradian and Moine. We believe that the Mona Complex should be equated with the Dalradian, for there is a general similarity between the Upper Dalradian succession and that of the Mona Complex and they occur in similar structural environments. If this correlation is true, then the evidence from the Highland Border requires re-interpretation. Read[2] suggested that the fossiliferous Cambrian limestones might be tectonic intercalations within the Dalradian. Alternatively it might be suggested that Lower Palæozoic outliers have been folded in with Dalradian basement rocks during the Caledonian evorogeny, and that Caledonian polyphase evorogenic structures are overprinted on earlier Pre-Cambrian polyphase recumbent structures along the Highland border.

In the working hypothesis at present favoured by us, two post-Lewisian orogenic systems are believed to cross Caledonian Britain. The earlier (possibly contemporaneous with the Carolinides of east Greenland, the Gardar Cycle of south Greenland and the Dalslandian of Sweden) culminated in a pre-(720–765) m.y. evorogeny which involved Moine, Dalradian and Monian rocks. On a basement consisting of the regional metamorphic rocks, migmatites and intrusives of this orogeny was deposited the Eo-Cambrian and Lower Palæozoic rocks of the Caledonian geosyncline. Over much of Northern Britain these deposits were thin, and both Eo-Cambrian and Cambrian strata are largely absent. Parorogenic disturbances of increasing intensity can be distinguished in various parts of this mobile belt, but the main evorogenic event that involved the whole Caledonian system did not begin until late in the Silurian. Paralic and terrestrial conditions appeared in western Ireland and in the Midland Valley of Scotland in the Upper Silurian (most probably in the Ludlovian, in rocks often erroneously referred to as 'Downtonian', but see the opinion of Westoll in the discussion on Rolfe[38]). Elsewhere Ludlovian troughs, slump beds and turbidites indicate crustal instability. Throughout the Caledonian mobile belt the evorogenic structures involve all strata older than Downtonian, including the high Ludlovian of the English Lake District and of South Wales. In many areas the Downtonian is missing, but where it is present, it rests unconformably on the older rocks (for example, at Stonehaven and on the south-east flank of the Towy anticline in South Wales). The Downtonian is followed conformably by the Lower O.R.S., and where it is absent, it is the Lower O.R.S. which is seen to be unconformable on the older Pre-Cambrian and Lower Palæozoic rocks of the mobile belt. In the Grampians the Lower O.R.S. is unconformable on Dalradian, in the Midland Valley it rests with strong unconformity on Ordovician and Silurian rocks. Angular discordance is absent at Lesmahagow, but in a regional synthesis George[39] finds evidence for major pre-Devonian orogeny with fold amplitudes of 10,000–15,000 ft. in the Midland Valley area. In the Southern Uplands the stratigraphical break is between Wenlock/?Ludlow and the Lower O.R.S.[37,40]. The Mell Fell conglomerate of the Lake District is rich in Ludlovian pebbles, and is regarded by Capewell[41] as being a Lower O.R.S. mountain piedmont deposit. In North Wales the earliest post-orogenic deposits are the presumed Lower O.R.S. rocks of Anglesey which accumulated in an intermontane basin unconformably on folded Ordovician and Silurian and were themselves folded and cleaved in what can only have been a mid-Devonian parorogeny. In Ireland a similar position is occupied by the unfossiferous Dingle Beds.

Only in the Welsh Borderland, outside the limits of the Caledonian mobile belt proper, was Ludlovian and Downtonian sedimentation undoubtedly continuous. Here Allen and Tarlo[42] refer to two extensive bone beds as indicators of major uplift in the adjacent geosyncline, suggesting its final transformation into a land area. The first of these bone beds occurs at the level of the top of the Aymestry Limestone. The disappearance of the graptolitic facies at this level and the presence of a major stratigraphical break with submarine gullying in the Leintwardine area[43] add support to the view that this bone bed represents the initiation of the culminating phase of the Caledonian evorogeny. The second bone bed is the well-known 'Ludlow Bone Bed' on the boundary between the Ludlovian and the Downtonian, after which deltaic, near shore and finally arid continental conditions rapidly ensued in the Welsh Borders and farther south.

Late Caledonian parorogeny is known to have been important after the deposition of the Lower O.R.S. in many parts of Britain. Middle and Upper O.R.S. can be seen in various places to be unconformable on Lower

O.R.S. and older rocks. Nevertheless, we believe that the major Caledonian structures in the Pre-Downtonian rocks of Britain reveal an overall tectonic unity that is distinct from the structural pattern seen within the Lower O.R.S. and Downtonian, and that the weight of isotopic and stratigraphic evidence suggests that these structures were produced in an evorogenic event that occurred in uppermost Ludlovian/earliest Downtonian times between (420 and 410) ± 10 m.y.

In Scotland it is suggested that the 420 m.y. ages are to be equated with the third phase (F_3) structures and metamorphism of the Highlands, the effects of which are superimposed and 'overprinted' on rocks folded and metamorphosed in earlier phases (bracketed letters refer to the terminology used in *The British Caledonides*[1]). The (F_3) movement phase of the Highlands is accompanied by retrogressive metamorphism in parts of central Perthshire, but elsewhere it is closely associated with the development of migmatites and with the magmatism responsible for early 'forceful' Caledonian granite bodies dated by Miller and Brown[16] as having been intruded around 420 ± 15 m.y. Evidence which suggests that the same episode of crustal stress was responsible for the major Caledonian folds (F_1 in the terminology of Helm, Roberts and Simpson[34]) of the Irish Sea area can be obtained in North Wales and Ireland. The post F_1 basic magmatism of North Wales has been given a minimum date of 411 ± 14 m.y. (ref. 19), and the grouping of five overprinted ages from Ireland between 424 and 414 m.y. suggests that the main Caledonian movements and metamorphism in both areas were around 420–410 m.y. Secondary chlorite produced by Caledonian dynamic metamorphism in the Pre-Cambrian granite-gneiss of the Gader Inlier on Anglesey revealed an age of 410 ± 16 m.y. (Table 1), thus defining a minimum age for the cessation of major Caledonoid dynamic deformation in the area (which includes both F_1 and F_2 in the terminology of Helm *et al.*[34]).

Thus it would appear that the F_1 episode of Britain south of the Highlands is to be broadly correlated with (F_3) of the Highlands, and that this event was the main Caledonian evorogeny of Britain, beginning in the Upper Ludlovian around 420 m.y. and continuing into the earliest Downtonian around 410 m.y. Major post-tectonic Lower O.R.S. volcanism and intrusive acid magmatism occurred in Scotland and elsewhere around 405 m.y., and a further episode of major granite intrusion is known around 395 m.y., still within the Lower O.R.S. From a consideration of the varying sedimentary pattern over Britain, it would appear that the growth and subsequent uplift of the major Caledonian structures in the geosynclinal belt south of the Highlands, although beginning in the Upper Ludlow, did not finally exclude the sea from all areas until Lower O.R.S. times, when continental conditions became general and Caledonian post-tectonic granite magmatism was at its height.

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TECTONO-MAGMATIC EVOLUTION OF THE SVECOFENNID CHELOGENIC CYCLE IN SOUTH GREENLAND

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RECENT compilation of isotopic age determinations from South Greenland[1], together with work on a newly discovered appinitic suite and the recognition of its orogenic significance[2], have led to the suggestion that the geology of South Greenland may best be understood in terms of a developing chelogen[3]. The information contained in this article is based on work of the Geological Survey of Greenland. The ideas expressed are our own and will be tested by relevant isotopic age determinations as they become available. A programme to provide these determinations has been started recently at the Mineralogical Museum, Copenhagen. In view of the renewed interest in continental drift and the key position of South Greenland in relation to Europe and America it seems worth while presenting an interpretation based on the detailed geological knowledge of the area combined with the few known age determinations.

As recognized by Wegmann[4] the rocks of South Greenland fall into three main groups: (*a*) an older gneissose basement preserved as cores to major structures and as conglomerate pebbles (the pre-Ketilidian); (*b*) a Ketilidian series of metasediments and metavolcanics lying unconformably on the pre-Ketilidian basement, together with gneisses and igneous rocks; (*c*) the Gardar—a younger

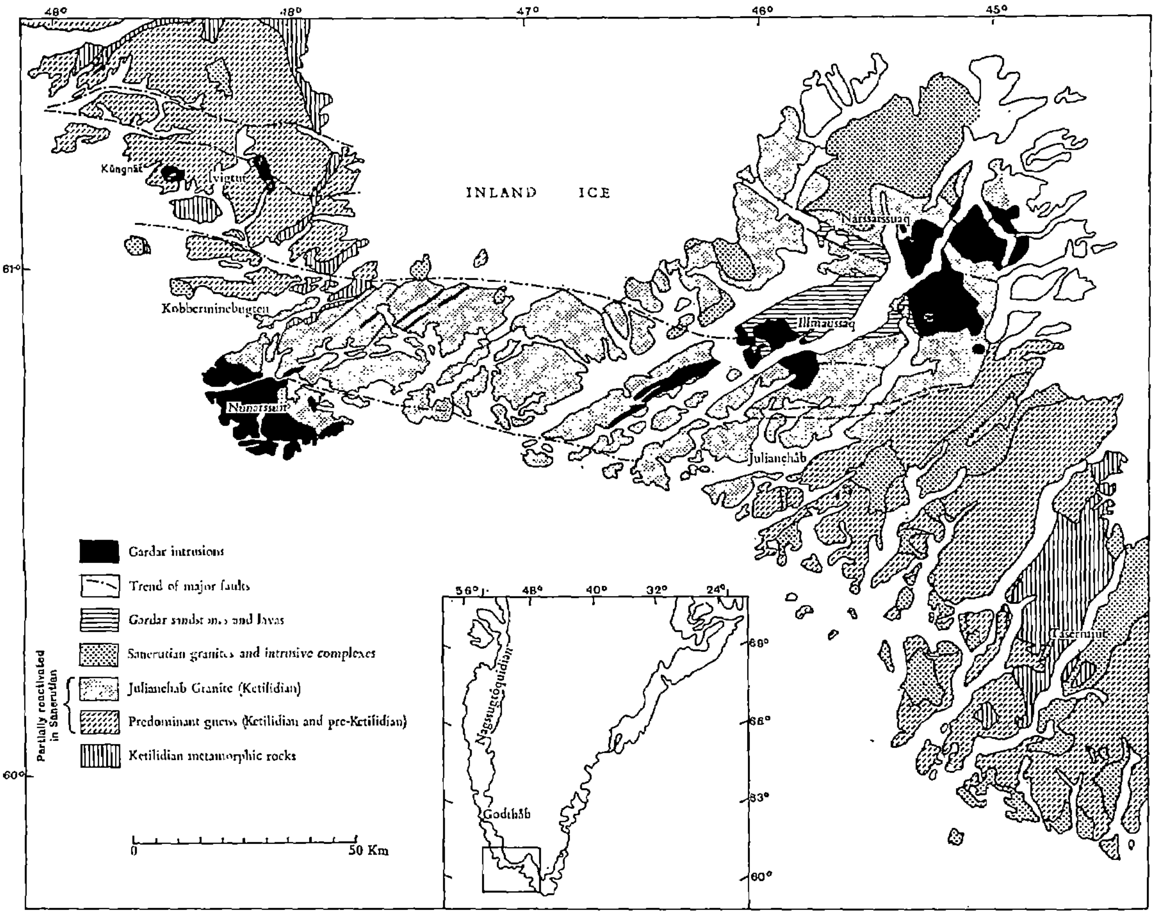
48°
48°
47°
46°
45°
61°
60°
INLAND ICE
Kûngnât
Ivigtut
Kobberminebugten
Nunarssuit
Narssarssuaq
Ilímaussaq
Julianehåb
Tasermiut
Gardar intrusions
Trend of major faults
Gardar sandstones and lavas
Sanerutian granites and intrusive complexes
Partially reactivated in Sanerutian
Julianehåb Granite (Ketilidian)
Predominant gneiss (Ketilidian and pre-Ketilidian)
Ketilidian metamorphic rocks
0
50 Km
56°
48°
40°
32°
24°
68°
66°
63°
60°
Nagssugtoqidian
Godthåb

Fig. 1. Map of the general geology of South Greenland

series of unmetamorphosed sediments (dominantly arkoses) and lavas with an alkaline to per-alkaline suite of igneous rocks.

The pre-Ketilidian basement which possibly forms a major part of the west coast of Greenland has not been recognized as mappable units south of Kobberminebugten (Fig. 1). Its only interest in this article is to note that some confusion over the term Ketilidian exists. This is due to the use of the term in the Ivigtut region for a series of reactivated gneisses and granites[5] some of which are now known to be below the Ketilidian basal conglomerate[6]. Age determinations of between 3,000 m.y. and 2,300 m.y. come from areas in which the rocks could be reasonably interpreted as being pre-Ketilidian.

The Ketilidian geosynclinal rocks preserved in the Ivigtut region show the following succession: basal conglomerate, quartzites, dolomites, pelites, greywackes, pillow lavas, pyroclastics and basic flows and sills. A second well-preserved area of supracrustal rocks exists around Tasermiut fjord where the upward (probably stratigraphical) succession is: pelitic schists, quartzites, and basic metavolcanics[7]. The main Ketilidian metamorphism varies from epidote-amphibolite facies in the north to granulite facies in the south. Three main episodes of folding occurred of which the dominant gave north-east trending axes. Syn- and late-tectonic granitization formed mainly oligoclase bearing gneisses. Further introduction of felsic material probably associated with re-mobilization of considerable amounts of pre-Ketilidian gneisses formed the Julianehåb granite, a variable mass covering at least 8,000 square kilometres between Kobnerminebugten and Julianehåb[8]. Relict structures within the granite generally correspond to the dominant north-east fold direction. The foliated granite and gneisses contain thin concordant or slightly discordant, foliated, folded and granitized basic sills and dykes which probably represent relics of the feeders to geosynclinal basalt activity[7,9]. More discordant to the granite are a younger series of basic and intermediate dykes (second period dykes). These were probably emplaced mainly as dolerites into cold rocks[9,10], but were later metamorphosed and granitized. The return to plutonic conditions which this indicates, called the Sanerutian and dated between 1,650 and 1,500 m.y., has previously been considered as a thermal event essentially independent of the main phase of granitization described here, and has been regarded as a marginal effect of the Nagssugtoquidian fold belt[11,12]. This supposition was based mainly on the correlation of the main phase of Ketilidian granitization with the gneisses dated from Godthåb at 2,700 m.y.[13]. However, the recognition of pre-Ketilidian basement north-east of Ivigtut reduces the likelihood of the correlation. It now seems possible that the main phase of Ketilidian plutonism may correspond to the widespread thermal activity in the Canadian shield at about 1,800 m.y. although no proved rocks of this episode in South Greenland have been dated. If it is true that the Ketilidian and Sanerutian plutonic episodes are closely spaced in time, the chronological significance of the period of basic dyking separating them is considerably reduced; this may only represent a temporary volcanic phase in the development of a single orogeny.

A factor which supports the idea that the two plutonic episodes belong to the same orogeny is the recognition of an appinitic suite of rocks intruded immediately following the second period basic dykes. These rocks vary from alkali-noritic gabbros to hypersthene–augite or hornblende bearing diorites and hypersthene monzonites. The most thoroughly investigated group of these rocks occurs north of Narssarssuaq[2]. In this area there is a close connexion between the intrusion of the large basic and intermediate masses and the formation of younger granite by reactivation of older granite and gneiss. Relict second period basic dykes are commonly present within the younger granite although parts of the granite show signs of having been mobile, for example, they contain a closely mixed assortment of angular xenoliths.

Elsewhere in South Greenland the granites formed in conjunction with comparable basic rocks are essentially allochthonous and not formed directly from the surrounding gneisses[14]. Other areas of reactivation of earlier rocks are widespread in the Julianehåb granite without recognizable connexion to the intrusion of basic masses. The reactivation is best recognized by its effect on the second period basic dykes although it can be suggested in areas where the granite has recrystallized after a period of shearing in non-plutonic conditions. Outside the main area of Julianehåb granite younger granites are commonly associated with belts of renewed movement parallel to the main Ketilidian structural trend[9].

An intrusive phase of microdiorite sheets occurred contemporaneously with the major diorite masses. Outside the main zone of granite reactivation there are also occasional closely spaced swarms of intermediate dykes. These may represent feeders to orogenic andesitic lavas which have been completely removed by erosion. Other minor basic and intermediate intrusions were emplaced after the peak of reactivation but before the end of plutonic conditions. Three main types have been recognized: dykes intruded into hot shear zones while movement was still taking place[9], dykes intruded into mobile granite[14] and sills and sheets the intrusion of which was closely followed by aplitic granite forming 'net-veined diorites'[15]. Some of the late microdiorite sheets grade into hornblende lamprophyres with interstitial calcite and locally minor phases approaching carbonatites are developed.

The appinitic intrusive complexes north of Narssarssuaq show many similarities to the type occurrences in Scotland. They also have a closely comparable position in the orogenic chronology[16,17], that is, they were intruded after the main periods of folding, metamorphism and granitization but just before the formation of the younger granites. Sutton[18] considers that in Scotland there is just one orogenic cycle represented (that is, Caledonian) and similarly in South Greenland the events from the Ketilidian sedimentation to the end of the second phase of plutonism could be regarded as one complete orogenic cycle. These appinitic rocks appear to form normal members of an orogenic calc-alkaline suite. They also show a sub-alkali development which corresponds to Vorobieva's[19] folded-structure association of alkali rock petrogenesis. In particular, large masses of hypersthene monzonite and locally syenite appear to have formed by diffusive potassium enrichment of the central parts of a basic-intermediate magma chamber consequent on the earlier crystallization of the margins and roof of the chamber. Examples of bodies believed to have been formed by similar processes have been described by Boone[20] and Vance[21]. The tectonic setting of these rocks can perhaps be explained by considering the possible later stages in the development of a root-zone of an orogenic belt. After the main compressional period of folding, metamorphism and granitization, down-dragging convection currents beneath the belt died down and eventually ceased. The orogenic pile cooled and started to rise up due to isostasy. Partial tensional conditions now allowed the introduction of simatic material (presumably tholeiitic) represented in South Greenland by the second period basic dykes. Because of the orogenic environment this basic magma became extensively contaminated by sialic material with the consequent formation of a high proportion of intermediate igneous rocks. Rising palingenetic acid magmas possibly supplemented with newly differentiated acid material would tend to follow the more basic intrusions. Abundant wrench faulting and locally the development of foliation and folding could be expected to accompany this phase.

If this interpretation is correct the Sanerutian events could be the logical epeirogenic uplift phase of the orogeny which started during Ketilidian time rather than the expression of a separate orogenic development as has been suggested previously. It should be noted, however, that it is perfectly possible that a younger orogeny was developing at this time further away on the flanks of the original orogenic belt. It is also possible that a minor orogenic belt comparable to the Labrador Trough[22] was superimposed on the epeirogenic uplift phase of the Ketilidian–Sanerutian orogeny.

Gardar sedimentation of mainly coarse arkosic sandstones and volcanism, the main phase of which started as fracture-controlled agglomerate vents followed by basic lavas with alkali tendencies[23], began in basins controlled by major east-south-east wrench faults[24]. These faults were probably initiated during the Sanerutian plutonic phase[25] and remained active at least under the initial stage of Gardar sedimentation. From comparisons with the post-orogenic sediments in other areas, for example, the Caledonides[17] and the Appalachians[26] it seems probable that these sediments were deposited soon after the end of plutonism and may have overlapped the formation of the younger granites elsewhere.

The Gardar now preserved in South Greenland consists dominantly of intrusive igneous rocks. This activity can be divided somewhat arbitrarily into three phases[27]: (*a*) Early Gardar in which nepheline syenites, carbonatites, lamprophyres and trachytes predominate[28]. (*b*) Mid-Gardar characterized by several generations of olivine dolerites, trachydolerites, microsyenites, larvikitic dykes and giant composite gabbro–syenite dykes. Many of the dykes contain large masses of anorthosite and labradoritic feldspar[29]. Tensional conditions leading to the formation of dykes trending north-east were dominant in the mid-Gardar. (*c*) Late-Gardar characterized by major alkaline complexes including Nunarssuit[30], Kûngnât[31] and Ilímaussaq[32]. The tectonic control of the late-Gardar appears to have changed from that of the mid-Gardar; instead of the dominant north-east trending fissures the late-Gardar complexes are approximately equidimensional and emplaced by stoping. Reliable age determinations are limited to late-Gardar rocks which range from the Ivigtut Granite at 1,255 m.y.[33] to the agpaitic rocks of Illímaussaq at 1,020 m.y.[34] (modified from Moorbath *et al.*). The Gardar igneous activity must therefore have lasted at least 250 m.y. and possibly lasted 300–400 m.y. Whether there is a major break between any of the phases is not known.

The early-Gardar alkali magmatism occurs in a similar setting to the igneous activity of the Midland Valley of Scotland, the Oslo province, and the White Mountain magma series of New Hampshire[17]. All are associated with fault zones which probably developed during the final epeirogenic uplift at the end of an orogeny. They agree with the general setting described by Vorobieva[19] for alkali magmatism of platform type in the U.S.S.R. She remarks that alkali magmatism occurs in unstable conditions in the late or last stages of a tectono-magmatic cycle. Yoder and Tilley[35] suggest that the initiation of an alkali basalt trend takes place at a greater pressure than a tholeiitic trend. They equate the difference in magma type to a difference in depth of formation.

Mid- and late-Gardar intrusions were emplaced between 250 and 500 m.y. after the end of plutonism in South Greenland. Similar alkali rocks were formed at a comparable time in the Canadian[36] and Scandinavian[37] shields immediately before the widespread orogenic activity which marked the beginning of the Grenville chelogenic cycle. At the present time, at the presumed end of the Grenville chelogenic cycle[8] alkali rocks are being formed along deep fracture zones, for example, the African rift valley, and have been formed in the Tertiary volcanism of Greenland and Scotland.

Sutton[3] suggested that with the development of a chelogenic cycle orogenies are progressively restricted to the margins of the continental masses probably consequent on the fusion of subcontinental convection cells. The same process results in disruption of the continental masses. This disruption might be expected to be the cause of exceptionally deeply penetrating tensional zones which in turn may result in alkali magmatism. From the global point of view, as Sutton has noted, continental disruption produces dominantly basaltic magmatism, but it seems likely that this leads to alkali rocks genesis in areas where post-orogenic fissures were developed to great depths.

Geological evidence suggests that the Sanerutian plutonic episode may represent the epeirogenic uplift phase of the orogeny which started in Ketilidian time. If the main Ketilidian metamorphism proves to have a date of about 1,800 m.y. this orogeny would then be an early event in the Svecofennid chelogenic cycle. The Gardar alkaline activity may be due to continental disruption during the later phases of the chelogenic cycle. The whole sequence of events forms a characteristic tectono-magmatic cycle[17,38]. The location of the Gardar alkali activity may be due to a fundamental weakness in the crust developed during the epeirogenic uplift phase of the Ketilidian–Sanerutian orogeny which continued to control magmatism marginal to the early phases of the Grenville orogenic belt, which probably passed just south of what is now Greenland.

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A METHOD FOR DESCRIBING THE PACKING TEXTURE OF CLASTIC SEDIMENTS

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PACKING is one factor determining the texture of a clastic sediment. Pettijohn[1] defines texture as the size, shape and arrangement (packing and fabric) of the component elements of a sedimentary rock. Arrangement is a rather neglected factor; Krumbein and Pettijohn[2], surveying the field of sedimentary petrography in 1938, gave detailed consideration to size and shape but did not mention packing.

Packing is usually considered in terms of systematic arrangements of equal spherical particles and reference is often made to the work of Graton and Fraser[3], who examined several simple regular packs and suggested that these had a geological significance. A much more complete investigation of simple systematic sphere packings has been made[4], but this approach is not likely to yield a realistic representation of the packing in a clastic sediment. With allowance made for the idealized particle the regular model suffers from one great defect: it is supposed to represent a random structure.

Two problems present themselves: (1) How is this random packing to be represented? (2) How can an applicable method be devised whereby determinations of packing textures of real clastic sediments can be made? An attempt has been made to solve problem (1) by using the radial distribution function developed to describe the structure of liquids[5]. A reference particle is chosen and the variation in density around it is represented on a graph of density against distance from the reference particle. This graph effectively shows the radial distribution of the particles around a representative particle and may provide a useful means of describing and comparing the packing textures of clastic sediments. Some fairly elaborate experiments have yielded radial distribution graphs for packs of equal spheres[6,7], the tricky part of these experiments being the measurement of the

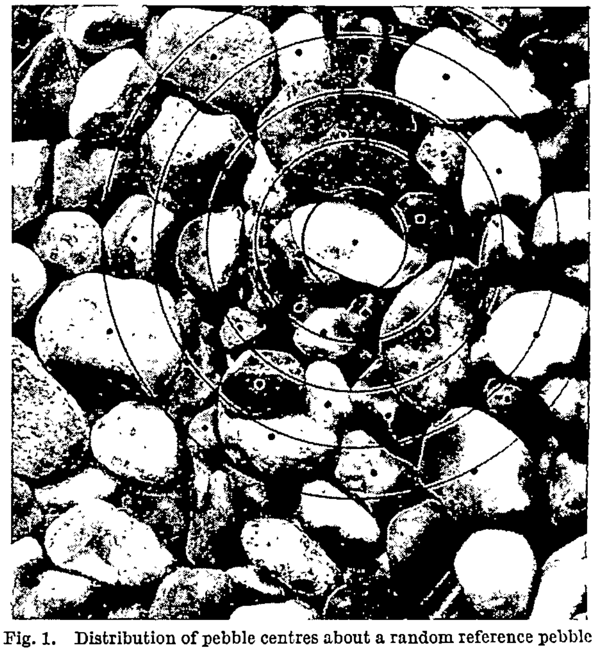
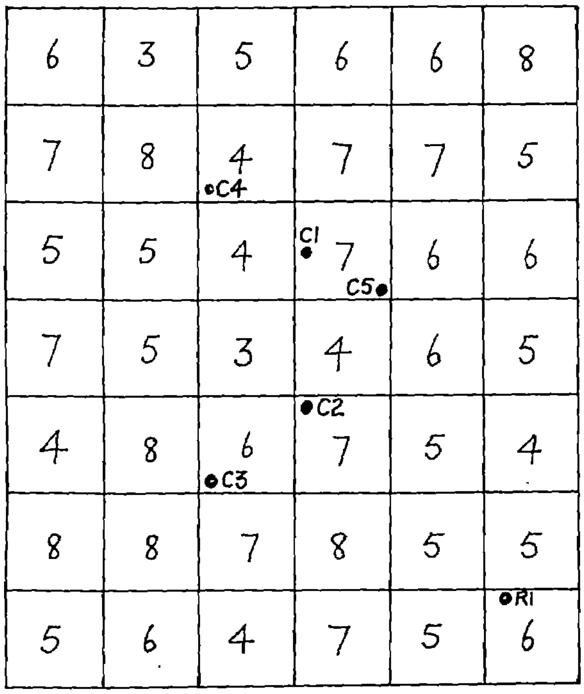


Fig. 1. Distribution of pebble centres about a random reference pebble



6	3	5	6	6	8
7	8	4	7	7	5
5	5	4	7	6	6
7	5	3	4	6	5
4	8	6	7	5	4
8	8	7	8	5	5
5	6	4	7	5	6

Fig. 2. Grid showing positions of pebbles $C1$–$C5$ and $R1$. The number of pebble centres in each cell is also shown

three-dimensional position of the particles. Most geological situations present only two-dimensional accessibility and to be useful a technique must allow for this. The method described here relies heavily on the assertion by Prins[8] that random two-dimensional structures have all the essential features of three-dimensional structures. He was referring to packs of similar particles and gave some experimental data.

This article describes three attempts to obtain indications of packing texture for clastic structures. Two of these attempts were on shingle beaches and the third on an orthoquartzite rock. The basic technique adopted for the beach material was to photograph the surface—this gives a two-dimensional representation of the pack—and then choose a random pebble and with it as centre draw concentric circles on an enlarged print. These circles increased in radius by a constant amount each time. The number of pebble centres within each annular zone was counted and tabulated. A structural factor related to the density was calculated by dividing the number of pebble centres in a zone (n) by $2\pi R$, where R was the number of the zone. Fig. 1 shows some pebble centres and zones marked on a print of pebbles from Hythe beach. A sample of pebbles was taken from one spot on Hyth Beach (National Grid reference 61/160340). These we later poured into a tray and photographed. The tec nique worked for this special case, so some photograp of a small area of actual beach surface were taken Dungeness (61/091167), just about at the spot marked on the Lewis and Balchin map reproduced by Steers[9].

The technique for the beach is quite straightforwar A photograph of the beach is taken, with the came carefully adjusted so that the film and the beach surfa are parallel, and an enlarged print with good contrast made. A square unit reference grid is drawn on the pri and each pebble is identified and its centre marked. a reference object is included in the photograph the sca can easily be indicated. The number of pebble centr in each square of the grid is counted; this gives som

Table 1. DISTRIBUTION OF PEBBLE CENTRES ABOUT FIVE RANDOM REFERENCE PEBBLES IN HYTHE BEACH SAMPLE

Numbers of pebbles in surrounding 2×2 cell groups are also indicated

Circle No.	R	1	2	3	4	5	6	7
C1.21*	n	0	7	6	13	18	18	22
	$\frac{n}{2\pi R}$	0	0·556	0·318	0·515	0·573	0·479	0·500
C2.20	n	0	5	4	11	13	16	19
	$\frac{n}{2\pi R}$	0	0·398	0·210	0·435	0·415	0·425	0·430
C3.29	n	0	9	6	13	16	18	22
	$\frac{n}{2\pi R}$	0	0·716	0·318	0·516	0·510	0·479	0·500
C4.21	n	0	7	5	11	13	18	21
	$\frac{n}{2\pi R}$	0	0·556	0·265	0·435	0·415	0·479	0·479
C5.23	n	0	7	6	11	14	15	21
	$\frac{n}{2\pi R}$	0	0·556	0·318	0·435	0·445	0·398	0·479

* Approximate

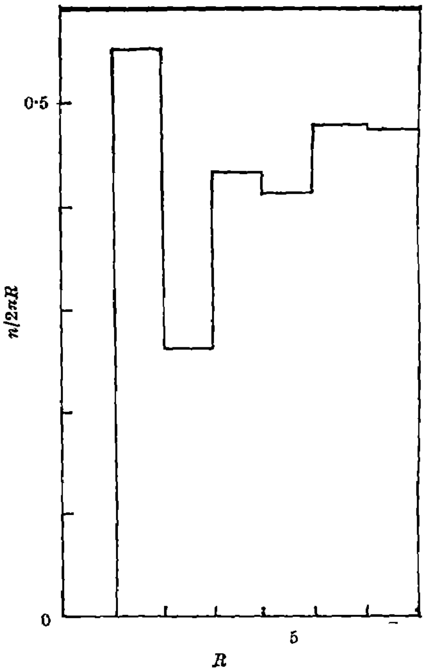


Fig. 3. Variation of $n/2\pi R$ with R around pebble $C4$

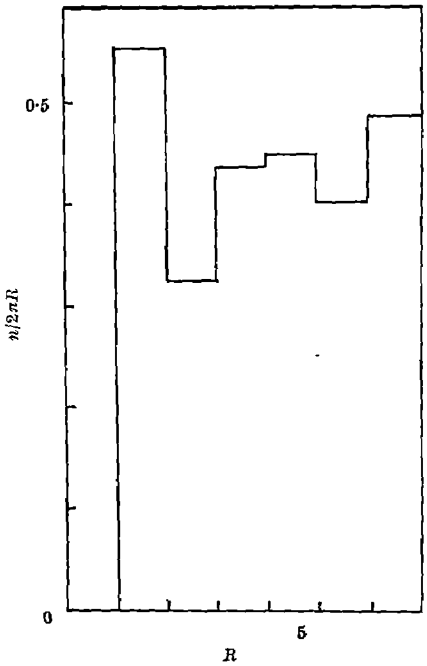


Fig. 4. Variation of $n/2\pi R$ with R around pebble $C5$

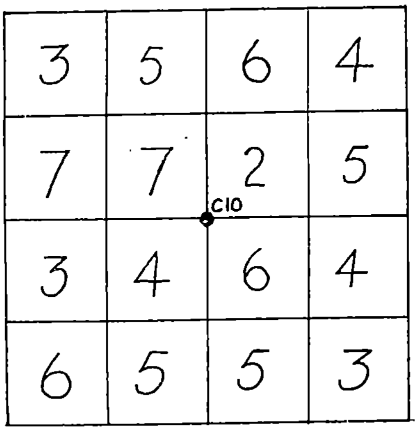


Fig. 5. Reference grid for a small part of Dungeness beach, around centre *C*10

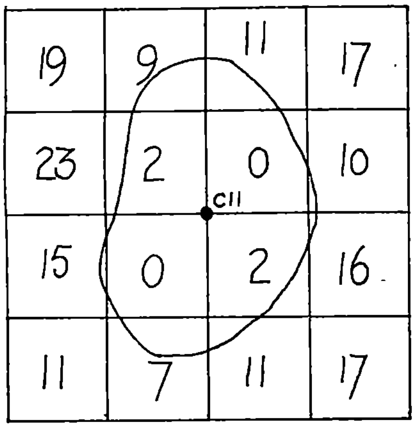


Fig. 6. Reference grid for a small part of Dungeness beach, around centre *C*11

idea of the evenness of the distribution and the sizes of the particles. Fig. 2 shows a reference grid for the Hythe pebbles with the number of centres marked. The basic cell of the grid is a square of side 2 cm (all measurements refer directly to the real packing).

The marked position *R*1 refers to the scale reference pebble; this one was noted and measured and the measurement compared to the photographic image. The positions of the reference pebbles used for the determination of packing variation are marked *C*1–*C*5. With one of these as centre concentric circles were drawn on the print, the radii of these circles increasing by a constant factor, in this case 6 mm. The number of pebble centres inside each successive annular zone were counted and tabulated; these provide values of n (Table 1). The circle number is indicated by R. If n is divided by $2\pi R$ a value is obtained which is related to the packing density; this value is plotted against R and the graph indicates the variation in packing density around a typical particle (Figs. 3 and 4). For the Hythe grid the mode pebble occupation of a cell is 5 (11 occurrences) and the mean is 5·8. A square (2 × 2) cell group containing about 22 pebbles was considered a reasonably representative area, that is, around *C*1, *C*4 or *C*5. *C*3 falls in a high-density area, and this is shown by the large value of $n/2\pi R$ for $R = 2$ in Table 1. The opposite is true of *C*2.

The mode pebble size in the sample examined was about 1 cm diameter although the pebbles were far from spherical. The sample was split into smaller samples and the pebbles were assigned to Zingg shape classes; the node shape was oblate (class 1).

Eleven photographs were taken at Dungeness, all fairly close together and near the water's edge. These showed a considerable variation in the particle size in a small patch of beach. Two were selected and the packing details are given here. Figs. 5 and 6 show reference grids, and Fig. 7 shows the comparative distributions. One of these illustrates an unusual case, a large pebble surrounded by much smaller pebbles. The large pebble has been chosen as the centre, and its outline is roughly indicated in Fig. 6. The reference grids have 4-cm cell sides and R increases in steps of 1 cm.

Two photographs in a paper by Heald[10] provide examples of packings in consolidated sediments. These are of two samples of St. Peter sandstone from Missouri. One of these has distinct rounded separate detrital grains and contains no clay; the other is a clay-bearing highly presolved rock with close sutured contacts between the grains; it was collected 2 in. below the first sample. Since the magnification was 55 times, drawing circles

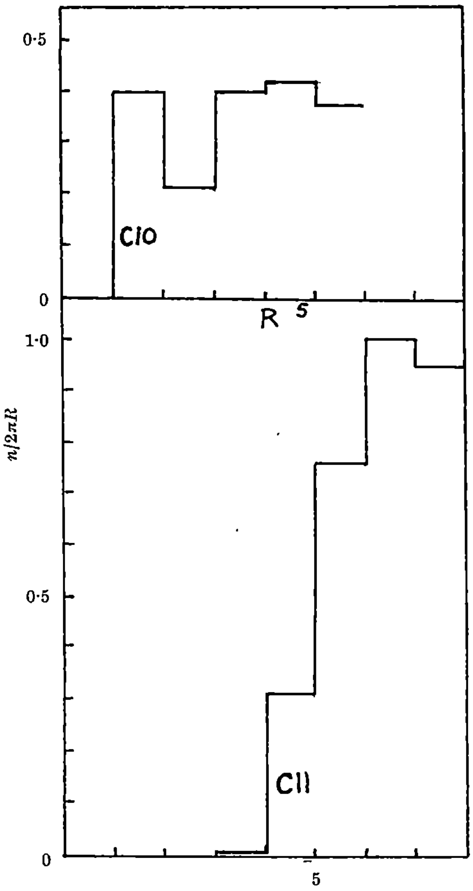


Fig. 7. Radial distributions around pebbles *C*10 and *C*11

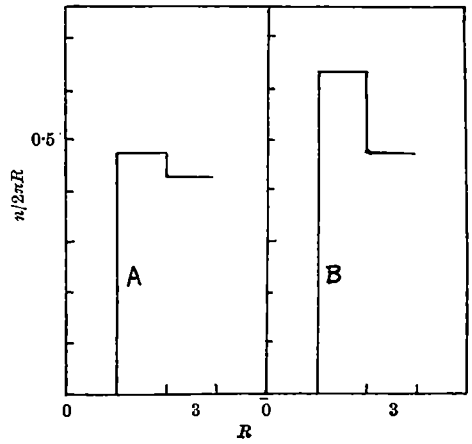


Fig. 8. Packing in sandstone samples; *A*, clay-free sandstone; *B*, sandstone containing clay

with radius increments of 11 mm gave a real value of 0·2 mm. Because of the small size of the photographs only a few circles could be drawn, but the results (Fig. 8) show that a tighter packing is produced in the clay-bearing sandstone. The photographs are too small to give very good results, but with sufficient centres chosen from a large picture and a careful choice of radius increment for the reference circles, packing textures could be easily and conveniently determined.

I thank Ted Willett and Valerie Smalley, both of this College, for their assistance.

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SEMICONDUCTION IN KAOLIN

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It has been reported that the electrical conductivity of kaolin increases with temperature[1]. This observation indicates that kaolin may be a semiconductor. In absence of usual confirmatory experiments, an attempt is made here to ascertain the semiconducting nature of kaolin from less direct evidence as adsorption of lime by kaolin in presence of water and rheology of dilute aqueous suspensions of kaolin. The possibility that kaolin may be a ‘*p*’ type semiconductor is indicated.

From the results of measurements on the adsorption of lime at ordinary temperatures by kaolin, kaolin heated at 800° C with or without addition of zinc oxide and of electrical conductivity of these materials at different temperatures, given elsewhere[1,2], it will be seen that at any temperature the electrical conductivity of kaolin, previously heated with zinc oxide, is lowest and that of unfired kaolin is highest[1]. Amount of lime chemisorbed from saturated lime water at ordinary temperatures, in a given time, is highest in kaolin fired with zinc oxide and least in unfired kaolin[2]. This antiparallel behaviour of lime adsorption and electrical conductivity means that the reaction between lime and kaolin in the presence of water is a semiconductor surface reaction which may either be an ‘*n*’ type reaction on ‘*p*’ type semiconductor or vice versa depending on the type of semiconduction of kaolin.

It is stated that, at the temperatures worked with, kaolin previously fired at 800° C has higher conductivity than kaolin which has been similarly fired with zinc oxide[1]. Thus on addition of an ‘*n*’ type semiconducting oxide (ZnO) the conductivity of fired kaolin is lowered. This may occur, if the semiconduction in fired kaolin is predominantly by ‘holes’, through the combination of these with the electrons introduced by ZnO. Again, kaolin fired at 800° C has lower conductivity than unfired kaolin[1]. This may indicate that the effect of dehydration is to remove ‘holes’ from unfired kaolin (dehydration taking place through transfer of ‘holes’ from the crystal). Thus, kaolin may be a ‘*p*’ semiconductor itself; effect of dehydration and heating it with ‘*n*’ type semiconducting oxide is to change it into a more and more ‘*n*’ type semiconducting oxide.

In a recent communication[3] the rheological behaviour of dilute aqueous suspensions of some reactive solids, as lime, portland cement and plaster of paris, has been explained on the assumption that the reaction between these materials and water is a chemisorption process. In such a process the position of the bulk Fermi-level in the particles controls the coverage of chemisorbed molecules. The coverage determines the volume over which stabilization of water lattice takes place and the viscosity of the suspension depends on the extent of the volume over which water structure is stabilized. It is concluded that the rheological behaviour of such suspensions is controlled by the position of bulk Fermi-level in the particles in suspensions. Again, plasticity of clay–water systems has been explained on the assumption that in such a system a layer of water is rigidly attached on the surface of the clay mineral[4]. The nature of the interaction between the water layer and the clay mineral surface is, however, uncertain. Assuming that a chemisorption process is occurring, hydroxyl groups may get attached on the surface of the clay mineral through a transfer of electrons from the crystal (‘*n*’ type reaction) as in the reactions between portland cement, clinker and water[6], and lime and water[7]. If it were so, then the rheological behaviour of dilute suspensions of kaolin in water would be controlled by the position of bulk Fermi-level in the crystal: viscosity will increase or decrease as the position of the Fermi-level is raised or lowered. For the present purpose one has to know what would happen to the viscosity of aqueous suspensions of kaolin which has been previously allowed to react with lime in the presence of water. It has been found that dilute aqueous suspensions of kaolin, so treated and then washed and dried, have lower plastic viscosity than similar suspensions of H-kaolin[5]. This means that, on reacting with lime, Fermi-level of kaolin has been lowered. This can only happen when the reaction between lime and kaolin in the presence of water is an ‘*n* type reaction. With the reaction type settled, the antiparallel behaviour of lime adsorption and electrical conductivity would mean that kaolin is a ‘*p*’ type semiconductor, provided the assumption regarding the nature of interaction between kaolin and water is correct.

The electrical nature of kaolin appears to be similar to that of portland cement clinker. Possibility of the latter behaving as a ‘*p*’ type semiconductor has been indicated elsewhere[6]. The hydraulic action of cement clinker also increases with the decrease of electrical conductivity of clinker[6] as in the case of adsorption of lime by kaolin in the presence of water. Parallelism in the nature of these reactions is clear. The Fermi-level appears to be the controlling factor.

It is concluded that there is a possibility that kaolin may be a ‘*p*’ type semiconductor and that some of its physical and chemical properties may be controlled by the position of bulk Fermi-level in it. The latter suggestion should also be true for other silicates which may prove to be semiconductors. This approach, then, will open a new aspect of chemistry and technology of such silicates.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Structure of the Solar Chromosphere

The suggestion of Biermann[1] and Schwarzschild[2], that the radially increasing temperature of the solar atmosphere is to be ascribed to propagation of non-thermal energy, such as acoustic waves, is now generally accepted. The effect of magnetic field, and the possibility that the energy-carrying waves are of a magnetohydrodynamic character rather than acoustic, has been considered seriously[3]. However, the fact that the density and temperature of the chromosphere are significantly affected by magnetic field only when the field attains the strength ($\sim$ 50 gauss) which is found only in localized regions (plages) on the Sun's surface indicates that magnetic field is not of primary significance. Hence one may attempt to understand the energy propagation and its effects on the assumption that one can ignore the magnetic field and so consider only acoustic waves.

Although the acoustic waves generated at the photosphere by granular convection are estimated to be of small amplitude[3,4], it has been recognized that these waves may build up into shock waves, which would bring about a drastic increase in the absorption of the acoustic-wave energy. In a uniform gas, discontinuities are developed in waves of arbitrarily small amplitude if the waves propagate sufficiently far, but one should not expect this phenomenon to occur in an atmosphere in gravitational equilibrium, with which we are concerned, with wavelengths comparable with the scale height. This is because the dispersion relation for plane acoustic waves propagating vertically[4] is:

$$\omega^2 = \omega_0^2 + a^2k^2 \qquad (1)$$

where a is the speed of sound given by:

$$a^2 = \frac{\gamma kT}{m} \qquad (2)$$

if the gas is behaving adiabatically, and the resonant frequency, ω_0, is given by:

$$\omega_0 = \frac{a}{2H} \qquad (3)$$

where H is the scale height given by:

$$H = \frac{kT}{mg} \qquad (4)$$

In an ionized gas we must give to m the value of the average particle mass, and take $\gamma = 5/3$. We see from equation (1) that waves which are harmonically related in frequency are not harmonically related in wave-number and vice versa; hence one would not expect that the non-linear interaction between waves which leads to the generation of shock fronts would occur for waves of arbitrarily small amplitude.

There is another important way in which waves propagating in a 'resonant' medium defined by the dispersion relation (1) differs from waves in a non-resonant medium for which $\omega_0 = 0$. In a non-resonant medium, k decreases with height if the temperature increases with height, and this leads to refraction of rays away from the vertical. In a resonant medium, we observe that ω_0 decreases with height if temperature increases with height, and this will lead to an increase in k if the wave is launched with a frequency close to the resonant frequency, with the consequence that the wave would be refracted towards the vertical. Since the maximum value of ω_0 is the lower bound of the frequency pass band, and since this frequency (corresponding to a 5-min period) for the solar atmosphere is high compared with the turbulence frequency spectrum, we expect that the acoustic energy is carried principally by waves close to the cut-off frequency. Hence one may expect that, in the medium and high chromosphere, the acoustic waves are propagating almost vertically. This picture is in agreement with observations on vertical motions in the chromosphere reported by Leighton[5].

We now consider the energy carried by vertically propagating acoustic waves. If the waves were purely sinusoidal with a maximum flow velocity v_f, the energy density would be $\frac{1}{2}nmv_f^2$, half in kinetic and half in potential energy. Hence the energy flux per unit area, s, is given by:

$$s = \frac{1}{2}\, nmM^2a^3 \qquad (5)$$

if we write $M = v_f/a$ (the 'Mach number' of the flow) and approximate the group velocity by a. This equation may be re-written as:

$$n^2T^3 = \frac{4m}{\gamma^3k^3}\,\frac{s^2}{M^4} \qquad (6)$$

or, approximately:

$$n^2T^3 \approx 10^{23.9}s^2 \qquad (7)$$

if we consider 'Mach 1' flow ($M = 1$). The observed values of n and T for the solar atmosphere[6] are compared with curves s = const. in Fig. 1.

We see from Fig. 1 that the temperature begins to increase when the curve (n,T) intersects the curve $s = 10^5$ ergs cm^{-2} sec^{-1}. This is what one would expect if the energy flux of acoustic waves in the chromosphere at 4,000 km height and above has this value. If the value of temperature did not increase, the waves would have M values in excess of unity, leading to the formation of shock waves and enhanced dissipation; this would lead to local heating of the chromosphere. If, on the other hand, the temperature were to increase significantly more than required by the curve s = const., the acoustic waves would have M values below unity, so that no shock waves would be formed and there would be no heating of the chromosphere.

My assumption that a wave forms a shock only if $M \sim 1$ is a departure from the usual assumption that acoustic waves of arbitrarily small amplitude will form shocks[4]. This difference was ascribed to the form of the dispersion relation (1), which implies that waves which are harmonically related in frequency are not harmonically related in wave number; so that the non-linear wave interaction responsible for the build-up of shocks from small-amplitude waves is inhibited.

The present model enables one to understand the sharp transition from the chromosphere to the corona. The dispersion relation for waves in a medium with thermal conductivity $\varkappa$ is found to be[7]:

$$\omega\left(\omega^2 - \frac{\gamma kT}{m}\,k^2\right) = -\,i\,\frac{2}{3}\,\frac{\varkappa}{k}\,\frac{k^2}{n}\left(\omega^2 - \frac{kT}{m}\,k^2\right) \qquad (8)$$

In most of the chromosphere $\varkappa$ is sufficiently small that there is no dissipation due to thermal conductivity.

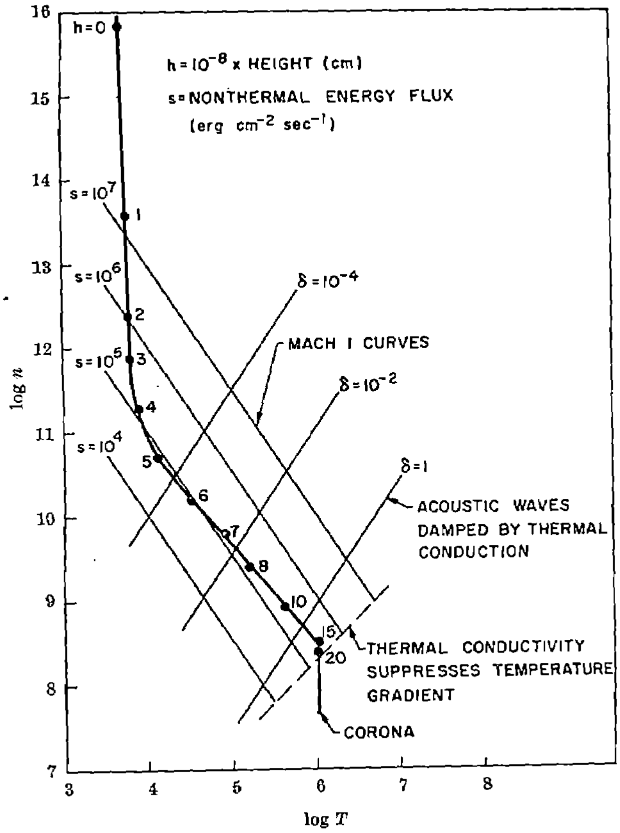

Fig. 1

It is clear from the form of equation (8) that the dissipation depends on the parameter δ, defined by:

$$\delta = \frac{2}{3}\frac{\kappa k^2}{nk\omega} \tag{9}$$

which is given approximately by:

$$\delta \approx T^{3/2} n^{-1} \tag{10}$$

if $\omega = 10^{2.3}$ and we adopt $\kappa = 10^{-6}T^{5/2}$, appropriate for a highly ionized gas[8]. The dissipation is small if δ is either small compared with unity or large compared with unity and is significant only if δ is near to unity. Curves δ = const. are shown in Fig. 1 and indicate that the transition from chromosphere to corona occurs just above the height at which thermal dissipation becomes significant.

Another effect of thermal conductivity is that the downward heat flux can become substantial. It will clearly be impossible to maintain the rise in temperature when the downward heat flux becomes comparable with the flux of acoustic energy. Hence it can be asserted that the transition to the corona must come about before the condition:

$$\kappa H^{-1}T = s \tag{11}$$

is satisfied. By using equation (7), this may be expressed as:

$$n = 10^{2.3}\, T \tag{12}$$

This curve also is shown in Fig. 1, and it is seen that the transition to the corona has occurred before this line is crossed.

The value $s = 10^5$ ergs cm^{-2} sec^{-1} represents a total power input of $10^{27.7}$ ergs sec^{-1} into the corona, a value which fits well with the estimates of energy loss from the corona made by Osterbrook[3]. We may note that the loss of energy by the solar wind is of the same magnitude if the plasma velocity is 500 km/sec and the plasma density about 10 protons cm^{-3} at the Earth's orbit.

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Gravitational Collapse

THE stellar-like radio sources ('quasars'), of which 3C48 and 3C273 are examples, appear to be objects with very small diameters yet the total energies of which lie in the range 10^{60}–10^{61} ergs. At the moment, the only source that has been imagined for the production of such high energies, is the Newtonian gravitational potential energy of a highly compressed mass. If a mass equal to some 10^8 solar masses could be compressed into a sphere the diameter of which equalled that of the orbit of Pluto, a gravitational potential energy of the required order of magnitude would become available. During the past thirty years a number of authors have shown that a compression of this kind is predicted by Einstein's equations of general relativity. It is postulated that the only force acting is the gravitational self-attraction of the material, that the density is uniform throughout the spherical mass at each instant of time and that the pressure is always zero. The last condition implies that the material remains at the absolute zero of temperature throughout the collapse to a small volume. These assumptions lead to the conclusion that, for such zero-pressure configurations, the motion continues until a singular state is reached in which the volume occupied by the material tends to a zero value whereas its density becomes infinite. The time of collapse is very short: at best it occurs in a few hundreds of thousands of years, but it may also be over in a matter of weeks or days, depending on the initial conditions.

I have been able to find a solution of Einstein's equations in which the restriction to zero pressure is removed. Both the pressure and the density are, however, uniform at each instant of time, but both rise to infinite values at the end of the collapse. When this happens the volume of the configuration is not zero, but has a small finite value. The time of collapse is also shortened as compared with the time for the corresponding zero-pressure case.

I have also shown how the zero-pressure solutions can be fitted to the external (vacuum) Schwarzschild solution of Einstein's equations. I have failed to establish this property for the solution with pressure. In order to examine this point, as well as a number of others which are mathematically too complicated to analyse through Einstein's equations, I have turned to a combination of classical gas-dynamics and Newtonian gravitational theory. It proved possible to establish the following results: (1) There are classical analogues of the zero-pressure general relativity motions which mimic them very closely and in which the motion is adiabatic. (2) Classical gas-motions with a non-zero pressure are possible but they are non-adiabatic. This means that energy must be extracted from the collapsing configuration if the motion is to continue. I conjecture that this is the reason why, in the general relativity case, the motions with pressure cannot be fitted to an external vacuum Schwarzschild field. The appropriate external field would presumably be one in which energy was present. (3) It is possible to solve with comparative ease the classical equations when pressure-gradients are present, in contrast to the situation in general relativity. The pressure-gradient, of course, provides an additional force which opposes the gravitational collapse. A specific example shows that the time of collapse is lengthened and that the configuration can finally come to rest with a volume of finite magnitude.

These investigations will be published in detail in the August 1964 issue of the *Astrophysical Journal*.

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GEOPHYSICS

Combined Effects of Drift and Diffusion in the *F*2 Region

The latitude distribution of f_0F_2 characteristically exhibits an anomalous equatorial trough which we shall refer to as the Appleton anomaly[1,2], although there is evidence[3] that earlier workers were aware of the dip angle dependence of $M_{\max}$ reported by Appleton. Results are available showing the variation of N with dip below the peak[4,5], and more recently satellite results have been reported for the topside electron density[6].

These satellite results have stimulated some theoretical interest[7], although, of course theoretical studies have been made continually[8–10] since Martyn's early proposal[11,12] *that the cause of the Appleton anomaly is a noonday upwards electrodynamic drift communicated from the dynamo regions. (In magnetohydrodynamics this drift may be re-interpreted as an outwards motion of the magnetic lines of force.)*

Theoretical studies have been hindered by the mathematical complexity of the fundamental $F2$ region diffusion equation[9,13–15], and we report here a simple examination of the problem of a self-maintained equilibrium layer in the absence of electron production and loss. It is sufficient to assume the electron density profile at the dip equator to be given, and we refer the reader to recent investigations of the $F2$ layer[16–18] which explain the method more fully.

We first observe that in the $F2$ layer N varies only slowly with respect to the dip angle I. Consider a small element of plasma being carried upwards by electrodynamic drift at right angles to a magnetic line of force as shown in Fig. 1. In the diagram, material is carried across the lines of force from A to B with speed $v^{\perp}$. But, provided AC is not large compared with AB, the electron density at C is the same as the electron density at A, where AC is horizontal. It follows that with this restriction, the significance of which will be discussed later, the drift at right angles to a magnetic line of force is equivalent to a drift v'', parallel to a line of force, such that if I denotes the dip angle:

$$v'' = v^{\perp} \cot I$$

The equilibrium diffusion-drift equation may then be transformed into an equation in one variable, and integrated under suitable boundary conditions. As in the absence of drift, individual lines of force may be regarded as isolated, using the equivalent drift v'', where the aforementioned approximation holds. In what follows we assume, in absence of other information, that $v^{\perp}$ is equivalent to a uniform drift w vertically upwards such that:

$$w = v^{\perp} \cos I = v'' \sin I$$

However, although we shall work in terms of the perpendicular height z above a fixed reference-level, the fundamental drift is the equivalent drift v'' and the fundamental equation is the diffusion equation along a line of force.

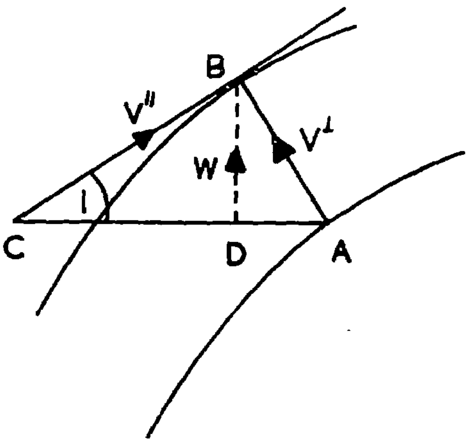

Fig. 1

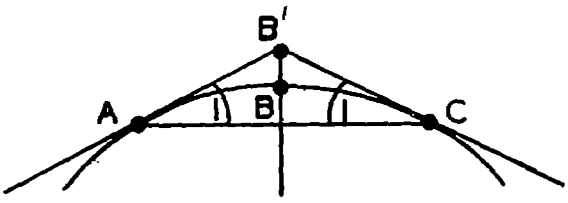

Fig. 2

Ferraro (private communication) has pointed out that the plane stratified $F2$ layer diffusion equation[19] is in fact of far wider application than is commonly supposed. This equation will be used here and the curvature of the Earth will be neglected. We suppose further that the electron density on the dip equator is given to be a Chapman profile with maximum electron density N_{m_0}, scale height H and height of maximum z_{m_0}, and that this boundary condition is to be applied to obtain a local solution of the fundamental equations under conditions of symmetry about the dip equator by replacing the problem at every field point by a straight field line problem. This is illustrated in Fig. 2. The solution at points A and C for the curved field line ABC is assumed to be approximated by the solution for the field line $AB'C$.

Denote by D the coefficient of diffusion, by H_1 the scale height of the neutral constituent and by H_2 the scale height of the ionized constituent. Then we define constants D_0 and w_0' by:

$$D = D_0 \exp(z/H_1)$$

and:

$$w_0' = wH_1/D_0$$

We also write:

$$w_0 = w_0' \operatorname{cosec}^2 I$$

Denote by x the horizontal distance from the dip equator, so that if a line of force crosses the equator at height z_0:

$$z = z_0 - x \tan I$$

Then we find, after reduction which will be reported elsewhere, that a solution of the equilibrium diffusion equation with drift is:

$$N = f(z + x \tan I) \exp(-z/2H_2 - w_0 e^{-z/H_1})$$

where f is an arbitrary function of $z + x \tan I$. Imposing the condition of a Chapman profile at the equator $x = 0$, evaluates f, giving:

$$\begin{aligned} N(x,z) = N_{m_0} \exp\tfrac{1}{2}[1 &- (z-z_{m_0})/H \\ &+ (H_2^{-1} - H^{-1})\, x \tan I \\ &- 2w_0\, e^{-z/H_1}(1 - e^{-x \tan I/H_1}) \\ &- e^{-(z+x \tan I - z_{m_0})/H}] \end{aligned}$$

Taking different equatorial profiles would, of course, give different solutions. To interpret these results for a curved field line model we put:

$$\tan I = 2 \tan (x/r_0)$$

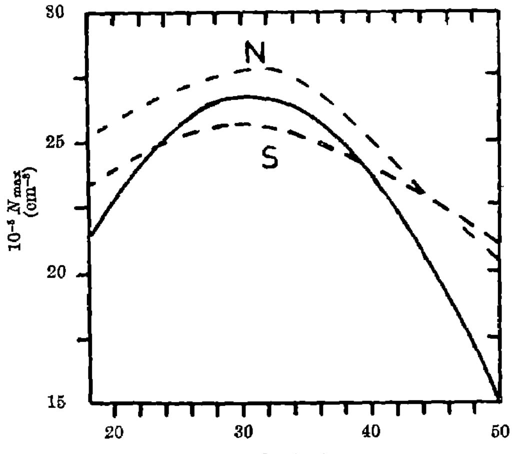

Fig. 3. The broken lines marked *N* and *S* denote measurements made by Croom *et al.* (ref. 4). The solid line represents our theoretical results

where r_0 is the mean geocentric distance of the *F*2 layer (= 6,550 km). Because the electron density varies appreciably over a vertical distance of 100 km and over a horizontal distance of 500 km we expect the aforementioned results to hold for:

$$\sin I \gg 0{\cdot}2$$

that is (say) for dip angles rather larger than 12°.

Fig. 3 shows a comparison of these theoretical results with measurements for:

$$H = H_1 = 100\text{ km}, H_2 = 107\text{ km}$$

The values of z_{m0} and w_0' are irrelevant since in the regions considered $x \gg H$ or H_1 and the curves obtained have the same shape for all values of z_m and w'. The theoretical curve was multiplied by a suitable constant, corresponding to N_{m0}, in order to give agreement in magnitude at the latitudinal maximum.

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Generation of Whistler Waves by Helical Electron Beams

SEVERAL investigations[1] published within the past few years deal with the interaction of a stationary plasma in the outer regions of the ionosphere (exosphere) with an assembly of charged particles trapped in the Earth's magnetic field. Such an interaction results in electromagnetic radiation within the audible range which is detected at the Earth's surface in the form of continuous white noise or whistlers. The trapped particles spiral around the lines of the magnetic field B_0 and form a 'helical beam', characterized by velocity components $\beta_\perp c$ and $\beta_\parallel c$ which are, respectively, perpendicular and parallel to B_0 (c is the velocity of light). A whistler wave thus produced and aligned in the direction of B_0 is usually described by the equalities:

$$\omega \approx \tilde{\omega} \equiv ck\beta_\parallel + \Omega_e(1 - \beta^2) \qquad (1)$$

$$F_p \equiv c^2k^2 - n^2\tilde{\omega}^2 = 0 \qquad (2)$$

in which ω is the frequency, k is the wave number, $\beta^2 = \beta_\parallel^2 + \beta_\perp^2$, Ω_e is the electron gyrofrequency, and $n = \omega_e^2/\omega(\omega - \Omega_e)$ is the refractive index for the whistler mode (ω_e is the plasma frequency).

The relationship (1) represents the condition of resonance, that is, the characteristic frequency $\tilde{\omega}$ of the whistler wave is equal to the Doppler shifted electron gyrofrequency. Equation (2) represents the dispersion equation for the stationary plasma which is valid only in the absence of the perturbation produced by the beam. The whistler waves described by equations (1) and (2) will be designated as whistler *P*-waves.

The purpose of this investigation is to point out that the *P*-waves described by equations (1) and (2) are not the only type of whistler waves which can be excited by a helical beam. There are also whistler *B*-waves which are resonant with the beam in accordance with equation (1) but do not satisfy the dispersion equation (2). Thus the plasma-beam interaction results in a *P*-instability and a *B*-instability. In a *P*-instability the growing waves (*P*-waves) result from the excitation of corresponding stationary waves in an undisturbed plasma. If the intensity of the beam is sufficiently small, the frequency of an excited whistler *P*-wave differs very little from the corresponding whistler wave in the undisturbed plasma. If the beam is linear, that is if the electrons in the beam have trajectories aligned along the direction parallel to that of B_0 ($\beta_\perp = 0$), there is only one type of instability[2] (*P*-instability). On the other hand, if the beam is helical ($\beta_\perp \neq 0$), there is an additional instability (*B*-instability) which gives rise to excited *B*-waves. A *B*-instability occupies a relatively wide frequency range in the neighbourhood of a *P*-instability and represents a continuation of the *P*-instability along the frequency axis.

The properties of the whistler *B*-waves are determined from the analysis of the dispersion equation for the plasma-beam system. This equation has the form[3]:

$$F_p + \sigma F_b = 0 \qquad (3)$$

in which $F_p \equiv c^2k^2 - n^2\omega^2$ represents the stationary plasma and σF_b is the perturbation produced by the beam. It is assumed that the perturbation is small, that is, $\sigma \ll 1$, where σ is the ratio of the beam density to the plasma density. One has[3]:

$$F_b = -\,\omega_e^2(1-\beta^2)^{\frac{1}{2}}\left[\frac{\omega - ck\beta_\parallel}{\omega - ck\beta_\parallel - \Omega_e(1-\beta^2)^{\frac{1}{2}}} + \frac{\beta_\perp^2(c^2k^2 - \omega^2)}{2[\omega - ck\beta_\parallel - \Omega_e(1-\beta^2)^{\frac{1}{2}}]^2}\right] \qquad (4)$$

Following the procedure of Zheleznlakov[3], one assigns in the dispersion equation (4) a real value of k and solves the equation for ω. The roots of equation (4) are represented as $\omega = \tilde{\omega} + \delta$, where $\tilde{\omega}$ is the frequency of a resonant wave defined in equation (1). An instability occurs when $\text{Im}\ \delta < 0$ and $|\text{Im}\ \delta|$ denotes the rate of growth of an excited wave.

It is usually assumed that mono-energetic electrons (characterized by fixed values of $\beta_\parallel$ and $\beta_\perp$) can excite only a single frequency *P*-wave described by equations (1) and (2)[1]. However, it follows from equation (4) that such a beam can excite not only a single frequency

P-wave but also a continuously distributed frequency spectrum of whistler B-waves.

When $\beta_{\parallel} \ll \beta_{\perp}$ the helical beam becomes 'almost circular'. This would occur, for example, near a mirror point for particles spiralling along the lines of the Earth's magnetic field. In such cases the possible occurrence of a P-wave within the range of whistler frequencies depends on the magnitude of the transverse velocity component $\beta_{\perp}$. A whistler P-wave can be excited only when electrons in the almost circular beam are relatively energetic. The transverse velocity component $\beta_{\perp}$ of such electrons must be larger than approximately 0·97. When $\beta_{\perp}$ is smaller than approximately 0·97, an almost circular electron beam cannot excite a whistler P-wave. It does excite, however, B-waves which occupy the entire whistler frequency range and are characterized by the rate of growth $|\text{Im}\ \delta|$, which is as follows:

$$|\text{Im}\ \delta| = \frac{\sqrt{2}}{2}\,\sigma^{\frac{1}{2}}\,\omega_e(1-\beta^2)^{\frac{1}{2}}\,\beta_{\perp} \tag{5}$$

It is noted that the rate of growth as given in equation (5) is independent of the frequency of the wave. The excited B-wave moves in the direction opposite to that of the beam.

It is generally recognized that a whistler P-wave moves in the direction opposite to that of the beam[1]. It represents, therefore, a 'counterstreaming instability'. It should be noted in that connexion that there are two whistler P-waves excited by an almost circular electron beam when $\beta_{\perp}$ is sufficiently large. One of these waves does indeed represent a counterstreaming instability. However, the other wave moves in the direction of the beam and its phase velocity exceeds the velocity of the beam. Therefore, it represents a 'sub-luminous instability'[2]. Both the counterstreaming and sub-luminous P-instabilities have frequencies in the immediate neighbourhood of $\Omega_e(1-\beta_{\perp}^2)^{\frac{1}{2}}$.

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PHYSICS

Simultaneous Sampling at Different Altitudes

A MODIFICATION of the technique reported earlier[1] for making simultaneous measurements at widely separated heights in the atmosphere is described. This promises to be more successful for heavier loads.

In the previous publication a balloon carried a cone of nylon cord with equipment to be exposed at two levels. At about 70,000 ft. one piece of equipment which was tied to one end of the cord was released and this fell freely, unwinding rapidly the string from the cone. The other end of the string was tied to the balloon which also supported the second piece of equipment. Some successful flights were obtained using nylon string lengths of 10,000–27,000 ft. supporting loads of 300 g wt. hanging from level balloons at about 90,000 ft. (The nylon used was 840 denier, which had a breaking strain without knots of 5,000 g wt.)

The new technique uses the same nylon cord but this is wound on a reel which has oil damping. Owing to the lower speed of fall of the equipment during this damped unwinding process compared with that for the previous flights, the magnitude of the impulse on the string at the end of the unwinding is reduced. One flight only has been performed using the modified equipment, and this tested the technique and recorded the pressure at both levels. In this case the supported equipment hanging from the lower end of the string consisted of a mass of about 900 g. The mass of string and load totalled 1,500 g. This equipment consisted of a double 30″ paper corner reflector and a clock with pressure recorder.

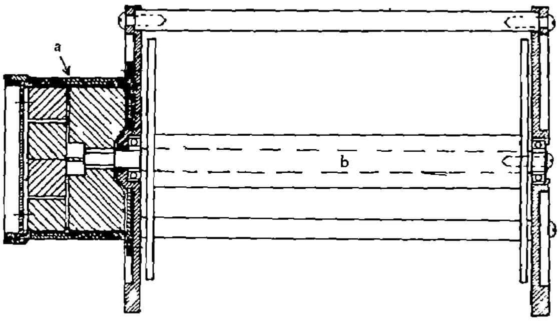

Fig. 1. Reel used for suspension of equipment. *a*, Oil damping chamber; *b*, axle of reel. Mass of reel 1,100 g. Mass of string 600 g

A diagram of the reel used is shown in Fig. 1. The oil damping device at (*a*) consists of six blades each of area 7·5 cm² rotating in oil of *SAE* 140 and set at an angle of 20° to the rotating axis (*b*) of the reel. Attached to the fixed outer part of the oil chamber are a further six blades each of area 4 cm² set parallel to the axis, but slightly off centre. A series of parallel grooves on the inside cylindrical wall of the oil container allows the oil to circulate in the chamber. This device was tested by releasing equipment from the top of a 150-ft. high building and recording rates of fall for temperatures of oil ranging from 20° to 27° C.

A string joint was made by tying the string to a 1-in. diameter intermediate ring made of aluminium rod 1/8 in. in diameter. It was found that the strain at breaking point using this practice could be increased by a factor of 1·3 compared with that for tying string to string using a clove hitch with 3 half-hitch knot. The weight of the reel was 1,100 g and an additional radar target and pressure recorder were tied to the balloon so that a record was available of variations of balloon height. Flight was terminated using the clockwork device described earlier[1]. The flight curve is shown in Fig. 2. It will be observed that the rate of fall of the equipment during the unwinding process was constant over most of the time of fall.

To carry heavier loads including, say, radiosondes, etc., it will be necessary to increase the string denier proportionally, and it is planned to proceed with applications of this technique to cosmic ray measurements.

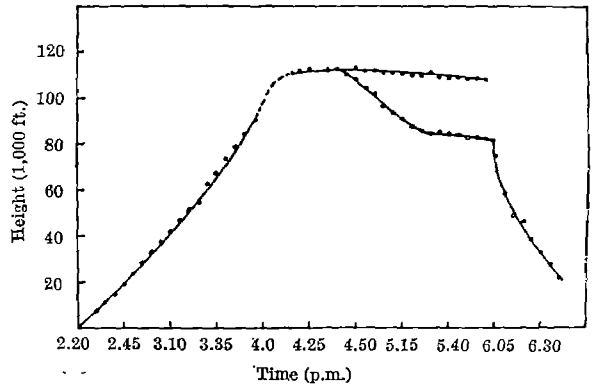

Fig. 2. Height–time curve of balloon and suspended equipment as measured by 3-cm radar (string flight 2.4.64, plastic balloon *WF*2)

We thank Mr. K. Maxwell, Mr. D. Arnold and Mr. W. Carruthers for assisting with the design and testing of the reel.

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Trapped Charges on Dielectrics

It has been proposed[1] that the charge produced on a dielectric surface by electrical discharges is the result of trapping of electrons at impurity or defect sites in the material. Although numerous experiments on surface charges have been reported[2,3], no direct evidence for a trapping process has been obtained. Assuming that the electrons are held in traps of thermal energy W eV, the probability of escape per second may be expressed as:

$$B = A \exp(-W/kT)$$

where A is a constant, k is Boltzmann's constant and T is the absolute temperature. The charge density decay rate is then given by:

$$q/q_0 = \exp(-Bt)$$

In order to determine whether the behaviour of a surface charge is consistent with a trapping process, the decay rate at different temperatures has been measured for several dielectric materials using an electrometer technique.

A schematic diagram of the apparatus is shown in Fig. 1. A disk of the dielectric, 25 mm in diameter and 4 mm thick, is fitted into a recess in a circular brass plate so that rotation of the plate causes the centre of the dielectric disk to pass under a point electrode and, later in the rotation, under an electrometer probe, which are both fixed vertically above the plate. The probe is a screened and highly insulated 0·8-mm diameter copper wire. Its measured capacitance, including the co-axial lead to the electrometer valve, type *ME*1403, is 104 *pF*. The probe, electrode and plate are in a temperature-controlled oven. The plate rotates at 2,850 r.p.m., so any charge on the dielectric induces a voltage pulse on the probe every 21 msec. The dielectric is charged when it is under the point electrode by applying a 100-μsec negative voltage pulse to the electrode, the timing of the pulse being controlled by an interrupted light-beam and a photocell. The resulting pulse train on the probe is amplified and displayed on an oscilloscope, which is triggered by the charging pulse. It is recorded photographically, using a time-scale of the same order of duration as the time-constant of decay of the charge on the disk. The experiment is repeated at several temperatures, and the rates of decay are afterwards determined by projecting the photographs on to a calibrated screen.

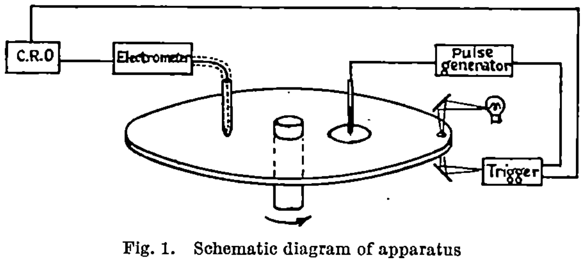

Fig. 1. Schematic diagram of apparatus

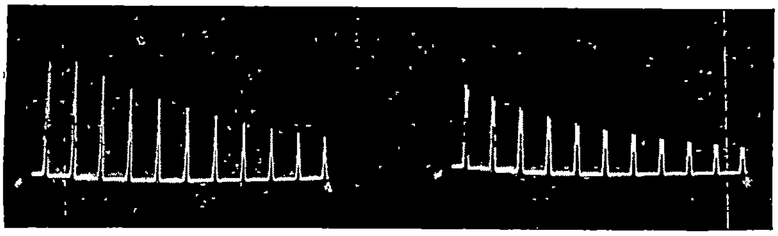

Fig. 2

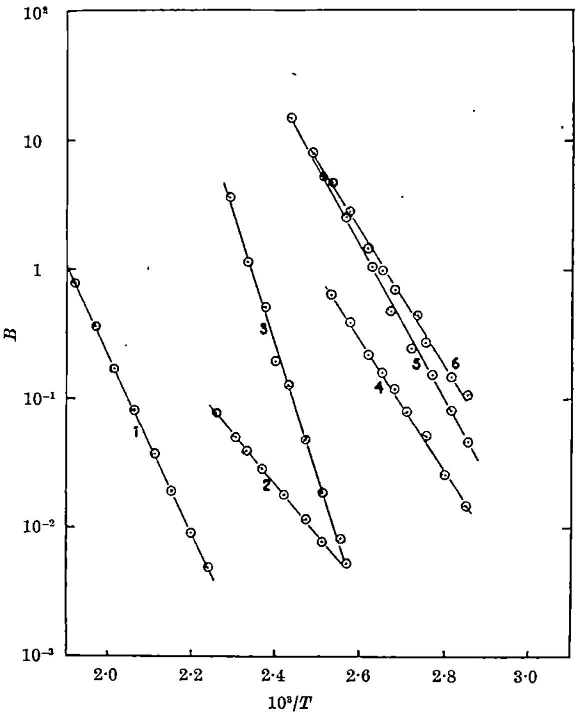

Fig. 3. 1, Polystyrene; 2, polythene; 3, polymethylmethacrylate; 4, potassium iodide; 5, sodium chloride; 6, potassium chloride

Fig. 2 shows traces obtained with a disk cut from a single crystal of sodium chloride at 129·5° C after a 5-kV and an 8-kV charging pulse. The decay characteristics are identical; but a spontaneous loss of charge has occurred after the 8-kV pulse, accounting for the smaller amplitude and more complex form of the record. The surface charge density may be calculated from the expression $q = KCV$, where C is the probe input capacitance, V is the voltage pulse measured on the oscilloscope and K is a constant. K is a function of the probe cross-section and the amplification of the electrometer and is about 35 cm^{-2}. The maximum signal in Fig. 2 is 0·4 V and so the charge density is approximately 10^{-9} coulomb cm^{-2}. This is nearly the maximum charge density that may be attained in air since the breakdown field of air under normal conditions is about 30 kV cm^{-1}. The loss of charge on increasing the charging pulse amplitude is thus probably the result of local discharges.

Experimental values of the coefficient B for disks cut from single crystals of sodium chloride, potassium chloride and potassium iodide, and disks cut from sheet polymethylmethacrylate, cross-linked polythene and cross-linked polystyrene are shown in Fig. 3, plotted as log B against the inverse of the absolute temperature. The linear plot obtained in each case indicates that a single activation energy is involved, and hence that the decay of charge may be described by diffusion of electrons through the volume of the material via the defect traps. An electron trapping and diffusion process has been shown to be operative in the alkali halides[4], the traps being vacant cation sites (Schottky defects) which capture electrons, forming *F*-centres. The electron traps in the plastic materials remain to be identified.

A surface charge is effectively a space-charge (in the absence of conducting films on the surface) and so the procedure described above affords a direct method of determining the thermal energy of the traps active in space-charge-limited solid state devices[5].

It is noted that for a positive frictional charge obtained in an experiment with polythene, by pressing a brush on to the surface while the plate was rotating, the decay parameters were the same as those obtained for the negative, electrically produced charge.

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Propagation of Stress Pulses across a Liquid-Steel Boundary

Experiments have been carried out in which pressure pulses, of about 40-μsec duration, are propagated across a plane liquid–steel interface. For this purpose a slightly modified form of the apparatus described by Bull[1] was used.

The liquid was contained in a vertical steel cylindrical tube fitted with a steel piston at its lower end and the upper end open. The tube was of length 3 ft., internal diameter ⅞ in. and external diameter 1¼ in.

A pressure pulse was generated in the piston by firing a lead bullet of 0·22-in. calibre so as to strike the lower end of the piston normally at its centre. As a first approximation it was assumed that the (pressure, time) curve of this pulse was rectangular in shape, of amplitude P, and of duration T (the time of impact of the bullet); in these experiments P was about 600 atm. and T about 40 μsec. When the front of this rectangular compression pulse reached the upper surface of the piston a fraction α was reflected and a fraction $(1-\alpha) = \beta$ transmitted into the liquid. Since the incident pulse was in the steel, this was a case of reflexion at a rarer medium because the acoustic impedance of the liquid was less than that of the steel. Thus the pulse, of amplitude βP, transmitted into the liquid was of the same sign as the incident pulse while the reflected pulse, of amplitude $-\alpha P$, in the steel was a pulse of tension. When this reflected pulse, of tension reached the lower surface of the piston it was reflected as a compression pulse of the same amplitude, and on its arrival at the liquid–steel interface a fraction α of it was reflected as a wave of tension and a fraction β transmitted into the liquid. The cycle was then repeated and so on. In this way a number of successive pulses of compression of amplitudes βP, $\alpha\beta P$, $\alpha^2\beta P$, etc., was propagated upwards through the liquid column. Each was of duration T in time and the fronts of the pulses followed one another at time intervals of $\tau = 2L/c$, where L is the length of the piston and c the velocity of the pulses in the steel. If $L < c\tau/2$ the individual pulses in the liquid would overlap in time. If, however, $L > c\tau/2$ the individual pulses would be propagated upwards without overlap in time; this was arranged to be the case by using a piston of length 13 cm.

The behaviour of these pulses was studied by means of a steel pressure bar of length 6 ft. and diameter ½ in. inserted into a 'porthole' about 2 ft. above the bottom of the liquid column. The recording technique was the condenser method developed by Davies[2], in which the (displacement, time) curve of the motion of the outer end of the bar was determined as an oscillogram; differentiation of this curve then gave the (pressure, time) curve of the events occurring in the liquid.

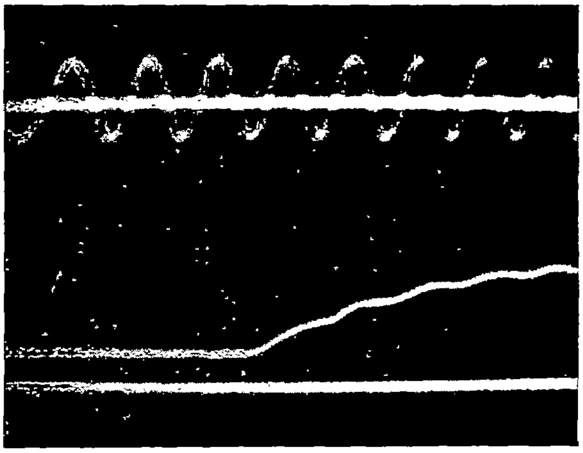

Fig. 1. Upper trace, timing wave, period = 50 μsec.; middle trace, condenser unit signal; lower trace, datum line

An example of the type of oscillogram obtained using water is shown in Fig. 1. The separate displacements due to the successive pulses are clearly shown on the 'staircase' type of trace obtained; the timing wave is of frequency 20 kc/s. An analysis of this trace enables two results to be obtained. First, the time of impact T of the bullet may be obtained by averaging over four pulses; this turns out to be 39·1 μsec, which agrees well with other methods of determination. Secondly, the velocity c of the pulses in the steel may be obtained by measuring the time lag τ between successive 'knees' on the oscillogram; averaging over four pulses it is found that τ = 51·9 μsec and thus:

$$c = \frac{2 \times 13}{51{\cdot}9 \times 10^{-6}} = 5{\cdot}01 \times 10^5 \text{ cm/sec}$$

which is in good agreement with the accepted value.

The next step which suggests itself is the use of calibrated strain gauges mounted on the pressure bar so that the (pressure, time) curves of the individual pulses may be obtained directly; this procedure should then enable a value of α, the reflexion coefficient between steel and the liquid, to be obtained.

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RADIATION CHEMISTRY

Effect of γ-Radiation on Deoxynucleoprotein acting as a Primer in RNA Synthesis

It has been found[1] that on irradiation of calf-thymus deoxynucleoprotein (DNP) the major part of the radiation damage occurs on the protein moiety of the DNP and that, in fact, the DNA itself is largely protected from the effects of radiation. This would be in agreement, for example, with the structure of DNP as proposed by Wilkins[2]. Since it has been suggested that the protein (histone) in DNP is a regulator of RNA synthesis and thus of protein formation[3], it seemed of interest to examine the effects of radiation on DNP and DNA when acting as primers for RNA synthesis.

DNP was prepared by the method of Zubay and Doty[4]. Calf-thymus DNA was a gift from Prof. M. H. F. Wilkins. The appropriate RNA polymerase was prepared from *M. lysodeikticus* by procedure *B* of Nakamoto, Fox and Weiss[5]. Radioactive labelled and unlabelled nucleoside triphosphates were obtained from Schwartz Bioresearch Inc. Irradiations of aqueous solutions of DNA and DNP (0·02 per cent. in DNA) were carried out with a 500-c. cobalt-60 γ-ray source and assayed using (^{3}H)CTP as a tracer. The acid-insoluble alkali-soluble products were estimated using a Packard Tri-Carb scintillation counter.

The results for the DNA primed synthesis show that under these conditions a dose of 2,000 rads is sufficient to reduce the priming activity by 50 per cent and a dose of 12,000 rads lowers it to 2 per cent of the original activity (Fig. 1). These results are similar to those obtained by Harrington[6] and are in contrast to previous experiments concerning the DNA primed DNA-synthesis[7,8].

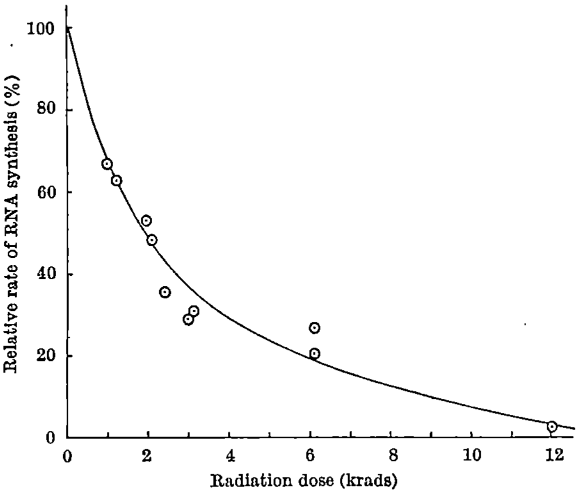

Fig. 1. Effect of radiation (cobalt-60 γ-rays) on DNA (calf thymus) acting as primer in RNA synthesis. The incubation mixture contained: 1 μM each of ATP, GTP, CTP, UTP; 20 μc. (^{3}H)CTP, 3·0 μM Mn^{2+}; 50 μM *tris* buffer (*p*H 7·4), 1·6 μM spermidine phosphate, 80 μg DNA, 60 μg protein (polymerase), in a final volume of 1 ml. Incubation was for 30 min at 30° C

However, with deoxynucleoprotein an entirely different response is obtained (Table 1). In unirradiated systems the priming activity of DNA when 'complexed' as deoxynucleoprotein is only about 25 per cent of that of an equivalent amount of 'free' DNA. Moreover, on irradiation of deoxynucleoprotein with a dose of 12,000 rads only a 30 per cent reduction in RNA synthesis is observed. However, on further irradiation, the priming activity of the deoxynucleoprotein rises until at 72,000 rads its activity is approximately 40 per cent greater than that of the unirradiated DNP.

Table 1. DEOXYNUCLEOPROTEIN-INDUCED RNA-SYNTHESIS

Effect of radiation (cobalt-60 rays) on the priming activity of calf thymus nucleoprotein (DNP)

Radiation dose (krads)	RNA synthesis (%)
0	100*†
12	72
18	82
24	81
36	112
48	133
72	138

Each tube contained: 1·0 μM each of ATP, GTP, UTP, 0·1 μM CTP (labelled with 20 μc. (^{3}H)CTP), 3·0 μM Mn^{2+}, 10 μM phosphate at *p*H 7·4, 160 μg DNP, 180 μg protein (polymerase), in a final volume of 1 ml. Incubation was for 30 min at 30° C.

* Corresponding to 200–300 μμM nucleotide incorporated depending on the experiment. Each result is the average of 4 or 6 experiments.

† Under the same conditions DNA-induced RNA synthesis corresponds to 408 per cent.

These results can be interpreted by assuming that in the normal state of DNP the protein (histone) is not attached to the DNA throughout the length of the molecule and it is the 'non-complexed' DNA which acts as primer for RNA synthesis as suggested by Huang and Bonner[3].

The radiation appears to attack the non-complexed DNA as indicated by the initial drop in the RNA synthesis, and further, as irradiation proceeds, fresh DNA is 'uncovered' to act as primer. This would suggest that one of the actions of radiation is to cause the protein sheath to become detached, to a lesser or greater extent, from the DNA.

If, in a living cell, the histone does, in fact, act as a gene controller, irradiation of the deoxynucleoprotein could cause two effects. First, the production of 'normal' messenger-RNA would be severely curtailed, because of the effect of the radiation on the free DNA; secondly, new messenger-RNA's could be produced which would not normally be formed. Such effects would cause severe imbalance of the cell's metabolism. It has also been suggested that the amount of histone complexed with the DNA varies during the cell cycle[9]; in that event, a mechanism such as outlined here could also account for the variability of radiation sensitivity during the cell cycle.

We thank Dr. S. B. Weiss for making available to us some unpublished results and also Mr. M. G. Robinson of this laboratory for preparation of nucleohistone.

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CHEMISTRY

Effect of the Spreading Solvent on Monolayer Properties

EFFECTS of the spreading solvent, rate of compression, and substrate water on monolayer properties have been the subject of much discussion[1–11]. Most attention has been directed at the spreading solvent. Volatile solvents are used to aid spreading and to facilitate accurate measurement of minute quantities of film-forming materials. One possible source of difficulty is retention of solvent in the monolayer; a second is effect of the solvent on the state of aggregation of the film material (size and shape of islands); a third is dissolution and spreading by the solvent of paraffin wax used in coating parts of the apparatus (float and foils).

The first and second sources are under investigation but present experimental difficulties. Significant information has now been obtained on the third source by measurements on a film balance completely free of wax and related contaminants. The trough and bridge are coated with unpigmented '*TFE* Teflon', the float is solid '*TFE* Teflon', and the thin flexible foils are made of '*FEP*

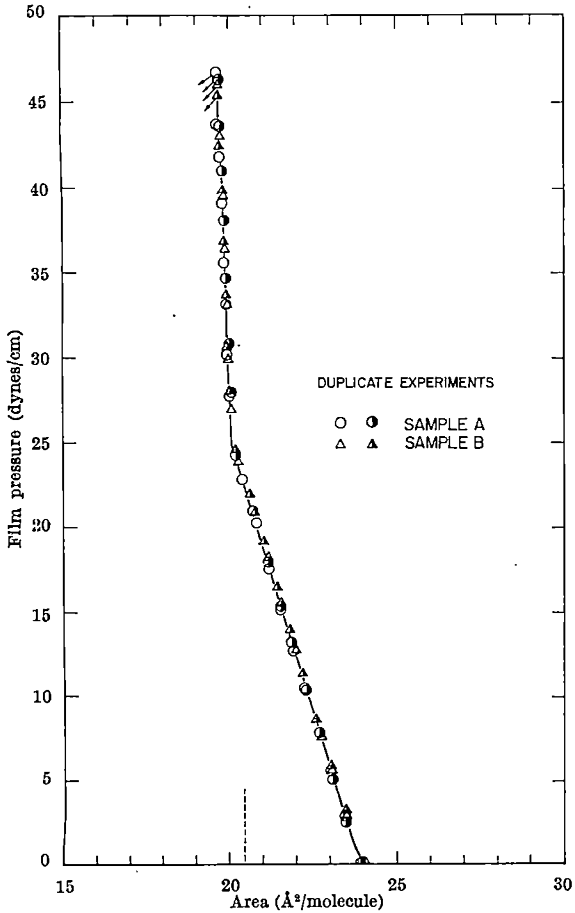

Fig. 1. Pressure-area isotherms for stearic acid spread from benzene

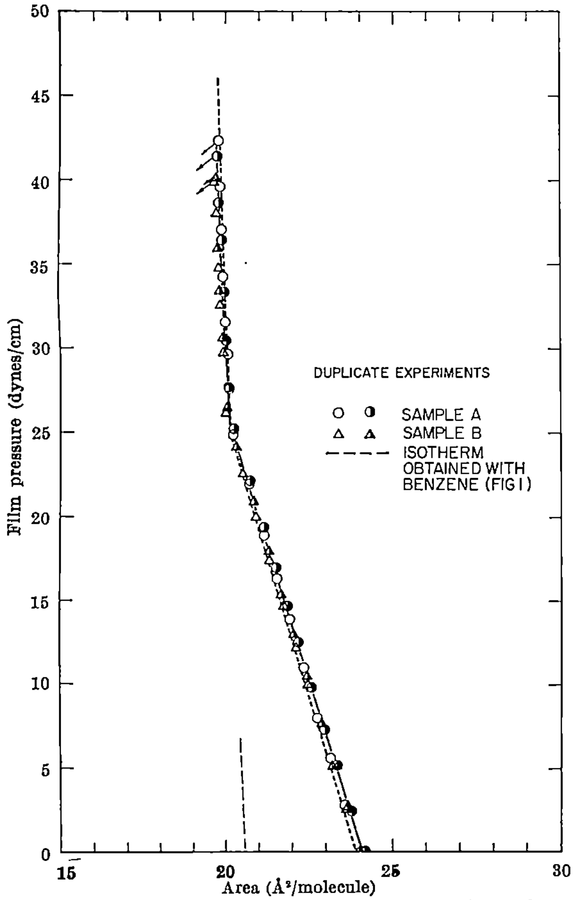

Fig. 2. Pressure-area isotherms for stearic acid spread from *n*-hexane

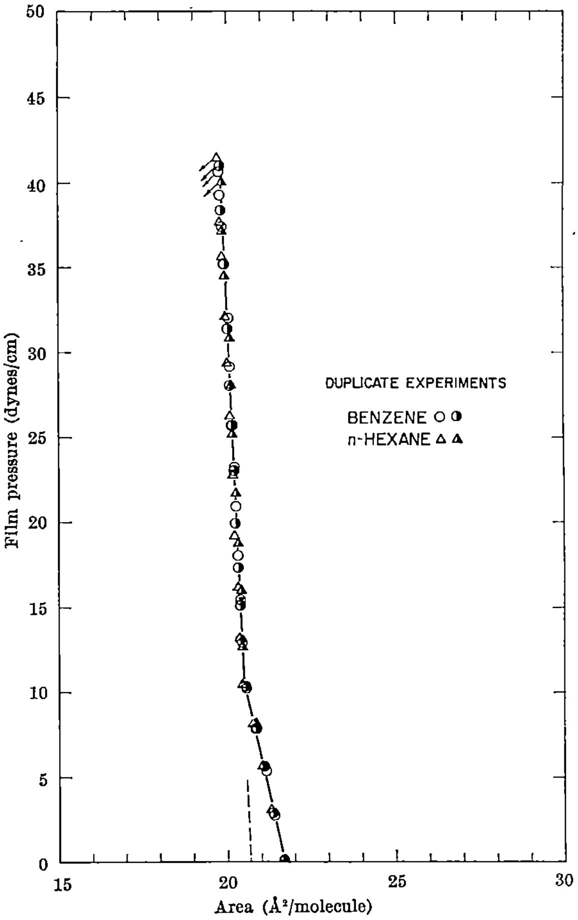

Fig. 3. Pressure-area isotherms for cetyl alcohol spread from benzene and from *n*-hexane

Teflon' heat-sealed to the float and bridge. Surface pressures were continually balanced during incremental compressions of about 3 dynes. Time intervals between readings were one minute throughout. The substrate water was held at 25° ± 0·1° C by internal thermostating of the trough.

Experiments were performed with two high-purity samples of stearic acid, the classical compound in monolayer studies, and with cetyl alcohol, the principal film-forming compound for retardation of water evaporation. Pressure-area isotherms were determined for these materials spread from benzene and from *n*-hexane that had been passed through silica gel and then distilled. The substrate water was twice distilled in quartz; it had a specific conductivity of about 0·5 μmhos.

Isotherms for stearic acid are shown in Figs. 1 and 2. They are identical for the two solvents except for a small difference in collapse pressure. Extrapolated areas for the two linear segments are 20·5 and 23·8 Å²/molecule. In earlier work with stearic acid, the linear segments at high pressures had coincided[3,5]. At low pressures, however, isotherms obtained with benzene had shown an appreciably larger area. Evidently small amounts of wax (from the float and/or foils) or other contaminants had been dissolved more effectively by benzene than by *n*-hexane. The wax occupies area at low pressures but is squeezed out at high pressures. Recent experiments in which small quantities of wax were introduced into the spreading solution support this interpretation.

There is a small difference in collapse pressure: with benzene, 46; with *n*-hexane, 42 dynes/cm. Isotherms obtained recently for stearic acid spread without a solvent show a collapse at 51 dynes/cm, but are identical in contour. Thus, at the highest pressures *n*-hexane may be interfering more than benzene, or benzene may be a better spreading solvent. Collapse pressure, however, depends somewhat on the rate of compression. Variation of collapse pressure with rate of compression for films spread from benzene indicates a collapse pressure close to 42 dynes/cm for a 1·5 min interval; this is in agreement with earlier experiments performed in this laboratory.

Isotherms for cetyl alcohol spread from the two solvents also coincide, as shown in Fig. 3. Extrapolated areas for the two linear segments are 20·7 and 21·7 Å²/molecule. Collapse pressures of about 41 dynes/cm agree within experimental error for monolayers spread from the two solvents.

In recent pressure-permeability experiments with the evaporometer developed in this laboratory[12], the data for stearic acid and for cetyl alcohol spread from the two solvents effectively coincide. However, reproducibility with benzene is superior. Details of the apparatus and procedure used to obtain both the pressure-area and pressure-permeability isotherms will be described later.

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Isotope Effect in Radical Reactions

THE first demonstration that molecules differing only in their isotopic constitution could be separated by the technique of gas chromatography was made by Wilzbach and Riesz[1], who used a packed column to separate deuterated and tritiated *cyclo*hexanes. Later, Cartoni *et al.*[2] showed that capillary columns were more efficient for this purpose, and achieved a separation of all the deuterated benzenes on a 500-ft. column. Cvetanovic[3] demonstrated the value of this technique in the examination of radical reactions, being able to separate the products of the combination of protonated and deuterated alkyl radicals.

In the present investigation, an 80-ft. cupro-nickel capillary column, coated with squalane, was used to separate the pairs, *cyclo*hexane–perdeutero*cyclo*hexane, benzene–perdeuterobenzene and toluene–toluene-αd_3. The separations achieved are illustrated in Fig. 1.

This technique was used to investigate the insertion of methylene into *cyclo*hexane by the photolysis of diketene in the presence of a mixture of *cyclo*hexane and perdeutero*cyclo*hexane. The mixture was divided into two fractions, one of which was passed immediately through the gas chromatography apparatus, and the second irradiated with light of wave-length 3130 Å from a medium pressure mercury vapour arc, so as to photolyse the diketene and produce methylene radicals. After the irradiation was completed (1 h), the products were passed directly to the inlet system of the gas chromatography apparatus. The *cyclo*hexane–perdeutero*cyclo*hexane ratios were thus determined before and after reaction and k_H/k_D calculated using equation 1.

In a second investigation, the effect of methyl radicals on the *cyclo*hexane–perdeutero*cyclo*hexane mixture was investigated by the photolysis of acetone with light of wave-length 3130 Å in the presence of the mixture in the gas phase. An average value for k_H/k_D is given in Table 1.

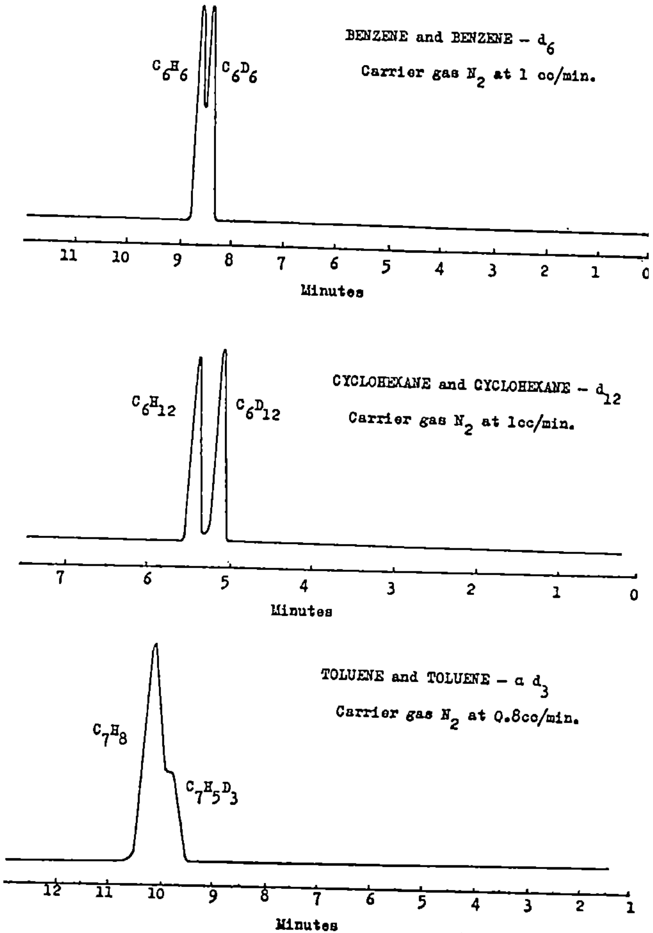

Fig. 1. Separation on the 80-ft. capillary column

Finally, the mixture was submitted to mercury photosensitization by irradiating the *cyclo*hexane–perdeutero*cyclo*hexane mixture alone with light of wave-length 2537 Å from a low-pressure mercury vapour arc. In this case, the results obtained from gas chromatography were checked by the use of a mass spectrometer (A.E.I. *MS* 2).

Table 1. ISOTOPE EFFECT

Reaction	k_H/k_D
Methylene insertion in *cyclo*hexane	0·7
Hydrogen abstraction from *cyclo*hexane	0·37
Mercury photosensitization of *cyclo*hexane	2·7
Reaction of methyl radicals with benzene	0·73

If the initial concentrations of *cyclo*hexane and perdeutero*cyclo*hexane are $[C_0^H]$ and $[C_0^D]$ and the final concentrations are $[C_1^H]$ and $[C_1^D]$, then the ratio of the rate constants for the disappearance of the two species k_H/k_D can be expressed as:

$$\frac{k_H}{k_D} = \log_{10}\frac{[C_1^H]\,[C_0^H]^{-1}}{[C_1^D]\,[C_0^D]^{-1}}$$

Before reaction, the ratio is C_0^D/C_0^H $(=A)$, and after reaction, in which n per cent conversion occurs, the ratio is C_1^D/C_1^H $(=B)$. A and B are the ratios which are determined by gas chromatography and also by mass spectrometry. But:

$$[C_1^H] = \left(1-\frac{n}{100}\right)[C_0^H]$$

and

$$[C_1^D] = \left(1-\frac{n}{100}\right)[C_0^H]\,B$$

therefore

$$\frac{k_H}{k_D} = \frac{\log(1-n/100)}{\log B/A(1-n/100)} \qquad (1)$$

The isotope effect has been calculated from this last equation and the results are given in Table 1.

It can be seen that a usual isotope effect is obtained in the case of the mercury photosensitized reaction, a finding which is consistent with the accepted concept of the process as a transfer of energy from the excited mercury atom followed by a scission of a C–H bond.

$$C_6H_{12} + Hg^3P_1 \rightarrow C_6H_{12}{}^* + Hg^1S_0$$

$$C_6H_{12}{}^* \rightarrow C_6H_{11} + H$$

In the methylene reaction and hydrogen abstraction by methyl radicals, an inverse isotope effect is indicated. In these two reactions, however, a secondary C–H bond is broken and a primary C–H bond is formed.

$$C_6H_{12} + CH_2 \rightarrow C_6H_{11}CH_3$$

$$C_6H_{12} + CH_3 \rightarrow C_6H_{11} + CH_4$$

Thus both these reactions are exothermic and if the exothermicity of the reaction for the C–D bond interchange is greater than that for the C–H bond interchange, then perdeutero*cyclo*hexane would be expected to disappear more rapidly from the reaction system.

Recently, Gray and Thynne[4] have determined the isotope effect in the abstraction of hydrogen atoms from methylamine by methyl radicals.

The isotope effect was also found to be reversed for the attack of methyl radicals on benzene and perdeuterobenzene. Here, however, the situation is more complicated because, although some hydrogen abstraction does take place at room temperature, the predominant reaction is addition of the radicals to the benzene ring to form a complex:

$$C_6H_6 + CH_3 \rightarrow C_6H_6CH_3$$

which may disappear by dimerization or by reaction with a second methyl radical.

It would be difficult to predict the value of the isotope effect for such a reaction.

The value of the gas chromatographic method in the determination of the isotope effect of chemical reactions lies in its extreme simplicity and accuracy. In all cases where the results of gas chromatographic analyses were compared with those produced by mass spectrometry, good agreement was obtained.

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Fluoroketone Hydrates: Helix-breaking Solvents for Polypeptides and Proteins

It is well known that compounds which form strong hydrogen bonds can disrupt the intramolecular hydrogen bonds in synthetic and natural polypeptides. For example, urea in large amounts (about 20 per cent by weight of solution) causes the helix–coil transition in aqueous solutions of ribonuclease[1]. Various organic compounds, particularly acids, have also been reported to effect these transitions. Notably, trifluoroacetic acid (TFA)[2] and dichloroacetic acid (DCA)[3] have been used with success. For synthetic polypeptides, the evidence for helix–coil transitions has been obtained from measurements of intrinsic viscosity[3] and, more conveniently, optical activity[4].

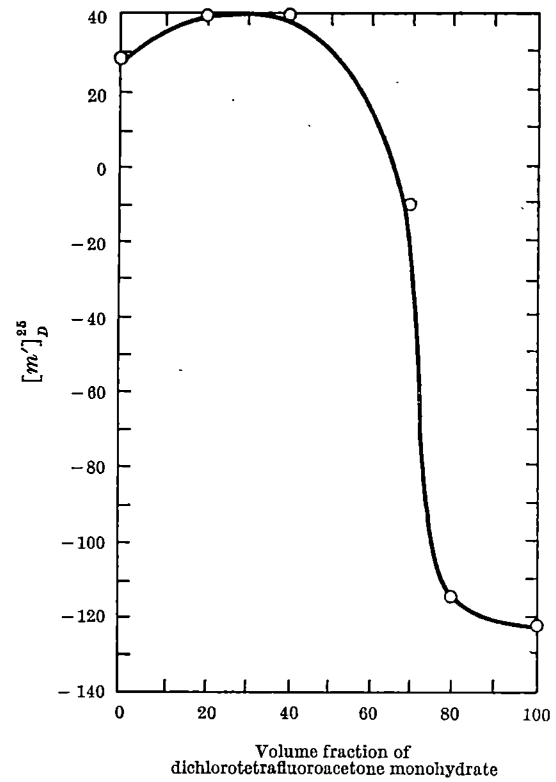

Fig. 1. Effective molecular rotations of solutions of poly-γ-benzyl glutamate

It appears that TFA forms more powerful hydrogen bonds than DCA[4,5], but tends to degrade the peptide bonds. The latter effect is borne out by a decrease in intrinsic viscosity[3] and fluorination of the polymer[6].

I have found that hydrates of fluoroketones are solvents for synthetic polypeptides and some proteins and apparently form more powerful hydrogen bonds with the peptide group than does TFA. However, the solutions do not suffer from the degradative effects of TFA and are comparatively stable over long periods of time.

Two fluoroketones have been investigated. Dichlorotetrafluoroacetone was converted to a monohydrate by adding the stoichiometric amount of water. (The precise structure of this hydrate is uncertain, and it probably exists in equilibrium with a dissociated, acidic form. This problem has been the subject of spectral investigations which will be reported elsewhere[7].) Hexafluoroacetone was dissolved in water to form a hydrate containing about 1·6 moles of water to 1·0 of ketone. (This mixture is liquid at room temperature and is more convenient to manipulate than the 1·5/1 hydrate, which is a solid at room temperature.)

The evidence for dichlorotetrafluoroacetone monohydrate effecting a helix–coil transition was obtained from measurements of the optical activity of solutions of poly-γ-benzyl glutamate (PBGA) in mixtures of the fluoroketone hydrate with chloroform. The results are shown in Fig. 1, in which we have plotted [*m'*], the effective molecular rotation (as defined by Moffit and Yang[8]) given by:

$$[m']_D^{25} = [\alpha]_D^{25}\left(\frac{3}{n_D^2 + 2}\cdot\frac{M}{100}\right)$$

where $[\alpha]_D^{25}$ is the specific rotation, n_D the refractive index appropriate to the particular solvent and M the residue molecular weight of PBGA.

The volume fraction of dichlorotetrafluoroacetone monohydrate necessary to effect the transition is about 65 per cent, which compares with 70 per cent of DCA required in mixtures of ethylene dichloride and dichloroacetic acid[4]. Furthermore, the specific optical activity ($[\alpha]_D^{25}$) of the solution of PBGA in the pure dichlorotetrafluoroacetone monohydrate is more negative than for other solvents. The value of −72·6° for ($[\alpha]_D^{25}$) may be compared with values of −17·5° and −46° for DCA and TFA, respectively[5]. Presumably this large negative value reflects the powerful solvating effect of the solvent for the peptide group.

The stabilities of these solutions are revealed by the inherent viscosity, defined as $\eta_{\text{inh}} = (\ln\ \eta_r)C$, where η_r is the relative viscosity at some concentration C g/dl. If C is small, then η_{inh} is not too different from the intrinsic viscosity $[\eta]$. At 60° C the inherent viscosity of a 0·4 per cent solution of PBGA in TFA fell to 50 per cent of its original value (1·8 dl/g) in 10 h, whereas over the same period of time the inherent viscosity of a solution in the dichlorotetrafluoroacetone monohydrate fell by only 7 per cent. After 30 h at 60° C, the η_{inh} of the TFA solution had decreased by 70 per cent, but that of the solution in the hydrate had decreased by only 11 per cent.

In a second series of experiments, silk fibroin was found to be soluble in and to exist as a random coil in the hydrates of dichlorotetrafluoroacetone and hexafluoroacetone. The results are shown in Fig. 2. The procedure was to measure the optical activity in the pure hydrate and then in mixtures with increasing amounts of a weaker solvent to the point of precipitation of the silk. The results are substantially similar to previous measurements with dichloroacetic acid[5].

Finally, ribonuclease was found to dissolve in dichlorotetraacetone monohydrate but to have been denatured when recovered. However, mixtures of the hydrate with water might be effective in breaking the helix without causing denaturation, but this point was not investigated.

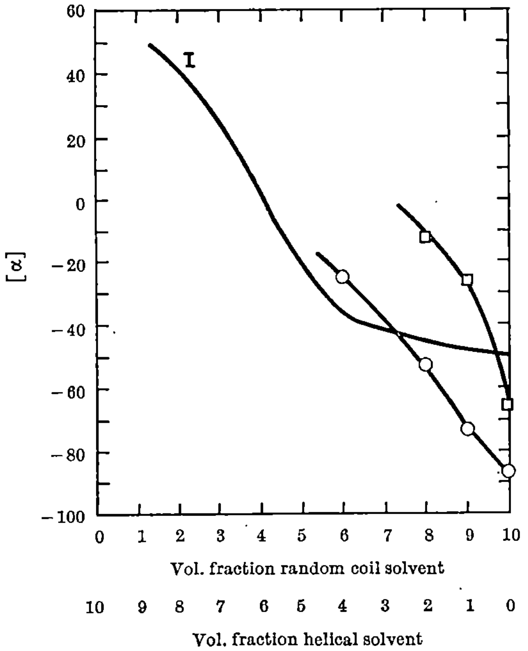

Fig. 2. Specific rotations of solutions of silk fibroin. I, Ethylene dichloride-dichloroacetic acid (ref. 4); ⊙ chloroform–dichlorotetrafluoroacetone monohydrate; □, dioxane–hexafluoroacetone-1,6-hydrate

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A Quaternary Alkaloid from *Croton turumiquirensis*, Stayerm.

A GREAT deal of work has been done on oil of *Croton*[1] but the genus as a whole has received little attention from the chemical point of view. Some alkaloids have been reported and the structures of some of these have been established[2]. Recently, in connexion with work in these laboratories on the volatile oils of Venezuelan species of *Croton*[3], we noticed that aqueous extracts of many of the local species gave positive tests with alkaloid reagents such as Maeyer's, Dragendorf's and Vassler's. No alkaloid could be extracted into organic solvents so the base present was assumed to be quaternary. The bark of the trees proved to be richest in alkaloid content.

The solution obtained by percolating very dilute aqueous acid through a column of the dried, ground bark of *Croton turumiquirensis* was concentrated at 40° to a manageable volume and then treated with Maeyer's reagent. The voluminous precipitate was collected by centrifugation and, after washing, the alkaloid was obtained from the Maeyer's salt with hydrogen sulphide, in the usual manner. From the concentrated solution pale yellow crystals separated, but even after many recrystallizations the material, although chromatographically homogeneous, showed no melting point but decomposed slowly from 130°. Analysis of this material showed in fact that it was a mixture of alkaloid iodide and chloride, but the analytical figures (organic portion only) suggested the molecular formula $C_{42}H_{52}N_2O_9^{++}$ (found, C, 69·2; H, 7·5; N, 3·7; O, 19·6 per cent; $C_{42}H_{52}N_2O_9$: requires, C, 69·4; H, 7·2; N, 3·8; O, 19·8 per cent).

Using ion exchange resin ('Amberlite *IRA* 400') the mixed salt was converted to the pure iodide, m.p. 235° (decomp.) (found, C, 51·1; H, 5·3; N, 3·2; O, 14·3; I, 26·3 per cent; $C_{42}H_{52}N_2O_9I_2$: requires, C, 51·3; H, 5·3; N, 2·9; O, 14·6; I, 25·8 per cent). The nitrate, m.p. 240°–241° (decomp.), which was prepared by the addition of dilute silver nitrate solution to an aqueous solution of the base iodide, agreed with the same molecular formula (found, C, 58·6; H, 6·0; N, 6·5; O, 28·9 per cent; $C_{42}H_{52}N_2O_9(NO_3)_2$: requires, C, 59·1; H, 6·2; N, 6·6; O, 28·1 per cent).

The ultra-violet spectrum of turumiquirensine (as we have provisionally called the base) is complex and probably arises from the superimposition of several chromophores ($\lambda_{\max}^{\text{EtOH}}$ 220 mμ (ε = 63,700), 265 mμ (ε = 18,700) and 300 mμ (ε = 15,600)). The spectrum changes on the addition of alkali ($\lambda_{\max}^{\text{EtOH·NaOH}}$ 225 mμ (ε = 69,000), 275 mμ (ε = 13,700) and 315 mμ (ε = 14 000)), suggesting a phenolic hydroxyl, and this is confirmed by the formation of a phenolic acetate in acetic anhydride/pyridine. The infrared spectrum of turumiquirensine iodide shows a broad peak attributable to hydroxyl groups (around 3,170 cm⁻¹) but does not show carbonyl absorption, while the acetylated material (as the iodide) shows additional peaks at 1,780, 1,760 and 1,200 cm^{-1} characteristic of phenolic acetates. At least one hydroxyl group is still present in the acetylated derivative.

The molecular formula and spectral evidence suggested that this base might belong to the *bis*benzylisoquinoline group of alkaloids, but none of the degradations usually effective in this group has afforded interpretable results. Almost all chemical reactions give rise to viscous, highly coloured oils. Similar alkaloids have been found in *C. rhamnifolius*, *C. xanthochlorus*, Croiz and *C. speciosus*, M. Arg.

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BIOCHEMISTRY

Fusicoccin: a New Wilting Toxin produced by *Fusicoccum amygdali* Del.

Our attention was directed by Dr. A. Graniti, of the Istituto di Patologia Vegetale of the University of Bari, to the observations of Grosclaude[1] and Graniti[2] that culture filtrates of *Fusicoccum amygdali* Del., the causative agent of a disease of the almond tree (*Prunus amygdalus* St.) common in Italy, exhibited phytotoxic activity. This manifested itself in bringing about the wilting of leaves on small branches of almond trees, such as occurs under natural conditions in the infected plant.

The original strain of *F. amygdali*, given to us by Dr. Graniti, was grown in shake flasks and stirred fermenters. A modified Czapek–Dox medium, containing 3 per cent glucose in place of sucrose, was found suitable for producing the active principle. The fermentations were carried out at 24° C for 3–4 days, when the culture medium showed maximal activity, detectable in a test on young tomato plants after 30–100-fold dilution. The biological assay was run according to the semi-quantitative techniques developed by Gaümann[3] in the course of his detailed studies of wilting phenomena. We are obliged to Dr. Graniti for advice concerning this assay method.

The phytotoxic principle was exhaustively extracted with *n*-butyl acetate from filtered batches of 50–300 l. culture medium, and purified by the following steps: (1) evaporation to dryness; (2) residue taken up in $CHCl_3$ (insoluble fraction discarded); (3) residue from $CHCl_3$ extract taken up in methanol (insoluble fraction discarded); (4) residue from methanol extract washed with hexane to remove lipoid impurities, then taken up in ethyl ether (insoluble fraction discarded); (5) residue from ether extract (usually 20 mg/l. culture fluid) dissolved in about 10 times the volume of chloroform containing 3 per cent acetone, and passed through a 'Florisil' column (1 g for each 3 litres of culture medium) on which the active material was firmly adsorbed. The column was washed with about three column volumes of the same solvent. Fractional elution was effected with chloroform containing 6–10 per cent acetone. The fractionation was followed at first by biological assay and later by thin layer chromatography with chloroform/*iso*-propanol (92:8), using a sulphuric acid spray. During the work it became clear that the phytotoxic activity was due to several substances. The present investigation is concerned with the component present in the largest amount, which proved to be the least polar and migrated faster in the chromatographic test. It was termed fusicoccin *A*.

The fractions containing this active substance were combined and evaporated. The residue consisted of biologically active crystals embedded in an oil which exhibited negligible biological activity. It was filtered through a coarse sintered glass plate to remove the oil and the solid was crystallized from ethyl acetate.

Crystalline fusicoccin *A* showed phytotoxic activity in the tomato plant test in concentration of 0·1–0·2 µg/ml. It is a white solid, m.p. 150°–152° C, soluble in alcohols, ether, chloroform, benzene, insoluble in hexane; it crystallizes readily from esters and *cyclo*hexane. The solubility in water is very low, but the compound can be brought into aqueous solution by first dissolving in a small amount of alcohol.

Elementary analysis indicated the formula of fusicoccin *A* to be $C_{38}H_{58}O_{13}$ (found: C, 63·07, 63·16; H, 8·03, 8·01 per cent; $C_{38}H_{58}O_{13}$ required: C, 63·10, H, 8·03 per cent). It contained one methoxy and two acetoxy groups (direct analysis and nuclear magnetic resonance spectrum), and at least one double bond (one mole of hydrogen taken up on catalytic hydrogenation). It is optically active, $[\alpha]_D^{25} + 73$ ($c = 0·6$, in chloroform). Molecular weight determination by X-rays (kindly carried out by Dr. M. Bonamico and Prof. A. Vaciago, C.N.R. Research Centre for Structural Chemistry, Rome) gave a value of 733·5 (± 2 per cent); isothermal distillation (kindly carried out by Dr. W. Simon of the Eidgenossische Technische Hochschule, Zürich) gave a value of 697·3 (± 4 per cent). The ultraviolet spectrum showed only strong end absorption. The infra-red spectrum showed the presence of hydroxy and ester (acetate) groups and of unsaturation, and the absence of aromatic structure. The mass spectrum (kindly carried out by Dr. J. S. Shannon, Sydney, Australia) showed a very complex pattern. A peak corresponding to the molecular ion was absent; the highest mass peak was at *m/e* 704, probably due to an ion formed through loss of water molecule from $C_{38}H_{58}O_{13}$ (mol. wt. 722). A similar behaviour was shown by dihydrofusicoccin *A* (prepared by catalytic hydrogenation of fusicoccin *A*, m.p. 151°–153° C), which exhibited the highest mass peak at *m/e* 706, the value expected for the ion derived by dehydration of a $C_{38}H_{60}O_{13}$ compound (mol. wt. 724). Dihydrofusicoccin *A* had about half the biological activity of fusicoccin *A*. On acid hydrolysis (in N sulphuric acid at 100° C) fusicoccin *A* liberated one mole of glucose (identified by paper chromatography and by reaction with glucose oxidase), demonstrating the glucoside nature of the substance. The structure of the aglycone is under investigation.

We have been informed by Dr. Graniti that crystalline fusicoccin *A* after injection into a wound made near the stem of a branch of an intact almond plant produced wilting of the leaves, as occurs after infection with the fungus under natural conditions.

We thank Miss Maria T. Simeoni for biological assays and Mr. B. Santurbano for his chemical assistance.

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Specific Inhibition of Anti-Rh Antibodies by Extracts from Twigs of Angiospermous Plants

INVESTIGATION of higher plants, bacteria and viruses for their content of substances related to the human blood groups has been in progress in this laboratory for more than a decade[1,2]. Substances possessing only Rh specificity have not been reported previously by us. Recent attempts to confirm reports on Rh_0(D) blood group specificity for an unidentified strain of *Pseudomonas* and a 'glycopeptide' prepared from it and for L-glucose, rutinose and sialic acids were not successful[3].

Extension of earlier work[1] has now shown that heat-stable non-dialysable substances, which specifically inhibit anti-Rh agglutinins, occur in twigs of the majority of Angiospermae, but they were not found among the Gymnospermae tested. Members of the family Oleaceae—such as *Syringa* (lilac, 9 species tested), *Forsythia* (5 species tested), *Fraxinus* (ash, 4 species tested), *Ligustrum* (privet, 1 species tested), *Chionanthus* (fringe tree, 1 species tested), *Jasminum* (jasmine, 1 species tested), *Osmanthus* (1 species tested), *Phillyrea* (1 species tested)—were more closely investigated.

Samples of twigs showed negligible bacterial or fungal contamination. The twigs were stored at − 20° C until they were worked up. The extraction procedure was based on one previously used in this laboratory[1]. It consisted of chopping the materials in a Waring blender for 10 min with 10–20 times their weight of water. Boiling the samples for 20 min did not affect their activity. The ground suspensions were filtered through gauze, then centrifuged at 2,500 r.p.m. for 15 min, toluene and chloroform were added, dialysis was carried out for 12 h against 25–100 vol. distilled water at 4° C and then for 24–48 h against running tap water. The materials were then concentrated under reduced pressure and their dry weight adjusted between 0·6 and 2·0 per cent with 0·85 per cent aqueous saline, containing 0·025 M phosphate buffer, *p*H 7·2. The final *p*H of all samples was between 6·8 and 7·3.

Human erythrocytes were stored and prepared as described previously[4]. They were used as a 0·5 per cent suspension in buffered saline. All anti-Rh sera listed in Table 1 were of human origin, monospecific and contained saline agglutinating antibodies.

The source and properties of the other agglutinins have been described previously[1,4] as have the serological procedures. All reagents were used in 0·1 ml. volume. Two-fold serial dilutions of test materials were made using a different pipette for each tube. The titrated suspensions were shaken and incubated with 4 minimum agglutinating doses of antiserum for 2 h at 23°–25° C. The appropriate erythrocytes were then added and the tests were read microscopically after one further hour of incubation at 23°–25° C. Each titration series included appropriate controls. An Rh_0(D) active preparation from human erythrocytes was included as standard in the assays with anti-Rh sera[3]. Active materials were tested more than 3 times with each serum. Substances which did not inhibit at concentrations of 5 mg/ml. were considered to be inactive. Activities are expressed in terms of dilution of inhibitor before addition of serum and erythrocyte suspension.

Lilac twigs were picked over a period of 8 months (May–January); there was no significant fluctuation in their content of active material. Table 1 shows representative results. Active preparations inhibited all anti-Rh_0(D) sera tested. In addition to most Oleaceae, strictly specific and similar Rh inhibiting activity was found in extracts from *Sassafras albidum*, which also possessed H(O) activity, *Betula* (birch, 1 species tested), *Platanus* (plane tree, 1 species tested), but *Chionanthus virginicus* and the gymnosperms *Taxus cuspidata* (1 species) and 1 *Pinus* sp. showed no anti-Rh agglutinin inhibiting activity. Also inactive were blood group specific lipopolysaccharides from *E. coli* O_{86} (B active) and *E. coli* O_{128} (H(O) active)[2] as well as 'infectious mononucleosis receptor' from beef and sheep erythrocytes[2]. The lack of Rh_0 specificity of blood group A, B and H(O) human ovarian cyst mucoids[5] was confirmed on specimens prepared in this laboratory. Most plant extracts weakly inhibited (2·5–5 mg/ml.) *Lotus tetragonolobus* extracts in their action on O erythrocytes and lilac also in the S-anti-S system. Boiled, non-dialysable lilac twig extracts were further treated by Sevag's method[6]. The active electrodialysed material, of unchanged activity, was in the aqueous phase.

Control experiments indicated that the inhibiting principle did not attach itself to erythrocytes; determination of the agglutinability showed that neither Rh positive nor Rh negative erythrocytes acquired the inhibiting material when incubated for 1 h at 23°–25° C. Also, no inhibiting material was removed from lilac extract solutions, as demonstrated by their unchanged potency before and after incubation with red cells. The active material had no absorption maximum between 230 and 320 mμ but a plateau between 260 and 285 mμ. Spectrophotometric determinations indicated < 1 per cent of hexosamine, sialic acid and methylpentose.

These results show that material of moderate activity and appreciable Rh specificity could be extracted from the twigs of the majority of the Angiospermae tested. The activity was equal to that reported for the most active preparations isolated from human erythrocytes[3,7]. In contrast to the erythrocyte material which is readily inactivated by heating for 1 min at 56° C or when exposed to 3 per cent ethanol for 24 h, the plant material resisted

Table 1. INHIBITION OF ANTI-RH AGGLUTININS BY SUBSTANCES FROM HIGHER PLANTS

Preparation from twigs of:	Fraction	Minimum amount of material (mg/ml.) giving complete inhibition of four agglutinating doses of human anti-										
		Rh_0*	rh′	rh″	hr′	hr″	P	M†	N‡	A_1	B	O§
Syringa sp.	Whole extract	1·2	5									
	Non-dialysable	0·2–0·6	5	2·5	2·5	± 5	NA‖	NA	NA	± 5-NA	NA	NA
	Dialysable	± 5	NA			5						
Phillyrea decora	Non-dialysable	0·3–0·6						NA	NA	NA	NA	NA
	Dialysable	NA	NA	NA	NA	NA						
Ligustrum japonicum	Non-dialysable	0·3–0·6	5	5	± 5	2·5	NA	NA	NA	NA	NA	NA
Jasminum nudiflorum	Non-dialysable	0·6–1·2					NA	NA	NA	NA	NA	NA
Forsythia giraldiana	Non-dialysable	0·3–0·6					NA	NA	NA	NA	NA	NA
Betula sp.	Non-dialysable	1·2	1·2	2·5	NA	1·2–2·5		NA	NA	NA	NA	NA
Chionanthus virginicus	Non-dialysable	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pine sp.	Non-dialysable	NA	NA	NA	NA	NA				NA	NA	NA
Taxus cuspidata	Non-dialysable	NA	NA	NA	NA	NA				NA	NA	0·002
Human blood group A, B or H(O)	Mucoid	NA	NA									
Human erythrocyte stroma	A.F.	0·3–0·6	5	NA	0·6	5	2·5	NA		NA	NA	2·5
E. coli O_{86}	Lipopolysaccharide	NA						NA	NA	5	0·001	NA

* Arithmetic average of results obtained with five sera.
† Human and rabbit anti-M.
‡ Human, rabbit and *Vicia graminea* anti-N.
§ Eel, *Laburnum alpinum* human and *Ulex europeus* anti-H(O).
‖ NA not inhibitory at concentrations of 10 mg/ml.

boiling and all concentrations of ethanol. Therefore, while activity and specificity are similar to the human receptor substance, as determined in the hæmagglutination inhibition test only, it is likely that the physical and chemical nature of the plant material is different from the human Rh agglutinogen.

The lilac preparations were non-antigenic to 6 rabbits and non-toxic when given (1·2 mg/kg) intravenously or subcutaneously.

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Antioxidants in Oats : Light-induced Isomerization

BY means of thin-layer chromatography on silicic acid we have shown that there are at least six phenolic antioxidants in oats[1]. These substances are esters of the hydroxycinnamic acids, caffeic and ferulic acids. When the preparation of larger quantities by column chromatography was attempted, it was found that additional compounds appeared. We have now shown that these arise from the action of light on the original antioxidants.

By working entirely in the dark or in subdued tungsten lighting, it has been possible to isolate antioxidant components IV and V (single spots on thin-layer chromatography plates); some of their properties are listed in Table 1. The other components have not yet been obtained in a pure state. If exposed to an ultra-violet lamp, or to daylight for as little as 10 min, substances IV and V each give a second spot on thin-layer chromatography (solvent system *A*). It is evident that a sample of the mixed antioxidants prepared in daylight will contain both the isomers of each component. Column chromatography using a solvent mixture similar to *A* may give a series of well-resolved peaks, but each will contain two substances: for example, peak IV will also contain the isomer of V. On further exposure of the eluate to light, equilibrium is re-established with the formation of V and the isomer of IV, and thus three spots appear on the thin-layer chromatogram of the peak. In consequence, the column appears to have a low-resolving power.

Table 1. PROPERTIES OF COMPONENTS IV AND V

Component	Solvent	R_F			M.p. °C	Anti-oxidant activity units/mg	Phenolic products of hydrolysis
		Dark (*trans*)	Irradiated (*trans*)	Irradiated (*cis*)			
IV	*A*	0·42	0·42	0·59	85–86	14	Caffeic acid
	B	0·54	0·51	0·51			Ferulic acid
V	*A*	0·29	0·29	0·41	140–141	39	Caffeic acid
	B	0·28	0·23	0·23			

Thin-layer chromatography on silicic acid containing 2 per cent calcium sulphate; solvent *A*, diethyl ether—light petroleum (b.p. below 40°), 3 : 2; solvent *B*, chloroform—diethyl ether, 4 : 1. Irradiation at 366 mμ, 5 min, 30 cm from 125-W Hanovia lamp, type 11. Antioxidant activity, defined as in ref. 1.

Table 2. THIN-LAYER CHROMATOGRAPHY OF CAFFEIC AND FERULIC ACIDS AND THEIR ESTERS

	Solvent	R_F		
		Dark (*trans*)	Irradiated (*trans*)	Irradiated (*cis*)
Caffeic acid	*A*	0·11	0·10	0·15
	B	0·06	0·05	0·05
Ethyl caffeate	*A*	0·28	0·29	0·41
	B	0·33	0·29	0·29
Ferulic acid	*A*	0·17	0·14	0·24
	B	0·21	0·19	0·19
Ethyl ferulate	*A*	0·43	0·43	0·57
	B	0·65	0·63	0·63

Thin-layer chromatography as in Table 1.

The effect of ultra-violet light on cinnamic acids and their derivatives is to convert them into equilibrium mixtures of their *cis*- and *trans*-forms[2,3]. These isomers can be separated by paper chromatography[4], and the separation of *cis*- and *trans*-ethyl ferulate on a thin layer of silica gel *G* has recently been reported[5]. We have confirmed that when caffeic and ferulic acids and their ethyl esters are isomerized by ultra-violet irradiation, the *cis*- and *trans*-forms are separated by our solvent system *A*, but not by system *B* (Table 2). It would seem that the oat antioxidants easily undergo the same type of isomerization.

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Distribution and Fate of 2-Aminoethylphosphonic Acid in *Tetrahymena*

THE recent discovery of 2-aminoethylphosphonic acid (AEP) and its isolation from certain ciliates and from the sea-anemone is of interest, for these reports[1–3] represent the only occasions on which a compound containing a C–P bond has been discovered in biological material. Nothing is as yet known of the biosynthetic pathway by which such a bond is formed. The present communication deals with the initial work on this problem, and is concerned with the distribution of AEP among the cellular components of *Tetrahymena* and the rate of incorporation of ^{32}P-orthophosphate into the molecule.

*Tetrahymena pyriformis Wh*14 grown in axenic culture at 28° in media containing proteose-peptone, yeast extract and glucose attained maximum cell density in about 40 h; 'log-phase' cells were collected at 30 h, and stationary phase cells at 100–120 h.

AEP assay was based on the resistance of the C–P bond to hydrolysis after 48 h exposure to 6 N hydrochloric acid at 110°. Hydrolysates of cells grown in the presence of inorganic phosphorus-32, when subjected to paper electrophoresis followed by autoradiography, contained AEP and inorganic phosphate as the only labelled compounds present. AEP was separated from orthophosphate by chromatography on either 'Dowex 50-*H*' or 'Dowex 1-acetate'.

Preliminary experiments indicated that AEP was present in both the free state and in a bound form, the latter largely in the lipid fraction. This confirmed the finding of Kandatsu and Horiguchi[3], who pointed out that the bulk of AEP in *Tetrahymena* was bound in the lipids. However, examination of the lipid-free residue has revealed considerable amounts of bound AEP in that fraction also; the AEP bound in this residue could not be extracted by a variety of boiling lipid solvents nor by mixtures of methanol and chloroform containing ammonia.

To gain some information about the metabolism of AEP, the rate of incorporation of phosphorus-32 into the molecule was followed under a variety of conditions. Inorganic phosphorus-32 was added to a culture at 17 h, that is, at the beginning of the log phase. Samples of the cultures were withdrawn at intervals and the AEP present in the acid-soluble pool, in the lipid fraction (chloroform-soluble) and in the lipid-free residue was isolated and its specific radioactivity determined. In the 6 h following addition of phosphorus-32, during which the cell density increased threefold, the specific activity of the AEP in all three fractions increased steadily (Table 1), but the increase was most rapid in the lipid-bound AEP and slowest in the free AEP, the residue-bound AEP being intermediate. While the two latter fractions continued to gain in specific activity after that period, the specific activity of the lipid-bound AEP did not increase further, although the cell density continued to increase for some 18 h or more. The reason for this is not known, but it is possible that the cells take up the labelled inorganic phosphate in preference to organophosphates, and only when the former becomes exhausted is the unlabelled organic phosphorus of the medium (yeast extract) used.

Table 1. Specific Radioactivities (10^3 c.p.m./μmole P) of Three AEP Fractions after Addition of ^{32}P-Orthophosphate to Log-phase Suspension of *T. pyriformis*

The cell density (mg dry weight/100 ml.) at the times shown, 1, 6 and 23 h, was 20, 55 and 141, respectively

AEP fraction	Time after ^{32}P addition (h) 1	6	23
Lipid-bound	19	62	65
Residue-bound	11	43	68
Acid-soluble	4·4	17	55

By contrast with the situation in growing cells, little radioactivity was incorporated into the AEP of stationary phase cells (Table 2), even though such cells were able to incorporate the isotope into the non-phosphonic phosphorus of the lipid and residual fractions.

Table 2. Total Radioactivity Incorporation into Some Phosphorus Compounds in Growing and Stationary Cultures of *Tetrahymena*

(Total counts per 25 ml. of suspension, 1 h after the addition of 100 μc. ^{32}P-orthophosphate.) The cell density (mg dry weight/100 ml.) of the log-phase and stationary suspensions was 204 and 272, respectively

Phosphorus fraction	Log phase	Stationary
AEP		
Lipid-bound	19,000	100
Residue-bound	2,500	13
'Ester-P'		
Total lipid P	—	46,000
Total 'residue' -P	—	41,000

The negligible degree of uptake of phosphorus-32 into AEP during the stationary phase confirmed that its synthesis proceeded only during cell growth. It was, therefore, suspected that this compound was associated with the structural elements of the cells, and was likely to represent an end-product of metabolism.

To test this, log-phase cells were labelled with phosphorus-32 and re-suspended in fresh medium containing excess unlabelled orthophosphate, in which growth continued. If AEP was an end-product, then the total label in the AEP per unit suspension volume would remain constant, irrespective of multiplication. However, if AEP was further metabolized, the phosphorus-32 in this fraction would be displaced by 'cold' phosphate and the total AEP radioactivity per unit volume of suspension could be expected to fall with time. The experiment illustrated in Fig. 1 shows that, after an initial rise (presumably due to incorporation of endogenous phosphorus-32), the radioactivity in the AEP per unit volume of suspension remained constant. The lipid- and residue-bound AEP is, therefore, not further metabolized[4].

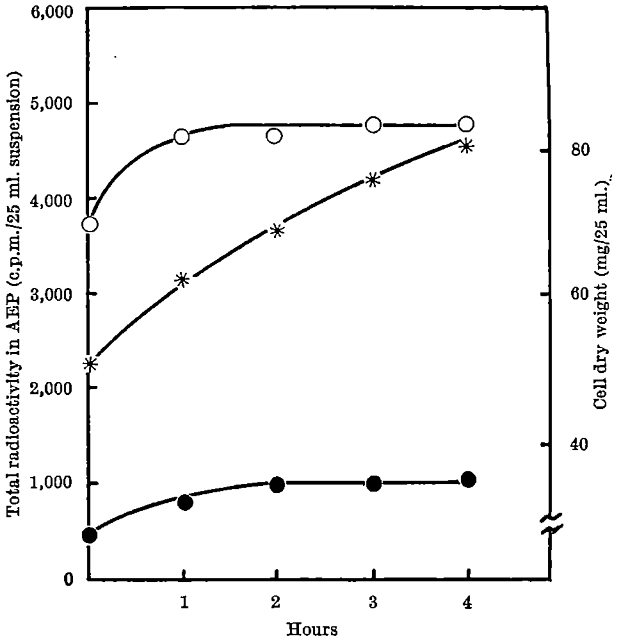

Fig. 1. Time–course of the level of radioactivity in the total lipid-bound (○), and residue-bound (●) AEP in growing *Tetrahymena* suspensions labelled with phosphorus-32 and re-suspended in phosphorus-rich, 'cold' medium. (✱) Cell density

The nature of the residue-bound AEP has been investigated. A portion of this residue, labelled with phosphorus-32 and yielding labelled AEP on acid hydrolysis, was subjected to proteolysis by 'Pronase', trypsin, or the consecutive action of both. The material was heated at *p*H 4·5 at 100° for 10 min prior to suspension in the appropriate buffer for proteolysis. After incubation for 6 h, a large portion of the material had dissolved and the insoluble residue was removed by centrifugation. Acid hydrolysis (48 h in 6 N hydrochloric acid at 110°) of portions of the precipitate and supernatant, followed by electrophoresis and autoradiography, showed that the bulk of the AEP was still bound in the residue which was insoluble after proteolysis. The acid hydrolysate of this residue contained a large variety of amino-acids. AEP is thus associated with macromolecular complexes which resist hydrolysis by proteolytic enzymes.

The results described here suggest that the synthesis of the large molecules containing AEP is not likely to proceed by a pathway involving the incorporation of the free amino-acid pool AEP. The characteristic pattern of phosphorus uptake, and the resistance of the residual material to proteolysis, suggest that the lipids and the non-lipid complex containing AEP are a part of the structural material of the cell, possibly the cell membranes.

The nature of these complexes is being further investigated.

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Absence of Bile Salts in the Gastric Juice of a Crab

THE digestive juices of several decapod Crustacea have been shown to possess a low surface tension and emulsifying properties indicating the presence of surface-active compounds[1]. Earlier claims[2,3] of the presence of taurodeoxycholic acid in the gastric juice of the crab and the crayfish have not been supported by recent investigations[4] which have demonstrated some differences in the properties of the emulsifiers and those of bile salts.

The aim of the work recorded here was to establish by means of chromatographic and mass-spectrometric methods the presence or absence of bile salts in crab (*Cancer pagurus* L.) gastric juice.

Gastric juice was collected as described by Vonk[4] from freshly caught crabs and the juice was freeze-dried. The dried juice was extracted with hot ethanol and the alcohol extract was evaporated to dryness under reduced pressure. The residue (4·25 g corresponding to 85 ml. of fresh juice) was hydrolysed with 2 M methanolic potassium hydroxide at reflux temperature for 1 h. The hydrolysis mixture was diluted with water, acidified to *p*H 1 with hydrochloric acid and extracted first with ethyl ether and then with water-saturated *n*-butanol. Both extracts were washed with water until neutral and evaporated to dryness under reduced pressure. The weight of the residue of the ether extract was 150 mg and of the butanol extract 1·9 g. The residue of the ether extract was chromatographed on a 13·5 g column of hydrophobic 'Hyflo SuperCel' using phase system *F* 2 (ref. 5). Titration of the effluent showed the presence of four peaks with a combined weight of 48 mg. After methylation with diazomethane an aliquot of each peak was subjected to gas chromatography using *QF*-1 as stationary phase and under the conditions described by Sjövall[6]. The main components emerged from the column at much lower temperatures than do bile acid methyl esters and bile sterols. Mass-spectrometry of the material in the four peaks showed that two of these consisted of decanoic acid and *n*-dodecenoic acid respectively, whereas the material in the other two peaks could not be identified. No fragments typical of steroids or bile acids were found. The alcohol eluate of the column (91 mg) was found to consist mainly of cholesterol identified by melting point (147°–148°) with no depression on admixture of authentic cholesterol, and by thin-layer chromatography (benzene/ethyl acetate, 4 : 1, as moving phase).

The residue of the butanol extract was chromatographed on a 45-g column of hydrophobic 'Hyflo SuperCel' using phase system *D* (ref. 7). A single peak appearing shortly after the solvent front was obtained. This material (913 mg) was somewhat less polar than taurodeoxycholic acid both on paper and on thin-layer chromatography[8,9]. Neither from the remainder of the effluent nor from the alcohol eluate of the column could any material be isolated that on gas chromatography or mass-spectrometry showed properties of bile acids or steroids. The infra-red spectrum of the unknown material isolated from the butanol extract as described here showed peaks at 6·0 and 6·5μ, indicating the presence of amide groups. Due to the low volatility no mass-spectrum could be obtained. The unknown material was hydrolysed with 1 M sodium hydroxide in 50 per cent aqueous ethanol for 8 h in a sealed steel bomb at 120°. After dilution with water and acidification, the hydrolysis mixture was extracted with ether. The residue of the ether extract weighed 359 mg. Gas chromatography of an aliquot of the ether extract after methylation showed the presence of a number of compounds emerging from the column at temperatures lower than those required for bile acid methyl esters and bile sterols. Direct mass-spectrometric analysis of the gas chromatographic effluent[10] showed that the material in the ether extract consisted of a series of saturated and unsaturated straight-chain fatty acids with chain-lengths of 10–24 carbon atoms. The water phase of the hydrolysis mixture was passed through a 'Dowex 50-*X* 8' column (hydrogen form; 200 mm × 10 mm). The column was washed with water until the effluent was neutral and then with an additional 50 ml. of water. The effluent was evaporated to dryness under reduced pressure. Crystallization of the residue from methanol/water yielded 132 mg of taurine identified by thin-layer chromatography (ethanol/ammonia/water, 90 : 5 : 5, as moving phase[11]) and by formation of *N*-acetyltaurine with m.p. 190°, not depressed on admixture of authentic *N*-acetyltaurine. Thin-layer chromatography of the mother liquor showed that in addition to taurine another compound moving as sarcosine was present. The compound could be isolated by alcohol extraction of the residue of the mother liquor, followed by crystallization from water/acetone/methylene chloride. The crystals had m.p. 168° with no depression on admixture of authentic sarcosine.

The results of the present investigation show that no bile acids or bile alcohols are present in gastric juice of the crab. However, material possessing emulsifying properties can be isolated from an alcohol extract of dried gastric juice. This material, which has the same properties as that earlier investigated by Vonk, has been shown to consist of fatty acids, taurine and sarcosine.

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Nuclear Magnetic Resonance of Phosphorus in Deoxythymidine Polynucleotides

NUCLEAR magnetic resonance signals from polynucleotides of thymidine-5′-phosphate have been investigated and the terminal monoester phosphate distinguished from the diester phosphate of the chain.

A mixture of 3′-O-acetylthymidine-5′-phosphate (1 m.mole) and thymidine-5′-phosphate (3 m.mole) was polymerized with di*cyclo*hexylcarbodiimide and the resulting mixture of polynucleotides was resolved on a DEAE-cellulose (carbonate) column using the method described by Khorana and Vizsolyi[1]. From the polymerization mixture, 26,500 optical density (OD) units at 267 mμ (1 m.mole of thymidine-5′-phosphate is equivalent to 9,600 OD units) were applied to the column and polynucleotides up to the dodecanucleotide were cleanly separated. Fractions were identified using paper chromatography with reference samples, prepared in the above manner by Khorana and Vizsolyi and characterized enzymatically by Dr. W. E. Razzell of Syntex Institute. Pooled fractions containing cyclic dinucleotide (3,300 OD units), linear dinucleotide (2,700 OD units), linear trinucleotide (2,600 OD units), linear tetranucleotide (2,300 OD units), and linear pentanucleotide (2,000 OD units) were evaporated to dryness and each dissolved in 0·5 ml. of methanol for the nuclear magnetic resonance measurements.

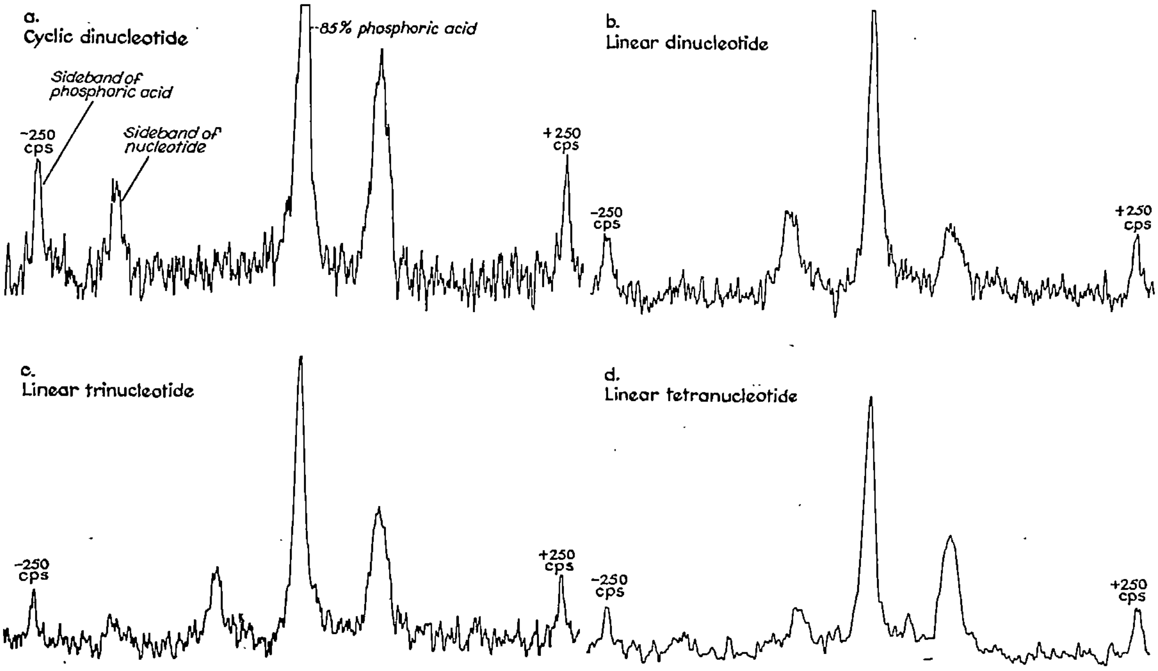

Fig. 1. Phosphorus nuclear magnetic resonance spectra taken at a sweep rate of 4 c/s/sec with a bandwidth of 0·5 c/s.

Nuclear magnetic resonance measurements on the phosphorus nucleus were made in a Varian spectrometer operating at 40·5 Mc/s. Chemical shifts were measured by the use of sidebands[2] with 85 per cent phosphoric acid as an external reference. The observed line width was of the order of 20 c/s. One can determine the number of nucleotides in short chains by the intensity ratio of the monoester to diester lines (Fig. 1). The chemical shifts of the monoester and diester lines are given in Table 1. Jones and Katritzky[4] have found the chemical shift of the phosphorus in trimethyl phosphate to be +2·0 p.p.m. in methanol and +2·7 p.p.m. in water. In the tetramethylammonium salt of adenosine triphosphate, Cohn and Hughes[5] find the chemical shift of α, β and γ phosphorus nuclei to be +11·2, +22·5 and +8·06 p.p.m. respectively at *p*H 7·0.

Table 1

Compound	Chemical shift (±0·1 p.p.m.) Monoester	Diester
Cyclic dinucleotide	—	+1·82
Linear dinucleotide	−1·90	+1·80
Linear trinucleotide	−1·90	+1·90
Linear tetranucleotide	−1·73	+1·93
Linear pentanucleotide	N.O.*	+2·00

*N.O., not observed because of poor signal to noise.

The phosphorus chemical shifts of the 5′-mononucleotides of DNA (100 mg of ammonium salt dissolved in 0·5 ml. of water) were also measured with 85 per cent phosphoric acid as an external standard and found to differ significantly. The purines, deoxyadenylate (−2·84±0·07 p.p.m.) and deoxyguanylate (−3·13±0·07 p.p.m.), were observed at higher fields than the pyrimidines, deoxythymidylate (−3·35±0·07 p.p.m.) and deoxycytidylate (−3·65±0·07 p.p.m.). If this difference in phosphorus chemical shift of the four nucleotides persists in the polynucleotides, it could provide a means of end-group analysis for short chains. To explore this further, oligonucleotides derived from the other three bases and mixed polynucleotides will be examined with more efficient signal-extracting techniques.

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PHYSIOLOGY

Vitamin-binding Capacity of Experimentally Injured Liver

There are variable results on the therapeutic effects of B-complex vitamins given during experimentally induced liver injury[1–4]. Such results could be due to differences in vitamin binding by the injured liver. Because of the availability of sensitive and specific microbiological techniques for assay of tissue vitamin-levels[5], the work recorded here was undertaken. With these techniques, a more reliable assessment of the vitamin-binding capacity of the normal, necrotic and regenerating rat liver could be made.

Sprague–Dawley rats weighing 220–350 g were housed in individual cages and fed a standard diet of 'Purina Lab Chow' and water *ad lib.* To induce hepatic injury, rats were given 0·2 ml. of carbon tetrachloride (CCl_4)/100 g body-weight intragastrically via a polyethylene catheter; saline was administered in the same manner to control animals. Litter mates were pretreated daily for 5 days with glucose and water, or a mixture of B-complex vitamins intramuscularly; medication was continued until the animals were killed. The vitamin-treated animals received a mixture containing 50 mg thiamine, 10 μg cyanocobalamin (vitamin B_{12}), 1·0 mg folic acid, 50 mg nicotinic acid, 5·0 mg vitamin B_6, and 5·0 mg calcium pantothenate; these vitamin-levels saturated all tissues

The liver was removed from decapitated rats 12, 24, 36, 40, 48, 72, 96 and 120 h after they received CCl_4; and simultaneously from litter-mate control animals. After removal, the liver was perfused and rinsed with cold saline to remove adhering tissue and fluids; a section was removed for histology. The individual liver was homogenized in a blender with water, lyophilized to dryness, and stored at − 15° C. Liver sections obtained for histological study were fixed in formalin; paraffin sections of 7–8μ thickness were stained with hæmatoxylin and eosin. Nitrogen content of specimens, determined with a Coleman nitrogen analyser, served as a reference standard for the level of parenchymatous tissue. The vitamin content of the liver was assayed as described[5]. Because folinic acid is metabolically interrelated with folic acid[5], its assay in liver tissue was included even though it was not present in the vitamin supplement.

Vitamin titres were expressed as concentration per mg dried tissue, since differences between the nitrogen content of the liver of control animals and CCl_4-treated animals at the various phases of liver injury and regeneration were not significant. Histological examination was normal in both saline and vitamin-treated controls. Necrosis and inflammation involving one-third of the hepatic lobule were noted 24 h after receipt of CCl_4. Regeneration, as reflected in liver cell mitoses, was characteristic 48–96 h after receipt of CCl_4 (ref. 6); complete restoration of normal architecture was noted at 120 h.

Table 1 shows the mean vitamin titres obtained in liver tissue; deviations in each group of 4 or 8 animals was less than 10 per cent. Administration of vitamins to control animals caused no significant changes in thiamine, nicotinic acid, and vitamin B_6-complex titres; and increase was noted in folic acid (140 per cent), folinic acid (130 per cent), vitamin B_{12} (380 per cent), and a decrease in pantothenic acid (43 per cent).

Table 1. EFFECT OF VITAMIN THERAPY ON LIVER TISSUE B-COMPLEX VITAMIN CONTENT*

$\frac{\text{Without}}{\text{With}}$ vitamin pretreatment

	Thiamine	Nicotinic acid	Folic acid	Folinic acid	Vitamin B_6-complex	Pantothenic acid	Vitamin B_{12}
Control animals (8/group)	$\frac{33}{32}$	$\frac{423}{398}$	$\frac{14\cdot1}{19\cdot9}$	$\frac{10\cdot3}{13\cdot3}$	$\frac{23}{24}$	$\frac{219}{126}$	$\frac{38}{145}$
Time (h)	Effect of CCl_4 on vitamin titres (4 animals/group)						
12	$\frac{14}{20}$	$\frac{537}{344}$	$\frac{6\cdot7}{16\cdot4}$	$\frac{6\cdot6}{9\cdot4}$	$\frac{16}{20}$	$\frac{113}{88}$	$\frac{27}{125}$
24	$\frac{17}{40}$	$\frac{358}{654}$	$\frac{7\cdot5}{13\cdot8}$	$\frac{4\cdot7}{10\cdot2}$	$\frac{16}{19}$	$\frac{77}{77}$	$\frac{59}{119}$
36	$\frac{14}{34}$	$\frac{254}{618}$	$\frac{1\cdot3}{10\cdot2}$	$\frac{1\cdot0}{6\cdot7}$	$\frac{14}{14}$	$\frac{49}{74}$	$\frac{54}{130}$
40	$\frac{20}{34}$	$\frac{367}{497}$	$\frac{4\cdot9}{10\cdot6}$	$\frac{3\cdot6}{6\cdot0}$	$\frac{14}{15}$	$\frac{61}{81}$	$\frac{27}{181}$
48	$\frac{25}{60}$	$\frac{395}{600}$	$\frac{12\cdot6}{16\cdot3}$	$\frac{6\cdot9}{9\cdot9}$	$\frac{19}{19}$	$\frac{106}{76}$	$\frac{50}{170}$
72	$\frac{42}{56}$	$\frac{392}{376}$	$\frac{11\cdot7}{16\cdot4}$	$\frac{8\cdot4}{11\cdot9}$	$\frac{19}{20}$	$\frac{161}{82}$	$\frac{31}{169}$
96	$\frac{38}{87}$	$\frac{423}{383}$	$\frac{7\cdot7}{15\cdot0}$	$\frac{5\cdot1}{10\cdot5}$	$\frac{20}{19}$	$\frac{202}{128}$	$\frac{26}{100}$
120	$\frac{28}{59}$	$\frac{383}{420}$	$\frac{10\cdot9}{13\cdot2}$	$\frac{8\cdot6}{13\cdot1}$	$\frac{18}{19}$	$\frac{141}{100}$	$\frac{23}{92}$

* Vitamin titres in mμg/mg; vitamin B_{12} values in μμg/mg

In animals not receiving vitamins, CCl_4-induced necrosis was associated with a significant decrease in thiamine (50 per cent), folic acid (90 per cent), folinic acid (90 per cent), vitamin B_6-complex (40 per cent), and pantothenic acid (80 per cent). During the early phases of liver injury, no significant changes occurred in the levels of nicotinic acid and vitamin B_{12}. Pretreatment with vitamin supplements had a variable effect on rats with CCl_4-induced liver necrosis: it increased nicotinic acid and vitamin B_{12} titres above those seen with vitamin supplementation alone; prevented a decrease in thiamine; minimized reduction in folic acid, folinic acid, vitamin B_6-complex, and unexpectedly lowered pantothenic acid to or below the titres seen in animals not receiving vitamins.

After inducing liver necrosis in animals not receiving vitamins, the ensuing regeneration was associated with a slow return of the normal tissue vitamin titres; in most instances, low tissue-levels persisted 120 h after receipt of CCl_4-vitamin therapy enhanced restoration to normal levels.

Results of this work show that supplemental thiamine, nicotinic acid, and vitamin B_6 do not significantly raise hepatic tissue-levels of these vitamins in normal animals, but there is a significant increase in folic acid, folinic acid, and vitamin B_{12} following vitamin B_{12} and folic acid administration; this effect persisted after supplementation was discontinued. The latter result may be due to the greater tissue-binding capacity by the liver for these vitamins.

Despite receipt of pantothenic acid, there was a reduction in hepatic tissue-level of this vitamin in both normal animals and in animals following CCl_4-induced liver injury.

It seems that increased catalytic activities resulting from administered vitamins increase coenzyme *A* and pantothenic acid turnover. The lowering of folic and folinic acids during the regenerative phase may be attributed to their participation in forming nucleic acids for new cell syntheses[5].

B-complex vitamins given to normal animals caused an increase in tissue vitamin B_{12}, folic acid and folinic acid, and no significant alteration in thiamine, nicotinic acid or vitamin B_6-complex. This indicates that normal rat liver is not fully saturated with vitamin B_{12}, folic or folinic acid. The necrotic liver had diminished vitamin-levels, indicating a decreased vitamin binding by the necrotic liver. Supplemental vitamins hastened restoration to normal levels during the regenerative phases—a time when vitamin-binding capacity was probably also returning.

These results suggest an inverse relationship between some vitamins, especially vitamin B_{12} and pantothenic acid. It has been shown that increase in hepatic pantothenic acid is induced by a vitamin B_{12} deficiency; the reverse was also true[7,8].

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Periodicity of Absorption Properties in Pigments based on Vitamin A_2 from Fish Retinæ

THE results presented here concern the absorption properties and vitamin A base of visual pigments extracted from the retinæ of eighteen species of fishes inhabiting North American waters. The methods used here have been fully described elsewhere[1]. All retinal extracts were

Table 1. DISTRIBUTION OF VITAMIN A_2-BASED PIGMENTS IN THE FISHES EXAMINED

Name	Habitat	λ_{max} (mμ) of pigments based on: Vitamin A_2	Vitamin A_1
Ameiurus nebulosus marmoratus (Holbrook)	fw*	534	—
Chaenobryttus coronarius (Bartram)	fw	524	—
Lepomis microlophus (Günther)	fw	525	—
Micropterus salmoides floridanus (Le Sueur)	fw	525	—
Lepomis macrochirus purpurescens Cope	fw	525	—
Lepisosteus platyrhincus De Kay	fw	523	—
Amia calva L.	fw	525	—
Umbra limi (Kirtland)	fw	526	—
Catostomus commersonnii (Lacépède)	fw	524	—
Notemigonus crysoleucas boscii (Valenciennes)	fw	529	502
Notemigonus crysoleucas auratus (Rafinesque)	fw	532	504
Pimephales notatus (Rafinesque)	fw	535	505
Hybopsis biguttata (Kirtland)	fw	535	505
Notropis cornutus frontalis (Agassiz)	fw	536	505
Balonesox belizanus Kner ♂, ♀	fw/m†	521	498
Mugil curema Cuvier and Valenciennes	fw/m	522	499
Mugil cephalus L.‡	fw/m	522	499
Dorosoma cepedianum (Le Sueur)	fw/m	521	ca. 500

* Fresh water.

† Fishes entering both marine and fresh waters, including brackish water dwellers; no indication of primary habitat, if any exists, is intended.

‡ Munz (ref. 8) found a single pigment with λ_{max} at 520 mμ and based on vitamin A_2.

tested for pigment homogeneity by the method of partial bleaching[2]. The results are listed in Table 1.

One striking feature seen in these results is that absorption maxima (λ_{max}) are situated, with one exception, at 523 ± 3 and at 534 ± 2 mμ. Prior to the work recorded here, the vitamin A_2-based pigments of only 15 species of fishes had been adequately characterized; if, however, these results are considered together with those presented here, it may be seen that pigments apparently occur in groups at certain 'preferred' positions in the spectrum[3]. As Fig. 1 shows, these are centred at 511·5, 523·5, 534 and 543 mμ. It may be noted that the vitamin A_2-based pigments of Amphibia fall into the group centred at 523·5 mμ (ref. 4).

This observation raises two interesting possibilities. (*a*) One possibility is that the molecular structures of visual pigments do not permit continuous variations of λ_{max}. Thus a visual pigment molecule might be envisaged in which the protein moiety can influence the λ_{max} in a series of steps due to the addition of certain units analogous to double bonds in a polyene chain (it being assumed that the isomeric configuration of the chromophore is the same in all instances). From this point of view, the fish is 'presented' with a series of pigments of fixed λ_{max} from which it must 'choose' one to suit its own peculiar requirements.

(*b*) The other possibility is that while there may be no reason why pigments should not continuously vary their λ_{max} throughout the spectrum, the 'preferred' positions have been gradually evolved by unrelated species in order to accommodate certain common visual requirements.

At present, it is not permissible to attempt to distinguish between these two possibilities, although the existence of an 'aberrant' pigment with λ_{max} at 529 mμ in Fig. 1 (this is the well-established pigment of *Notemigonus crysoleucas boscii*[1] in Table 1) lends slight favour to the second, suggesting that pigments of intermediate λ_{max} are structurally possible.

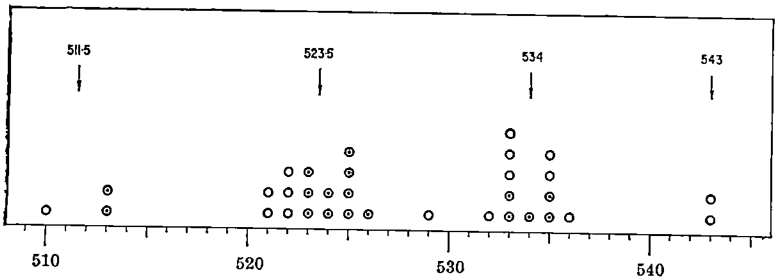

Fig. 1. Distribution of vitamin A_2-based pigments in fishes. λ_{max} are derived from Table 1 and refs. 4–8. The pigment with λ_{max} = 533 mμ of *Alburnus* (ref. 9) is regarded as paired and that of *Tinca* (ref. 2) as single. ⊙, single pigments; ○, pigments paired with corresponding ones based on vitamin A_1

The pairing of vitamin A_2-based pigments with others based on vitamin A_1 (in half the species of Table 1) and its probable function will be discussed elsewhere[10].

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PHARMACOLOGY

Blood Salicylate during Pregnancy

In the course of investigating the effects of aspirin on pregnancy in the rabbit and rat, we have observed a difference in the free salicylate blood concentrations in pregnant and non-pregnant animals. Such a difference has been reported previously by Hartiala *et al.*[1] following oral administration of salicylamide to rabbits. Their work showed that concentrations of free salicylamide in the portal blood were much lower in pregnant than in non-pregnant animals, and that there were also small differences in the concentrations of the glucuronide conjugate of salicylamide.

In our experiments aspirin was given as an aqueous solution of the sodium salt adjusted to *p*H 7·0 at a dose of 120 mg/kg. Female rabbits in late pregnancy and non-pregnant controls received a dose volume of 1 ml./kg body-weight introduced into the mouth through a blunt needle. The animals were restrained in stocks, and blood samples collected into heparinized tubes by puncture of the marginal ear vein at various times up to 6 h after dosing. Female rats on day 19–23 of pregnancy and non-pregnant controls received a dose volume of 0·1 ml./kg body-weight introduced into the stomach by intubation. At each time-interval three different rats in each group were anæsthetized by ether inhalation and blood collected by cardiac puncture into a heparinized syringe. The free salicylate concentrations in the samples of whole blood from rabbits and rats were estimated by Trinder's method[2]. The values obtained from three rabbit experiments and from two rats experiments are presented in Table 1.

These results clearly indicate that the concentrations of free salicylate in whole blood are smaller in the pregnant than in the non-pregnant groups in both rabbits and rats. Statistical analysis shows that the mean free salicylate concentrations are significantly less in the pregnant groups in experiment 2 ($P < 0·05$) and experiments 4 and 5 ($P < 0·001$). The differences in experiments 1 and 3 taken alone are not statistically significant, but in all cases the mean values are smaller in the pregnant groups. In rabbits the relative difference in concentrations was greater with increase in time after dosing. For example, at 0·75 h the salicylate concentration in the pregnant

Table 1. FREE SALICYLATE CONCENTRATIONS (MG/100 ML.) IN WHOLE BLOOD OF PREGNANT AND NON-PREGNANT ANIMALS AFTER ORAL ADMINISTRATION OF ASPIRIN (120 MG/KG BODY-WEIGHT)

Groups of individual values are given followed by the mean value in parentheses

Species	Exp. No.	Pregnant or not	Day of pregnancy	Time after dose (h)			
				0·75	1·5	3·0	6·0
Rabbit	1	Pregnant	23	15·1 (15·1)	14·0 (14·0)	9·3 (9·3)	4·0 (4·0)
		Not		15·7, 21·1, 10·2, 21·3, 12·8 (16·2)	14·9, 20·8, 8·0, 18·4, 17·0 (15·8)	11·8, 18·7, 5·9, 16·3, 17·1 (14·0)	2·8, 15·3, 7·1, 13·9, 16·7 (11·2)
	2	Pregnant	28	15·8, 14·4 (15·1)	17·1, 14·1 (15·6)	11·5, 11·1 (11·3)	6·4, 10·8 (8·6)
		Not		14·1, 19·7, 12·0 (15·3)	14·5, 18·6, 15·3 (16·1)	18·5, 18·8, 16·0 (17·8)	8·9, 14·5, 17·9 (13·8)
	3	Pregnant	30			10·0, 17·5 (13·7)	
		Not				17·3, 20·9, 21·6 (19·9)	
Rat	4	Pregnant	20–22	19·3, 17·6, 15·1 (17·3)	18·0, 18·2, 17·6 (17·9)	16·4, 17·8, 13·2 (15·8)	15·1, 13·6, 16·8 (15·2)
		Not		27·0, 21·5, 24·0 (24·2)	22·8, 20·9, 21·1 (21·6)	20·3, 18·9, 18·3 (19·2)	16·5, 15·9, 17·5 (16·6)
	5	Pregnant	19–23	21·7, 22·2, 18·4, 15·2 (19·4)	21·6, 16·7, 17·4, 16·1 (18·0)	17·7, 17·5, 15·5, 12·6 (15·8)	15·4, 13·0 16·1, 12·8 (14·3)
		Not		24·7, 31·1, 24·3, 21·9 (25·5)	21·7, 24·3, 20·8, 18·7 (21·4)	17·2, 22·6, 17·8, 20·3 (19·5)	18·3, 19·7, 17·7, 15·6 (17·8)

groups was on average 96 per cent of the controls, while at 6 h the average value had fallen to 49 per cent of the controls. This would indicate a shorter biological half-life for free salicylate in the pregnant animals than in the non-pregnant controls. In the rats, however, this effect was not apparent.

These observations suggest that the rate of metabolism and/or elimination of salicylate is more rapid in pregnancy. Booth and Gillette[3] have found that some steroid hormones affect the rates of drug metabolism so that changes in maternal hormones during pregnancy could explain the above findings. Another possible explanation would be accumulation of the drug by the fœtus; but if this were the case the drug must be stored selectively by the fœtal tissues because we have found that the salicylate concentrations in fœtal blood are about three-quarters of the maternal concentrations at various times after a dose of aspirin (120 mg/kg) to rabbit and rat.

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Stimulant Action of Barium on the Adrenal Medulla

BARIUM has been reported to release catecholamines from the adrenal gland in intact animals, and this action has been attributed to its setting up discharges in the splanchnic nerves[1]. A re-examination of the action of barium seemed to be called for in the light of more recent evidence that calcium, a closely related alkali earth, is intimately involved in the secretory process within the adrenal chromaffin cells. This evidence has suggested that the chemical transmitter liberated by the splanchnic nerves, acetylcholine, stimulates the chromaffin cells to secrete by increasing the permeability of their membranes so that there is an inward movement of calcium. In this view the appearance of an excess of calcium ions at some site in the chromaffin cell provides the immediate physiological stimulus for the release of the stored catecholamines[2–4].

We have examined the action of barium in acutely denervated cats' adrenal glands perfused through their arteries with Locke's solution, as previously described[2]. The perfusate escaping from the adrenal vein was assayed for catecholamines by bioassay[2], or by the photofluorometric ferricyanide method[5,6].

When barium was added to the perfusion medium in a concentration of 5 mM, the rate of catecholamine release increased greatly in every experiment. The increase was commonly more than 100-fold, for example, from 60 ng/min to 14·4 μg/min. Similar effects were obtained when the perfusion medium contained hexamethonium in a concentration (5×10^{-4} g/ml.) sufficient to block indirect actions through the splanchnic nerves which, although severed, were still present in the acutely denervated preparation. The stimulant effect of barium must therefore be attributed to some direct action on the chromaffin cells.

Unlike acetylcholine (or excess potassium), the stimulant action of which is abolished by removing calcium from the extracellular environment[2], barium continued to exert strong actions when given during perfusion with calcium-free 'Locke's solution'; and, indeed, its action persisted when the gland was perfused for 40 min or longer with a calcium-free 'Locke's solution' containing 3 mM EDTA as the disodium salt. This stimulant action of barium in the calcium-deprived gland mimics the action of calcium given in similar circumstances[2], and, like the corresponding effect of reintroducing calcium to the medium[3], was strongly inhibited by magnesium. Barium thus seems to be able to activate the 'secretory mechanism' just as does calcium. But barium has clearly some additional action which is not shared by calcium, for, as described here, it stimulates secretion when added during perfusion with Locke's solution, which contains 2 mM calcium. This is not true of calcium. The addition of excess calcium during perfusion with Locke's solution has little or no effect on secretion : calcium stimulates secretion only when the chromaffin cells are rendered unduly permeable to it either by a preceding period of calcium-deprivation or by exposure to acetylcholine or excess potassium[2,3]. Barium, however, appears to gain access to the 'secretory mechanism' without such aids. Since its hydrated ion radius is somewhat larger than that of calcium[7] it seems unlikely that barium is reaching this critical site simply by penetrating 'pores' in the chromaffin cell membrane more easily than calcium does. It seems necessary to suppose that barium has some action on the cell membrane which facilitates its own entry (and perhaps the entry of extracellular calcium). One possibility might be that barium promotes entry of sodium and by thus depolarizing the chromaffin cell membrane increases its permeability. Excess potassium, for example, is known to increase uptake of calcium in the adrenal medulla[8], and the effect of barium on the adrenal gland is reminiscent of that of calcium given along with a depolarizing concentration of potassium[3]. But against this possibility we have evidence that barium continues to act powerfully when added during perfusion with a sodium-free Locke's solution (Fig. 1). Further evidence that depolarization is not important for barium's action came from experiments in which barium was first added to Locke's solution to produce a sustained secretory response and then excess calcium was added while barium was still present. We argued that if barium did indeed owe its effect to some depolarizing action then the addition of excess calcium during exposure to barium should increase secretion as it does during exposure to excess potassium[3]. Excess calcium, however, had the opposite effect in these experiments and inhibited the barium-evoked secretion. This observation directed our attention to the possibility that barium might be acting to alter permeability by exchanging with the calcium bound to the membrane. We have earlier presented evidence that permeability of the chromaffin cells is increased when calcium is removed from their membranes by perfusion with a calcium-free medium[2,3]—and have suggested that the physiological secretagogue, acetylcholine, may increase the permeability of the chromaffin cell by displacing or prizing off this critical calcium fraction in its membrane[9]. It is all the more attractive to suppose that barium, which is more closely related in its chemical properties to calcium than is acetylcholine, is acting in a similar way and displaces membrane calcium by competition. This would imply that the replacement of calcium by barium renders the chromaffin cell membranes more permeable to these ions. Further evidence of antagonism between barium and calcium came from experiments in which brief secretory responses to barium were evoked at regular intervals either in Locke's solution or sodium-free Locke's solution, and where excess calcium was added along with the barium on alternate tests. It can be seen from Fig. 1 that increasing the calcium concentration inhibited the stimulant effect of barium on catecholamine secretion. Since the effects of calcium on secretion are excitatory whenever calcium is able to traverse the cell membrane, its inhibitory effect in this experiment indicates an action at an earlier stage and supports the idea that the two ions are competing for some membrane binding site.

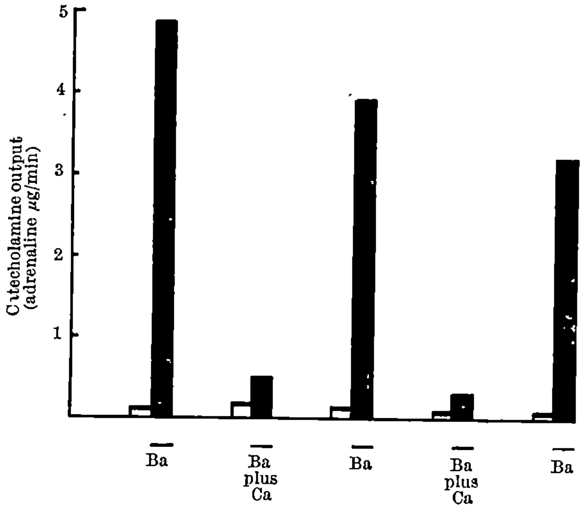

Fig. 1. The stimulant effect of barium on catecholamine secretion and its antagonism by calcium. The five successive pairs of responses were obtained at intervals of 14 min from a cat's adrenal gland perfused with sodium-free Locke's solution containing 2 mM calcium and hexamethonium 5×10^{-4} g/ml.: the tonicity of the solution was maintained by sucrose. The black columns indicate the secretory responses on adding barium (5 mM) alone or barium (5 mM) along with calcium (6 mM) for 2 min: the open columns indicate the control rates of catecholamine secretion in the 2 min preceding each test

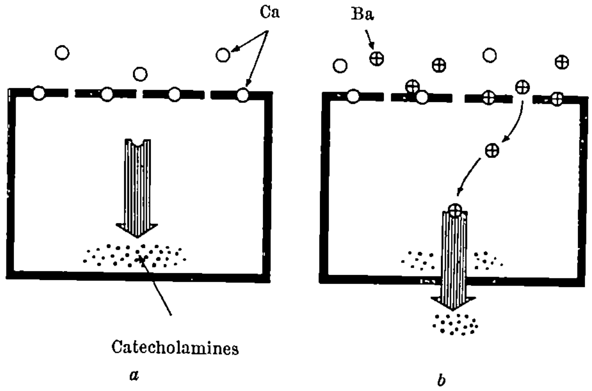

Fig. 2. A model of the suggested mechanism of action of barium on the adrenal chromaffin cells. In (*a*) the extracellular fluid contains a normal amount of calcium ions (○). The cell membrane is shown saturated with calcium, the function of which is to maintain a relatively impermeable state to ions including calcium itself. This is indicated by the small diameter of the 'pores' in the membrane. In (*b*) barium (⊕) has been added. It is depicted competing with calcium for the membrane sites of binding. Where it succeeds in displacing calcium the permeability of the membrane increases—as indicated by the enlarged pores—and barium (or calcium) enters the cell to activate the 'secretory mechanism' and induce extrusion of catecholamines. For simplicity, the 'pores' (channels of entry of calcium or barium, etc.) are shown distinct from the sites of calcium binding. This is not intended to imply that they are separate. The same comment applies to the site of barium (or calcium) entry and catecholamine extrusion

In summary, then, we suggest that barium acts on the chromaffin cell membrane to displace calcium, and that the barium–membrane complex is more permeable than the calcium–membrane complex; that this allows barium to traverse the cell membrane; and finally, that barium, once inside the cell, mimics the action of the normal secretagogue, calcium, and sets in motion the apparatus for catecholamine extrusion. These events are depicted in diagrammatic form in Fig. 2, which is based on a scheme presented at the second International Pharmacological Meeting in Prague in August 1963 to illustrate the supposed mechanism of action of acetylcholine[9].

In addition to barium, we have tested other alkali earths and various divalent ions. Strontium (2 mM) was almost indistinguishable from calcium in its effects on the adrenal gland. Magnesium (2–80 mM) did not itself evoke secretion in any of our experiments and antagonized secretory responses to other alkali earths, acetylcholine and potassium. Cobalt, manganese, nickel and zinc (each in a concentration of 2 mM) were without stimulant effect when added during perfusion with calcium-free Locke's solution and did not restore the responsiveness of the gland to acetylcholine. This spectrum of activity may possibly offer a clue to the nature of the unknown 'calcium-dependent' (or 'calcinergic') process involved in catecholamine extrusion.

Finally, we find it of great interest that our analysis of the effect of barium has led us independently to a mechanism of action on the chromaffin cell which resembles that advanced to explain its well-known stimulant action on smooth muscle[10]. We see in this a further extension of the already remarkable parallelism between the processes of 'stimulus-secretion coupling' in glands and 'excitation-contraction coupling' in muscles to which we have already directed attention[2-4].

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Anomalous Reactions of the Rat to Ganglion Blocking Agents

In work which will be reported elsewhere it was found that certain extracts of the solanaceous plant *Cestrum parqui* caused a pronounced and prolonged elevation of the blood pressure of the anæsthetized rat. Since this reaction did not occur in the pithed animal, attempts were made to block the pressor effect at the autonomic ganglia. When hexamethonium failed to block the effects of *C. parqui* extracts it was decided to examine the effects of ganglion blocking agents *per se* in the rat.

Male albino rats of an inbred line originating from the Wistar strain and weighing 210–245 g were anæsthetized by intraperitoneal injection of urethane or intravenous injection of pentobarbitone sodium. Blood pressure was recorded from the left common carotid artery. The right common carotid artery was cleared and made available for clamping at intervals for 30-sec periods. Intravenous injections were made into the tail veins and washed in with 0·9 per cent saline. The doses which follow refer to amounts of base in each case, the substances used being hexamethonium (as the bromide, iodide or tartrate), pempidine tartrate, mecamylamine hydrochloride and 1-noradrenaline base.

Initially, hexamethonium was injected intramuscularly in a dose of 10 mg/kg, this being the dose used by Kärki[1] to stabilize the blood pressure and sensitize the animal to catecholamines. This produced a slight fall in blood pressure, but was without effect on the rise resulting from clamping of the right carotid artery. In subsequent experiments, intravenous hexamethonium in doses ranging from 1 to 10 mg/kg caused a marked blood-pressure fall, but whether or not an additional 5 or 10 mg/kg has been given intramuscularly or subcutaneously, there was never complete suppression of the pressor response to carotid clamping. This response, although markedly diminished during the first minute or two after intravenous injection, rapidly returned to its previous level. Subsequent repetitive dosage with hexamethonium intravenously had less and less blocking effect on the response, and after 3 or 4 doses of 10 mg/kg the response was markedly enhanced. This pattern continued with further doses, the animals usually dying after a total of 70–80 mg/kg had been administered over a period of 1·5–2 h.

Similarly, pempidine intravenously in doses ranging from 0·1 mg/kg to 5 mg/kg, and mecamylamine intravenously in doses of 0·5–5 mg/kg never completely blocked the pressor response to carotid clamping. With each drug, repetitive dosage at the 5-mg/kg level eventually enhanced the response.

The sensitizing effect of doses of hexamethonium 'too small to influence ganglionic transmission' in the rat submitted to carotid occlusion and noradrenaline injections has been reported by Sultan Mawji and Lockett[2]. The very large doses used in the present experiments, as well as enhancing the carotid reflex, also enhanced the response to injected noradrenaline. Furthermore, hexamethonium itself in some circumstances produced a marked pressor response. This was observed in the pithed rat preparation, after several doses of noradrenaline and three or four successive doses of hexamethonium 10 mg/kg. Afterwards, similar injections of hexamethonium caused a blood-pressure rise comparable in height and duration with the effect of 50 ng of noradrenaline. Injection of saline controls eliminated the possibility of the pressor effect being due to venous stagnation of earlier doses of noradrenaline.

It is of interest to note that examples of quaternary, tertiary and secondary amine ganglion blocking drugs were unable to block the pressor response to carotid occlusion in the rat, and that in fact all three agents in high dosage enhanced the response. Of the procedures used, only pithing the animals completely obliterated the pressor response.

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PATHOLOGY

Resistance against Carcinomas of the Skin induced by Dimethylbenzanthracene (DMBA) in Mice of the Strain XVII/*Bln*

Past investigations in many laboratories concerning the antigenicity of experimental tumours have revealed that almost all the sarcomas induced by carcinogenic hydrocarbons contain specific antigens. For this reason a certain pretreatment of isologous recipients with these tumours can produce resistance against cell inocula of the tumour originally taken for immunization. Using this test system specific antigens were demonstrated in sarcomas which were induced by administration of methylcholanthrene[1-7], benzpyrene[5,8,9], dibenzpyrene[6], dibenzanthracene[10], and dimethylbenzanthracene[11].

Dependent on the mode of application the same substances are capable of producing carcinomas. Those tumours develop if the carcinogen acts on tissue of epithelial origin.

The demonstration of host resistance against grafts of the induced carcinomas would mean that the development of specific antigens is not only a characteristic of the sarcomas, that is, of tumours of mesodermal tissue origin, but also of tumours which arise from epithelial tissue under influence of the carcinogen.

First evidence for a tumour-specific immunizing capacity of epithelial tumours was obtained by Prehn[12], who has shown that methylcholanthrene-induced breast tumours (adenoacanthomas) of the *BALB/cAn* strain were antigenic in isologous hosts.

In this communication experiments on the induction of resistance against isologous cell inocula of DMBA-induced carcinomas of the skin are described.

Experimental animals were female mice of the strain XVII/*Bln*. For the purpose of tumour induction an acetone solution containing 0·01 per cent DMBA was prepared. Twice a week for a period of 10 weeks one group of mice (about 3 months old) was treated with the carcinogen-containing solution. Using a glass capillary to each mouse 3 drops of the solution were given to the dorsal region of the skin. The animals received a total amount of 120γ DMBA per mouse. The two tumours tested developed after latencies of 202 (DMBA 592) and of 267 (DMBA 788) days. Histologically they have been identified as keratinizing squamous epithelium carcin-

Table 1. RESULTS OF THE IMMUNIZATION AGAINST ISOLOGOUS CELL INOCULA OF DMBA-INDUCED CARCINOMAS OF THE SKIN

Mouse strain	Carcinoma of the skin	Pretreatment	Challenge dose	No. of days after challenge injection 25 Immunized group	 25 Control group	 32 Immunized group	 32 Control group	 39 Immunized group	 39 Control group
XVII	DMBA 502	Injection of irradiated tissue suspensions*	6 × 10^5	10/10‡	0/6	10/10	0/6	10/10	0/6
		Injection of living cells (1·6 × 10^5)*		8/8		8/8		8/8	
XVII	DMBA 788	Injection of irradiated tissue suspensions†	3 × 10^5	13/30	1/30	11/30	1/30	5/30	1/30
			Total:	31/48	1/36	29/48	1/36	23/48	1/36
			Percentage of resistant animals:	65	3	60	3	48	3
			χ^2	= 33·2		= 30·0		= 20·6	
			P	$< 10^{-7}$		$< 10^{-7}$		$< 10^{-5}$	

* Tumour material of the third up to the fifth generation of transplanting.
† Tumour material of the second generation of transplanting.
‡ Number of resistant animals over the total number inoculated.

omas. Because of the slow growth of the first transplant generations the immunization experiments were performed after 2–5 generations of transplanting the tumours in isologous hosts. Contamination of the primary tumour with bacteria was eliminated by suspending the cells in penicillin (100 I.U./ml.) and streptomycin (100 μg/ml.) containing saline solution following trypsination of the material.

According to previous experiments[7], isologous recipients were immunized by subcutaneous injections of the X-irradiated (14,500 r. *in vitro*) tumour suspensions prepared in a glass homogenizer. The animals received two injections in doses of 0·25 ml. per mouse at an interval of 14 days. The tumour material was diluted 1 : 4 in phosphate-buffered physiological saline solution. One day after the last immunizing injection a certain number of living cells was injected in the contralateral subcutaneous region of the mice to test the induced resistance. The cell suspension used for challenge was prepared by trypsination (0·15 per cent solution) of the tumour tissue. After vital staining of the cell suspension with trypan blue the dose required was adjusted. Control animals were mice of the same age and sex without prior immunization. The growth of the tumour implants was examined at weekly intervals.

The results obtained 25, 32 and 39 days after challenge injection are summarized in Table 1. The number of resistant animals in each group is presented over the total number inoculated. In the first experiment, in which DMBA carcinoma 592 was tested, all the animals pretreated were found to be resistant against this tumour if 6 × 10^5 cells were given subcutaneously. Eight animals in this group were immunized by a subcutaneous injection of 1·6 × 10^5 living tumour cells which did not lead to tumour formation. Six control mice developed tumours.

In the second experiment X-irradiated tissue suspensions of the carcinoma 788 were used for pretreatment. This tumour apparently induces a weaker resistance in the recipients although the immunization method used was the same as for carcinoma 592. 25 days after challenge inoculation (3 × 10^5 cells) only 13 of 30 animals in the experimental group were resistant. The number of resistant animals decreased to 5 mice 39 days after challenge injection and thereafter remained constant for an observation period of 90 days. In one animal of 30 control mice the tumour implant did not grow.

The final results of both experiments, presented as percentages of resistant animals in the experimental series in comparison with those in the controls, indicate that in mice a state of relative resistance against isologous cell inocula of carcinogen-induced carcinomas of the skin can be produced. The results of 65, 60 and 48 per cent resistant animals 25, 32 and 39 days after challenge injection in the test group as compared with 3 per cent in the control group are statistically significant.

From these experiments and considering Prehn's experiments[12], the conclusion can be drawn that tumour-specific antigenicity is not only a characteristic of carcinogen-induced sarcomas but also of carcinomas induced by carcinogenic hydrocarbons.

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Susceptibility of Syrian Hamsters to Induction of Fibrosarcomas with a Single Injection of 3,4,9,10-Dibenzpyrene

THE carcinogenic activity of polycyclic hydrocarbons administered subcutaneously to hamsters was first described by Gye and Foulds[1]. Later, Halberstaedter[2], Crabb[3], and Lutz *et al.*[4] reported the sarcomagenic activity of other polycyclic hydrocarbons in hamsters. These investigators were primarily interested in the question of transplantability and metastases and used multiple injections for tumour production.

The experiments reported here were designed to determine the susceptibility of the hamster to a single subcutaneous dose of 3,4,9,10-dibenzpyrene (3,4,9,10-DBP). For 3,4,9,10-DBP the range was 2–0·25 mg/hamster. The carcinogen was dissolved in tri-*n*-caprylin ('Trioctanoin') at different concentrations such that the desired dose was contained in 0·4 ml.

Five groups of male hamsters weighing 55–75 g were randomly assigned to treatment and control groups, and were injected subcutaneously at the nape of the neck to determine the susceptibility and the dose necessary to induce tumours in a substantial proportion of the hamsters. At the highest dose of 2 mg/hamster, four animals died before the appearance of the first tumour.

Microscopically, the sections revealed typical fibrosarcomas of the subcutis which were fairly uniform in their histological pattern. The tumours were classified as typical spindle-cell sarcomas with closely packed spindle-shaped tumour cells. Solid masses of cells alternating with large and small whorls and interlacing bundles of fibrous connective tissue were observed. The tumour cells ranged in size and varied in shape from blunt to narrow

Table 1. Incidence of Subcutaneous Tumours in Male Syrian Hamsters given a Single Subcutaneous Injection of 3,4,9,10-Dibenzpyrene

Agent	Dose (mg/hamster)	Effective No. of animals	No. of tumours per week and time (in weeks) 5	6	7	8	9	10	11	12	13	14	15	16	17	No. of tumours	Tumour incidence (%)	Average latency period (weeks)	Carcinogenic index
3,4,9,10-DBP	2·0	6				2		2	1	1						6	100	9·8	146
	1·0	10	1				2	2	2	2	1					10	100	10·2	140
	0·5	10							2	1	1	5				9	90	13·0	99
	0·25	9						1			1		2		1	5	55	14·0	56
'Trioctanoin'		10														0	0	-	—

Table 2. Subcutaneous Tumour Production in Syrian Hamsters induced with Single Injection of 1·0 mg 3,4,9,10-Dibenzpyrene, Hamster

Exp. No.	Effective No. of animals	Sex	No. of tumours per week and time (in weeks) 6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	No. of tumours	Tumour incidence (%)	Mean latency period (weeks)	Carcinogenic index
1 and 3	157	♀		5	6	19	40	32	23	18	4	5	4						1						157	100	11·0	130
2 and 4	139	♂			6	15	17	37	23	16	11	5	4	3	2										138	99	11·6	123

spindles. Mitoses were common, and occasional giant cells were present.

The incidence of tumours, mean latency period and carcinogenic index are recorded in Table 1. The percentage incidence is the number of tumours divided by the number of hamsters inoculated times 100. The mean latency periods were computed by multiplying the number of tumours each week by the time weeks after injection and dividing the sum by the total number of tumours. The carcinogenic indices, a value suggested by Iball[5], were determined by the formula $P/T \times 100$, in which P is the percentage of hamsters developing tumours, and T the average latent period of the experimental group in days.

It is evident from the data that 3,4,9,10-DBP is sarcomagenic for the hamster. The results also indicate that the tumour incidence and carcinogenic index decreased and the latency period increased as the dose decreased. Similar findings in mice were obtained for benzpyrene, dibenzanthracene and for methylcholanthrene[6–8]. As this was a pilot investigation to determine the susceptibility of the hamster, only a small number of animals was used in each group, but since the completion of this experiment an additional 169 female and 151 male hamsters have been injected subcutaneously with a single injection of 3,4,9,10-DBP at a dose-level of 1 mg/hamster. Twelve female and 13 male hamsters died without tumours during the course of the experiment.

The results in Table 2 show that the incidence of tumours, mean latency period and carcinogenic indices were similar for both male and female hamsters.

With the knowledge that the hamster can be utilized for the experimental induction of fibrosarcomas, detailed investigations are now in progress to gain some insight into the biological characteristics of the hamster fibrosarcoma resulting from a single subcutaneous injection of 3,4,9,10-dibenzpyrene.

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Distribution of Intravenously Administered Saccharated Iron Oxide in the Popliteal Lymph Node of the Rabbit

Labelled thoracic duct lymphocytes transfused into host rats have been shown to migrate across the walls of the post-capillary venules of the lymph node, through the cortex into the medullary lymph sinuses[1,2]. In the present investigation, the fate of non-cellular particles in the lymph node of rabbits has been examined using intravenous

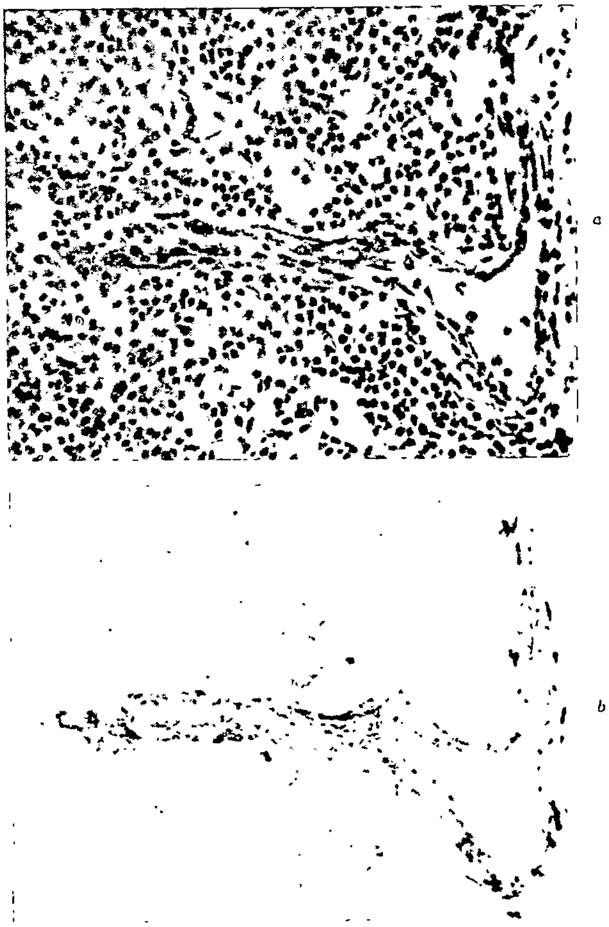

Fig. 1. Cortex of popliteal lymph node of rabbit after 20 days' intravenous 'Ferrivenin' administered as in text. *a* and *b* are of the same section stained with Prussian blue-nuclear fast red. By selective colour filtering *a* shows a venule with numerous intramural lymphocytes, while *b* demonstrates iron aggregates in the wall of the same vessel. Sections cut at 3μ ($\times c.$ 300)

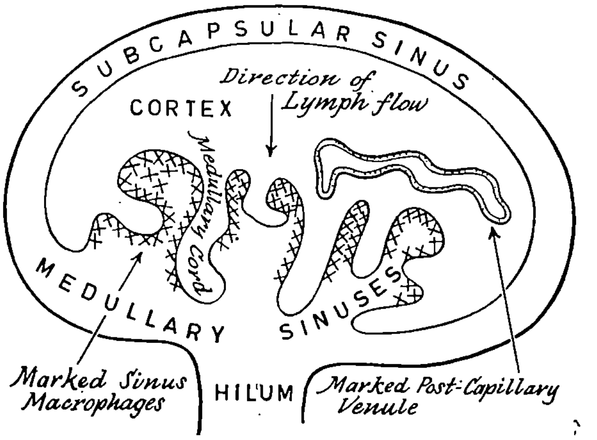

Fig. 2

saccharated iron oxide ('Ferrivenin', Benger) as the marker.

The marginal ear vein of adult rabbits of both sexes was injected with 1 ml. of 'Ferrivenin' daily for 1–20 days. One animal was killed each day, 24 h after its last injection, and popliteal lymph nodes were removed. Tissues were fixed in 10 per cent formolsaline (buffered with calcium carbonate) for 48 h and embedded in paraffin. Sections were cut at 3μ, stained by the Prussian blue reaction and counterstained by nuclear fast red[3].

Blue granules were found in the cytoplasm of medullary sinus macrophages after only one injection of 'Ferrivenin'. In the lymphatic cortex the few granules present were in the walls of venules. These vessels were considered to be analogous to the post-capillary venules in the lymph node of the rat, because of the frequent presence of lymphocytes in their walls (Fig. 1*a*). The venular Prussian blue marking increased progressively with subsequent injections and was intense on the twentieth day (Fig. 1*b*).

The combination of marking in cortical venules and medullary sinuses (Fig. 2) raises the possibility that saccharated iron oxide particles traverse the cortical venular walls and are carried by the lymph flow into the medulla, to be ingested by macrophages. The exact position of these particles in the wall of the venules will have to be investigated by electron microscopy.

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HÆMATOLOGY

Conditions affecting the Glucose-6-phosphate Dehydrogenase Activity and Glutathione Stability of Human Erythrocytes

Table 1. EFFECT OF *p*H OF ACD ON THE G-6-PD ACTIVITY AND GSH STABILITY IN NORMAL HUMAN ERYTHROCYTES

G-6-PD activity (units/g Hb/min)	*p*H of glycylglycine buffer	*p*H of ACD	G-6-PD activity (units/g Hb/min)	GSH (mg/100 ml. erythrocytes) Initial	After incubation at 37° C 24 h	24 h and 2 h with APH
15	6·5	6	17·3	120	65	43
22	7	7·2	22	107	75	67
33	7·6	7·6	25	92	74	83
21	8	8	19·4	116	91	44
—	—	9	19	120	80	43

Table 2. G-6-PD ACTIVITY AND GSH STABILITY IN HUMAN ERYTHROCYTES STORED AT 4° C IN ACD OF *p*H 7·6

Erythrocytes	Time (weeks)	G-6-PD (units/g Hb/min)	GSH (mg/100 ml. erythrocytes) Initial	After incubation at 37° C 24 h	24 h and 2 h with APH
Fava-sensitive	0	2·4	72	66·5	17
	1	0	76	68	16·8
	2	—	74·5	72	23
	3	—	62·5	34	22
Normal	0	23	100	89	67
	1	19·8	102	93	68
	2	17·5	100	87	70
	3	15·8	104	68·4	21·1

Table 3. G-6-PD ACTIVITY IN HUMAN ERYTHROCYTES STORED AT 4° C IN ACD OF *p*H 7·6

Time (days)	G-6-PD (units/g Hb/min) Normal erythrocytes 1	2	3	*Fava*-sensitive erythrocytes
0	22·5	29·8	21·8	2·5
1	22·5	28·0	20·1	2·5
2	21·5	29·0	20·0	1·4
3	20·5	28·0	18·1	0·5
4	19·5	—*	—*	0·0
5	19·5	—*	—*	0·0

* No blood sample taken.

ALVING *et al.*[1] have shown that primaquine-sensitive erythrocytes are deficient in glucose-6-phosphate dehydrogenase (G-6-PD) activity and a similar abnormality has also been found in *fava*-sensitive erythrocytes[2]. Consequent on their diminished G-6-PD activity, such cells have an unstable reduced glutathione-level (GSH) when exposed to various oxidizing agents[3,4]. Estimations of GSH stability are defined more clearly when glucose is added to the test system[5]. Under these conditions, little reduction in the GSH-level occurs when either *fava*-sensitive or normal erythrocytes are incubated at 37° C for 24 h, whereas a significant decrease, limited to the *fava*-sensitive cells, is found after incubation for a further 2 h at 37° C with acetylphenylhydrazine (APH). This final post-incubation GSH-level has been used to distinguish *fava*-sensitive from normal erythrocytes[5].

Beutler[6] has suggested that specimens for such tests might be stored at 4° C in acid-citrate dextrose (ACD) since during storage the G-6-PD activity and GSH-level and its stability in both *fava*-sensitive and normal erythrocytes remain unchanged for up to 30 days[7]. In addition the ACD would provide adequate glucose for the GSH stability test.

Preliminary examination by us of blood stored in ACD showed, however, an excessive loss of enzyme activity, while blood stored for transfusion purposes, also in ACD, gave suspiciously low GSH-levels even after as short a period as 7 days.

For estimations of G-6-PD activity and GSH stability in *fava*-sensitive and normal subjects, 7-ml. aliquots of blood were added to 2 ml. ACD of varying *p*H. G-6-PD activity was estimated by the method of Marks[8] and GSH by the method of Thompson and Watson[9], and its stability by a modification of the technique of Szeinberg *et al.*[5].

Using these techniques, the optimum *p*H of the ACD for G-6-PD and GSH stability was found to be 7·6 (Table 1), the same as that found for enzyme activity in heparinized blood using glycylglycine buffer of varying *p*H. Presumably the unsatisfactory results obtained in our preliminary experiments were due to partial deterioration of the enzyme with an associated drop in GSH stability, while the difference between the optimum *p*H range of 8–9 found by Marks *et al.* and that now reported may result from the purified material used in their system. All subsequent experiments were performed, therefore, using ACD, the *p*H of which had been adjusted to 7·6.

Despite correction of the *p*H of the ACD, the G-6-PD activity declined during storage. In the *fava*-sensitive cells the initial level was very low, none being detected after a week, whereas in the normal cells the initial level was high, 87 per cent of the activity remaining after one week (Table 2). Closer examination of the early storage period showed that after three days the level in the sensitive cells was negligible while in the normal cells it remained at about 90 per cent of the original value (Table 3). In contrast the GSH-level and its stability, although lower in the sensitive cells, were unaffected by storage of the samples of blood at 4° C in ACD at pH 7·6 for up to two weeks. During the third week the GSH stability of the normal cells fell, the final post-incubation value reaching the low, but relatively constant, level found in the sensitive cells (Table 2). Clearly ACD is satisfactory as an anticoagulant for field studies only if its pH is corrected to 7·6. Although storage of the blood under optimal conditions maintains the GSH-level and its stability for up to two weeks, the sample should be examined at once to determine its full G-6-PD activity. As the decline in G-6-PD activity is approximately linear the results of tests performed during the first 48 h may be corrected to indicate the original level. Later, owing to the rapidity of the decline in activity, results are less reliable so that *fava*-sensitive and normal subjects might be confused.

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Differences of Bone Marrow Cells in Sheep and Man

Authors working on bone marrow of sheep have generally considered the cells sufficiently similar to those in man to disregard the necessity of their closer morphological examination. However, recent examinations of normal cells have disclosed differences which should not be neglected, particularly when changes of the bone marrow due to pathological conditions are to be examined. The detailed results are being published in monograph form, with comprehensive colour illustrations of all the cell types[1], and the following account summarizes the differences between (the cells of) sheep and man.

Using human material as a yardstick, the first impression gained of smears from sheep is apart from differences in shapes and sizes, a less brilliant display of colours, giving a duller and technically almost unsatisfactory picture. Differences between certain cells are less pronounced, making identification more difficult. The cells differ from their better known human counterparts mainly by the following characteristics.

In the erythrocytic series, the cells are smaller, due to less abundant cytoplasm, and develop into erythrocytes of an average diameter of only 4·5μ. The cytoplasm is more basophilic, which is particularly evident in the more mature stages where normoblasts show only a narrow rim of bluish cytoplasm around the nucleus. After denucleation, young erythrocytes are still bluish-grey, and only fully mature erythrocytes become more acidophilic, yet noticeably less so than in man. The failure of the erythocytes to stain a brighter pink contributes significantly to the duller appearance of the preparation. This cannot be improved by staining at a more acid pH as this would render the colour of the nuclei unsatisfactory.

In the neutrophilic series the cytoplasm becomes, with maturation, chromophobic rather than acidophilic. While it is distinctly blue in the youngest cells, it becomes less intensely stained with maturation and is pale blue or almost unstained at the metamyelocyte stage. In band cells it is even less stained, and in segmented cells, usually unstained, otherwise very pale pink. An outstanding feature of the neutrophilic series is the difficulty of demonstrating the characteristic granules. In segmented neutrophils small granules can usually be shown by certain conventional hæmatological dyes, in a narrow pH range, and a few granules are occasionally seen in band cells, but not in still younger cells. However, the presence of these granules, which are smaller than in man, can be demonstrated as far back as the myelocyte stage, by certain fat stains and the peroxidase reaction[2]. Segmented eosinophils rarely show two lobes, as usually seen in man, but three or four and occasionally more.

Monocytes are more difficult to recognize in sheep and may be confused with neutrophilic myelocytes, metamyelocytes or lymphocytes. The nucleus varies in shape from round to highly indented and resembles in texture that of a more mature metamyelocyte. The main difficulties are caused by the colour of the cytoplasm which is similar to that of neutrophilic myelocytes or metamyelocytes. The cytoplasm of the monocytes is less basophilic than in man, while that of the neutrophils is more basophilic than in the corresponding cells in man. Furthermore, the young neutrophils do not show granules. The 'ground-glass' appearance of the cytoplasm, usually characteristic for monocytes, is less pronounced, which makes differentiation from lymphocytes more difficult.

A myelogram of normal sheep based on 122 counts from 18 healthy animals, with an insignificant parasite burden[3], showed the proportions of erythrocytic and eosinophilic cells to be 60·8 and 8·76 per cent respectively, and thus several times higher than in man. The average granulocytic/erythrocytic ratio was 59·4 per cent and the average granulocytic maturation ratio 53·4 per cent. It is argued, however, that this form of calculation as practised in man, is not suitable for sheep. While in man granulocytes other than neutrophils amount to a relatively insignificant number, in sheep, with their larger proportion of eosinophils, such treatment of granulocytes as a uniform group would give valid results only if alterations of the eosinophils due to pathological conditions closely followed alterations of the neutrophils. As this is unlikely, neutrophils and eosinophils have been separated and the following average ratios have been found: neutrophilic/erythrocytic, 41·8 per cent; eosinophilic/erythrocytic, 16·37 per cent; neutrophilic/eosinophilic, 317·0 per cent; neutrophilic maturation, 49·2 per cent; eosinophilic maturation, 66·18 per cent.

These findings, representing the normal bone marrow of sheep, are now being used as a basis for studying alterations due to various pathological conditions; in the first place acute and chronic post-hæmorrhagic anæmia and helminthic anæmia.

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High-potency Antihæmophilic Factor Concentrate prepared from Cryoglobulin Precipitate

INVESTIGATIONS made to determine the causes of loss of antihæmophilic factor (AHF) during the Blombäck process[1] for preparing AHF-rich fibrinogen from large volumes of plasma have revealed that when the starting material is frozen plasma instead of fresh plasma there is a presumed liability which can be converted to an advantage.

The well-known susceptibility of AHF to denaturation by heat, storage, freezing and thawing has led to attempts both in Sweden[1], and in the United States[2], to arrange for prompt processing of freshly collected plasma whenever possible. If large-scale production is contemplated, however, such a requirement is a distinct handicap, and so our efforts have been directed to maximizing the yield from frozen plasma.

In order to investigate the causes of poor final product activity after standard Blombäck processing, samples were taken at each step to determine where the largest losses were occurring. As previously reported[3], the 'starting material' was found to have on the average only 54 per cent of the activity of the mean of normal individuals, and this low level was attributed to the freezing, thawing, and storage involved. Essentially all the remaining activity was recovered in Cohn Fraction I, but the citrate–ethanol–glycine extraction used to obtain the Blombäck Fraction I–O regularly resulted in large and variable losses of AHF activity. Still further decreases occurred during sterile filtration and ultra-violet irradiation. These latter procedures are not necessary in Sweden and England, where it is possible to process small batches under sterile conditions. Our final product, at that time, when prepared as a 2 per cent solution, typically had only the same AHF activity as normal plasma. More recent lots have approximately twice as much AHF activity, but even though the protein concentration is about one-third that of plasma, this is clearly a far from ideal therapeutic material.

A recent review of this project revealed that the 'starting sample' for analysis had in each case been taken after a preliminary step. This step, 'Clarification', involved centrifuging the plasma which had been thawed at 10°–12° C, pooled, and re-cooled to 1°–2° C, in order to remove any materials which had failed to dissolve. When six new batches of approximately 1,000 l. each were studied, it was found that the samples taken before clarification (the cryoglobulin material dissolves on warming for assay) averaged 90 per cent AHF activity, whereas after clarification the average was only 46 per cent. It thus became apparent that storage losses were not so great as previously thought, but that almost half the plasma AHF was being discarded in the clarification residue. This finding should not have been a great surprise since one of us reported previously that undissolved cryoglobulins in plasma include large amounts of AHF[4]. Attention now turned to this material, a gelatinous paste, of which approximately 2 kg were obtained from a 1,000-l. plasma pool. On a wet weight basis, between 10 and 20 per cent assays as protein, thus representing only about 0·4 per cent of the original plasma proteins. However, about 20 per cent of the original plasma AHF (half the AHF lost during the clarification) can be recovered from this material. Therefore, on a protein basis, a 50-times concentration of AHF activity has been achieved with this step alone.

Table 1. CIRCULATING AHF RESPONSE TO FRACTION *PR* 2018 IN FOUR PATIENTS WITH SEVERE CLASSICAL HÆMOPHILIA

Patient	^{51}Cr—Det'd. Plasma volume (ml.)	Fraction transfused (mg.)	Post-transfusion AHF-level (%)	Calculated AHF activity of fraction (units/g)
T. T.	2,500	375	16·5	870
R. P.	2,300	750	40	945
J. G.	2,360	750	35	848
S. F.	2,100	750	30	638
				Average = 825

Subsequent studies have dealt with further purification and sterile filtration of this material to prepare a concentrate for clinical use. A report on the details of this process is in preparation. The final product at present studied in clinical trials has an activity of 825 units of AHF (1 unit = AHF activity in 1 ml. of normal human plasma) per gram of protein, as determined *in vivo* after transfusion into hæmophilic patients. Actually our *in vitro* determination gives approximately twice this value in the Pool and Robinson AHG assay[5] following dilution of 0·1 ml. of the fraction with 0·9 ml. of hæmophilic plasma prior to the adsorption step. At this time there is no way of telling whether the true activity is in the range of 1,650 units/g, and, for some reason, only half of it is recovered after transfusion, or whether the true activity is 825 units/g, and we have not yet solved the problems of *in vitro* assay of very high potency materials.

Table 1 gives the data on the four patients with severe classical hæmophilia who received this material after determination of their chromium-51 plasma volumes had been carried out. The result on patient R. P., who received 750 mg of material, shows that a solution which can be administered with a 50-ml. syringe is capable of raising the circulating level of such a patient to 40 per cent of normal.

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IMMUNOLOGY

Specific Precocious Protective Action of Toxoids

THERE have been several reports of a specific precocious protective action, not involving antibody response, of tetanus toxoid against tetanus toxin[1-8]. Massive doses of tetanus toxoid injected into mice protected them against a few lethal doses of tetanus toxin injected at the same time, or shortly before, or shortly after. Diphtheria toxoid, given in doses comparable on a basis of antibody-combining power units, had no such protective effect against tetanus toxin[5,6]. Suggestions have been made that the protective action of tetanus toxoid may be connected with its ability to prevent the fixation of tetanus toxin by nervous tissue[7-9] and ganglioside[10].

We have tested whether other toxoids have a similar protective action against their homologous toxins. A number of toxins were titrated by injecting 0·5 ml. volumes of serial two-fold dilutions into control and toxoid-treated animals. Toxoid and toxin were injected in immediate succession intramuscularly in the same hind-limb. In the case of tetanus, toxoid and toxin were also injected intraperitoneally and intravenously. The toxins and toxoids (in crude form) were kindly supplied by Dr. R. O. Thomson of the Wellcome Research Laboratories. In each case, the largest practicable dose of toxoid was used, which was 0·5 ml. of the solutions as

Table 1

1	2	3	4	5	6	7	8	9
Toxin and toxoid	Toxoid values per dose before toxoiding		No. of animals per dilution of toxin	Total No. of animals protected in titration	LD_{50}/ml. toxin		LD_{50} against which protection achieved $(x-y)/y$	Molecules toxoid protecting against one molecule toxin (mean)
	$L+$	LD_{50}			Control animals	Toxoid-treated animals		
					x	y		
Cl. welchii Type *A*	3·25	81	2 2	1 0	96 48	64 64	0·5 −0·25	?
Cl. welchii Type *B*	90	900	2 2	1 1	96 64	64 48	0·5 0·25	$2{\cdot}4\times10^3$
Cl. welchii Type *C*	120	$2{\cdot}4\times10^5$	2 2	2 2	96 96	48 48	1·0 1·0	$2{\cdot}4\times10^5$
Cl. welchii Type *D*	155	$3{\cdot}1\times10^4$	2 2 2	3 2 4	320 240 320	120 120 80	1·6 1·0 3·0	$1{\cdot}7\times10^4$
Cl. oedematiens	6·5	$4{\cdot}4\times10^3$	2	0	24	32	−0·25	$>4{\cdot}4\times10^3$
Diphtheria	100	$3{\cdot}5\times10^3$	2	0	24	24	0	$>3{\cdot}5\times10^3$
Tetanus, *IP*	600	$2{\cdot}7\times10^7$	6 6	9 3	16 8	8 6	1·0 0·33	$4{\cdot}0\times10^7$
IV	600	$2{\cdot}7\times10^7$	2	2	12	6	1·0	$2{\cdot}7\times10^7$
IM	600	$2{\cdot}7\times10^7$	2	3	12	4	2·0	$1{\cdot}4\times10^7$

provided by Dr. Thomson. Mice were used in all the experiments except those with diphtheria toxoid and toxin, where guinea pigs were used. The number of animals used for each dilution of toxin are shown in Table 1. Deaths were recorded for 7 days after the injection of toxin. All the animals that died did so within two days, except those injected with tetanus toxin, which died on all the days between the first and seventh day after injection.

The results of these experiments are shown in Table 1. The values given in column 9 were obtained by dividing the values in column 3 by the means of those in column 8; they are based on the assumptions that the toxin preparations used for making toxoid contained no toxoid before toxoiding and that homologous toxins and toxoids have the same molecular weights. *Cl. oedematiens* and diphtheria toxoids in the doses used had no protective effect; *Cl. welchii* Type *A* toxoid in the comparatively small dose used had a very doubtful protective effect; *Cl. welchii* Types *B*, *C* and *D* toxoids appear to have had specific protective effects greater than that of tetanus toxoid by several orders of magnitude. Column 5 shows that in some cases these conclusions are based on the survival of very few animals, and the values in column 9 must be regarded as qualitative rather than quantitative; nevertheless, we believe they support the conclusion that the specific precocious protective action of tetanus toxoid is not unique. Additional experiments showed that *Cl. welchii* Types *C* and *D* and diphtheria toxoids in the doses shown in Table 1 failed to protect against *Cl. welchii* Types *D* and *C* and tetanus toxins respectively.

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Homotransplantation Antibodies detected by Mixed Agglutination with Cell Culture Monolayers

FORMATION of humoral antibodies as a result of tissue homotransplantation in mice was shown by the hæmagglutination technique introduced by Gorer[1,2]. Later, other serological methods such as lysis[3], cytotoxicity[4], leuko-agglutination[5], and complement fixation[6] have been used.

The purpose of this communication was to detect homotransplantation antibodies by means of the mixed agglutination technique on tissue culture monolayers[7–9].

The mice used in this work were purchased from Roscoe B. Jackson Laboratories. Full thickness skin grafts approximately 18 mm in diameter were exchanged between mice of *C3H/He* and *C57BL/6J* strains. In addition, some *C57BL* mice were grafted with skin of other mice of the same strain. The transplantation technique described by Hauschka[10] was followed; however, cleaning of the graft by dissection was omitted. The mice were bled from the retro-orbital plexus at various time-intervals and sera from the individual bleedings were tested.

Strain 929 '*L*' cell fibroblasts obtained originally from a *C3H* mouse by Earle[11] was used as the *in vitro* antigen. Eagle's minimum essential medium in Hanks' balanced salt solution (BSS) with antibiotics and 10 per cent calf serum was used for cell growth. Cell cultures which formed complete monolayers were used in the test.

The mixed agglutination test was performed in a similar way as described in a previous communication[9]. Cell cultures were washed three times with LH medium (0·5 per cent lactalbumin hydrolysate in BSS). The sera to be tested were inactivated for 30 min at 56° C. They were incubated with the cultures for 2·5 h at room temperature. Then the cultures were washed five times with LH medium and the indicator system was added. In order to prepare this, human blood group O erythrocytes were mixed with mouse anti-human erythrocyte serum. The resulting small agglutinates were washed and incubated with a rabbit antiserum against mouse serum. The red blood cells were washed again and prepared as a 0·25 per cent suspension.

Table 1 summarizes the results obtained from one experimental series. As is to be seen, all sera of *C57BL* mice transplanted with *C3H* skin acted on the '*L*' cells, which was to be expected, inasmuch as '*L*' cells are of *C3H* origin. The sera of *C57BL* mice grafted with skin of their own strain gave virtually negative results. Sera of *C3H* mice grafted with *C57BL* skin gave negative or

Table 1. MIXED AGGLUTINATION TEST WITH '*L*' CELL MONOLAYERS AND MOUSE SERA OBTAINED IN THE THIRD WEEK AFTER SKIN GRAFTING

	No. of sera with a titre of 1:					
	<10 and 10	30	90	270	810 and more	Total
C57BL grafted with *C3H* skin	0	0	1	2	9	12
C57BL grafted with *C57BL* skin	5	0	0	0	0	5
C3H grafted with *C57BL* skin	11	2	0	0	0	13

only weakly positive reactions. One could expect positive reactions of the latter sera on tissue cultures derived from *C57BL* mice; however, these experiments have not yet been performed.

It was our experience that antibodies could be detected as early as 9 days, and persisted at least 48 days after grafting. It was also found that re-transplantation of *C3H* skin on *C57BL* mice would result in an increased titre of the mixed agglutination test.

It is felt that the described procedure may be a very useful tool in detecting humoral antibodies accompanying homotransplantation. For convenience, an established cell line was used in this work; however, preliminary experiments indicated that primary cell cultures would give similar results. The titres of hæmagglutinating antibody reported by others may sometimes equal or even exceed those observed in this work; however, most of the antisera which have been tested by hæmagglutination were obtained by extensive immunization with erythrocytes, normal tissue or tumour tissue rather than by a skin graft.

It is hoped that the mixed agglutination technique may be useful in elucidating the mechanism of homograft rejection and in the delineation of transplantation antigens.

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RADIOBIOLOGY

Production by X-rays of Petite Colonies in Yeast and their Radiosensitivity

THE effect of X-rays on the viability of cytochrome a_3-deficient yeast cells, which form petite colonies, has previously been examined and compared with that of the wild type, which forms normal-sized, large colonies[1-5]. The reported results differ to a certain extent. Thus Moustacchi[3], O'Brien[5] and Gonzales and Barron[2] observed an increase in radiosensitivity of petite cells, while Raut and Simpson[1] and Wood[4] found no such difference. This discrepancy may be due to strain differences, to the use of either naturally occurring petite mutants or of colonies produced by chemical means, such as acriflavine or gentian violet, or to the use of cells not genetically related and, therefore, not directly comparable.

Production of petite colony formation by X-irradiation has also previously been reported[1,6], but with greatly differing results. Thus, at approximately identical X-ray doses, 100–150 kr., the yield of petite colonies among survivors in the experiments by Raut and Simpson[1] never exceeded 5 per cent while it reached 40 per cent in the work of Patrick *et al.*[6]. No attempts have been reported at increasing the yield of petite colonies by repeated irradiation of survivors nor has the sensitivity of radiation-produced petite cells been examined. The present work intends to fill this gap.

The yeast used was a diploid strain of Distillers Co., Ltd., baker's yeast, maintained as stock culture on wort-agar slopes. For the purpose of X-irradiation, the cells were grown in a synthetic medium[7]. The irradiation procedure was the same as previously described[8] at a dose rate of 8 kr./min. For aerobic irradiation, oxygen was bubbled through the cell suspension during irradiation.

Production of petite colonies. The number of petite colonies among survivors increased with increasing X-ray dose in an irregular manner, the highest yield being 25 per cent, after which it began to decrease to almost zero on further increasing the X-ray dose. The dose giving the highest yield of petite colonies, however, was not constant, varying in the present experiments between 32 and 96 kr. No explanation for this was found. If a single petite colony was streaked on wort-agar, the resulting colonies were a mixture of petite and large colonies. This indicates that a number of cells reverted to the parent type. A similar result was obtained when cells from a single colony were inoculated into liquid medium and plated on wort-agar after having grown to approximately 10^7 cells/ml. The percentage of re-appearing large colonies was variable in both cases, but, as a rule, did not exceed 30 per cent. No relation between X-ray dose, percentage of petite colonies and reversion to large type could be established.

In order to test the radiosensitivity of radiation-produced petite cells it was, therefore, necessary to obtain a strain of petite cells which breeds true. This was effected by repeated subculture and irradiation of successive (petite) survivors. The procedure was as follows:

(1) A yeast suspension, approximately 1×10^7 cells/ml., is irradiated with 32 kr. in oxygen and, suitably diluted, plated on wort-agar.

(2) A single petite colony of the survivors is inoculated into liquid synthetic medium and allowed to grow at 30° with aeration for about 18 h.

(3) 0·1 ml. of this culture, suitably diluted, is spread on a wort-agar plate.

(4) After 3–4 days' growth at 30°, a single petite colony is again subcultured in liquid medium, as (2).

(5) This suspension is irradiated with 64 kr. and plated for survivors.

Steps 2–5 were repeated five times and followed by two cycles in which the X-ray dose was doubled (128 kr.).

After 5–6 irradiations the number of large colonies which reappeared on the plates became decidedly less, the seventh and eighth cycle showing none. Subcultures from different colonies of the last cycle remained petite throughout, without further interposition of irradiation. The strain has now been maintained as 'petite colonies' for eighteen months.

Spectroscopic examination. Assessment of cytochrome content revealed absence of cytochrome a_3; cytochrome b appeared to be present only in traces while cytochrome c was richly present. The absence of cytochrome oxidase (a_3) is typical for petite yeast and agrees with the findings on chemically produced petite mutations[9,10].

Metabolism. The respiratory rate, measured manometrically, with glucose as substrate was very low ($Q_{O_2} = 1$–2 at 30°), while aerobic fermentation was almost as high as the anaerobic fermentation:

$$Q^{O_2}_{CO_2} = 145, \; Q^{N_2}_{CO_2} = 150$$

Radiosensitivity. No difference was found between the cells of large and petite strains. On plotting viability against X-ray dose (Fig. 1), the curves for both types of cells were almost identical, having the same initial slope followed by a short plateau, which is characteristic for this and other yeasts, individual variations falling within normal limits[8]. The oxygen-effect of irradiation in petite cells was equally not altered, the oxygen-enhancement ratio being approximately 2, as in the parent strain[8]. This latter point is in agreement with previous results by Moustacchi[3] and confirms her conclusion that the magnitude of the respiratory rate of a cell is not one of the factors which determines the oxygen-effect of irradiation. However, this does not entirely support her implied rejection of the theory put forward as the basis for the oxygen-effect[11], namely, that it is due to the irreversible reduction of an enzymatic cell component present during irradiation in the oxidized state. A similar view has previously been expressed by Tahmisian and Devine[12].

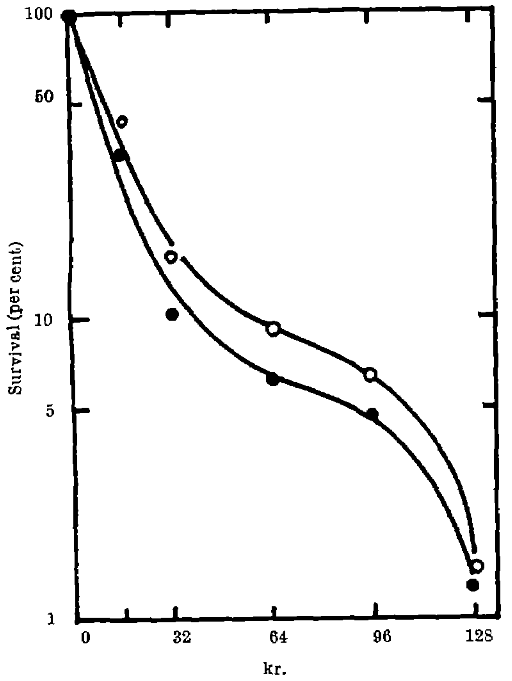

Fig. 1. Effect of X-rays on the survival of large, wild-type (●) and of petite (○) yeast

The experiments, finally, touch on the unresolved question, which may be of practical importance, namely, whether repeated irradiation of survivors may change their radiosensitivity and produce either more sensitive or more resistant cells. The former possibility appears, *a priori*, almost incapable of experimental verification except within narrow limits, because the more sensitive the cells might become the more easily they are rendered non-viable, thereby escaping the practical test. One case only has been reported[12] where a bacterium (*E. coli*[13]) showed a slightly increased susceptibility to radiation damage after repeated exposures to γ-irradiation, while two other *E. coli* strains, on the contrary, became more resistant. The technique used was to use massive inocula of irradiated cells for consecutive subcultures. The same procedure has been used in the work of *S. faecalis* and *E. coli* by Erdman *et al.*[14], who obtained a 1·2–1·9-fold increase in resistance after eight repetitive cycles. Further irradiation failed to raise the degree of resistance.

The results of the work recorded here indicate that the initially produced petite cells cannot be due to genetic difference of the two strains, because of the very high incidence (25 per cent) of petites and their facile reversion to the wild type. On repeated irradiation of petite survivors, however, the cells seem to have lost their ability of spontaneous reversion and to have gradually evolved into a genetically stable strain, indistinguishable from a true mutant. Although the changes involve the conversion of a fast-growing aerobic into a slow-growing anaerobic cell, the radiation sensitivity, nevertheless, remained unaltered.

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Influence of Chronic Gamma-irradiation on the Yield of Chromosomal Aberrations in Rat Liver Cells

It has already been shown that radiation damage to chromosome is conserved for several months after an acute X-irradiation. Due to this, dividing of a dose into two fractions with a seven-day interval between them had the same effect as a single irradiation[1–4]. Similar results have been obtained by other authors[5,6]. Results of the induction of chromosome aberrations during chronic irradiation are presented here. Preliminary results have been published elsewhere[7].

Female albino rats weighing 160–180 g received from the Animal Farm of the U.S.S.R. Academy of Medical Sciences were exposed to 150 rads of cobalt-60 γ-rays under the following conditions: (1) acute irradiation; (2) chronic irradiation. All animals were placed at various distances from the source and irradiated concurrently for 24 h. Partial hepatectomy was carried out with the standard method[8] 24 h after irradiation. Animals were killed 30 h after the operation. In each animal, the number of cells with chromosome aberrations was assessed among 100 late anaphases and early telophases on slides stained by the Feulgen method and with light green. The same was performed on non-irradiated rats. The mitotic index in non-regenerating liver was determined in 6 normal rats and in 6 irradiated with 150 rads (26 rads/min). The number of mitoses among 100,000 cells was counted in each animal.

The percentage of cells with chromosomal aberrations diminishes with the dose-rate decrease (Table 1). This phenomenon may be caused by: (1) selective death of cells with chromosome aberrations during interphase; (2) death of such cells as a result of division; (3) restitution of chromosome damages. Interphase death of liver cells does not occur even after their exposure to much higher doses[9]. There are grounds for suggesting that interphase death of cells in mouse bone marrow and in intestinal crypts is not related to chromosome aberrations[3,10]. That is why the first hypothesis seems to be less probable. Let us assume that the percentage of cells with aberrations increased linearly. In the absence of cell division it would then reach $A_0 = 22{\cdot}4$ per cent (the

Table 1. PERCENTAGE OF CELLS WITH CHROMOSOMAL ABERRATIONS IN RAT LIVER CELLS AFTER γ-IRRADIATION COBALT-60 WITH 150 RADS

Irradiation time	5·8 min	5·8 min	15 days	15 days	30 days	45 days	60 days	90 days	180 days
Dose-rate (rads/min)	26	26	7×10^{-3}	7×10^{-3}	3.5×10^{-3}	2.3×10^{-3}	1.7×10^{-3}	1.15×10^{-3}	0.58×10^{-3}
Exp.	29·3 ± 1·1 (6)*	30·0 ± 1·0 (5)†	21·8 ± 0·7 (6)	23·0 ± 0·8 (5)‡	21·2 ± 1·2 (6)	19·2 ± 0·8 (8)	16·2 ± 0·5 (6)	13·9 ± 0·5 (9)	11·8 ± 0·5 (8)
Non-irradiated control	6·9 ± 0·3 (20)	7·2 ± 0·5 (6)	6·5 ± 0·6 (6)	11·6 ± 0·4 (7)	7·2 ± 0·5 (6)	7·7 ± 0·6 (6)	7·5 ± 0·5 (6)	8·1 ± 0·6 (8)	11·6 ± 0·4 (7)

* Figures in parentheses show the number of experimental animals.
†, ‡, Hepatectomy 3 and 5·5 months after irradiation, respectively.

Table 2. DISTRIBUTION OF ACENTRIC FRAGMENTS AND BRIDGES IN RAT LIVER CELLS AFTER γ-IRRADIATION COBALT-60 WITH 150 RADS (PERCENTAGE TO THE TOTAL NUMBER OF CELLS WITH CHROMOSOME ABERRATIONS)

Irradiation time	1 fragment	2 fragments	3 and more fragments	Bridge and acentric fragments	Bridge	Average number of fragments per damaged cells
5·8 min	57·0 ± 3·5	17·0 ± 2·3	11·0 ± 2·4	11·0 ± 1·8	4·0 ± 2·0	1·3 ± 0·05
15 days	68·0 ± 3·5	15·0 ± 2·3	6·0 ± 1·6	5·0 ± 1·5	6·0 ± 1·0	1·2 ± 0·04
30 ,,	48·0 ± 6·7	19·0 ± 3·0	13·0 ± 5·9	12·0 ± 4·6	8·0 ± 1·8	1·4 ± 0·1
45 ,,	56·0 ± 3·4	17·0 ± 2·4	7·0 ± 2·4	14·0 ± 2·6	6·0 ± 1·3	1·3 ± 0·06
60 ,,	58·0 ± 3·3	20·0 ± 2·4	8·0 ± 2·4	7·0 ± 1·5	7·0 ± 1·4	1·3 ± 0·04
90 ,,	57·0 ± 3·7	12·0 ± 2·3	6·0 ± 1·8	14·0 ± 1·6	11·0 ± 2·5	1·1 ± 0·06
180 ,,	68·0 ± 2·8	16·0 ± 3·1	7·0 ± 1·6	9·0 ± 2·1	0	1·3 ± 0·04
Non-irradiated control	80·0 ± 2·9	10·0 ± 2·4	4·0 ± 1·4	6·0 ± 2·3	0	1·1 ± 0·03

effect of acute irradiation without normal 'background') after accumulation of $D_{\max} = 150$ rads for T days. Let us also assume that the division of cells with and without chromosomal aberrations has equal probability. Then the increase in the number of cells with aberrations may be described by the equation:

$$\frac{dA}{dt} = k\frac{D_{\max}}{T}(I-A) - qA = k\frac{D_{\max}}{T} - \left(k\frac{D_{\max}}{T} + q\right)A$$

where q is the rate of cell division. When solved, this equation shows that the number of cells with aberrations at any time t after the onset of chronic irradiation is:

$$A_t = \frac{k}{k\frac{D_{\max}}{T} + q} \cdot \frac{D_{\max}}{T}\left(I - e^{-\left(k\frac{D_{\max}}{T} + q\right)t}\right)$$

In T days, that is, after accumulation of $D_{\max}$:

$$A_T = \frac{kD_{\max}}{kD_{\max} + qT}(I - e^{-kD_{\max} - qT}) =$$

$$\frac{I}{I + \frac{qT}{kD_{\max}}}(I - e^{-kD_{\max} - qT}) =$$

$$\frac{I}{I + 3.92\,qT}(I - 0.776e^{-qT})$$

since $A_0 = A_{T=0} = I - e^{-kD_{\max}}$; $A_0 = 0.224$;

$$e^{-kD_{\max}} = I - 0.224 = 0.776, \frac{I}{kD_{\max}} = 3.92.$$

A_T values can be determined in Table 1. While solving the equation for T values from 15 to 90 days, the average $\bar{q} = 0.0435$ is obtained. The mitotic indices in non-regenerating liver in normal and irradiated animals were 0·0058 and 0·0048 per cent, respectively. Taking into consideration diurnal changes in mitotic rhythm[11], let us assume that the mean index is 0·01 per cent and that the duration of mitosis is 30 min. Then $q = 0.0048$, that is, at least one order less than $\bar{q}$. Therefore, the data presented cannot be interpreted only by the elimination of cells with chromosome aberrations as a result of their division.

This conclusion is confirmed by the results of experiments in which hepatectomy has been carried out 3 months after a single irradiation (dose-rate, 26 rads/min) and 5·5 months after chronic irradiation (dose-rate 7×10^{-3} rad/min) with 150 rads (Table 1). If the decrease in the percentage of cells with chromosome aberrations were to be caused only by their division then the percentage of such cells in these experiments could be expected to be equal to that after chronic irradiation for 3 and 6 months. Actually it was respectively 3 and 10 times higher. Thus, in these cases, there is a conservation of chromosome damage after irradiation in agreement with earlier published data[1-5]. The observed decrease in the percentage of cells with chromosomal aberrations under conditions of chronic irradiation can be suggested to be caused, apart from elimination, by the restitution of chromosome injury during irradiation. Probably, the reparation of chromosome damage may take place during fraction γ-irradiation with small doses[6] which does not occur under neutron treatment[12].

About 60 per cent cells with aberrations have one acentric fragment (Table 2). One break in one chromosome results in one or two acentric fragments. Therefore, it is possible to assume that approximately 60 per cent of damaged cells have one break in one chromosome. The distribution of cells with one, two and more acentric fragments, with bridges, etc., did not significantly change at various irradiation times (Table 2). If the hypothesis on the restitution of chromosome damage were correct, then the results presented in Table 1 would suggest that reparation took place not only in cells with two injured chromosomes but in those with one damaged chromosome as well. A break of a chromosome seems to be a multi-hit process. It may be that all or none of the damaged chromosomes undergo recovery. Further experiments are required to check up on these hypotheses.

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BIOLOGY

An Inhibitor of Adrenal Steroid 11β-Hydroxylase

Trans-1,4-*bis* (2-chlorobenzylaminomethyl) *cyclo*hexane dihydrochloride ('AY–9944')[1] has been shown to represent a novel class of cholesterol biosynthesis inhibitors which act by interfering with the enzymatic conversion of 7-dehydrocholesterol to cholesterol[2,3]. In laboratory animals, 'AY–9944' significantly depressed serum sterol-levels[4]. In view of its potential use as antihypercholesterolemic agent[5], we have studied the effects of this agent on adrenal steroid metabolism. The inhibition of 11β-hydroxylase by 'AY–9944' in rat adrenal homogenates is reported herewith.

The effect of 'AY–9944' on the conversion of progesterone, 11β-hydroxyprogesterone and 11-deoxycorticosterone to corticosterone by rat adrenal homogenates[6] was determined by the fluorometric method of Guillemenin *et al.*[7]. As indicated in Table 1, 'AY–9944' inhibited the conversion to corticosterone of both progesterone and 11-deoxycorticosterone without affecting that of 11β-hydroxyprogesterone respectively. Hence, in rat adrenal homogenates 'AY–9944' depresses 11β-hydroxylation without affecting 21-hydroxylation. The inhibitory action of the agent on 11β-hydroxylase was corroborated by measuring its effect on the conversion of 11-deoxycorticosterone-4-^{14}C to corticosterone-4-^{14}C by rat adrenal homogenates; at a final concentration of 1 × 10^{-4} M the inhibition was 60 per cent.

The effect of 'AY–9944' on 11β-hydroxylation was compared with that of 'SU–4885' ('Metopirone'), a known 11β-hydroxylase inhibitor[8] (Fig. 1). The concentration of 'AY–9944' sufficient to cause a 50 per cent inhibition of 11β-hydroxylation, that is, 6·6 × 10^{-5} M, was significantly lower than that of 'SU–4885', that is, 9·6 × 10^{-4} M.

Table 1. EFFECT OF 'AY-9944'* ON THE CONVERSION OF PROGESTERONE, 11-DEOXYCORTICOSTERONE AND 11β-HYDROXYPROGESTERONE TO CORTICOSTERONE BY RAT ADRENAL HOMOGENATES

Substrate	Inhibition of corticosterone formation (%)†
Progesterone	73
11-Deoxycorticosterone	63
11β-Hydroxyprogesterone‡	2

* 1 × 10^{-4} M final concentration.
† Each value is the mean of three separate determinations.
‡ Final concentration, 1 × 10^{-3} M 'AY-9944'.

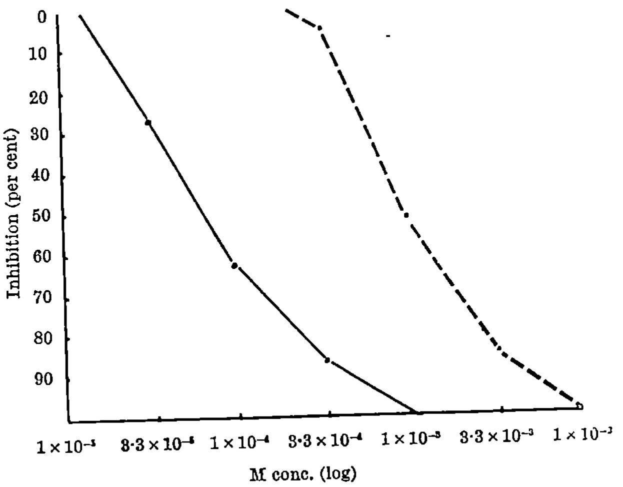

Fig. 1. Effects of 'AY-9944' (——) and 'SU-4885' (– – –) on the conversion of 11-deoxycorticosterone to corticosterone by rat adrenal homogenates

It is interesting to note that 11β-hydroxylase activity was not affected in adrenal homogenates obtained from rats given orally 10 μmole/kg of 'AY–9944'. In contrast, in liver homogenates obtained from the same animals the metabolism of 7-dehydrocholesterol, as measured by the method of Kandutsch[3,9], was completely blocked. The finding that, *in vivo*, 'AY–9944' does not affect rat adrenal 11β-hydroxylase parallels the experience with 'SU-4885' which, *in vivo*, was inactive in rats[10] and an effective 11β-hydroxylase inhibitor in dog[11] and man[8].

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Variations in Flow of Blood within the Epididymis and Testis of the Sheep and Rat

THE total venous outflow from the testis and epididymis of conscious rams has been measured using the Fick principle with tritiated water as the dilution substance[1]. Because of their different functions the two organs might have quite different blood flows, and it cannot be assumed that each contributed blood to the total venous outflow in proportion to its weight. The technique of Sapirstein[2] has been used to determine the partition of blood between the testis and epididymis.

Twenty male rats (290–430 g) were anæsthetized with pentobarbitone sodium and given 5 μc. of 4-^{131}I-iodo-antipyrine into the jugular vein. Sapirstein's method depends on the ratio of organ content to body content of isotope becoming constant soon after injection. The ratio of the amount of iodine-131 in the testes and in the epididymides to that in the rest of the body was constant between 10 and 60 sec; the same was true for brain. In a second experiment, using 12 rats, the epididymides were divided into six portions that were intended to correspond with zones 1–6, as described by Reid and Cleland[3]. For each portion the ratio of its isotope content to that in the rest of the body was constant during the period 20–40 sec after injection, indicating that the technique was valid for the portions as well as for the whole epididymis. The variation in flow of blood between the parts is shown in Fig. 1. The flow was always highest in the first portion of the head of the epididymis with a smaller peak in the body portions 4 and 5.

Assuming a cardiac output for rats[2] of 20·5 ml./100 g/min, the flow of blood through the whole epididymis was 14 ml./100 g/min in both experiments and through the testis 21 ml./100 g/min in both experiments; the latter value is much higher than the 14 ml./100 g/min previously reported for testis[4]. The mean flow of blood through the epididymal fat pad was 8·8 ml./100 g/min in the first experiment and 3·8 ml./100 g/min in the second.

Sapirstein[5] later applied his technique to the human brain and head using external counting equipment. A similar technique has been applied to the sheep; one scintillation probe was directed at the head and one at the scrotum of four rams anæsthetized with pentobarbitone sodium and 100 μc. of ^{131}I-iodoantipyrine was injected intravenously. The concentration of iodine-131 in the brain became constant between about 60 and 90 sec and that in the testis between about 90 and 120 sec after injection. Four rams were therefore killed, 90 sec after isotope administration, by the rapid intravenous injection of saturated potassium chloride containing pentobarbitone sodium (10 g/100 ml.). The heart stopped within 10 sec. At the same time as the injection of the potassium chloride, the blood flow to the testes and epididymides was arrested by clamping the spermatic cords. The various organs were then dissected out, and portions weighed and counted.

Assuming a cardiac output for anæsthetized sheep of 11 ml./100 g/min[6–10], the blood flow through the testis was 11 ml./100 g/min and, unlike the rat, that through the epididymis as a whole, 11·4 ml./100 g/min, was similar. These values are close to those previously found[1] for the testis and epididymis together, using the Fick principle and tritiated water. The blood flow in eight sites in each testis was quite uniform. The blood flow in the epididymis was low in the first portions of the head, increasing to a peak in portions 8 and 9, just beyond where the head turns back on itself. There was a sharp decrease in flow in the last portion of the head and the first portion of the body of the epididymis, a slight increase in lower portions of the body and a further decrease in the tail (Fig. 1).

This pattern is different from that in the rat but the anatomical arrangement in the two species differs also[11]. The cells lining the duct of the first segment of the rat epididymis are tall and secretory, but they are low in the first portion of the ram epididymis, which corresponds to the efferent ducts of the rat. Taller cells are found in portions 3–8 of the ram epididymis. In both species, tall cells are also found in the distal parts of the body[3,12]. Thus the highest segmental blood flow in both species roughly coincides with the portions of the epididymis that have the tallest epithelial cells. It has also been shown that the incorporation of ^{32}P-phosphate into glycerylphosphorylcholine is higher in the head than in the tail of the epididymis of the rat[13], that the head of the epididymis has more alkaline phosphatase than the tail[14], and that absorption of dye from the lumen is greatest in the head of the epididymis[15].

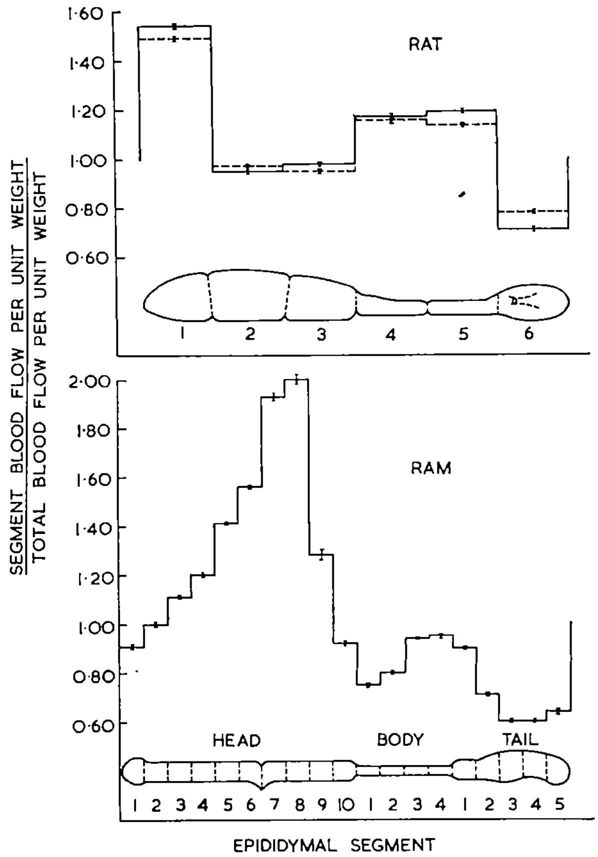

Fig. 1. Variations in blood flow in different parts of the epididymis of the rat and ram. Flow is expressed as the ratio of blood flow per unit weight in the segment to flow of blood per unit weight of the whole epididymis. For rat, estimates 20 sec (——) and 40 sec (– – –) after injection of isotope. Means for 24 rat and 7 ram epididymides with their standard errors are shown. Drawings of the epididymides are diagrammatic

It would, therefore, appear that the region of highest blood flow is also a region of high metabolic activity, but as we have noted that the venous blood from the head of the epididymis of the ram was more oxygenated than that from the tail, it is tempting to postulate that the high blood flow has some specific function other than supply of oxygen.

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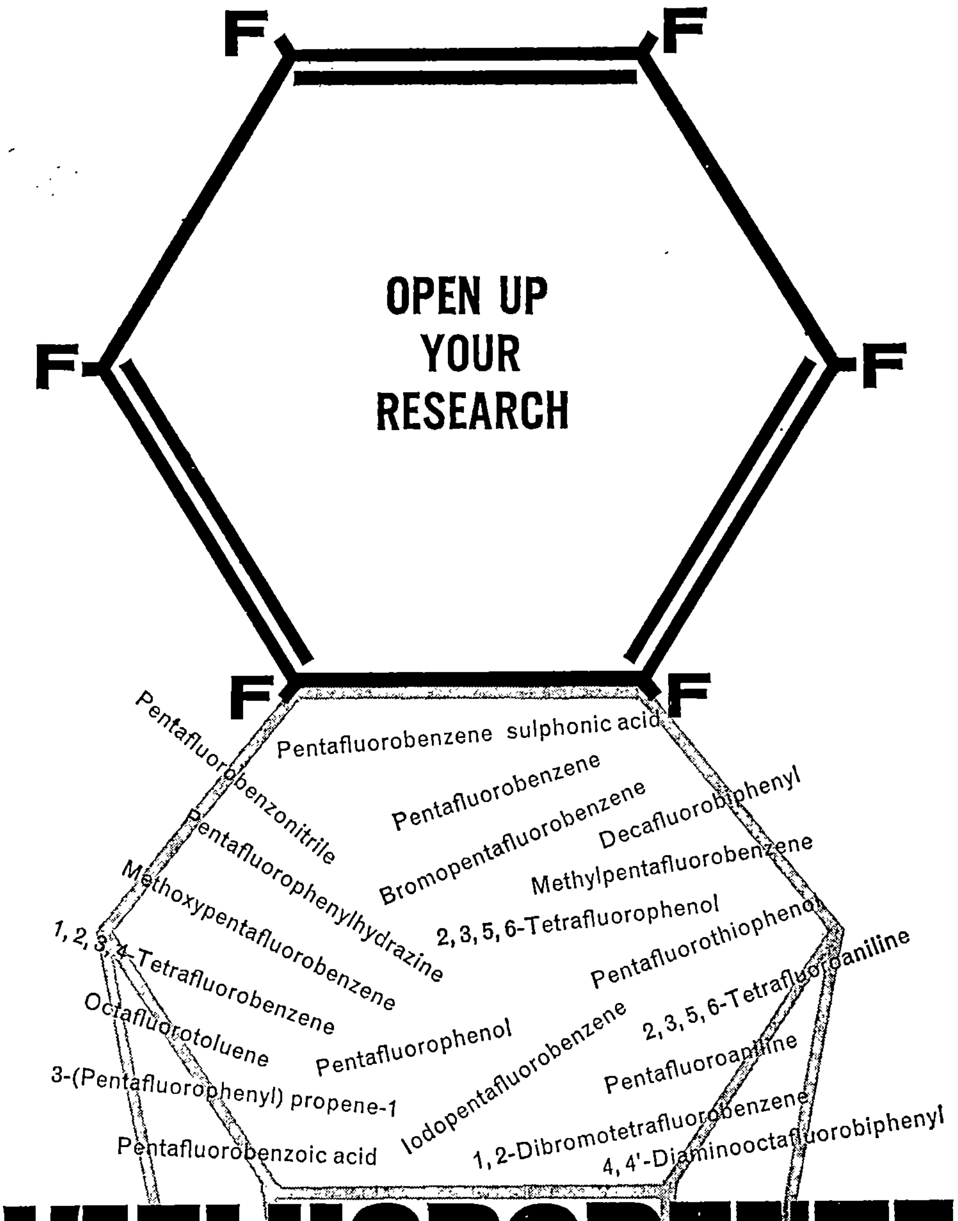
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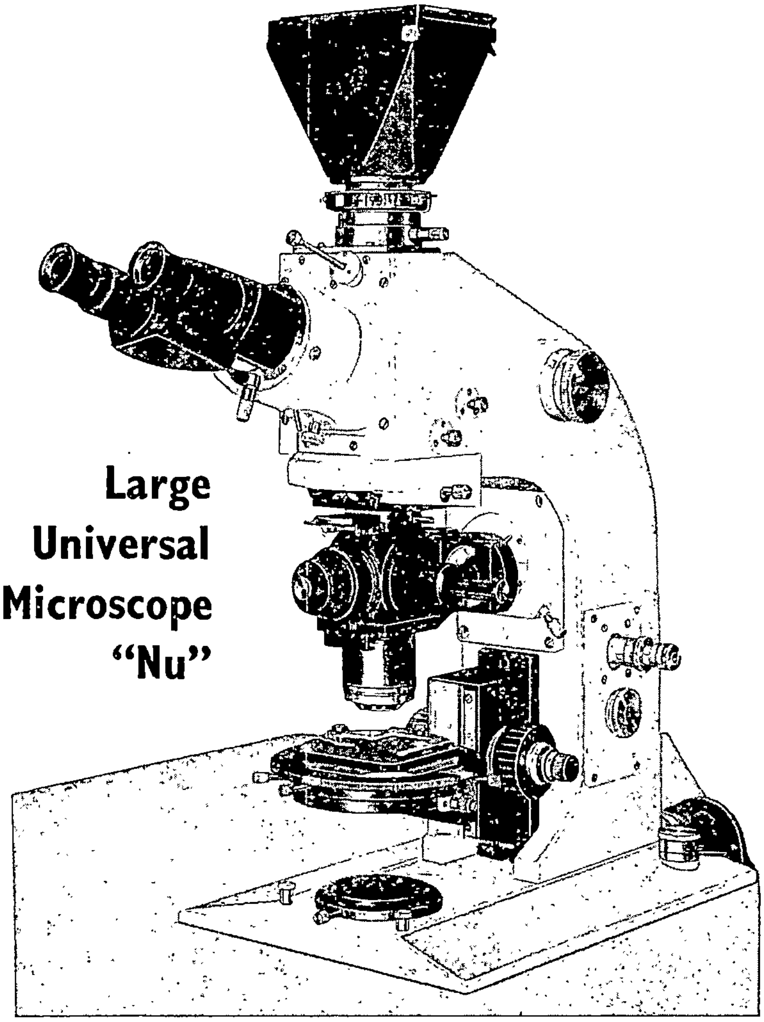

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Removal and Dissociation of Epithelial Cells from the Rodent Gastrointestinal Tract

WITH advances in the study of gastrointestinal physiology, knowledge of functions unique to the intact organ has accumulated. To facilitate investigations at a cellular level, attempts have been made to obtain a pure suspension of epithelial cells for *in vitro* investigations. In procedures so far reported, there is contamination of the cell suspension by mesenchymal elements[1-3]. Additionally, abundant flora in the postnatal gut has hampered cellular maintenance and study. The following method largely overcomes these difficulties and provides a reliable cell sample for *in vitro* biochemical, anatomical, and immunological analysis.

Male Sprague–Dawley rats weighing 100–200 g were used in this work. Seventy-two hours prior to death, neomycin (10 mg/30 ml.) is added to their drinking water. Forty-eight hours prior to death, all food is withheld with the final 24 h under complete oral restriction. Sixteen hours before death, each rat receives by gastric tube 24,000 units of 'Nystatin', 500 mg of streptomycin, and 200,000 units of procaine penicillin *G*. Our bacteriological investigations confirm the fact that this procedure reduces organisms to an extremely low level[4].

Of the methods available for dissociation of cells, trypsinization as described by Moscona is used[5,6]. Rats are anæsthetized with pentobarbital sodium, and the bowel is removed under aseptic conditions. The stomach and colon are removed *in toto*, but the small intestine is divided into two equal portions for ease in handling. To obtain a closed system, in which both wash and enzymatic solutions come in contact only with epithelial elements, lengths of vinyl tubing are inserted intraluminally at distal and proximal ends of the organ and tied into place. The epithelial surface is washed several times with room temperature calcium magnesium free (CMF) Tyrode's solution introduced through a syringe attached to the tubing. The bowel segment is then filled with fresh CMF Tyrode and incubated for 15 min at 37° C. This solution is discarded and replaced by 3 per cent trypsin/pancreatin (3 : 1) in CMF Tyrode and incubated again for 15 min at 37° C and pH 7·0 ± 0·10. Longer exposures to trypsin reduce cell viability to 50 per cent or less, and shorter exposure times reduce the cell yield considerably. During and after incubation, the bowel segment is mechanically agitated to facilitate cell removal. A medium composed of Eagle's solution, 10 per cent horse serum, glutamine, penicillin, streptomycin and vitamin A is then washed through one end of the organ as the cells are collected in a syringe at the opposite end. This suspension is adjusted to yield a 4 : 1 ratio of medium to trypsin and, afterwards, forced through a wire mesh of 0·086-millimetre pore size to disrupt cell clumps. Further dispersion is obtained by repeatedly forcing the suspension through a 14-gauge needle. The cells are washed twice with medium by centrifugation at 300g for 5 min and resuspended for counts and culturings. Cell yields have averaged 175 × 10^6 for the small intestine, 30 × 10^6 for the stomach, and 47 × 10^6 for the colon with viabilities of 76 per cent, 78 per cent, and 87 per cent, respectively, as determined by the nigrosin stain[7].

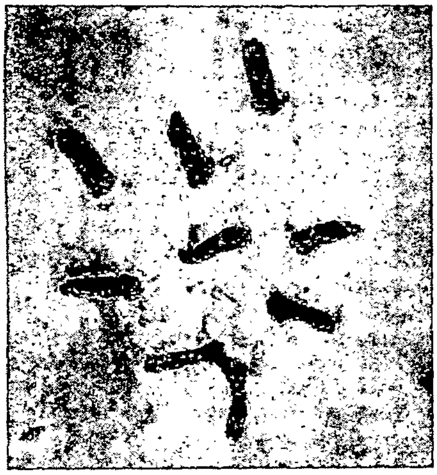

Fig. 1. Epithelial cells after removal from small intestine (× 565)

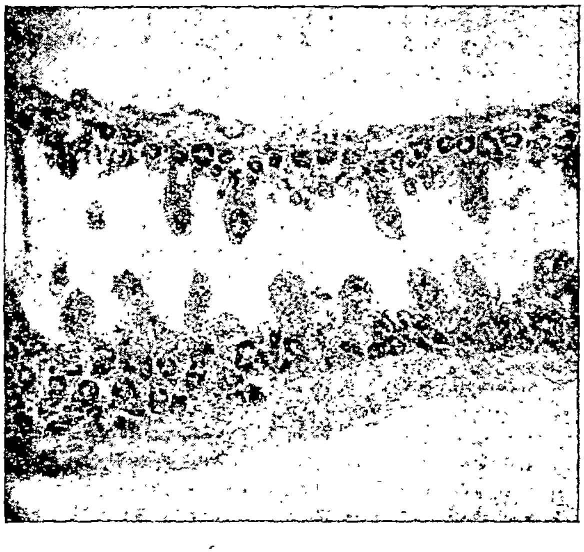

Fig. 2. Section of small intestine following trypsinization (× c. 94)

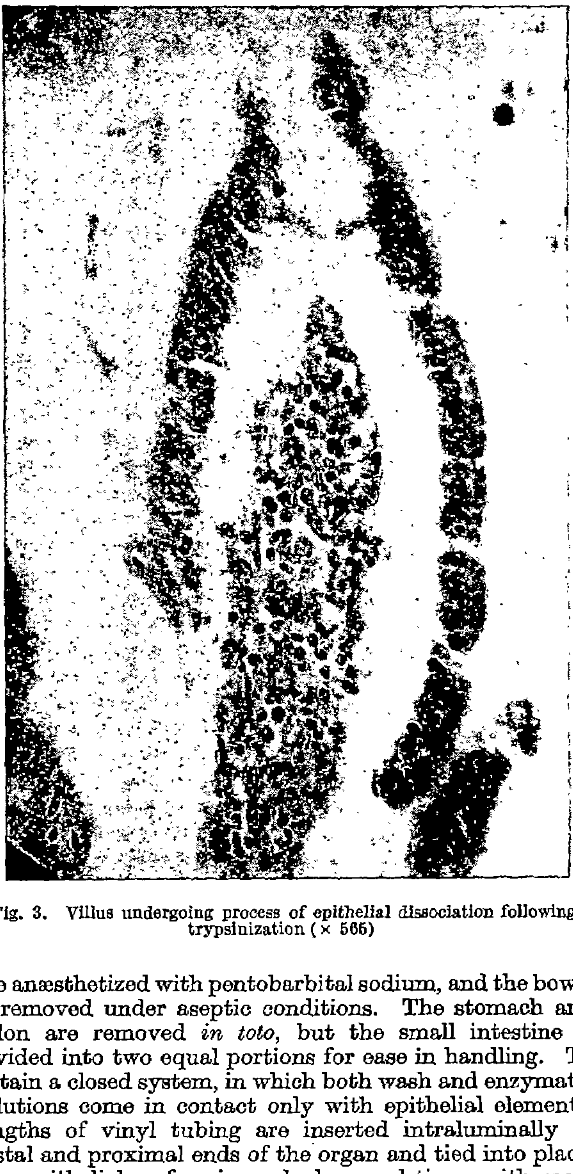

Fig. 3. Villus undergoing process of epithelial dissociation following trypsinization (× 566)

Smears of cell suspensions stained with azure-eosin show a collection of well-dispersed cells. Differential counts of these smears give an average of 81 per cent epithelial cells, 13 per cent lymphocytes and granulocytes, and 6 per cent bare nuclei. From the small intestine, two types of epithelial cell may be distinguished: a large columnar or sometimes slightly rounded cell with brush border and a smaller rounded form seen individually or within isolated crypts (Fig. 1). The stomach yields both dark staining chief cell types and large eosinophilic parietal cells. Cells from the colon are basophilic, varying in size and cytoplasmic content, and of ovoid or goblet shape. Following trypsinization, sections from all levels of the gut show a loss of epithelial cells with an apparently intact basement membrane (Figs. 2 and 3). It is of interest that a tryptic digest method similar to the one described has been used successfully to separate epithelial cells from other hollow organs such as aorta and Fallopian tubes[8,9].

Preliminary work done on gastrointestinal cells removed by trypsinization includes metabolic investigations and *in vitro* cultivation. A report of the characteristics of respiration of dissociated gastrointestinal epithelial cells will soon be forthcoming[10]. As yet a culture line has not been established, although small intestinal cells have been maintained in continuous culture for 3 weeks. An opportunity is now afforded for definitive investigation of metabolic processes peculiar to gastrointestinal epithelial cells.

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Growth Stasis and Tryptophan Deficiency in the Very Young Chicken

Considerable work has been reported concerning the consequences of underfeeding poultry and animals in the early stages of growth[1,2]. Quite typically, growth in terms of weight gain is markedly reduced, but some tissues of the animal, particularly the connective tissues, cartilage, etc., continue to develop[1]. Basal rations used to examine amino-acid requirements usually do little more than slow down growth or maturation rates. However, we have now found that for the chick, depending somewhat on the strain used, tryptophan-deficient diets fed to chicks weighing less than 80–100 g completely stopped gain in weight and all, or nearly all, visible signs of subsequent maturation. Therefore, it was of interest to observe whether prolonged tryptophan deficiency would affect subsequent maturation or physiological development. Reproduction (egg production) was chosen as a very sensitive indicator of successful maturation in the chicken.

Chicks were fed semi-purified diets of the type shown in Table 1. These basal diets contained adequate levels of all essential amino-acids and of all known nutrients, when compared to accepted standards[3], except tryptophan. Chicks were maintained in electrically heated brooders, with wire mesh floors, in air-conditioned rooms. Food and water were available *ad libitum*. After hatching, the chicks were fed a commercial 'starting' ration for 6–9 days, or until they weighed 90–100 g. The chicks were then distributed at random into groups of 10 chicks each and fed diet 3. Initially, quadruplicate groups received each treatment. By the third experimental day, significant differences were found between the tryptophan-supplemented and tryptophan-deficient groups. By the end of 21 days, it was noted that birds receiving the basal diet virtually had not grown at all. These chicks appeared grossly unchanged from a chick of 7–10 days of age; that is, no further development (feet enlargement, comb growth) had occurred. When fed a tryptophan-supplemented diet, growth immediately resumed. However, it was observed that once the chicks weighed 180 g or so, when they were fed the basal diet again their growth was not stopped, but continued at quite a slow rate (4–5 g per day rather than 14–17 g on the tryptophan-replete diet). In fact, a stair-stepping effect, achieved by stopping and starting growth by feeding alternately tryptophan-deficient and tryptophan-supplemented diets, could be demonstrated for chicks if they weighed less than the 180–200 g. So long as the birds were maintained on the basal diet and weighed less than 125 g, they did not grow appreciably, nor did any signs of partial maturation occur as is usually observed when chicks are deliberately starved or the level of total protein or total calories is drastically reduced.

Table 1. Tryptophan-deficient Diets

Ingredient	Diet 1 (*T*–04)* %	Diet 2 (*T*–06)* %	Diet 3 (*T*–08)* %	Diet 4 (*TC*–06)* † %
Casein (purified)	1·67	3·67	5·67	—
Gelatin	15·59	15·59	15·59	—
Acid-hydrolysed casein	6·00	3·00	—	—
Zein	5·52	5·52	5·52	—
DL-Threonine	0·30	0·30	0·30	—
DL-Phenylalanine	0·50	0·50	0·50	—
DL-Valine	0·40	0·40	0·40	—
Methionine hydroxy analogue (MHA)‡	0·60	0·60	0·60	—
Salts *A*§¶	6·00	6·00	6·00	—
Vitamin mixture *S*–35§¶	0·50	0·50	0·50	0·50
Vitamin A (10,000 I.U./g)	0·10	0·10	0·10	0·10
Vitamin D (7,500 I.S.U./g)	0·03	0·03	0·03	0·02
Choline chloride	0·20	0·20	0·20	—
Procaine penicillin (50%)	0·002	0·002	0·002	—
Nitrofurazone (*MF* 180)	0·05	0·05	0·05	0·05
Corn oil	4·00	4·00	4·00	—
Santoquin‖	0·015	0·015	0·015	0·015
Glucose monohydrate ('Cerelose')	*q.s.* 100	*q.s.* 100	*q.s.* 100	—
Corn				*q.s.* 100
Dicalcium phosphate				2·40
Calcium carbonate				1·50
Potassium phosphate (dibasic)				0·25
Magnesium carbonate (U.S.P.)				0·10
Trace minerals mixture ('Delamix')**				0·10
Iodized salt				0·50
Animal fat (tallow)				0·05
Aureomycin concentrate (25 mg/lb.)				

* Code number under diet indicates percentage of tryptophan included (for example, *T*–04 contains 0·04 per cent tryptophan). For maximal growth, 0·50 per cent *dl*-trytophan or 0·25 per cent *l*-tryptophan is added to the basal diets.

† A diluted version (*TC*–05) of this corn diet can be formulated by replacing 15 per cent corn with 13 per cent glucose ('Celerose') and 2 per cent animal fat (tallow).

‡ Registered trade-mark of the Monsanto Chemical Co. for calcium *dl*-2-hydroxy, 4-methylthiobutyrate.

§ Machlin, L. J., and Gordon, R. S., *Poultry Science*, **37**, 1460 (1958).

¶ Nutritional Biochemicals Co., 21010 Miles Avenue, Cleveland, Ohio.

‖ Registered trade-mark of the Monsanto Chemical Co. for 6-ethoxy, 1,2-dihydro, 2,2,4-trimethylquinoline (an anti-oxidant).

** Limestone Products Corp. of America, Newton, New Jersey.

A longer term experiment was conducted to determine if chicks could be held for a greater time at the 100-g stage and to see if this period of growth stasis would adversely affect the maturation process. Pullets were used, because it was felt that normal egg production could be used as an index of satisfactory development and maturation. Twenty-five 'Hy-line' female chicks were fed the basal diet after being grown to a weight of 95 g (8 days) using a commercial chick-growing ration. As in the previous experiment, none of these chicks grew at all.

Table 2. EFFECT OF TRYPTOPHAN DEFICIENCY ON ONSET OF EGG PRODUCTION

	Control	Experimental
No. of hens	9	10
Days fed deficient diet	10	180
Weight at: day 204	2,080 g	111 g
day 397	—	2,130 g
Average No. of days to lay 10 eggs/hen	204	397
Net days after repletion for 10 eggs/hen	180	193

After 10 days, 10 pullets were fed the tryptophan-supplemented diet. They immediately began growing at a rate (14 g gain/day) similar to the rate observed in the first experiment. After 14 days, when they weighed 280 g, these birds were fed commercial rations and observed until they had produced 10 normal eggs each. This took an additional 180 days, 204 days after the experiment was started (the 9 surviving hens thus averaged 212 days of age when they had laid 10 eggs each).

During this time the remaining 14 pullets (1 had died of the remaining 15) were fed the tryptophan-deficient diet only. On the 204th experimental day these birds weighed, on the average, 111 g; they had thus gained some 16 g during this period. When fed the tryptophan-supplemented diet, they immediately began growing at the rate previously observed for the control group of chicks, about 14 g/day. After 14 days (the 218th experimental day) they weighed 302 g. At this time, 10 of the chicks were fed the commercial growing ration and four were returned to the basal diet. After 20 days, the chicks fed the basal diet had gained only 60 g, while the pullets receiving the commercial ration had gained 180 g. The 4 chicks fed this basal diet were then discarded, and the remainder were observed until they, too, had laid 10 eggs each. This took 193 days (the 397th experimental day), on the average, after they had been permitted to resume growth. Table 2 summarizes this experiment.

In subsequent experiments with other strains of chickens, diets containing lower levels of tryptophan than diet 3 (see Table 1) had to be used in order to obtain similar results, that is, baby chicks from early-feathering broiler strains (some of which actually have well-developed feathers at the time of hatching) fed diet 3 exhibited some feather development, cartilage formation and connective tissue growth, although they did not gain in body-weight. Investigations are in progress to see if birds of this type are adversely affected by being held six months at the 100 g stage. We are finding with such early-maturing strains that successful stasis is attained by feeding the deficient diets very shortly after hatching.

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Symbiotic Association between Zooxanthellae and Two Marine Sponges of the Genus *Cliona*

THE association between Zoochlorellae and some fresh-water sponges of the family Spongillidae is well known for its constancy and has been well studied[1]. In the case of similar associations of Zoochlorellae with the fresh-water hydroid *Clorohydra viridissima*[2] and of Zooxanthellae with the sea-anemone *Anthopleura elegantissima*[3], the occurrence of a direct transfer of material from the autotrophic symbiont to the tissues of the Coelenterates has been demonstrated.

On the contrary, the occurrence of Zooxanthellae in marine sponges is a rare event and up to now it has not been sufficiently studied. Zooxanthellae have been found only occasionally in the superficial parenchyma layers of *Ircinia variabilis*, *Myxilla* sp., *Merlia normanni*, *Reniera* sp. and in tubes of *Clathrina* sp.[4].

Our observations have been performed on two species of marine and boring sponges of the genus *Cliona*, *C. viridis* and *C. copiosa*. *C. viridis* generally lives at a depth of some metres while *C. copiosa* is an allied species living in lightened caves near the surface. The external colour of these two species is variable; in general, *C. viridis* shows a dark green colour which changes to brown in the case of *C. copiosa*. The internal part of both sponges is yellowish to greenish. Specimens of both species were collected in different Mediterranean stations (Bay of Naples and along the coast of Ligury and Puglie) as well as at different depths and at different times of the year. In all cases a great number of Zooxanthellae were found embedded in extracellular arrangement in the sponge parenchyma (Fig. 1) and more densely near the surface of the sponges. These Zooxanthellae exhibit a yellow-greenish colour with a blood-red fluorescence under Wood's light. In addition, they possess two flagellæ and measure 7·5–9μ in diameter.

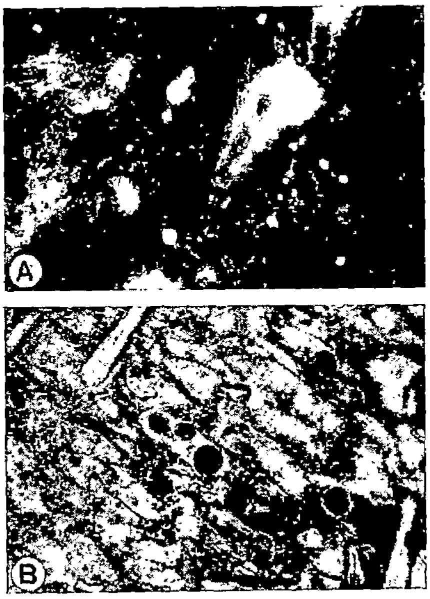

Fig. 1. Zooxanthellae in the sponge tissue observed (*A*) in Wood's light and (*B*) after fixation in formol and staining in Heidenhain hæmatoxylin–eosin

In the case of the associations between Zooxanthellae and Coelenterates, many investigators have considered that carbon dioxide, as well as other animal catabolites. may be essential for the metabolism of the autotrophic symbiont and that, on the other hand, the removal of carbon dioxide may be of some use for the associated animal[5]. The possibility of relationships between the occurrence of Zooxanthellae and the behaviour of *Clionidae*, boring in calcareous rocks and shells, must, therefore, be taken into consideration and deserves to be further investigated in greater details.

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ENTOMOLOGY

Inhibition and Excitation in the Acoustic Behaviour of *Pholidoptera*

EXPERIMENTS on the 'bush cricket', *Pholidoptera griseoaptera* (De Geer), using artificial signals[1] have shown that these have an inhibitory effect on chirping. Further work has shown that the chirping of one male has a similar inhibitory effect on other males. In addition to their inhibitory effect, artificial signals increase the overall chirp rate and alter the timing of chirps so that they tend to occur in the first second after the end of the inhibitory period. These excitatory effects are shown when the total basic chirp rate is below 30 per min; the signals actually depress the chirp rate when it is high.

Inhibition during the signal and an overall increase in chirp rate are shown when consecutive periods of 5-sec signal and 5-sec silence are used (pure tone signals 10–15 kc/s, 65–80 db rel. to 0·0002 dyne/sq cm). In addition to these effects, the alteration in timing of the chirps can be seen in experiments using a 1-sec signal every 5 sec. Fig. 1 shows the pattern obtained when the results of five 2-min experiments of this type are added together. The number of chirps occurring in the first sec after the end of the signal is much greater than could be expected if the signals were purely inhibitory. There is evidence from other experiments that in the range 40–80 db both the inhibitory and the excitatory effect of the signal are increased by an increase in intensity.

The chirp rate of each male is very variable and seems to be influenced by a number of factors such as time of day, temperature, reproductive state and auditory stimuli. Thus two males singing together may have very different chirp rates. When one or both are chirping at a high rate (total chirps 50-80/min) the song of one can be seen to have an inhibitory effect on the other. Fig. 2 shows examples of the interaction of two males. In the upper oscillogram one male is more active than the other, and in the lower they are equally active and chirp in phase for a short period during alternation. It is noticeable that they are not perfectly in phase and that the change from equal alternation to singing in phase and back is very sharp.

Measurements taken from 10 min of this recording (temperature 18° C) show that for a male chirping without intervention by the other male the interval has a mode of 0·9 sec (interval = end of one chirp to beginning of next). If the other male sings during the interval the mode is increased to 1·6 sec. The interval between consecutive chirps by different insects has a mode of 0·7 sec with a minimum of 0·5 sec. During this recording the two males sang in, or nearly in, phase more than 50 times. It is presumed that this happened because one had started to chirp before it had been inhibited by the chirp of the other. The maximum period between two 'in phase' chirps was in the region of 40–50 msec. This suggests that at 18° C the inhibitory reaction time is in the order of 50 msec. Two males with the same chirp period may sing in phase for as many as 4 consecutive chirps before the chirps become sufficiently out of phase for one to inhibit the other, so that they return to alternation.

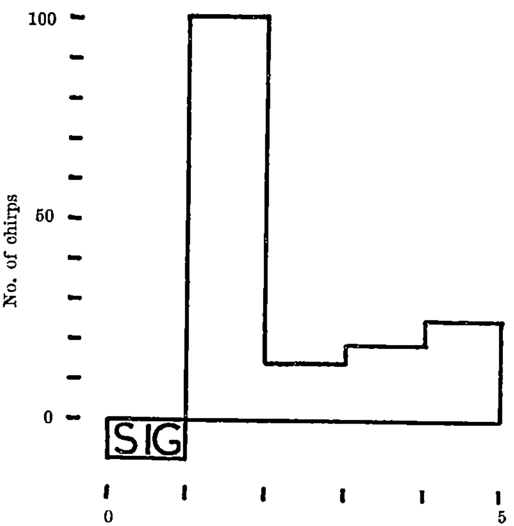

Fig. 1. Pattern of chirp emissions when a 1-sec signal is repeated every 5 sec. The results of five 2-min experiments are added together

The lengths of the intervals between chirps which are not in phase suggest that certain preparatory events must occur, perhaps in the central nervous system, before a chirp is produced. If the insect is inhibited during this preparatory period, the whole process has to be repeated when the inhibition is over.

Singing in phase has been heard a number of times, but so far only one recording containing it has been made. Other recordings of active males show a similar relation between the other intervals measured, but the actual length of these varies from experiment to experiment. Although the interaction of 2 males which are chirping at a high rate tends to decrease the rate of both, there is evidence that interaction has a long-term excitatory effect.

The interval between the ending of a 1-sec signal (10 or 12 kc/s, 70 db) and the beginning of the following chirp (mode 0·3–0·5 sec, minimum 0·2 sec at 17° C) is shorter than the corresponding interval between alternating chirps. It seems probable that an increase in the length of the inhibitory period increases the excitatory effect and thus there is a shorter preparatory period before the chirp.

Inhibition appears to play an important part in the interaction of singing males. A period of inhibition seems to be followed by a period of increased excitation (or

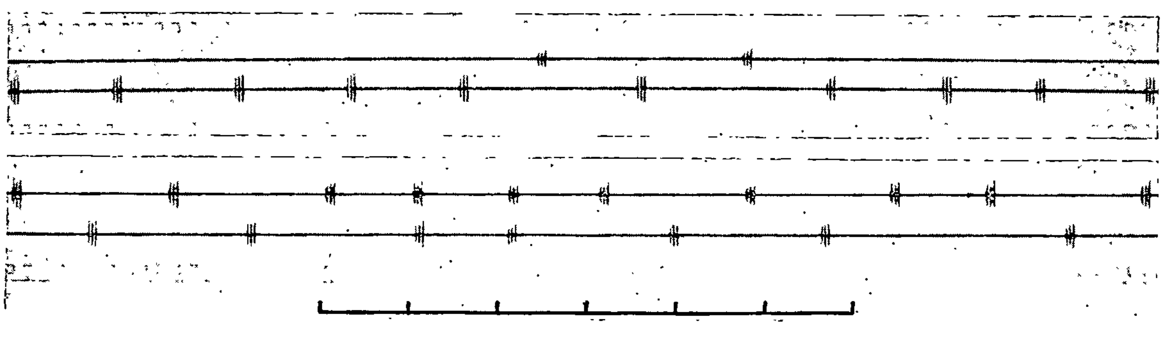

Fig. 2. The upper record shows the inhibitory effect of one male on the chirping of another. The lower record shows a change from equal alternation to 'in phase' and back again. Time marker, 1 per sec.

lowered threshold) and this may be an example of what Kennedy and Booth[2] have called "antagonistic induction". It is possible to interpret in this way a great deal of the information given by Busnel *et al.*[3] on the behaviour of *Ephippiger* males.

I thank Mr. W. B. Broughton for his advice.

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Some Effects of Decapitation on Metabolism in *Rhodnius prolixus* Stål.

The failure of oocytes to develop in decapitated female *Rhodnius* has been attributed to the failure of protein synthesis[1], and Vanderberg[2] has shown reduction of incorporation of radioactive leucine by several tissues after decapitation. Further experiments have been performed on the fat body and hæmolymph to investigate this.

In the normal insect there are two main adult-specific hæmolymph proteins, formed in the fat body, which are absorbed by the oocytes and form the bulk of the soluble oocyte protein. Absorption has been demonstrated by oocyte uptake of hæmolymph proteins labelled with fluorescein, and the proteins examined by immuno-electrophoresis. Decapitation results in the failure of yolk deposition[3], and also results in the presence of only small quantities of the 'yolk' proteins in the hæmolymph, whereas after ovariectomy large accumulations of these proteins occur. No 'yolk' proteins are found in the fat body after decapitation, even though the protein content of this tissue is increasing and is higher than normal. Other hæmolymph proteins are formed; it appears, therefore, that the action of the corpus allatum hormone is on the specific synthesis of 'yolk' proteins by the fat body. This does not exclude the possibility that the hormone may also affect the uptake of protein by the ovary. Digestion is not a limiting factor, since high concentrations of amino-acids are found in the hæmolymph.

This makes the finding of Vanderberg[2], that synthesis of DNA occurs normally in the decapitated insect, but that synthesis of RNA is inhibited, very relevant, and implies that the gonadotrophic hormone acts at the level of RNA synthesis. This would fit in with the theory of Karlson[4], who thinks that the moulting hormone acts on the chromosome to produce puffs, and that these puffs are the site of synthesis of RNA. It is relevant to note here that Wigglesworth[5] suggested that the juvenile hormone, which appears to be identical with the gonadotrophic hormone[6], must act in the larval instars on the gene-controlled system of the epidermal cell.

The demonstration of the possible action of the gonadotrophic hormone helps in the understanding of the control of metabolism in the fat body of the fifth instar. Wigglesworth had noted that the fat body grows more slowly in the decapitated larval insect than in the normal one[5], but that this is not due to the moulting hormone[7]. Investigations of concentration of amino-acid in the hæmolymph suggest that, on decapitation in the fifth instar, digestion is not limiting. Changes were followed over the first nine days after decapitation, and it was found that, on decapitation at 0–1 h after feeding, net protein release into the hæmolymph (mostly from the fat body) is normal. However, the relative amounts of the two main hæmolymph proteins, as separated by cellulose acetate electrophoresis nine days after feeding, depend on the time of decapitation. Accumulation of protein in the fat body occurs, but more slowly than normal. Glycogen is also stored in the fat body, in small quantities after decapitation 0–1 h after feeding, but in larger quantities 24 h after decapitation. On the other hand, the fat content of the fat body remains low after decapitation at either 0 or 1 day after feeding. It is suggested that the most economical hypothesis to explain these results is that the brain hormone is acting on the chromosomes of the fat body, and that different genes are responding to different concentrations of the hormone.

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Effects of Low-temperature Storage on the Eggs of *Spodoptera littoralis* (Boisd.)

In September 1963 a number of growers reported severe damage to the flowers and foliage of all-year-round chrysanthemums by a new caterpillar which, when more than half-grown, was apparently resistant to DDT. The larvæ were later determined as *Spodoptera littoralis* (Boisd.) (*Prodenia litura F.*) (Lepidoptera: Noctuidae), an important pest of cotton and many other crops in African and Mediterranean countries. Experiments showed that dichlorvos at 24 c.c. of 50 per cent emulsifiable concentrate per gallon per 600 sq. ft. of bed could kill larvæ up to the third instar. The older larvæ were susceptible to 0·04 per cent dieldrin as a stomach poison. The pest was afterwards eliminated from the nurseries on which it had appeared by the combined use of these two materials.

When the history of the individual outbreaks was examined they could all be traced back to cuttings which had been taken in the Canary Islands. *S. littoralis* is a serious pest of tomatoes in the Canaries after being introduced there many years ago following an abortive attempt to cultivate cotton. The production of cuttings in the Mediterranean area to take advantage of high winter light intensity is becoming an important feature of chrysanthemum production in north-west Europe. Some quarantine method to ensure that *S. littoralis* is not spread by the trade in cuttings is therefore highly desirable. As cuttings are frequently cold-stored for several days to facilitate handling procedures it seemed that the survival of *S. littoralis* under these conditions should be investigated.

In the laboratory egg-masses obtained from cultures of *S. littoralis* reared on chrysanthemum were divided into two so that every replicate had its own control. The eggs were placed on plastic gauze suspended over wet filter-paper within plastic pill-boxes with tightly fitting lids. The controls were kept at 75° F but the boxes to be treated were placed in a laboratory refrigerator maintained accurately at 35° F. After the requisite periods of treatment the boxes were returned to 75° F for at least one week to test viability.

Other tests were made in commercial cold-stores with eggs, which were obtained directly from the Canary Islands. Within a few hours of landing each egg-mass was divided at the Glasshouse Crops Research Institute and placed in pill-boxes as before. The controls were incubated at 75° F while the boxes due for treatment entered the cold-stores about 1 h. later. Each pill-box was placed among chrysanthemum foliage within the polythene sheets used to surround each batch of cuttings in the standard cardboard storage packs. As before, viability was tested by returning eggs to 75° F for one week.

Temperature records were taken over a period of one week in the stores with six recording thermographs (loaned by Meteorological Office), and throughout the period the temperature remained between 30°–35° F.

From Table 1 it is apparent that exposure at 35° F for longer than 5 days reduces the viability of the eggs and that after 10 days none remains viable. The 2–6 week exposures to cold were made to ensure that no induction of diapause had been caused by preliminary exposure to 35° F. The prolonged period at low temperature would normally end such diapause and the eggs therefore resume development at 75° F. No such effect took place and it seems quite clear that the eggs are definitely killed by prolonged exposure to low temperatures.

Table 1. VIABILITY OF EGGS OF *Spodoptera littoralis* FOLLOWING STORAGE AT 35° F IN THE LABORATORY

Age when introduced to cold (days)	Duration of storage (days)	No. eggs		% hatch	
		Treated	Control	Treated	Control
1	5	94	32	100	100
2	5	83	24	100	100
2	7	70	81	8·6	100
2	7	251	63	0·4	100
1	10	117	17	0	100
2	10	112	19	0	100
0–3	10	448	632	0	91
? (Field collection Canary Is.)	10	194	160	0	100
2	14	187	21	0	100
2	21	69	84	0	100
2	42	142	27	0	100

The viability of eggs which experienced low temperatures in the commercial stores (Table 2) confirmed that unless chilled for 10 days some hatching might occur. The highest hatch recorded for 7 days' exposure was 18·4 per cent, so that the complete mortality found after 10 days might, in fact, also have been found at 9 days had this been tested.

Table 2. VIABILITY OF EGGS OF *S. littoralis* FOLLOWING LOW-TEMPERATURE STORAGE IN COMMERCIAL CUTTING STORES

No. egg masses tested	Duration of storage (days)	Mean temp. (°F)	No. eggs		% hatch	
			Treated	Control	Treated	Control
15	10	30–35	1,766	1,012	0	100
11	7	30–35	889	629	13·5	100
3	7	31–34·5	187	102	0	100
1	5	31–34·5	58	42	3·5	100
1	5	31–34·5	118	94	12·0	100
1	6	31–34·5	128	35	3·9	100
1	7	31–34·5	127	67	0·8	100
23	7	31–34·5	2,320	1,831	18·4	100

The results obtained by exposing eggs at high humidity both in refrigerators and within boxes of chrysanthemum cuttings in cold-stores approximates to the conditions eggs would experience if actually laid on the leaves of cuttings which were subsequently cold-stored. No viability was detected in eggs kept at 35° ± 1° F or 30°–35° F for more than 10 days, but as 18 per cent hatched after 7 days' exposure it is unlikely that exposures below 10 days could be relied on to prevent hatching completely. As a practical quarantine practice, therefore, holding plants, subject to oviposition by *S. littoralis* for 10 days at temperatures of 35° F or below, should safely eliminate infestation. So long as the cold-rooms used for storage had suitable continuous temperature recording equipment, shipments treated in this manner could be safely cleared.

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CYTOLOGY

Autopolyploidy in *Asplenium*

Asplenium rutamuraria L. and *A. septentrionale* (L.) Hoffm. are two very distinct and well-defined species of ferns, generally distributed throughout Europe, and also possessing wide but interrupted ranges around the northern hemisphere. Previous to this communication, all the chromosome counts in these two species known to me, whether from Europe[1,2] or elsewhere[3,4], have indicated that both are exclusively tetraploid, with $n = 72$ chromosomes.

A report was recently published[5] concerning the surprising chromosome pairing found at meiosis in an example of a wild hybrid between these two species. This plant, originally collected in Borrowdale, Cumberland, showed as many as 64 bivalents out of a possible maximum of 72. More recently, fixations from another example of this hybrid growing wild in Canton Ticino, Switzerland, have been available for investigation, provided by Prof. T. Reichstein of Basle. A high proportion of cells from this plant show complete or virtually complete pairing (Fig. 1). It seems very likely, in view of the fact that *A. rutamuraria* and *A. septentrionale* are two very different species, that all the bivalents observed in these hybrids are produced by autosyndetic pairing. Evidence in support of such a conclusion can be adduced from investigation of chromosome pairing in hybrids formed between *A. septentrionale* and other species of *Asplenium*[6]. This conclusion clearly involves the implication that diploid forms of both *A.*

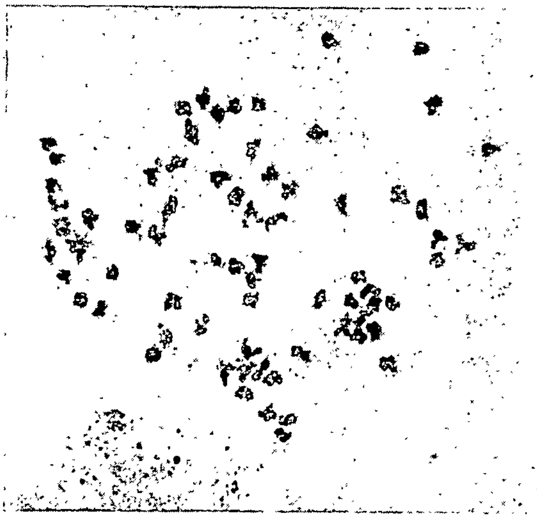

Fig. 1. Diakinesis from wild fixation of *Asplenium rutamuraria* × *septentrionale* at Bigorio, Ticino, Switzerland, showing $n = 72$ bivalents (× 1,000)

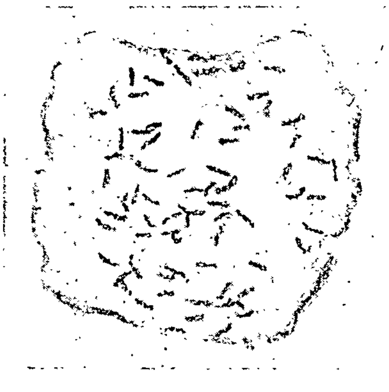

Fig. 2. Root-tip mitosis in *A. septentrionale* from the Western Caucasus, showing $2n = 72$ chromosomes (× 1,000)

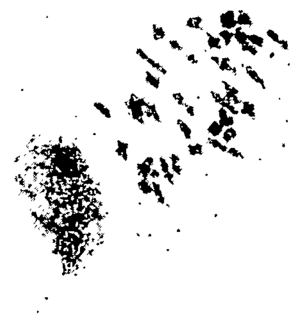

Fig. 3. Meiotic metaphase in *A. rutamuraria* from the Buco di Vela, North Italy, showing $n=36$ bivalents (× 1,000)

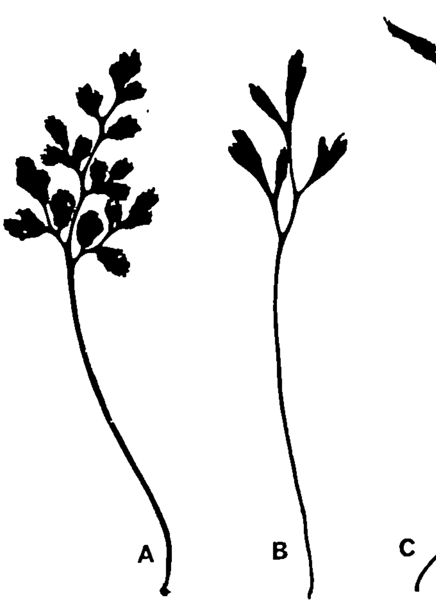

Fig. 4. Silhouettes (natural size) of fronds of the tetraploid hybrid *Asplenium rutamuraria* × *septentrionale* (B), and its parent species, *A. rutamuraria* (A), and *A. septentrionale* (C), all pressed from the wild. Bigorio, Ticino, Switzerland (September 12, 1963)

rutamuraria and *A. septentrionale* must have existed in the past, and might indeed still be alive to-day. Search has consequently been made for such plants, with the result that extant diploid forms of both *A. rutamuraria* and *A. septentrionale* have now been found.

The diploid form of *A. septentrionale* is as yet known to me only as a culture raised in Leeds from dried fronds obtained by exchange with the Moscow Botanic Garden. This material originated directly as part of a wild collection made in the Western Caucasus. Chromosome counts have been obtained both from meiosis and mitosis (Fig. 2).

The probable existence of an extant diploid form of *A. rutamuraria* became evident after a putative hybrid between *A. lepidum* C. Presl (another species known to be tetraploid[6,7]), and *A. rutamuraria* detected by Prof. Reichstein in the possession of Herr Th. Egli of Canton Zurich, Switzerland, had proved to be triploid, and not tetraploid, as is usual for this hybrid[6]. This plant had originally been collected by Herr Egli from the Buco di Vela near Trento in North Italy. Material of *A. rutamuraria* from this locality was already in cultivation in Basle and proved on cytological examination to be diploid (Fig. 3). Since then, diploid plants of *A. rutamuraria* have been collected by Prof. Reichstein and myself from several localities in the Italian Dolomites.

In both *A. rutamuraria* and *A. septentrionale*, the diploid cytotype is morphologically very similar to the tetraploid, but can be distinguished, particularly by use of micro-characters. A cursory examination of the herbarium material available to me has not yet produced any extension of the very limited known ranges of either diploid, and it seems that both are in fact rare plants, now occupying distributions of a relict character. It follows that the two tetraploids, with their wide but disjunct distributions around the northern hemisphere, are probably of relatively ancient origin.

There appears to be little doubt that both tetraploid *A. rutamuraria* and tetraploid *A. septentrionale* are of effectively autopolyploid origin. The case of *A. septentrionale* is especially interesting, since this is a particularly distinct and extremely isolated morphological type, scarcely at all variable, and with no known close relatives. The facts clearly suggest that, at least in *A. septentrionale*, the tetraploid form may indeed have arisen by simple direct autopolyploidy according to the more restricted application of the term (that is, by doubling of the chromosome complement without the intervention of any act of hybridization), a process now believed[8,9] to be only very rarely a successful mode of speciation in Nature.

Taxonomic and cytogenetic investigation of the newly discovered diploids is now in progress.

I thank Prof. T. Reichstein for his help, Prof. I. Manton for her advice, and Herr Th. Egli for his co-operation. I also thank the Moscow Main Botanical Garden for the material of *A. septentrionale* from the Caucasus.

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Measurements of Deoxyribonucleic Acid on Mammalian Germ Cells using the Integrating Microdensitometer

MICRODENSITOMETRIC determinations of the deoxyribonucleic acid (DNA) content of normal diploid cells and of tumour cells have already been reported[1-4]. In these measurements, cells known to be haploid, for example, spermatozoa, have been used as a baseline for ploidy measurements of other cells.

This communication deals with preliminary investigations of DNA measurements on Feulgen-stained rabbit germ cells using the Barr and Stroud *GN2* integrating microdensitometer. Incorporated into this instrument is an automatic scanning device with a small moving aperture 0·5μ in diameter. As the field area is scanned, instantaneous values of absorption are automatically integrated and the total field absorption is recorded as an integrated voltage on a relative absorption meter. By taking absorption measurements on fields with and without the specimen, and then taking the simple numerical difference between the two readings, an absolute absorption of the specimen, in arbitrary units, is obtained.

Follicular ova and ejaculated spermatozoa which have a theoretically constant DNA content were selected for the examination. Follicular eggs remain in the first meiotic prophase until just prior to ovulation, and thus theoretically contain a tetraploid (4*n*) amount of DNA. Ejaculated spermatozoa, having completed both meiotic

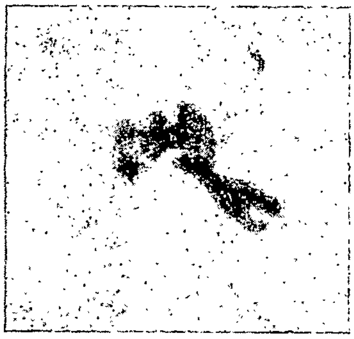

Fig. 1. Feulgen-stained nucleus of a follicular ovum

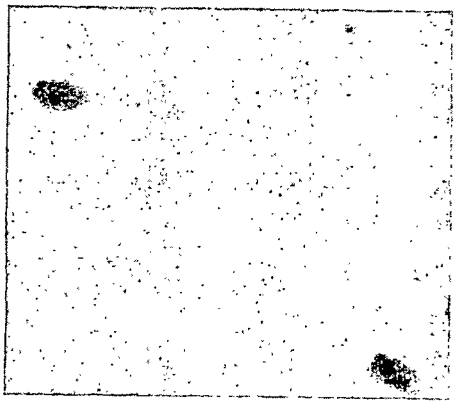

Fig. 2. Feulgen-stained nucleus of a spermatozoon

divisions, contain a haploid (1*n*) amount of DNA. The selection of these two particular cell types has the further advantage of comparing the measurements of regular- and irregular-shaped nuclei (Figs. 1 and 2).

Follicular eggs and ejaculated sperm were collected from New Zealand White rabbits according to methods previously described (Burks and Davis, in the press). The cells to be measured were squashed in 45 per cent acetic acid, fixed in acetic-alcohol, and stained by the Feulgen reaction. Multiple DNA measurements were taken on individual nuclei of six follicular eggs and six sperm at a wave-length of 5460 Å.

Comparing measurements made on follicular eggs and ejaculated sperm one would expect to find a DNA ratio of 4 : 1. The actual measurements approach this theoretical ratio. The follicular ova measure 14·7 ± 1·3856 arbitrary units of DNA; the sperm, 3·8 ± 0·3286 arbitrary units of DNA, giving a measured ratio of 3·9 : 1 (Table 1).

Table 1

Germ cell type	Ploidy	DNA (arbitrary units)	*S.D.*	Theoretical	Observed
Sperm	1N	3·8	± 0·3286	1	1·0
Follicular egg	4N	14·7	± 1·3856	4	3·9

In order to obtain maximum accuracy of measurement, the readings of clear field absorption (background) should always be the same. The nature of the present material makes it impossible to take consistent background readings. As the sperm have the more regular-shaped nuclei, it was decided to standardize the background absorptions and resulting DNA content of the ova with reference to the background readings obtained from the sperm. The proportionate size of the sperm nucleus in relation to the measured field area is more nearly constant than is that of the ovum. Our correction factor consists of the ratio of egg background absorption divided by sperm background absorption multiplied by the original calculated DNA content of the follicular egg. The density of the clear areas in the field was standardized when measuring the specimen, thereby leaving only one variable to be read by the microdensitometer, that is, the absorption of the specimen. In doing so, it was found that the estimated DNA content of the eggs and sperm fall into the theoretical ploidy ratio of 4 : 1.

Any system is of value only in so far as it is reproducible. To test this property of the system, it is irrelevant whether one takes one measurement on each of many cells containing the same DNA content or whether one takes multiple measurements on a single cell. The accuracy and reproducibility of the system will be apparent in either case. In these preliminary investigations, multiple measurements were taken on six of each type of germ cell. The results indicate that the measurements are reproducible. The correction factor allows one to use this system of measurement to obtain accurate readings of DNA content within a small range of standard deviation. Thus, it seems that the integrating microdensitometer which provides a reproducible method of comparison of DNA content in irregularly distributed nuclear material and in regular ovoid nuclei may be satisfactorily used for the assessment of the ploidy of germ cells for examination of germ cell maturation and *in vitro* fertilization. Work is in progress measuring the ploidy of polar bodies and pronuclei in order to follow the maturation of the female germ cell and to ascertain whether or not eggs have been fertilized *in vitro*.

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SOIL SCIENCE

Soluble Phosphate in Soil and Uptake by Plants

THE phosphate potential, defined by Schofield[1] as $\frac{1}{2}p\mathrm{Ca} + p\mathrm{H_2PO_4}$, has proved useful in describing the solubility of soil phosphate. However, Schofield went further and made out a case for the phosphate potential as one parameter of phosphate availability to plants, the other being the pool of labile phosphate measured by isotopic exchange. There is little doubt that plants may get insufficient phosphate because the concentration in the soil solution is too low, but it has not been shown whether this is due to concentration *per se*, or to a too-low potential (using Schofield's terminology)[2]. The following experiment, which was carried out as part of a broader investigation, suggests an answer.

Italian ryegrass ('Aberystwyth *S*22') was sown in pure sand at the rate of 0·25 g per 4·5 in. diam. pot. After a germination period of 8 days, nutrient solution was dripped on to the surface of the sand at a rate of 500 ml. per 24 h, the excess liquid running to waste through holes in the bottom of the pot. Phosphate as $\mathrm{NaH_2PO_4}$ was made to concentrations of 0·1, 0·5 or 2·5 p.p.m. phosphorus, which covers the range normally found in soil solutions. With each phosphate concentration, calcium + magnesium concentrations of $1{\cdot}65 \times 10^{-2}$ M or $3{\cdot}12 \times 10^{-3}$ M were used, which again approximate to that found in soil solutions. The molar ratio of Ca : Mg was 3 : 1. All solutions contained 2 m.equiv. $\mathrm{K^+}$ per l., 12 m.equiv. $\mathrm{NO_3'}$ per l., and micronutrient elements as recommended by Hewitt[3]. Additions of $\mathrm{Na_2SO_4}$ were made so as to give a total salt concentration of 2×10^{-2} M. The *p*H values were 5·40–5·89. Treatments with high calcium concentrations were replicated six times, those at low concentrations three times.

Table 1

P concentration (p.p.m.)	Ca concentration (10^{-3} M)	P potential	D.M. tops (g)	P in tops (mg)
0·10	3·1	6·97	0·347 ± 0·027	0·32 ± 0·017
	16·5	6·64	0·349 ± 0·013	0·32 ± 0·024
0·50	3·1	6·27	0·757 ± 0·043	1·68 ± 0·069
	16·5	5·94	0·707 ± 0·010	1·45 ± 0·045
2·50	3·1	5·57	1·229 ± 0·063	5·44 ± 0·066
	16·5	5·24	1·152 ± 0·021	5·52 ± 0·100

After 21 days of treatment the grass was cut, dried at 80° C and weighed. It was dry-ashed and analysed for phosphate by the vanadomolybdate method. The roots were not weighed or analysed.

The results in Table 1 show that where phosphate is the limiting growth factor a five-fold change in the concentration of calcium has no significant effect ($P = 0\cdot05$) on the yield of dry matter. At the low and high phosphate levels the phosphate content of the leaves is not affected by the calcium concentration, but at 0·50 p.p.m. phosphorus it is significantly reduced ($P = 0\cdot05$) by the high concentration of calcium. This latter result, which is conceivably due to precipitation of calcium phosphate, is in the opposite direction to that which would be predicted by the phosphate potential. It is, therefore, concluded that, with solutions of about the same composition as those in soil, it is the phosphate concentration and not the potential which determines phosphate uptake by plants, though it is not certain whether the concentration should be expressed as the total inorganic phosphate or as H_2PO_4' ions.

In analysing soils for soluble phosphate, ideally the soil solution should be used, but because the solution is difficult to obtain in sufficient amount for analysis the soil is sometimes shaken with a dilute solution of a calcium salt, for example, 0·01 M $CaCl_2$ (refs. 4 and 5). As an alternative this should be satisfactory so long as the soil solution itself is about 0·01 M in calcium; but there seems no case for expressing the results as potentials when considering uptake by plants.

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Effect of Chemical Weathering on Parent Material

THE summary by Keller[1] indicates that little progress has been made recently in the qualitative assessment of chemical weathering, since the equations of soil property by Jenny[2] and weathering rate by Jackson *et al.*[3] have not been developed. Recently, Loughnan[4] discussed several important aspects of weathering; but his paper is neither complete in itself nor intimately related to the main equations. The purpose of this communication is to elaborate on the last variable, 'parent material', in the equation of Jenny[2] with the aid of the recent data of Bayliss and Loughnan[5,6], Craig[7], Loughnan and Bayliss[8], and Loughnan *et al.*[9,10].

The variable of 'parent material' is subdivided into three factors: first, porosity and permeability—the quantity of fluid able to enter and flow through a rock; secondly, surface area—the extent of the area, which the fluid may attack; and thirdly, mineral structure—the susceptibility of the minerals in a rock to chemical weathering. The factor, 'mineral structure', is equivalent to the 'leaching index number' of Jackson *et al.*[3] and the 'persistence series' of Pettijohn[11]. These series are consistent with the results obtained from an approximately neutral oxidizing environment, although important exceptions are noted if the prevailing habitat is either acid (pH 4) or alkaline (pH 10), especially if the conditions are reducing rather than oxidizing.

To account for these exceptions in the equation the factor 'mineral structure' is further subdivided into the components of bonding energy, solubility, electrostatic stability and steric hindrance. The bonding energy for each of the main silicate groups, which is essentially controlled by the percentage of the strong silicon to oxygen bonds, has been calculated by Keller[12]. This series is shown by Keller[1] to be similar to the stability series of Goldich, the order of crystallization by Rosenbush, the reaction series of Bowen and the classification of silicates by Strunz. The second component, solubility, which is markedly affected by both the pH and Eh, is taken from a solubility product. If, however, this information is unavailable, then the ionic potential of Goldschmidt[13] gives an approximate value. This factor is important because it controls the differential movement of the various ions after release from the structure. Electrostatic stability of a crystal, the third component, depends on the local distribution of charge, which is a resultant of the intensity and position of the individual atoms. Any charge concentration will provide a nucleus for chemical attack. The fourth component, steric hindrance, is created by the structural configuration. A framework structure traps a highly mobile ion compared with that of an open structure.

An example of chemical weathering, that is, illite compared with vermiculite (another 2 : 1 layer lattice silicate), is taken to illustrate the four components of the factor 'mineral structure'. First, from Keller[12], the bonding between oxygen and aluminium in illite is stronger than that between oxygen and magnesium or ferrous iron in vermiculite. Secondly, from Loughnan[4] the aluminium in illite is less soluble than the magnesium or ferrous iron in vermiculite. Thirdly, the interlatered cation is shown by Bassett[14] to occupy a more stable electrostatic environment in illite compared with vermiculite. The fourth and final feature is the more difficult penetration of percolating fluids through the gap between the 2 : 1 layers of 1 Å in illite compared with 5 Å in vermiculite.

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PSYCHOLOGY

Taste Sensitivity and Food Deprivation, Blood Sugar Level and Composition of Meal

IN a recent communication, Berg *et al.*[1] cite Dell[2] that "a hungry animal is considered to be in a state of high nervous irritability leading to a heightened level of sensory and motor excitation. As glycogen stores are depleted, blood glucose levels decrease, circulating epinephrine rises and stimulates the reticular formation of the brain, which in turn produces cortical arousal and facilitation of motor

Table 1. Mean Values of Blood Sugar Levels and Thresholds for 'Sweet' and 'Salt'

Taste quality	Condition	No. of days	Subject H				Subject R			
			Threshold in scale steps		Blood sugar (mg/100 ml.)		Threshold in scale steps		Blood sugar (mg/100 ml.)	
			10 a.m.	12 p.m.	10 a.m.	12 p.m.	10 a.m.	12 p.m.	10 a.m.	12 p.m.
Sweet	Fasting	3	12·3	12·0	82	85	10·8	10·4	83	81
	Glucose—60 g at 9.30 a.m.; *S* fasting	3	13·9	12·9	123	74	12·5	11·0	126	71
	Insulin—12 units regular at 9 a.m.; *S* fasting	3	12·1	11·8	72	62	11·3	11·1	51	61
	C.P.F.-breakfast* at 8.45 a.m.	3	14·6	12·0	98	86	13·6	11·4	90	80
	P.F.-breakfast † at 8.45 a.m.	3	13·3	13·1	76	78	12·8	12·3	62	77
Salt	Fasting	3	21·9	21·0	82	86	17·4	16·3	76	81
	Glucose—60 g at 9.30 a.m.; *S* fasting	3	22·4	21·0	121	83	17·6	15·9	122	66
	Insulin—12 units regular at 9 a.m.; *S* fasting	3	22·3	20·6	66	64	16·8	15·1	66	57
	C.P.F.-breakfast* at 8.45 a.m.	2	24·1	20·9	101	85	20·8	18·3	102	82
	C.P.F.-breakfast* at 8.45 a.m. + glucose – 60 g at 11.30 a.m.	2	24·0	21·5	100	138	21·1	18·5	93	173

* Caloric value 508 (subject H); 759 (subject R). Composition: approximately 54 per cent carbohydrate; 9 per cent protein and 37 per cent fat.
† Caloric value as above. Composition: approximately 40 per cent protein and 60 per cent fat.

and sensory activity, lowering the threshold for all external sensory stimuli". This implies that with increasing hours of food deprivation sensory acuity in all modalities increases and, further, that blood glucose level is an important factor in this relationship. However, with respect to taste sensitivity it appears probable that neither of these implications is correct.

Certainly, a number of investigations have shown that, in human beings, taste sensitivity increases from soon after breakfast to just prior to the mid-day meal and tends to increase further if no lunch is taken[3–6], or to decrease after lunch depending on the caloric value of the meal[5,6], followed by a tendency to increase again in the late afternoon[3–6]. These results may be interpreted as suggesting a direct relationship between time since ingestion of food and taste sensitivity, and an inverse relationship between blood sugar level and taste sensitivity.

However, while the results of Goetzl *et al.*[3] show a further tendency to increased sensitivity in the late afternoon on 'no lunch' days, the results of Hammer[5] and Yensen[6] show the reverse tendency under this condition. Meyer[7] investigated taste sensitivity at 3-hourly intervals over a period of 34 h of fasting and reported no systematic changes. Yensen[6] noted certain defects in Meyer's investigation and found that when subjects fasted from the previous evening meal until after the late afternoon test session on the following day, taste sensitivity at 9 a.m. was significantly higher ($P < 0·01$) than the 9 a.m. levels on days when breakfast was eaten. On fasting days sensitivity varied significantly ($P < 0·01$) with time of day for all taste qualities except 'bitter' with one subject. In all cases the 11.30 a.m. thresholds were the lowest, with the 2.15 and 4.45 p.m. thresholds tending to increase. Even though reports of increasing hunger were made towards the end of the 21-h fasting period, taste sensitivity tended to decrease after the first 16 h of fasting. Thus it appears that up to a certain time taste sensitivity may increase with increasing hours of food deprivation, but that hereafter it decreases, at least up to 21 h of fasting.

During the course of a wider investigation of the influence of certain factors on taste sensitivity, it was necessary to look briefly at the relationship, if any, between blood sugar levels and taste sensitivity[8]. Absolute thresholds for 'sweet' (sucrose) and 'salt' (sodium chloride) were assessed with two subjects under conditions of widely varying blood sugar levels (Table 1). Test sessions, involving four threshold determinations per session, were conducted at 10 a.m. and 12 p.m. over 2 or 3 days for each condition. Details of overall control of subjects and of the test procedures used have been reported elsewhere[8,9]. Capillary blood samples were taken immediately before each session. Blood sugar determinations (Hagedorn ar Jensen's method[10]) were made daily by arrangement wi Mr. S. E. Davison at the John Bonnett Clinical Labor tories, Addenbrooke's Hospital, Cambridge. The me thresholds and the mean blood sugar levels for each under each condition are presented in Table 1.

Inspection of Table 1 reveals no evidence of a systemat relationship between blood sugar levels and tas thresholds. For example, in each of the six sessio directly following glucose ingestion, blood sugar levels 121 mg/100 ml. and over are accompanied by threshol which are lower (significantly so in five cases; $P < 0·0$ than those under the 10 a.m. C.P.F.-breakfast conditio which yielded blood sugar levels between 90 and 1 mg/100 ml. On the other hand, all thresholds followi the C.P.F.-breakfast decrease significantly ($P < 0·01$) fro 10 a.m. to 12 p.m. with the variation in blood sugar leve following the same trend.

Finally, it is interesting to compare the effects of t carbohydrate–protein–fat and the protein–fat breakfas Although both are of equal caloric value, blood sugar leve increase following the former and decrease after the latt as compared with fasting levels. Under the C.P. breakfast conditions the 10 a.m. thresholds are signi cantly higher ($P < 0·01$) than the 12 p.m. thresholds, b this is not so under the P.F.-breakfast conditions. Furth at 10 a.m. the C.P.F.-breakfast thresholds are significant higher than the P.F.-breakfast thresholds, while 12 p.m. they are significantly lower ($P < 0·01$).

Since these two are of the same caloric value, the fo going results suggest that the composition of the me (that is, carbohydrate–protein–fat versus protein–f influences sensitivity to sweet. The P.F.-meal does n raise the sweet threshold as high as the C.P.F.-meal, b its elevating influence is still present 3 h after the ingesti of the meal, by which time the influence of the C.P. meal has largely disappeared.

Roy Yensen

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FORTHCOMING EVENTS

Monday, July 20

Zoological Society of London and the Anti-Locust Research Centre (at the Zoological Society of London, Regent's Park, London, N.W.1), at 10.30 a.m.—Symposium on "The Endocrine Control of Metabolism and Maturation in Insects".

Tuesday, July 21

Parliamentary and Scientific Committee, General Committee (in Committee Room 12, House of Commons, Westminster, London, S.W.1), at 5.30 p.m.—Prof. B. R. Williams and Mr. Robert Nield: "Relationship of Technological Research and Development to Economic Growth".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer or Assistant Lecturer in Applied Mathematics in the Faculty of Science—The Registrar, The University, Manchester, 13 (July 23).

Assistant Lecturer (with an honours degree in agricultural chemistry, biochemistry or chemistry, and preferably experience in animal nutrition) in Agricultural Biochemistry—The Secretary, School of Agriculture, West Mains Road, Edinburgh (July 24).

Lecturers (2) (suitably qualified and able to teach for the B.Pharm. (honours) degree and at postgraduate level) in Pharmaceutical Chemistry—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (July 24).

Post-Doctoral Research Assistant in the Department of Pure and Applied Chemistry, to join in polymer research with Prof. M. Gordon—The Registrar, The University of Strathclyde, George Street, Glasgow, C.1 (July 24).

Biochemist (with an honours degree in chemistry or biochemistry) at the Area Laboratory, West Park Hospital—The Group Secretary, West Park Hospital, Epsom, Surrey (July 25).

Lecturer (with a good honours degree and teaching or research experience and preferably an interest in computers) in Mathematics; and an Assistant Lecturer, Grade B (graduate, preferably with an interest in microbiology or food technology) in Biology at Grimsby College of Further Education—The Director of Education, Education Office, Grimsby (July 25).

Lecturer (with teaching and research experience in some branch of human or regional geography, preferably with interests in a tropical area) in the Department of Geography—The Registrar, The University, Newcastle upon Tyne, 2 (July 25).

Reader (preferably with a particular interest in electromagnetic phenomena and experience in the application of advanced mathematics to the problems of the electrical engineering industry) in Mathematical Engineering—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, quoting Ref. 375/6 (July 25).

Research Assistant in Aeronautics (the field of research will be in aeronautics or aircraft structures)—The Secretary of University Court, The University, Glasgow (July 25).

Assistant Lecturer or Lecturer in the Department of Botany—The Registrar, The University, Liverpool, quoting Ref. CV/238/N (July 26).

Junior Research Fellow in Botany—The Registrar, The University, Sheffield (July 27).

Research Assistant (graduate in agriculture or agricultural science with a knowledge of nutrition, growth and development of farm livestock) in the Meat Animal Investigation Unit, Leeds University Farm, for work in connexion with an investigation of body composition by gamma radiation and ultrasonic methods—The Registrar, The University, Leeds, 2 (July 27).

Lecturer in Hydrogeology, and a Lecturer in Geology—The Secretary, University College, Gower Street, London, W.C.1 (July 30).

Part-time Demonstrators (2) in Geology (one post for geology graduates specializing in palæontology and stratigraphy, and one post for those specializing in petrology)—The Secretary, University College, Gower Street, London, W.C.1 (July 30).

Experimental Officer or Senior Experimental Officer (with a university degree or Higher National Certificate, and preferably a special knowledge of digital electronics and data handling systems) in the Department of Physics of the Faculty of Science, to take part in the experimental programme in nuclear physics associated with the heavy ion linear accelerator and other accelerators—The Registrar, The University, Manchester, 13 (July 31).

Lecturer (Grade II) in Physiology (for dental students)—The Registrar and Secretary, The University, Bristol (July 31).

Research Assistant (with a degree in pharmacology, physiology or a related subject) to join a team concerned with the identification of a urinary polypeptide which inhibits gastric secretion—Prof. H. O. Schild, Department of Pharmacology, University College, Gower Street, London, W.C.1 (July 31).

Assistant Lecturer (with an honours degree in physics and an interest in biology and electron microscopy) in Biophysics in the Department of Anatomy—The Registrar, The University, Manchester, 13 (August 1).

Research Fellow in Pure Mathematics—The Master, Corpus Christi College, Cambridge (August 1).

Assistant Lecturer in Biochemical Pharmacology—The Registrar, The University, Manchester, 13 (August 5).

Lecturer in Pharmacology—The Registrar, The University, Manchester, 13 (August 5).

Assistant Lecturer/Lecturer (with an interest in one or more of the following: meteorology, climatology, biogeography or cartography) in Geography—The Registrar, The University, Keele, Staffordshire (August 6).

Demonstrator in Geography—The Registrar, The University, Keele, Staffordshire (August 6).

Research Assistant (preferably with experience in the interpretation of air-photos and a degree in geography) in the Geography Department, to work on the organization and classification of the air-photo library—The Registrar, The University, Keele, Staffordshire (August 6).

Assistant Lecturer (with special interest in the application of techniques of physical chemistry to pharmacy) in Pharmacy—The Registrar, The University, Nottingham (August 8).

Lecturer or Assistant Lecturer (veterinary surgeon) in the Department of Veterinary Anatomy—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (August 8).

Lecturer in Pure Mathematics—The Registrar, The University, Manchester 13 (August 10).

University Demonstrators (2) in the Department of Metallurgy—Secretary of the Appointments Committee of the Faculty of Physics and Chemistry, Chemical Laboratory, Lensfield Road, Cambridge (August 10).

Lecturer (with a special interest in genetics and cytology) in Botany in St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Scotland (August 15).

Research Fellow (with a Ph.D. and research experience, preferably theoretical, in elementary particle physics) in Elementary Particle Physics—The Secretary and Registrar, The University, Southampton (August 15).

Lecturer in Mathematical Statistics at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 21).

Reader in Experimental Psychology—The Registrar, The University, Hull (August 22).

Senior Lecturer or Lecturer in Geography at the Australian National University—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 24).

Associate Professor in Biology (in the field of statistical plant ecology); a Lecturer/Senior Lecturer in Biology (Genetics); a Lecturer/Senior Lecturer in Biology (Animal Ecology); and a Lecturer in Biology (mainly for first year teaching) in the School of Biological Sciences, University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 27).

Lecturer or Assistant Lecturer (specializing in either genetics or biochemistry) in Biology at the University of Basutoland, Bechuanaland Protectorate and Swaziland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (August 30).

I.C.T. Fellow (with a Ph.D. or similar experience) in the Oxford University Computing Laboratory, to perform unsupervised research in any field relevant to computing machines—The Director, Oxford University Computing Laboratory, 9 South Parks Road, Oxford (August 31).

Lecturers in Psychology—The Secretary, Institute of Experimental Psychology, The University, 1 South Parks Road, Oxford (August 31).

Senior Research Officer (with special knowledge, preferably with research experience in some branch of numerical or non-numerical analysis)—The Director, University Computing Laboratory, 9 South Parks Road, Oxford (August 31).

Assistant Biologist/Sealing Officer (single, with a B.Sc. and aged between 22 and 45) in South Georgia, to carry out research work on seals, and supervise sealing—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC289/60/01.

Biologist (national of the United Kingdom or the Republic of Ireland, aged 24 to 35, with an honours degree in zoology and postgraduate experience in animal ecology) in Tanganyika, to carry out investigations connected with the management of wild life and to advise the Chief Game Warden—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC275/173/01.

Entomologist (with a degree in science or agricultural science, preferably with honours in zoology and experience in agricultural entomology) in Fiji, for biological control, experimental and advisory services, and other duties assigned by the Senior Entomologist—The Appointments Officer, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC213/62/03.

Lecturer or Assistant Lecturer in Geography—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

Lecturer/Senior Lecturer (with a good honours degree and research/lecturing experience, and preferably with special qualifications in one or more of the following: meteorology, climatology or biogeography) in Geography—The Registrar, Portsmouth College of Technology, Commercial Road, Portsmouth, Hampshire.

Lecturer (well qualified candidate with teaching or industrial experience, preferably a combination of both) in the Department of Electrical Engineering—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 20/G.

Lecturers or Senior Lecturers (2) in Food Processing and Engineering; an Assistant Lecturer or Lecturer in Physical Chemistry; and an Assistant Lecturer or Lecturer in Industrial Management and Liberal Studies—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

Natural History Assistant with the Area Museum and Art Gallery Service for the Midlands—The Secretary, Area Museum and Art Gallery Service for the Midlands, Council House, Birmingham, 1.

Professor of Mathematics and Head of the Department of Mathematics; and Professor of Physics and Head of the Department of Physics—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 17/G.

Research Assistant (suitable for graduate with a degree in biological sciences, chemistry or medicine) in Genetics, to take part in programmes designed to study (1) human chromosome abnormality and associated disorder; and (2) chromosome behaviour in normal and abnormal karyotypes—The Registrar, The University, Sheffield.

Senior Lecturer (preferably with administrative experience and a personal record of successful research in an industrial environment) in Chemistry—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 16/G.

Specialist Pathologist (with medical qualifications registrable in the United Kingdom, and a recognized postgraduate qualification in pathology with a minimum of five years experience in the speciality) in the Medical and Health Department, Hong Kong—The Appointments Officer, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC205/81/018.

Statistician (Biometrician) (national of the United Kingdom or the Republic of Ireland, under 50, with an honours degree in mathematics, followed by postgraduate training in mathematical statistics and biometry) with the East African Agriculture and Forestry Research Organization, to be in charge of the statistical (biometrical) division, to carry out research under the general direction of the Director on statistical problems of importance to the research workers in the biological natural resources field, and to advise these research workers—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC213/214/02.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Manchester Public Libraries. What's Available in the Technical Library: a Guide to some of the Resources in Addition to Books. By F. R. Taylor. Second edition. Pp. 29. (Manchester: Libraries Committee, 1964. Available from Technical Librarian, Central Library.) 2*s*. 6*d*. [225

Forestry Commission. North Yorkshire Forests. Edited by Herbert L. Edlin. Pp. vi + 116 + 36 plates. 7*s*. 6*d*. net. Bulletin No. 36: Mycorrhizal Associations and Calluna Heathland Afforestation. By Dr. W. R. C. Handley. Pp. iii + 70. 8*s*. net. Leaflet No. 27: Poplar Cultivation. By. J. Jobling. Pp. 26. 2*s*. net. Leaflet No. 47: Birds and Woodlands. By Dr. Bruce Campbell. Pp. 24. 2*s*. net. (London: H.M. Stationery Office, 1963 and 1964.) [225

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Department of Scientific and Industrial Research. Forest Products Research 1963: Report of the Forest Products Research Steering Committee with the Report of the Director of Forest Products Research. Pp. vi + 65 + 8 plates. (London: H.M. Stationery Office, 1964.) 5*s*. 6*d*. net. [225

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TECHNICAL ASSISTANCE FOR OVERSEAS MINING AND GEOLOGY

THE long-awaited report of the Committee on Technical Assistance for Overseas Geology and Mining under the chairmanship of Sir Frederick Brundrett was published on May 7 accompanied by a statement of Government policy on the Committee's recommendations*. The Committee had been appointed in April 1962 to report on the type of technical assistance which the United Kingdom could provide in geology and mining, and in particular to look into the functions and organization of a number of United Kingdom scientific establishments concerned with these fields.

The most important recommendation of the Committee, that the functions of the Geological Survey of Great Britain should be expanded to cover overseas work in geology and mineral assessment and that this should be accompanied by amalgamation with the existing Directorate of Overseas Geological Surveys, has been accepted by the Government. It is also agreed that as soon as possible the Extraction of Metals Group of the National Chemical Laboratory should be transferred to the Mineral Processing Division of Warren Spring Laboratory with the programme of which its research is already closely linked.

These steps towards the integration of organizations dealing with geology and mineral exploration on the one hand, and with mineral dressing and extraction metallurgy on the other, should be warmly welcomed. The expanded Geological Survey of Great Britain will in future fall into line with the United States Geological Survey, the French Bureau de Recherches Géologiques et Minières and the West German Reichsamt für Bodenforschung in concerning itself not only with the geology and mineral resources of the home country but also with those of overseas territories. The Geological Survey of Great Britain, founded in 1835, has provided the country with a set of geological maps second to none in the world and with a series of memoirs describing both the general geology and the mineral resources of the United Kingdom. In the production of these, the field geologists have been aided by a relatively small but highly skilled group of specialists in such fields as petrology, mineralogy, palæontology, geochemistry, geophysics and hydro-geology. Overseas Geological Surveys was set up in 1947 as the Directorate of Colonial Geological Surveys with the object of advising and assisting the Geological Survey Departments in the various Colonial territories, by whom the bulk of the field geologists were employed, and of affording them certain specialist services through its Mineral Resources, Geophysical and Photogeological Divisions. Under the proposed reorganization these specialist services would be reinforced by the Atomic Energy Division of the Geological Survey of Great Britain, the only branch of that organization which concerned itself to any great extent with overseas geology.

The often-quoted dictum that the primary aim of a geological survey is to produce geological maps is one which, though indisputable, can be open to misconstruction. The production of maps is indeed the basic long-term research of a geological survey, since geological maps are all in some measure interpretations which must be continually revised in the light of advancing knowledge and new techniques. A comprehensive geological survey to-day should involve a variety of specialist studies, some of them, geophysical, structural or sedimentological, carried out largely in the field, others dependent on the provision of up-to-date chemical, mineralogical, spectrographic, X-ray and electronic laboratory facilities. This is a reflexion of the fact that the past 20 or 30 years have seen a revolution in the techniques and instrumentation of geology, as was pointed out in the 1963 presidential address to the Institution of Mining and Metallurgy by Prof. K. C. Dunham.

The geological maps and accompanying memoirs provide the national store-house of information regarding the geology of any territory and indicate the areas in which more detailed exploration for mineral resources is worth while. The prosecution of these more detailed investigations is as important a duty of a geological survey as the production of the basic maps and memoirs. Much excellent work of this kind has been carried out both at home and in present and former Colonial territories overseas, but in general the geological surveys have been prevented by limitations of staff and of resources from conducting such investigations on anything like the necessary scale. Opinions differ as to the stage to which a State organization should prosecute mineral exploration before handing over to private enterprise, but it is significant that many of the countries in which mineral exploration is most active—Canada, Australia, the former French territories in Africa and, nearer home, the Irish Republic—are those where the State organization goes further in the exploration of areas of potential mineral wealth than has been customary either in the United Kingdom or in British Colonial and ex-Colonial territories.

The authors of the Brundrett Report clearly recognize the need for an extension in the activities of the expanded Geological Survey of Great Britain, in particular to provide mobile teams of geologists and mining engineers, skilled in economic assessment, to carry out technical assistance overseas. It may be added that were the fiscal climate at home a little more favourable to mining there would be opportunities to use these teams in the United Kingdom as well. These activities will call for a very considerable increase in staff, and perhaps the most disquieting aspect of Government reaction to the Committee's recommendation is the suggestion that this can be satisfied by the addition of four new senior posts on the complement of the Overseas Geological Surveys while the need for further appointments to the home establishment is kept under review. It cannot be too strongly emphasized that nothing less than a doubling of the present size of the combined home and overseas organizations will meet the needs of the present time. So great an expansion, entailing an increase both in field-staff and in specialists, including in particular geologists with economic experience, must be carried out over a period of years if the high standards of scientific ability which have been demanded by the geological surveys in the past are to be maintained.

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* Department of Technical Co-operation. Report of the Committee on Technical Assistance for Overseas Geology and Mining. Pp. 32. (Cmnd. 2351.) 2*s*. net.
Technical Assistance for Overseas Geology and Mining: Policy on the Recommendations of the Committee. Pp. 7. (Cmnd. 2352.) 8*d*. net. (London: H.M.S.O., 1964.)

However, at the present time there are able and experienced geologists from the staffs of the ex-Colonial surveys, not all of whom will wish to stay on under contract in the newly independent territories. An immediate and reassuring Government statement about the scale and scope of the new Geological Survey should lead many of these to transfer to its service at the same time as attracting a steady flow of the most able young graduates from the universities.

Apart from personnel, the reorganized Geological Survey of Great Britain will need enhanced laboratory facilities if it is to carry out the type of work, much of it of high economic importance, which is done by the United States Geological Survey. Among these facilities equipment for radiometric age determination and for electron micro-analysis may be mentioned since both can contribute significantly to understanding the origin and hence the distribution of ore deposits. In view of the spread of mineral exploration beyond the land masses some facilities for marine geology may also prove necessary.

The combined budgets of the existing Geological Survey of Great Britain and Overseas Geological Surveys are of the order of £1·75 million. Even double this figure would still compare poorly with the £7·25 million spent annually by the Bureau de Recherches Géologiques et Minières, three-quarters of it on work directly comparable with that envisaged for the expanded Geological Survey of Great Britain, let alone with the annual budget of the United States Geological Survey of more than 70 million dollars. In the light of the statistics quoted in the Brundrett Report, showing that in 1961 sixteen territories in which Overseas Geological Surveys have operated produced minerals to a value of more than £440 million, an annual expenditure of a few million pounds might seem an excellent investment.

Several recommendations of the Brundrett Committee are concerned with the general matter of publicizing the technical services available from the reorganized Geological Survey of Great Britain both to governments of developing countries and to the mining industry at large. British geologists and mining engineers have experience second to none to offer the newly independent nations, and, in return, Britain might hope to gain good will, markets for mining machinery and equipment and security of sources of mineral supply. Government reaction to these recommendations while acquiescent is largely unspecific as to how the object is to be achieved. It is suggested that opportunities should be afforded to political and official heads of departments visiting Great Britain to see the work being undertaken in the geological and mining fields, but it is ominous that now, when it is more necessary than ever before that the reorganized Geological Survey should be easily accessible, there has been talk of its headquarters being moved out of London. While it might well prove desirable to open somewhere in the south of England a branch office on the lines of those in Edinburgh, Belfast and Leeds, it would seem essential that the Directorate together with the bulk of the specialist staff, the headquarters of the mobile teams and the Museum of Practical Geology should remain in London.

From the aspect of publicity no less than of general policy, it is most regrettable that the Government has not been able to accept the Brundrett Committee's recommendation that a highly qualified advisory committee be established to consider problems of technical assistance and how they can best be met. Such a Committee would have to be in touch with the needs of developing countries, with long-term trends in demand for minerals and with all sources of technical assistance in geology and mining in the United Kingdom. It might go far towards framing, for the first time, a coherent mineral resources policy for Great Britain. The establishment as proposed by the Government of a committee of the Geological Survey Board, inevitably limited to considerations on a departmental level, would be no substitute for a top-level advisory committee. Any committee which is to be effective must be in a position to survey not only its own department but also all the facilities of the universities, the mining houses and the consultants.

The extent to which geological surveys should invite the co-operation of university staff in their work has always been a controversial problem. The Service de la Carte Géologique de France and the Service Géologique de la Belgique both rely largely on university personnel for their routine geological mapping. This system has disadvantages. It does not lead to the building up, within the organization, of the stock of experience and knowledge which the staff of a good Survey need. The Surveys do not have full administrative control of their collaborators or of their programme. Although there is much mapping ability in the universities many academics tend to be specialists in certain aspects of their science and would be reluctant to give all facets of the geology the impartial attention they would get from a professional surveyor. In specialist fields, however, the universities have much to offer, and in the reorganized Geological Survey with its greater emphasis on short-term work this will be more than ever the case. Overseas Geological Surveys have for a number of years made use of university personnel for special investigations, and their relations with the Centre for Geochemical Prospecting at the Royal School of Mines have been a particularly happy example of Survey–university collaboration. Both the British and Overseas Surveys have had fruitful co-operation with consultant firms in, for example, aerial or geophysical surveys or deep drilling.

On the subject of the provision of professional and technical training the Report might perhaps have laid greater emphasis on the importance of developing facilities for this in the newly independent countries. Many of the universities in these lands have close links with British universities, and it should be a matter of high priority to encourage the establishment and expansion of departments of geology and mining in them. While it is to be hoped that students from overseas will continue to attend British universities and schools of mines, the competition for places is likely to be severe for some time and the number that can be accepted must inevitably be limited.

The Brundrett Committee envisaged the combined Geological Surveys and the enlarged Mineral Processing Division of the Warren Spring Laboratory as both coming under the control of the Department of Scientific and Industrial Research. The recommendations of the Trend Report on the Organization of Civil Science, however, threaten to separate them and indeed to give rise to a disastrous fragmentation of what may be called the 'earth sciences', a coherent group including all aspects of geology, mineralogy, geochemistry, geophysics and physical oceanography. There is perhaps reason to hope that the original Trend proposals may be modified in such a way as to preserve the unity of the earth sciences, and it would facilitate the provision of technical assistance if geological surveys and mineral processing, even if not within the same organization, were at least controlled by the same department or research council. Many geologists will regret the passing of the Department of Scientific and Industrial Research which in the past has served the earth sciences well.

The Brundrett Committee interpreted its terms of reference as excluding any problems of financing mineral development. While no doubt it was right in this, there seems substance in the comment of a critical editorial in the *Mining Journal* of May 15 that the failure to direct attention to the consequences of divorcing financial from technical assistance is the Report's major weakness. Some mechanism must be developed whereby, when an area with economic possibilities is delimited, whether at home or overseas, Government can endeavour to interest private enterprise in its exploitation. The Bureau de Recherches Géologiques et Minières spends about a quarter of its £7·25 million annual budget on shares and partnerships in companies to exploit newly proved resources. The Irish Mianrái Teoranta carried development to a stage far beyond that hitherto envisaged by the British or Overseas Geological Surveys and financial assistance to mining companies was given by the Industrial Credit Co. on behalf of the Department of Industry and Commerce. In his 1962 presidential address to the Geological Society, Prof. S. E. Hollingworth speculated as to what the attitude in France or Germany would have been to the discovery of the substantial potash deposits of north-east Yorkshire, which, after ten years, are still undeveloped for technical reasons which do not appear insurmountable. In Great Britain the National Research and Development Corporation exists to develop and to exploit inventions where the public interest so requires, and of recent years the Department of Scientific and Industrial Research has placed contracts in industry for the development of new and technically advanced products. Is it too much to hope that in the reorganization of the facilities for technical assistance in geology and mining the opportunity may be taken to make comparable provision for the development of newly discovered mineral resources?

PLANT TAXONOMY SEEN AS A SCIENCE

Principles of Angiosperm Taxonomy

By Dr. P. H. Davis and Dr. V. H. Heywood. Pp. xx + 556. (Edinburgh and London: Oliver and Boyd, Ltd., 1963.) 95*s*. net; paper-back version, 75*s*. net.

THIS most welcome book is the first major work of recent times devoted to the theory of plant taxonomy. As a first approach to it, I recommend the reading of the four pages of conclusions that bring the text to an end. Not before has there been a clearer or more objective statement of the principles and issues involved in taxonomy to-day, nor rarely has there been a more categorical dispelling of humbug. For those who care for humbug in taxonomy—and these include both makers and users—there is much to shock and incense in these pages. If this impels them to a thorough study of the earlier chapters of *Principles of Angiosperm Taxonomy*, all to the good, for there they will find a rational and dispassionate exposition of a set of aims, methods and concepts for taxonomy which, when generally adopted, will bring it much nearer towards being a science than it has managed to be heretofore.

The authors' stance is revealed by their first three principles: "(1) Classification is a fundamental activity and is undertaken for primarily practical purposes. (2) Biological classification is an extension of general classification and is governed by the same basic principles. (3) Different classifications are needed for different purposes; no one classification can serve all purposes." Well said! It may be damaging to the mystique cherished by many who practise taxonomy to have these points so clearly put, but the more widely it is acknowledged that taxonomy is concerned first and foremost with the establishment of systems of classification with maximum utility as data storage and retrieval agencies, the better for biology as a whole. The corollary is that romantic notions about the place of phylogenetic speculation in taxonomy are overdue for abandonment. The authors make no bones about this issue, either. "In the Angiosperms," they say, "classifications based upon phylogeny are unattainable except for special cases, and then mostly at or near the species level. Because of the complexity and multi-dimensional nature of evolutionary relationships, what pass for such classifications vary from unconsciously phenetic classifications to distorted special classifications produced by unjustifiable weighting of characters." The phenetic classification which they themselves urge as the primary aim of taxonomy is one based on maximum correlation of attributes; that is to say, a natural classification in the original, pre-Darwinian sense. That natural classification should not mean automatically phylogenetic classification seems still to be a conception quite beyond assimilation for many, especially those with no very deep acquaintance with evolutionary mechanisms. Yet, as Davis and Heywood say, the conversion of true phenetic classification into something that might approach a phyletic one involves a whole series of problems of interpreting and weighting characters. The construction of phylogenetic shrubs and trees is an intriguing activity: nevertheless it is not taxonomy, but merely one use of the data of taxonomy.

Linked with their views on the basic function of taxonomy is the attitude of the authors towards the newer sources of taxonomic evidence, including cytology and plant-product chemistry. Their balanced appraisal of the potential role of the new kinds of data in general taxonomy merits attention from all who would over-value the classificatory significance of one or a few specialized criteria. Neither karyological nor phytochemical data, they say, have any special *a priori* claim to importance for comparative taxonomic purposes; surely an entirely valid conclusion, when both constitute merely expressions of the genotype in no way different from the more readily accessible morphological and anatomical characteristics.

The special significance sometimes assumed by cytological data—when, for example, a difference in chromosome number indicates a sterility barrier—is to be considered alongside experimental genetical observations bearing on the extent of breeding groups. The early enthusiasm for species concepts based on intersterility criteria never really impinged seriously on general taxonomic practice, and Davis and Heywood show once more why it cannot. They underwrite the view now gaining wider acceptance that classifications based on such criteria as gene exchange must be regarded as artificial, and as such to be designated by special terminologies.

I have emphasized in the foregoing paragraphs those characteristics which give *Principles of Angiosperm Taxonomy* its special and refreshing flavour; but in fact they constitute no more than one aspect of a very rich text. Sections are devoted to the history of taxonomy and taxonomic thinking and to the thoughtful discussion of the concepts of taxonomic categories and characters, while matters of taxonomic technique are treated as comprehensively and expertly as might be expected from two practitioners engaged themselves in major floristic research. The last five chapters (comprising rather more than 150 pp.) are devoted to experimental taxonomy and to the consideration of microevolutionary phenomena in plant populations. One can foresee a reviewer's point being made of the location of these chapters ("Can it be that the authors are looking at their subject upside down ? Can it be that they are actually unaware of the basic importance of genetics for their subject ?"), but the fact is that the taxonomic implications of population genetics and associated disciplines cannot be appreciated without an understanding of what taxonomy is all about in the first place.

An admirable book, then, destined to become a standard text in its own special field. For the practising taxonomist it is certainy obligate reading, whatever his present degree of enlightenment. From the non-taxonomic user of the products of taxonomy it deserves serious attention as a lucid and thoroughly documented treatment of the purposes and philosophy of a discipline frequently misunderstood and often misrepresented. For the student concerned to know what taxonomy has to offer as a discipline, it provides a stimulating and challenging introduction and guide. In this last connexion, it is gratifying to know that the authors are each to be concerned with new postgraduate courses in taxonomy in their respective universities, Edinburgh and Liverpool; with this text, their students should have a flying start.

Production is excellent, and the price, especially of the paper-back version, low enough to ensure that the volume, massive though it is, will have the circulation it deserves.

J. Heslop-Harrison

CLASSICAL PEDOLOGY

Treatise on Soil Science

By K. D. Glinka. Fourth (posthumous) edition, revised by the soil scientists of the Dokuchaev Soil Institute, Academy of Sciences of the U.S.S.R. Translated from the Russian by Prof. A. Gourevitch. Pp. v + 674. 126*s*.

Chemical Analysis of Soils

By K. K. Gedroits. Translated from the Russian by B. Toker and J. Schmorak. Pp. v + 602. 126*s*.

(Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1963.)

GLINKA'S *Treatise on Soil Science* is one of the classics in the literature of pedology, and as such its translation into English must be welcomed.

Bibliographical and other material omitted from the third edition was restored in the latest Russian edition, but even so, such references as "cf. objections by Borggreve, by Wollny, by Dokuchaev" are common, and the book suffers from the incomplete documentation so common in Russian works on soil science.

The fourth edition was published in 1931—about the time that the application of X-ray diffraction to the investigation of soils overthrew many of the views then held in soil science; it follows that the first half of the book, which deals with "Soil Formation and General Properties of Soils", has little more than historical interest to-day. The chief value of the book lies in the section on "Soil Types and Soil Geography", which contains definitive descriptions of the great world groups.

Glinka makes no concessions to the novice; names of soil types are introduced without definition, and even in the chapter devoted to a particular soil type it is usually necessary to read most of the chapter before finding a passing reference to the soil's distinguishing characteristics. As in much present-day literature on soil, most of the information is given in the form of profile descriptions, which even dedicated specialists appear to find hard to follow. It would be a great help to the reader if a statement of the significant features of a profile were appended.

Glinka's essentially descriptive approach to the subject is exemplified by the statement on p. 170 "... one should not forget that the main task resides in the elucidation of the process which resulted in the formation of any given soil type...". One can only commend this, but the paragraph continues: "Laboratory research should be carried out in such a way as to disclose the loss and gains of each of the several horizons ... each and every horizon in the soil profile must be studied separately and its properties compared with those of the parent rock". Laboratory research is thus considered as no more than the detection of gains and losses, but the fact remains that one has done no more than define the problem by establishing that translocation has occurred. It is no criticism of Glinka's book to say that at present we must ask how and why translocation takes place; when we ask this question, the extent of our ignorance of soil genetics is all too apparent.

Just as in the development of the older sciences, pedology has reached the stage at which the descriptive approach initiated by Dokuchaev, and developed so ably by the Russian school, needs to be supplemented by experimental investigation of the fundamentals of soil-forming processes.

Units are often omitted from tables of analytical data, and the basis on which percentages are calculated is not always specified. The table on p. 142 contains a typographical error carried over from the Russian edition, as well as several minor errors in calculation. The translator directs attention to several similar errors in the Russian version.

Despite a slightly un-English syntax the translation reads smoothly, although the word 'chemism', which is used throughout the book, caused some hesitation; it seems to mean "chemical composition-cum-chemical properties". Misprints are few and, with the exception of the omission of the latter part of a sentence on p. 402, do not obscure the meaning.

Gedroits's *Chemical Analysis of Soils* must have been a very good book in 1932 when it was last re-edited, but its value to-day is questionable. About one-third of the book consists of a supplement of newer methods compiled after the death of Gedroits, and which gives references up to about 1953. However, apart from descriptions of the use of some Russian optical equipment, the supplement contains little that is not already available in the literature.

Gedroits's original section is characterized by an extremely thoughtful approach and he emphasizes the need for selecting the method most appropriate for the particular purpose. For this reason the book has some value as background reading, but it is unlikely to find much use on the bench of the practising analyst.

C. Bloomfield

PHARMACEUTICAL ANALYSIS

The Quantitative Analysis of Drugs

By Dr. D. C. Garratt, assisted by Dr. L. Brealey, C. A. Johnson, K. L. Smith and G. Sykes. Third edition. Pp. xiii + 925. (London: Chapman and Hall, Ltd., 1964.) 126*s*. net.

THE appearance of a revised edition of this hardy perennial of the analytical field is an event that will be welcomed by all practising pharmaceutical analysts. On this occasion Dr. Garratt has been assisted in the marathon task by a team of specialist colleagues and the result is one that must bring satisfaction to readers and authors alike. The last revision of the book appeared in 1955, and the fact that the authors have found it necessary to increase the size of the present volume by some 250 pages reflects the rapid growth in complexity of the subject over this period.

The manner of presentation of material remains the same as in previous editions. Drugs are dealt with by means of monographs arranged in alphabetical order, except, in cases such as antibiotics, steroids, sugars, sulphonamides, etc., where the similarities of the analytical methods used make group treatment the logical mode of presentation.

Each monograph commences with a brief introduction outlining the principle on which the assay of the drug is based, and is followed by a detailed description of a recommended method. The limitations of the method are then discussed together with any modifications known to be necessary to cater for specific forms of interference. The monographs are studded with a multitude of critical observations and tips culled from the author's wide personal experience over many years as a practising analyst, and these are among the most valuable facets of the book.

Methods described in the previous volume that have been superseded in the interval between revisions have been ruthlessly discarded and replaced by their modern counterparts, as evidenced by the wider use of physical techniques such as ultra-violet and infra-red absorption and the various forms of spectroscopy and chromatography. The author makes reference to phase solubility analysis, electrophoresis and polarography and predicts their increasing application in pharmaceutical analysis. In this I am sure he is correct and to these could usefully be added nuclear magnetic resonance and mass spectrometry.

Much absorbing information is included in the twenty-one appendixes to the volume, many of which make their appearance for the first time. These range from practical hints on the elimination of emulsions during solvent extraction with immiscible liquids; a very real problem in the analysis of galenical preparations such as tinctures of *Hyocyamus* and *Cephaelis ipecacuanha*, to brief monographs on the application of infra-red spectroscopy and gas-liquid chromatography in quantitative analysis. These latter are not intended to be comprehensive treatments but include sufficient references to enable the interested reader to explore the subjects further.

The authors are to be congratulated on a formidable undertaking carried to a successful conclusion, and I am sure that the present volume will achieve a wide distribution among all those engaged in the field of pharmaceutical analysis.

A. HOLBROOK

ASTRONOMY, PRESENT AND PAST

Watchers of the Skies

An informal History of Astronomy from Babylon to the Space Age. By Willy Ley. Pp. xiii + 528. (London: Sidgwick and Jackson, Ltd., 1964.) 45*s*.

MANY histories of astronomy have been published during the past few years. All have followed much the same pattern; some have been poor, some mediocre, and a few good. This new book by Willy Ley is, however, distinctly different. Written in what the author evidently regards as an informal style, it nevertheless imparts a great deal of information not found in other books of its kind.

Part 1, "A Science Grows Up", describes the beginnings of astronomy, and its gradual development up to the eighteenth century. Without pretending to be exhaustive it covers a wide field, and gives the reader an excellent idea of how astronomy changed from casual star-gazing into an exact science. Part 2 deals with "The Solar System", and represents the major part of the book; a chapter is devoted to each planet, not forgetting the mythical Vulcan, and many little-known facts are brought out. The final section of the book, "Approximate Infinity", is briefer, and deals with developments in stellar astronomy and cosmology.

The author owes much to his writing style, which is remarkably clear and lucid throughout. Though the book is essentially popular, and can scarcely fail to interest the beginner, it is also valuable to the serious student; evidently an immense amount of work has gone into its compilation. There are useful references, together with a general bibliography, while the line drawings are very adequate. Wisely, Ley has resisted the temptation to include the usual spectacular but hackneyed photographs.

So far as the Solar System is concerned, the historical account could scarcely be bettered. It seems, therefore, to be a pity that the author has not dealt with stellar investigations in equal detail. His account is first-class so far as it goes, and suffers only in comparison with the treatment of the planets; to extend it would mean making the book very long, but such a course would be worthwhile, despite the inevitable difficulties of production. The account of theories of stellar evolution is, for example, somewhat sketchy, while radio astronomy is relegated to a brief note in the appendix.

As the author has stressed in his foreword, no attempt has been made to write a history which is exhaustive in every field of astronomy. The book, as it stands, is far superior to any of its predecessors. The criticisms are made here only because there has long been a need for a standard work on historical astronomy, and *Watchers of the Skies* so nearly fulfils all the requirements. A suitable extension of the astrophysical section would set the book in a class of its own, and there should be every opportunity for this, since future editions will certainly be called for.

The text is extremely accurate. There are a few minor misprints, such as 'Encke' for 'Hencke' on p. 321, and one or two slips; on p. 347, for example, it should be made clear that the 'South Tropical Disturbance' on Jupiter no longer exists (it has not been observed since 1941), while E. J. Reese, who has directed particular attention to the surface features of Jupiter, is American and not British. In Part 1 it is stated that Newton invented the reflecting telescope, but there is no mention of Gregory, who had proposed the principle earlier; and on p. 461 it is said that most galaxies are spiral in form, whereas in fact spirals seem to be in the minority. Errors are, however, very few and far between.

It is always easy to suggest improvements to a book, but it is safe to say that the reader who is anxious to learn something about the story of astronomy cannot do better than study Ley's account. Serious students will benefit equally, since they will find many facts not readily available elsewhere in collected form.

PATRICK MOORE

SOVIET AMMONIA MASER RESEARCH

Soviet Maser Research

(Transactions (Trudy) of the P.N. Lebedev Physics Institute, Vol. xxi.) Edited by Acad. D. V. Skobel'tsyn. Authorized translation from the Russian. Pp. 186. (New York: Consultants Bureau, 1964.) 27.50 dollars.

IN 1954, groups working at the Lebedev Institute and at Columbia University announced the first working models of the maser; both used a microwave inversion transition in the ammonia molecule. This paperbound translation of a volume of the *Transactions* of the Lebedev Institute contains four papers which summarize subsequent work at the Institute on the ammonia maser, mainly in connexion with its use as a stable oscillator and standard of frequency. The title of the English translation is unfortunate, as it could be taken to imply a far wider scope for the contents than this very restricted field. A bibliography lists papers published by members of the Oscillation Laboratory of the Physics Institute of the Academy of Sciences from 1935 to 1961; these papers have only an incidental connexion with masers, and cover subjects as diverse as electron paramagnetic resonance and the flight orientation of birds.

Under the title "Characteristics of Masers" G. M. Strakhovskii and I. V. Cheremiskin report experimental work on the relative stability and reproducibility of the oscillation frequency of a maser using the 3,3 line of $^{14}NH_3$, and its dependence on various operating parameters. N. G. Basov, V. S. Zuev and K. K. Svidzinskii describe similar work on the ND_3 maser, which produces a frequency near 1,600 Mc/s, significantly lower than that of the NH_3 maser which operates in the region of 24,000 Mc/s. Theoretical aspects of the frequency characteristics of the ammonia maser are considered by A. N. Oraevskii, who includes proposals for the use of oppositely directed beams and the reduction of beam velocity to improve the performance of the maser. The fourth paper, by K. K. Svidzinskii, is a comprehensive theoretical treatment of a subject related to the field of molecular beam maser research: the hyperfine structure of the rotational spectra of molecules.

The ammonia maser as a reproducible frequency source has been superseded in turn by the cæsium atomic beam standard and by the hydrogen maser. As a low-noise amplifier the solid-state maser is more practicable and versatile. In spite of the dwindling interest in the ammonia maser itself there is a good deal of instructive material in these translations, and the bibliography may reveal papers the existence of which could not previously be easily discovered. J. H. SANDERS

ELECTRON AND VACUUM PHYSICS IN EASTERN EUROPE

Proceedings of the Symposium on Electron and Vacuum Physics, Hungary, 1962

Chief Editor: Ernö Winter. Pp. 506. (Budapest: Akadémiai Kiadó, Publishing House of the Hungarian Academy of Sciences, 1963.) 11 dollars.

IN the present-day unhappy division of Europe into the Western and Eastern spheres of influence, it is always of interest to gain knowledge of scientific activity in Eastern Europe. In this respect, this excellently printed volume is valuable in presenting fifty-five papers read at the Budapest Conference in 1962, which was devoted to electron and ion physics and optics, vacuum physics and techniques and gaseous discharges. All except six of the papers are from Hungary, Roumania, Poland, Czechoslovakia and East Germany, with the majority from the first of these countries, but with virtually no influence (except in references) from the U.S.S.R. Admittedly six of the papers are in the Russian language, but they are chiefly by Czechs, the remaining forty-nine are in English or German with the exception of that, in French, by M. Degras (from Saclay) on the investigation of sorption by electron bombardment. Moreover, the many references collected in the Eastern European papers are primarily concerned with American, British and German work. Indeed, two factors are noteworthy: the apparently small influence from the U.S.S.R. in this field of activity and the indications that the Eastern European countries suffer a paucity of modern scientific equipment.

The direct British contribution is small and represented by papers from L. Holland, on surface contamination in vacuum evaporation systems, and by D. Gabor, on gas discharge work arising out of his experiments on thermionic generators. The well-known names of D. Alpert from the United States and P. Redhead from Canada appear, but otherwise the influence is dominantly Hungarian.

As the content of the papers of the few authors from the West is more readily available in recent American and British journals, the chief interest here is to survey briefly the topics covered within the papers from Eastern Europe. Electron and ion dynamics and optics are well represented, including two theoretical papers on quadrupole mass filters, one in Russian by C. J. Javor and a second in German by G. Otto. Among the papers on mass spectrometers is one by K. András, A. Dallos and J. Erdélyi in German, giving preliminary details of a new type of mass spectrometer, in which the ions move in spiral trajectories and selection is made at a specific angle of rotation in the track. Field emission cathodes and microscopes are clearly popular research topics, as is also secondary electron emission, particularly from the theoretical point of view. Other topics include oxide-coated, cermet and hollow cathodes, cæsium alloy photocathodes, conduction in gases including cæsium plasma, breakdown initiation and Penning discharges. As in Western Europe, investigations of gas sorption on filaments appear and the omegatron is a popular research tool for gas analysis observations, but one wonders whether the many papers published on the vagaries of this instrument might not eventually condemn it as a commercial device compared with the miniature mass spectrometer of the deflexion type. The hot-cathode ionization gauge in relation to ultra-high vacuum measurement is the subject of four papers, including one giving details by C. Brieman and R. Leyniers in English on a transistorized power supply for emission regulation. One paper on a 2-kW experimental electron-beam welder appears in English by E. Fóti and one on vacuum-tight windows for infra-red radiation detectors.

This volume is chiefly of use to the scientist engaged with research on electron tubes; it contains little of vital interest to the vacuum physicist able to consult the much vaster Western scientific literature. Perhaps one should be cautious here, however, after reading in German the opening paper by E. Winter which surveys the Hungarian research results in the fields of electron and vacuum physics within the period 1926–50, because he claims that several techniques in electron physics have originated in Hungary. Though this volume is unlikely to have much influence in Britain and America, it is nevertheless warmly welcomed as a record of research in countries with a growing scientific tradition. The excellence of the English used in many of the papers by Hungarian authors is, moreover, a tribute to their linguistic powers and to the first-class editorial work in preparing this book. J. YARWOOD

'OLD NICK'S' METAL

Nickel: an Historical Review

By F. B. Howard-White. Pp. xiii+350. (London: Methuen and Co., Ltd., 1963.) 45*s*.

THIS is no ordinary historical review, in the sense of scholarly detachment, but a man writing on the subject of his working life. There is perhaps a shadow of 'Pygmalion' in such devotion to one grey metal of less universal application than iron and of much inferior appearance to chromium. Nevertheless, nickel as an alloying element has played perhaps the most dominant part in the improvement of steels (based on iron), provided the ideal undercoating to chromium plate, and furnished the cutler with an amazingly good but cheaper substitute for silver. The author of this book concludes, in fact, with the attainment of manned space flight in a capsule sheathed in nickel-bearing materials.

The earliest known uses of nickel are the fortuitous occurrences in copper and bronze articles found in Antioch, Ur, and Kish, dating back some 3,500 years. The source of the material may be coincident with that of similar objects found in China. Undoubtedly, a favourite source of nickel was that of meteoric iron, and the word for iron used in Babylonia, Egypt, and Syria in ancient times signified 'heaven-sent'. Nickel was, therefore, a ubiquitous material long before it was isolated by Cronstedt in 1751. As almost every schoolboy knows, the name arose as a result of attempts to smelt the nickel-arsenide ore occurring in the Erzgebirge, and which was mistaken for copper ore. The association with 'Old Nick' arose partly because no copper resulted, partly by virtue of the arsenical fumes (injurious to health), and partly because the roasted material, required by local potters to colour their products, sometimes failed to do so.

The commercial exploitation of the metal provides a most fascinating story. The initial interest arose from the non-ferrous alloy known as German or nickel silver (known earlier to the Chinese as 'white copper'), and as demands increased, the quest for improved nickel ores spread farther, and the rich deposits in Canada and New Caledonia began to enter a phase of intense rivalry. The French source contained no copper or precious metals, and was comparatively easy to smelt, whereas the Canadian source required an economical process for the separation of copper and other metals. Needless to say, a method was found, relying principally on the immiscibility of two molten sulphide layers. Purification of the nickel became possible from the discovery of nickel carbonyl, and its development by Ludwig Mond.

The subsequent use of nickel in electro-plating, its incorporation into steel, particularly for armaments, and the development of new non-ferrous alloys based on reduction of the Canadian 'matte' containing copper, to give 'Monel' and a host of derivatives, created an unprecedented demand. (The First World War caused a trebling of production during a decade.) New alloys appeared with the discovery of so-called 'stainless' steel, and between the two World Wars, nickel became an alloying element in cast irons, and a constituent of many magnetic materials. Perhaps the most romantic story of many new uses for nickel is that of the gas turbine and later rocket engines.

The books contains some useful notes on each chapter, and five appendixes, the fifth being a key to the Annaberg Altarpiece by Hans Hesse, 1521, a reproduction of which forms the frontispiece. The style is easy to read; the author, as an engineer, pays full homage to the chemist and metallurgist, and provides an absorbing series of portraits of men in the industry. A professional historian could scarcely have done better. C. R. TOTTLE

A SHORT HISTORY OF METALS

A Hundred Years of Metallurgy

By W. H. Dennis. Pp. ix + 342. (London: Gerald Duckworth and Co., Ltd., 1963.) 35*s*. net.

DESPITE a history extending over some six or seven millenia, more progress has been made in metallurgy during the past century than in the whole period during which metals have been known to man. It is no easy task, therefore, to compress these into some 330 pages, and the skill with which this has been done and the ground covered reflect great credit on the author. The picture painted is, however, in the main concerned with that branch of the subject which deals with the extraction of the metals from the ores, and the subsequent processes to which they are subjected to render them fit for use are dealt with somewhat briefly.

In many cases, an essential preliminary to the actual smelting process is the removal of the waste material from the ore, and ore-dressing has assumed a greater importance as it has become necessary to utilize leaner ores; the flotation process and sintering are emphasized especially, so far as the latter is concerned, in connexion with iron and steel. Roasting is a necessary preliminary to the subsequent extraction of the metal in the case of many sulphide ores, and the introduction of the fluo-solid roaster in which the finely divided feed is held in suspension by a stream of gas is one of the more outstanding advances in this field.

The electro-magnetic discoveries of Faraday have had repercussions in two ways and, indeed, there are few better examples of the mutual interaction of discoveries in one field of knowledge on another. The demand of the electrical engineer for copper and aluminium as conductors and the fact that the electrical industry has become a major customer for lead and other metals have had immense economic influence on metallurgy, while the use of electric power in arc and induction melting has rendered possible techniques of which the metallurgist himself has taken full advantage. Hydrometallurgy—the process of recovering a metal by solvent action as, for example, in the cyanidation of gold and silver ores or in the Bayer process for the extraction of aluminium from bauxite—is then considered. After this general introduction the progress made in connexion with the production of iron and steel, the major non-ferrous metals, the 'newcomers' and the precious metals are dealt with in turn. A short chapter is devoted to subsequent shaping process, and the book ends with a very brief account of the fundamentally important question of metallography.

Within the limits which the author has been allowed this is done well, the descriptions are clear and the material well chosen. It is inevitable, however, in such a widespread survey that there are minor errors, one of which in a historical treatment may be mentioned. It is stated (p. 279) that the earliest separation of gold from silver was effected by the use of nitric acid. The earliest Greek coins were made from natural electrum containing up to around 30 per cent silver. It was not until the reign of Croesus in the middle of the sixth century B.C. that a pure gold currency was introduced—the method of removing the silver being still unknown, though it is not improbable that it may have been done by prolonged heating with sulphur or a sulphide. This would eliminate the silver as a molten silver sulphide slag: it was certainly long before nitric acid was available.

The author has done an excellent job of work, and his book maintains well the reputation of the series in which it appears. F. C. THOMPSON

SEMICONDUCTORS

Progress in Semiconductors

Vol. 7. Edited by Alan F. Gibson and Prof. R. E. Burgess. Pp. 238. (London: Heywood and Co., 1963.) 65*s*.

THE first and last of the four articles in this volume are by American authors, and the other two are by British; but there is little or no sign of this distinction in the presentations or styles of writing. Indeed, there is a marked similarity between the first two articles, on bismuth and bismuth telluride respectively, in that the best available data on physical properties are selected and used to test present-day theories of the band structure of each substance.

Some licence has been taken with the definition of a semiconductor in including the article on bismuth, but that strange semi-metal with its rare and even unique properties deserves attention. At low temperatures, the surface scattering of carriers is specular rather than diffuse, and several properties, notably magneto-resistance, which result from the application of a magnetic field show a periodicity in the reciprocal of field strength. For a substance the lattice of which departs so little from simple cubic, bismuth has many properties which are highly anisotropic and not easy to interpret.

Bismuth telluride is noteworthy because its figure of merit as a material for thermoelectric generation or refrigeration is among the highest recorded. Exact stoichiometry (Bi_2Te_3) in single crystals seems always to result in p-type conductivity; not until the tellurium content is increased to 63 per cent (atomic) is intrinsic behaviour noted. The article on this substance concentrates on the galvanomagnetic and thermoelectric properties and thermal conductivity, without neglecting the elastic constants and optical properties, and reports some hitherto unpublished measurements of specific heat.

The two elemental semiconductors, germanium and silicon, dominate the rest of the book. In the third article they are the vehicles for studies of the interactions of impurities with dislocations, a subject which has been attacked from several sides with some success even though some very obvious simplifications have been made in the models assumed. The elastic and electrical interactions and the kinetics of migration are briefly described before more detailed attention is directed to precipitation, particularly of aluminium in silicon crystals containing much oxygen ($\sim 10^{18}$ atoms cm^{-3})—which may be sited interstitially or be largely combined in the form of silica precipitates—and of some fast diffusers in both germanium and silicon. The role of carbon in silicon, only recently appreciated in this context, is not overlooked.

The fourth and longest article examines the effects of high hydrostatic pressures (10,000 kg cm^{-2} and upwards) on the electronic properties of the two elemental semiconductors and of one or two intermetallic compounds. Most interest has centred around the band structure, including, particularly, changes in the energy gap as deduced from measurements of resistivity and optical absorption, and the scattering of carriers as reflected in the drift mobility. The influence of pressure on the effects introduced by impurities, donors and acceptors and those giving rise to deep lying levels receives due attention. The authors do not fail to point out the more serious gaps in present knowledge: thus the pressure dependence of permittivity and refractive index deserve more attention and many measurements already made with temperature as a second variable have yet to be given satisfactory quantitative explanation.

Many physicists and materials scientists can profitably explore this volume despite its specialist appeal; they will find the descriptive portions of each article easy to absorb. J. R. TILLMAN

NEVANLINNA THEORY OF FUNCTIONS

Meromorphic Functions

By Prof. W. K. Hayman. (Oxford Mathematical Monographs.) Pp. xiv+191. (London: Oxford University Press, 1964.) 63*s*.

PROF. HAYMAN'S monograph deals with that part of the theory of functions of a complex variable which discusses the roots of an equation $f(z) = a$, where $f(z)$ is either regular in the whole plane (an integral function) or regular save for poles (a meromorphic function), with related results when the domain is the unit circle. The theory has a fairly long history, since it may be said to have begun with Picard's famous theorems, that if $f(z)$ is an integral function, $f(z) = a$ has roots for all values of a with at most one exception, and that if $f(z)$ is meromorphic, there cannot be more than two exceptional values of a. Hadamard developed the theory of integral functions, using the order, which is ρ if the maximum modulus on $|z| = r$ behaves like $\exp(r^\rho)$. The connexion between order of growth and zeros is crudely that the more zeros, the greater must be the order.

The modern theory, to which Hayman has himself made substantial contributions, arises almost entirely from the fundamental work of Rolf Nevanlinna, begun about 1924, described in Nevanlinna's 1929 tract in the Borel collection and in one of the Springer 'yellow-backs' in 1936. On the basis of Jensen's generalization of Poisson's formula, Nevanlinna derived his central 'characteristic function', T, which differs boundedly from a sum $m + N$, where N measures the number of times the function $f(z)$ takes the value a, and m measures the intensity of the approach of $f(z)$ to a; this is in fact the first fundamental theorem. The second asserts that in general N is the dominant term in the sum $m + N$. From a study of the properties of T, a host of special results can be deduced, such as Picard's theorems and extensions; for example, if $f_1(z)$, $f_2(z)$ assume 5 distinct values at the same points, they are identical (and 5 cannot be replaced by 4). The methods extend to results about the derivatives of $f(z)$.

A somewhat similar line of thought is due to Ahlfors, expressed in terms of lengths and areas in mappings on to a Riemann sphere; here the outstanding result so far appears to be Ahlfors' 'five islands' theorem. When the general theory is applied to functions meromorphic in the unit circle, it yields as special cases the famous theorems of Schottky, Landau, Bloch and Bohr.

As a lucid and connected account of recent work in this field, the book will be of immense value to the young research worker, since it appears that the methods are far from being exhausted, either in their power to afford new results or in co-ordinating a variety of special cases into a simpler and more general theory. The novice, however, might be well advised to acquaint himself with Nevanlinna's Borel tract as a prolegomenon to Hayman's masterly work, a work which will surely be the standard text in this field for the present generation.

T. A. A. BROADBENT

MODERN ALGEBRA: A POWERFUL ENGINEERING TOOL

Matrix Algebra for Electrical Engineers

By R. Braae. Pp. xi+162. (London: Sir Isaac Pitman and Sons, Ltd., 1963.) 30*s*.

NOW that matrix algebra is finding its way into sixth-form mathematics we can expect the next generation of electrical engineers to be fairly conversant with it by the time they are professionally qualified. Many of them know little to-day of its elegance and power, and find mathematical text-books, admirable as they are, faintly repellent. Such electrical engineers will find Mr. Braae's work clear, readable and interesting. He is himself an electrical engineer and has written with the peculiar needs and difficulties of his fellow engineers in mind.

The scope of the book is wider than the title suggests. The author's declared aim is the modest one of enabling a beginner to reach a standard where he can read engineering articles that use matrices without too much difficulty. Any reader who absorbs this text and tackles the many exercises scattered throughout the work should be in a position to 'originate' articles using matrices as well as read them.

In addition to the main subject of determinants and matrices some of the topics dealt with, and dealt with competently, are vectors and vector spaces, co-ordinate transformations, group theory, bilinear and quadratic forms, invariance, linear programming, applications to geometry and mechanics, linear networks, diakoptics (that is, the piece-wise solution of large systems, not to be confused with the partitioning of matrices), and an excellent final chapter on tensor analysis which takes the reader as far as covariant and intrinsic derivatives. Clearly the book is not about matrices alone but about their place in engineering mathematics and their relation to allied subjects, and the ground is prepared for a deeper study of tensors and topology. Since the book is designed for self-study there are many exercises and at the end of each chapter a set of problems for the reader to solve. There is also a good bibliography designed to guide readers with their further reading in the allied subjects and the applications.

In a disarming introduction, Mr. Braae disclaims any attempt at rigorous treatment, but in fact the treatment is more rigorous than electrical engineers need and willingly accept. The matter is presented so concisely that much ground is covered in the 160 pages of text. Yet the style is remarkably fluent and pleasant. It is certain that the author has a deep affection for mathematics. To him it is not just an engineering tool but a joy, and he seeks to arouse in his readers his own enthusiasm for a fascinating subject. His book can be warmly recommended to all electrical engineers.

W. J. GIBBS

NON-AQUEOUS SOLUBILITIES

Solubilities of Inorganic and Organic Compounds

Edited by Prof. H. Stephen and Dr. T. Stephen. Vol. 1: Binary Systems, Part 2. Pp. viii + 963–1933. (London and New York: Pergamon Press, 1963.) 250*s*. net.

THIS book is the English edition of a compilation of solubilities prepared under the direction of a panel of scientists appointed by the Soviet Academy of Sciences. It deals with non-aqueous binary mixtures and completes the first volume of a larger work, which will ultimately include ternary and multicomponent systems. It is a useful collection of data which is well arranged, for the most part in a tabular form, although graphical presentation of data is occasionally used. Molecular formulæ and name indexes for the whole of the first volume are provided and these are easy to use and enable the information to be readily located. Nomenclature is said to follow the recommendations of the International Union of Pure and Applied Chemistry and the indexing arrangement to be that of *Chemical Abstracts*.

This last-mentioned claim is not entirely true, in that elements in the inorganic section of the formula index are arranged by order of Periodic classification and not alphabetically. In addition to being indexed by name and by formula, the solubility tables are arranged according to the chemical formula of the component with the smaller number of carbon atoms. There are nearly 3,000 tables of solubilities in all and each table is provided with a reference to the original work from which the data were obtained. It is apparent from these references, of which there are more than 2,000, that the literature has been widely surveyed. The references to Russian literature are conveniently numbered 1–208. An examination of the references themselves is not without interest and reveals that about a third of them relate to work completed in 1914 or earlier. The very oldest reference is indeed old; it is a reference to the work of Gay-Lussac and is dated 1819. In contrast, only about one-tenth of the references relate to work completed since 1945. This is, of course, no criticism of the authors, who can only collect information from available sources, but when one considers the prodigious number of new compounds isolated or synthesized since 1945, this paucity of information on such a mundane property as solubility seems almost unbelievable and surely reflects an unsatisfactory situation.

On the debit side of this book, there are a number of omissions and misprints which although minor in themselves should not appear in a reference work and especially one at this price.

There are also some untranslated Russian words such as trace, insoluble, completely miscible, components, etc. These occur mostly in tables of figures and their presence is so obvious that it is difficult to see how they were missed during all the stages of preparation. Certain entries in the book relate to compounds existing in isomeric forms, and the nature of the isomer or mixture of isomers is not specified. Reference to the original journals, which were by no means readily accessible in all examples, revealed that in some cases additional information was available.

No doubt in the first edition of a work translated from Russian, errors can more easily occur than in other works. One hopes, however, that in future editions the various mistakes will be eliminated so as not to detract from an otherwise valuable collection.

The book will obviously not appeal to individuals, but it should prove a useful reference work for the library.

P. J. COOPER

TECHNOLOGY FOR SCHOOLS

Technology

Man remakes his World. Editorial Board: Dr. J. Bronowski, Sir Gerald Barry, James Fisher and Sir Julian Huxley. (The Macdonald Illustrated Library, Vol. 5.) Illustrated and designed by Hans Erni. Pp. 367. (London: Macdonald and Co. (Publishers), Ltd., 1963.) 55*s*. net.

THIS is a solid and well-produced book, worthy of the Verona printers who made it. In it are presented, in American style in two page units, the achievements of modern technology. The field which it ploughs is wide, even if the furrows are not deep. It is difficult to think of a technological subject which is not mentioned and illustrated in the book. It is an exciting book, and no doubt much of it will be relevant in five years time.

Technology is the fifth volume of a library of knowledge "for the family". It can be used as an encyclopædia or a background book for a fifth- or sixth-form pupil. To the older student of science it might be useful as a book of applications of his fundamental principles. The arts student should find it useful because it tells him of the forces which, whether he likes it or not, are shaping his environment. The illustrations, of which many are in colour, are of high quality and would make good teaching aids.

Apart from the possibility of the destruction of man by the atomic bomb, the tone of the book is optimistic. "Our planet," says the book, "will become what the British poet, William Blake, called 'a green and pleasant land'. Those who feel the need for a more exciting life will find it as pioneers and developers of the other planets in the solar system." The realistic critic, not to say the cynic, may feel that there are few signs of this at present. Perhaps it is not the fault of technology that it deals with means rather than ends, with techniques rather than values; but one might pause before this catalogue of human ingenuity and ponder on the increasing slaughter on the roads and of cancer of the lung, on high wages, leisure and adolescent crime, the starvation of a large part of the world, the transistor-set and noise nuisances, and the insidious tendencies to the erosion of human individuality, as a start.

It is understandable that the sales of this book will be greater in the United States than in Britain, but it is a pity that the English reader has to endure such spellings as "sulfa", "aluminum", "fiber", "center" and so on. Since communication theory is one of the subjects of the book, an intelligent sixth-former might ask why a common use of English for scientific communication in English speaking countries is not yet vouchsafed us!

W. L. SUMNER

POPULARIZING SCIENCE

"POPULARIZATION", Dr. Martin Green[1] has said, "is one of those words that goes round doing harm: it is one of the prime agents in a conspiracy to divide and enfeeble the modern mind: for it implies that the thing popularized has been reduced in value, and that the people reading it have been fobbed off with a substitute. It divides by implying that modern science, for instance, is so remote from the literary man's understanding that his idea of it is only half-true; and it enfeebles by implying that the only things we really know are a handful of specialities (if we are lucky enough to be specialists in something) and that the rest of our intellectual life is a game of blind-man's buff, if not an exercise in deliberate fraud".

Bad popularization is bad, of course, wherever one meets it: science is not singled out for special attention in this respect, although, no doubt, the education of journalists does not conduce to accurate reporting in this field. That scientists should make a scornful distinction between knowledge and half-truth is both natural and necessary, but those who persist in regarding popularization as an unprofessional activity do both science and culture a disservice. For, at its best, popularization of science can be an interpretative art-form in its own right, comparable perhaps to literary criticism, with a culturally valuable integrating function: or so Mr. Maurice Goldsmith (Science Information Service) claimed when the Scientific Publications Council met at the Ciba Foundation, London, on April 23 to discuss the subject. But Mr. Goldsmith sought for a word to replace 'popularization' in vain.

Prof. A. S. Parkes (Physiological Laboratory, Cambridge), introducing the subject, distinguished two possible meanings of popularization: making science popular as such among non-scientists, and making it intelligible to them. We undoubtedly have to make science popular, since scientific research is increasingly financed by public funds. Just as society now depends on science for its livelihood, so science depends on society for men and money, and both are impossible without public support. More complex are the questions of whether and how science can be made intelligible; the value of attempting to convey the changing detail which science accumulates is dubious, but it is important to communicate broad scientific ideas and more scientific and analytical ways of thinking. Of the several media available, Prof. Parkes commended the semi-specialist periodicals in particular, describing how he had been attracted to reproductive biology—as an academic pursuit—by an article in *Discovery* by Julian Huxley. Prof. Parkes imputed neither praise nor blame to Huxley for this, but he did draw a moral: that popular science is a great recruiting sergeant. This is important because the 'brain drain' is not only from the Old World to the New—it can also flow into unproductive forms of scholarship. It is interesting, however, that medicine, which receives the lion's share of publicity, is nevertheless among the last in the queue for public funds (although it is among the best provided for by private foundations, no doubt because of its high moral tone and philanthropic connotations). Perhaps familiarity breeds contempt, and maybe a certain mystique is necessary for respect.

From the early days of modern science there have been two major strands to the thread of popularization. Ever since Fontenelle (secretary to the French Academy of Sciences) wrote *Entretiens sur la pluralité des mondes* in 1686 there have been those who interpreted science to the public from within; and there has also been the equally honourable tradition of exposition from without, beginning perhaps with Voltaire's *Elements de la philosophie de Newton*, which appeared in 1738. More recently, specialization has added to these a third strand, that of interpreting biology to the physical scientist and vice versa, which has given rise to new and valuable channels of communication—*Endeavour*, *Scientific American* and Penguin *New Biology*, for example. For a while the popular and semi-popular press (such as *Discovery*) and the professional science writer—including eminent men like Waldemar Kaempfert—seemed likely to dominate the scene, interpreting science, as it were, from outside. But the newer mass media, broadcasting in sound and vision, have restored the possibility of immediate personal presentation by the scientists themselves, that was once confined to the limited audiences of the local learned societies and the annual meetings of the British Association.

Speaking from a personal point of view, Mr. Gordon Rattray Taylor (B.B.C. Television Science Service) took up the challenge on behalf of his particular mass medium. He distinguished five facets of popularization, namely, (1) news of recent advances in science; (2) knowledge of the great intellectual foundations of science, such as the theory of evolution or quantum theory; (3) appreciation of the scientific method and attitude; (4) the history of science; (5) the politics and economics of science—the way it is organized and financed. No doubt public finance necessitates popular support and may, indeed, impose an obligation on scientists to account for themselves, both individually and in general, before the public of which in all other respects they are themselves a part. But in the last analysis the justifications for popularizing science are the justifications for science itself: that it is an expedient and indeed necessary activity, that it is fun, and, most important of all, that it is a high intellectual enterprise and that any culture is flawed that is without insight into this great ferment of our age.

Mr. Rattray Taylor spoke of the philosophy and the technique of presenting science on television. At one time the approach had been to ask the scientist direct questions about science. There is perhaps a certain incongruity in asking the Astronomer Royal to discuss, say, the cause of the rainbow, and, although audience figures are not wholly reliable indexes of quality, such programmes did not attract wide acclaim. A more successful approach has been to take the television camera into the laboratory in order to show scientists as people with jobs and ways of living, rather as one might visit a factory and be fascinated by other men's lives and their environment, absorbing the general flavour without straining after detail. More recently, it has become clear that a very large section of the public seeks from the scientist not so much explanations as some sort of orientation towards his activities and attitudes and their place in the wider scheme of things, and programmes surveying whole areas of science have attracted audiences as large as ten million people.

Scientists are not infrequently rather inhibited in dealing with people—which may indeed have influenced them to go into science—and quite often poor speakers, but they are always to be preferred to professional actors in broadcast feature programmes because of their essential and total involvement. (Prof. Parkes thought they should be paid at Equity 'star' rates.)

There are, of course, considerable conceptual difficulties wrapped in the jargon of scientific discourse, and what constitutes an 'explanation' is itself a philosophically interesting question. As Mr. Rattray Taylor pointed out, Warren Weaver[2] has distinguished two modes of explanation: *horizontal explanation* in terms of familiar physical analogies (radio propagation in terms of waves on water), and *vertical explanation* by analysis into simpler but related concepts (radio propagation in terms of electromagnetic oscillations). Whether the vertical or horizontal mode is appropriate in any context depends partly on the level and aim of the explanation. The vertical mode is always involved at the level of quantification and is, therefore, often thought of as the scientific explanation. Horizontal explanation, on the other hand, provides imaginative insight and is often the best basis for popularization, but the scientist sometimes feels embarrassed to be asked to speak in these terms, as though he were betraying a trust. However, vertical explanation carried to its conclusion also arrives at a set of irreducible concepts, which then have themselves to be explained horizontally (for example, by reducing electromagnetism to 'forces' which are ultimately metaphors derived from muscular tensions). So Schon[3] argues that science advances by displacement of concepts from familiar to unfamiliar contexts, by what is essentially an appeal to metaphor. Scientific explanation thus depends on both horizontal and vertical components. Horizontal explanation is perfectly valid for relating the unfamiliar to the familiar, the unseen and conceptual to the visible and tangible. To some extent familiarity is a matter of bodily size and sensory acuity, and the social role of explanation is simply to relate unusual phenomena to common experience. It has been one of the great achievements of science to bring the mysterious and magical into the sphere of the rational and physical by this sort of process.

Colin A. Ronan (London) agreed that it is one of the functions of popularization to discourage magical thinking. It is a great tragedy that incomprehension so frequently breeds not respect, but fear, and one of the historical triumphs of science has been to cast out fear, as Halley did, for example, when he brought comets within the Newtonian scheme. Popularization helps not only to dispel fear and to undermine the power of charlatans, especially in medicine, but also to offer an alternative to the uncontrolled mythopoeic imagination which is characteristic of primitive thought[4].

The history of science affords a great window for popularization (though not perhaps on television, as Arthur Garratt reminded the Council, where the business of fancy dress and writing with feathers tends to obtrude), probably because it deals in people with problems rather than the problems *tout court*. The International Geophysical Year and now the International Biological Programme offer perfect opportunities to display science as a very human activity, a co-operative venture cutting across all the frontiers of tradition and politics. Here television comes into its own again.

The difficulties to be overcome in popularization are not only, or even mainly, the conceptual ones involved in 'explanation', or those which arise when the physical scientist dares to explain matters of biological moment and vice versa. They are frequently semantic. William Molyneux was perhaps a little optimistic when he wrote to Hans Sloane in 1697, "I hear Mr. Newton's *Phil. Nat. Prin. Math.* is out of press [print], and that he designs a 2nd Edition. Pray advise him to make it a little more plain to Readers not so well versed in Abstruse Mathematicks, a few Marginal Notes and references and Quotations will doe the business". But mathematics apart, ever since science abandoned the 'close, naked, natural' use of language exacted from the founder members of the Royal Society, and substituted the 'didactic deadpan' of modern usage, it has been the foremost task of popularizers simply to translate from the hieratic into the vernacular[5]. Mr. Ronan recommended clear thinking and plain speaking as a salutary discipline for scientists and writers alike.

At the same meeting the Scientific Publications Council elected a new chairman and executive committee as follows: *Chairman*, Dr. R. K. Callow (National Institute for Medical Research, Mill Hill), in succession to Prof. A. S. Parkes (Cambridge); *Committee*, Mr. A. L. Bacharach, Prof. N. F. Maclagan, Prof. J. Rotblat and Dr. C. C. N. Vass. Mr. A. V. S. de Reuck (Ciba Foundation, 41 Portland Place, London, W.1) was elected honorary secretary.

A. V. S. DE REUCK

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A NEW FOSSIL HUMAN POPULATION FROM THE WADI HALFA AREA, SUDAN

By DR. G. W. HEWES, H. IRWIN, M. PAPWORTH and A. SAXE

University of Colorado Nubian Expedition,
University of Colorado, Boulder

DURING 1962–63 the University of Colorado Nubian Expedition, participating in the international campaign to salvage ancient remains in that section of the Nile Valley to be flooded by the waters impounded by the new high dam at Aswân, reported the discovery of a fossil human mandible from a presumably Upper Palæolithic site about 650 m west of the Nile, opposite Wadi Halfa, Republic of the Sudan[1]. In early 1964 another site was investigated, 1·5 km farther west, and 2·5 km from the River, which has yielded the permineralized skeletal remains of 38 individuals, associated with fossil bones of large bovids, and a flint tool complex of Mesolithic character. The skeletal remains, while obviously *Homo sapiens*, have several strikingly robust 'primitive' features, and appear to belong to a hitherto undescribed North African physical type.

Traces of ancient human occupancy at this place, now designated Site 6*B*36, were noted in December 1962 by Hewes[2], who picked up a few worked flints, but failed to observe the human and animal bones which lay a hundred metres or so south of his traverse. In mid-January 1964, Irwin, of the 1963–64 season's party, discovered burials eroding on the surface along with abundant faunal remains (large mammals, birds, reptiles), flint artefacts and hearths. Excavations were begun soon afterwards by Papworth, Saxe and the Expedition's physical anthropologists, G. Armelagos, G. Ewing and D. Greene, who will prepare the detailed analysis of the skeletal materials. The first task was to determine whether the mineralized human remains were contemporaneous with the fossil animals, flints and hearths. Fluorine, uranium oxide and nitrogen tests of the human and animal bones are now being carried out at the British Museum (Natural History), although the evidence from the excavations suggests that there is no difference in time between the deposition of the faunal, artefactual and human remains. The total absence of pottery is also indicative of a date prior to 4000 B.C., since cultures using pottery are known to have been in existence along the Nile both to the north and south by that time[3]. Age-determination by carbon-14 may present difficulties, since suitable charcoal has not been recovered from the hearths, though there is burnt animal bone which may be usable.

The site is located at about 21° 57′ 28″ N., 31° 19′ 10″ E., with a military grid reference of 918900 N., 647500 E., on the 1 : 25,000 topographic map of the area[4]. The site surface lies 32–35 m above the present mean level of the Nile at Wadi Halfa, or 152–155 m above mean sea-level at Alexandria. It will be subject to eventual flooding from the waters impounded by the new High Dam at Aswân. The site extends 450 m north-west–south-east, and 300 m north-east–south-west, occupying a gently sloping desert plain north of a large wadi or dry wash. In several places this surface is cut by gullies and beds of clean white sand, and is veneered on its higher spots by gravels. According to K. Butzer[5], such gullying reflects local episodic rainfall as the increasingly dry climate replaced pluvial conditions which had prevailed during the late Pleistocene. Destructive rainstorms still occur in this region of usually zero precipitation, as W. Churchill, C. B. M. McBurney and H. Kees have described[6]. Beginning around 5000 B.C., a moister climate returned to Nubia for the next 2,500 years. When the wadi still carried water down to the Nile, it presumably supported a local gallery forest, and what is now the surrounding desert, totally devoid of vegetation, was a grassland, probably with brushy patches which are indicated by extensive fossil root-systems on what are now quite barren plains. The moisture and vegetation had led to soil formation, indicated by a layer of *terra rossa* up to 30 cm thick in places, in which artefacts, human burials, faunal remains and hearths occur *in situ*. This relict soil is now overlain by gravels and wind-drifted sand. The bovid bones also testify to a moister climate, since these large herbivores would have required sufficient pasture.

The edges of the gullies which cross the site and 'drain' toward the wadi are marked by coarse dark sand and lensed fine gravel, derived from the low sandstone-capped escarpment to the north, which reaches elevations of 165–176 m above sea-level. The central gully channels contain white sand, which also underlies the *terra rossa*, and in turn overlies an ancient grey-brown Nile silt. Some hearths have been cut by the gullies. The surface gravel seems to have resulted from sheet-erosion from farther up-slope, formed during sudden rainstorms which at rare intervals even now afflict this normally rainless region. On the old silt, which is at the surface east of the site, are numerous molluscan shells—of the snail *Cleopatra* and the bivalve *Corbicula*. The Columbia University Expedition obtained during 1961–62 carbon-14 dates from these shells of around 9000 B.C. (ref. 7).

When the skull fragments of the first burials were examined in the field laboratory, it was apparent that the ancient population at 6*B*36 differed sharply from later pre- and proto-historic Upper Nile Valley peoples, who do not seem to differ much from the modern inhabitants of Nubia. Detailed presentation of cranial and other measurements and morphological observations must await further laboratory study and the reconstruction of fragmentary skulls, but the outstanding physical features of the 6*B*36 population can already be described in general terms.

Burial 2, typical of those on the site, lay on its left side with legs flexed, hands close to the face, head to the west, and shallow grave. The upper parts of the skeleton had been somewhat eroded, damaging the skull. Some other burials were multiple, with two or more persons interred in the same grave. In Burial 11, the head had turned downward, preserving the facial mask almost completely (Figs. 1 and 2). Judging from the pelvis, this rugged, muscular skull belonged to a female. Compared with recent Nubian skulls of the past 2,500 years, from Meroitic, *X*-Group, Christian Nubian, and later times, the supra-orbital area is massive, the nasal root depressed, the molars thick and greatly expanded at their lower margins, the orbits rectanguloid and the cranial vault low. The mandible has wide ascending rami, which flare outward at the gonial angles; the chin is rounded rather than sharp, the symphysis high and thick. Yet this mandible is not the most massive; that of Burial 14 (Fig. 3) is more so, and has an even broader ascending ramus. The stature of the presumed female of Burial 11 has been estimated from long bones to be 160 cm. (5 ft. 3 in.). The crania intact enough to measure (and there are several in excellent condition) indicate that the population tended toward brachycephaly, but a definitive statement must be postponed until more of the skulls can be reassembled.

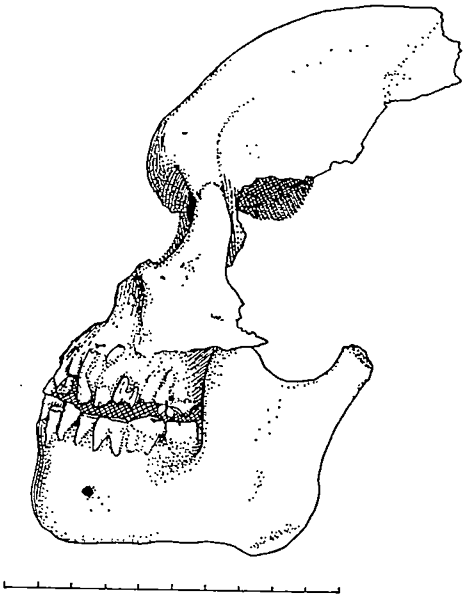

Fig. 1

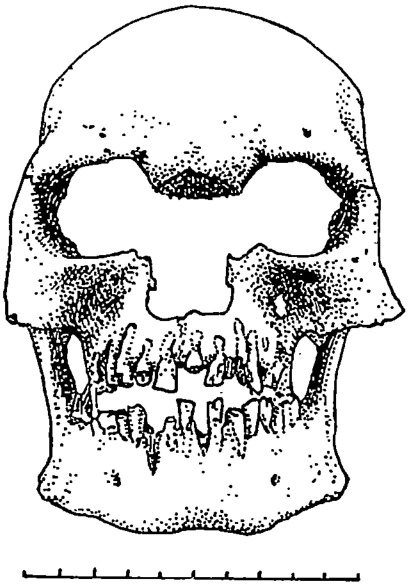

Fig. 2

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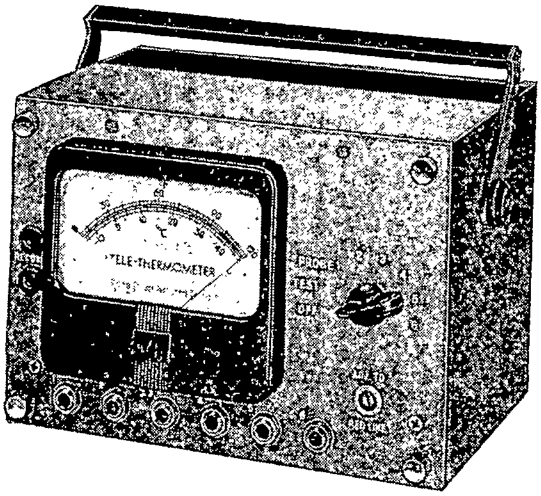

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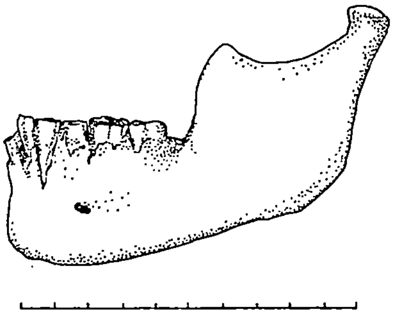

Fig. 3

The ancient population of 6*B*36 as known so far do not appear to be Bushmanoid, as has been claimed for the isolated Singa skull, found on the Blue Nile 320 km south of Khartoum[8], nor are they much like the Mesolithic Caucasoid populations of the Maghreb, such as Afalou bou-Rhummel, Taforalt, Mechta al-Arbi, and so on, who had flaring, massive jaws, short faces, but high cranial vaults and rather sharp chins[9]. If the 6*B*36 people were Negroid, they did not closely resemble either the man of Asselar, found in the western Sahara, or the inhabitants of the Early Khartoum site[10]. Surprisingly few skeletal remains have been reported for times prior to the immediate Predynastic Period in the Nile Valley[11]. Fossil human remains of apparent antiquity, but undated and undescribed, have been reported from Kau el-Kebir, south of Asyût, by Vignard in 1923, from Gebel Silsileh (a single frontal bone), from Kom Ombo Plain (also by Vignard), and from the east bank of the Nile near Wadi Halfa by the Columbia University Expedition during 1961–62. One or more of these finds may turn out to be approximately contemporaneous with the 6*B*36 population, and perhaps even physically similar to it.

Tools at 6*B*36 are mostly chipped uniface flint points and scrapers, amounting to about 1,000 specimens, plus about ten times that number of cores and scraps. The chipped implements were prepared from pebbles which occur in profusion on the higher terraces and hilltops. There are mullers or manos of reddish sandstone conglomerate, which takes a rough finish, and which must have contributed to rapid tooth wear among those who ate seed meal ground on them. Small flake points were made by a backing technique, leaving a steeply faced edge on one side, a sharp flake edge on the other, and part of the striking platform on the base. There are crescent flakes, 'geometric' flakes of microlithic proportions, side-scrapers, perforators, some real burins, and notched flakes which may have been used as shaft smoothers. The absence of bifacially flaked stemmed or concave-based projectile points, which are quite common in North Africa and the Sahara, and which occur in the Egyptian Neolithic–Copper Age sequence, is noteworthy. Ostrich egg-shell beads were also found.

Until more data are available, including radiocarbon dates from burnt bone on the site, and the results of more thorough analysis of the lithic industry, we can only make a guess at a date for Site 6*B*36, namely, about 6000 B.C. The Site was almost certainly occupied and used as a burial-place prior to 4000 B.C., or we should have found pottery. Site 6*B*27, which lies 1·5 km east, has yielded a carbon-14 date of 7312 ± 600 B.C., and its stone tools appear to represent a slightly earlier stage in the development of the local sequence. Site 6*B*36 implements are notably different from those on still another nearby ancient campsite, 6*B*29, which has been dated by carbon-14 at 13,150 ± 750 years. The radiocarbon tests were performed by Geochron Laboratory, Inc., Cambridge, Massachusetts.

The analysis of the local lithic tool sequence is being carried out by H. Irwin; the detailed site report for 6*B*36 is being prepared by A. Saxe. M. Papworth served as field director for the Expedition in the 1963–64 Season, with R. H. Lister and J. B. Wheat as senior research associates. G. W. Hewes was administrative director for the project. We thank Sayed T. H. Thabit, commissioner for archæology, Sudan Antiquities Service, and his staff, for assistance in the field. The work was supported by the U.S. Department of State under Public Law 480 Funds, and by U.S. National Science Foundation grants.

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A NEW CHROMOSOME MODEL

By Dr. DEEPESH N. DE

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SEVERAL attempts have been made to construct a workable model of chromosome based on the structure of DNA. Taylor[1] proposed a 'centipede' model in which the DNA molecules are arranged in a lateral fashion on a protein backbone. Later, Taylor[2] proposed another model in which DNA segments are attached to protein blocks on two sides. Schwartz[3] proposed the side-chain structure with DNA strands interlinked by protein for its integrity. Kaufmann and De[4] proposed that a chromosome is made of pairs of pairs of fine strands. Later, Steffensen[5] supported the structure. Recently, Cole[6] has proposed a complicated structure which needs large mechanical activity for replication and function. This model is based on a successive series of helical condensations of a linear thread composed of DNA segments covalently joined by short segments of either protein or specialized DNA. Unfortunately none of these models can explain all the established biochemical and cytological facts about a chromosome. Here I propose a simple model of the structural and functional unit of the chromosome, which conforms with all the cytological, genetical and biochemical data.

The proposed model is based on a large number of circumstantial and experimental evidence. Frey-Wyssling[7], examining the scattering of nucleic acid chains of chromosomes, concluded that the nucleic acid molecules are at right angles to the long axis of the chromonema. Mirsky and Ris[8] consider the backbone of the chromosome to be a protein framework. Removal of the DNA–histone complex by sodium chloride solution does not destroy the integrity of the chromosome, and they therefore consider the residual chromosome to be the basic unit. Enzyme digestion studies with DNase, RNase, pepsin and trypsin on animal chromosomes have led Kaufmann *et al.*[9,10] to conclude that, both structurally and functionally, the chromosomes of higher organisms represent an integrated fabric, in which histone and non-histone proteins combine with RNA and DNA. Wilkins[11] and Zubay and Doty[12] concluded that histone polypeptide chains are wrapped around both the small and the large grooves. From the works of Bloch and Godman[13], Rasch and Woodward[14] and others, it can be concluded that the DNA–histone ratio is maintained 1 : 1 throughout the cell cycle. With the aid of autoradiographic techniques, De[15] has shown that DNA and histone are synthesized concomitantly. Dounce *et al.*[16] have shown that residual proteins are firmly bound to DNA by covalent bonds at the end of DNA chains. Kirby and Frearson[17] also examined the DNA–protein bonds. On the degradation of DNA–protein they noticed that some glutamic acid, alanine and serine remained with the polynucleotide fragments. Steffensen[5] has indicated that divalent cations are bound to the chromosomes and they have a stabilizing effect on the chromosomes. Kaufmann and De[4] have shown from electron microscope examinations of chromosomes of *Tradescantia* that the most prominent unit of the fibres in the chromosomes is about 1000 Å in diameter. Gall[18] has shown that the diameter of the chromatid in the lampbrush chromosomes is about 500 Å. Gall[19] has also shown recently that the identifiable nuclear material from red blood cells of newt, human and grasshopper cells consists entirely of long fibres having a diameter of approximately 400–600 Å.

The proposed model is of a chromonema—the structural and functional unit of the chromosome. It follows the structural principle of Watson–Crick DNA model[20]. According to this model, the chromonema is made of two separate sub-units, each with a backbone of residual non-basic protein which is continuous along the length of the chromonema (Figs. 1 and 2). Several polypeptide chains are bound to each other by divalent cations Ca^{++}. The two protein backbones are so constituted they are unlike in some structural organization that restricts reunion of like strands when exchanges occur. The DNA segments are approximately at right angles to the length of the chromonema axis and are parallel to each other. For a given chromonema, the sequence of the DNA segments along the length of the chromonema is constant and species-specific. Each strand of the DNA duplex is attached by covalent bonds to one of the protein backbones at one end, the other end remaining free and the complementary strand to the other protein backbone in the same fashion. The DNA segments are wrapped around with histone. The length of the DNA segment across the chromonema could be in the order of 500–1000 Å, which may vary from species to species. The spacing between consecutive DNA–histone segments may not be constant, and the segments may be grouped in closer proximity to act as functional units. The chromonema is not structurally rigid and its dimensions would vary with degree of coiling and folding, and presence and absence of additional products of chromosomal activity. Nevertheless, the integrity is always maintained. The ionic environment would play a great part in coiling of the chromonema. In the proposed structure, RNA is not involved, but it may be associated with it as a metabolic product.

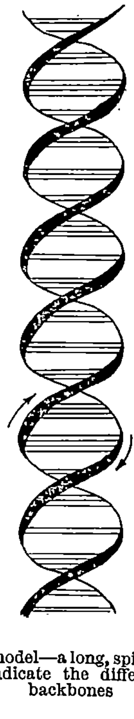

Fig. 1. General view of the model—a long, spirally coiled, double-stranded structure. The arrows indicate the difference between the protein backbones

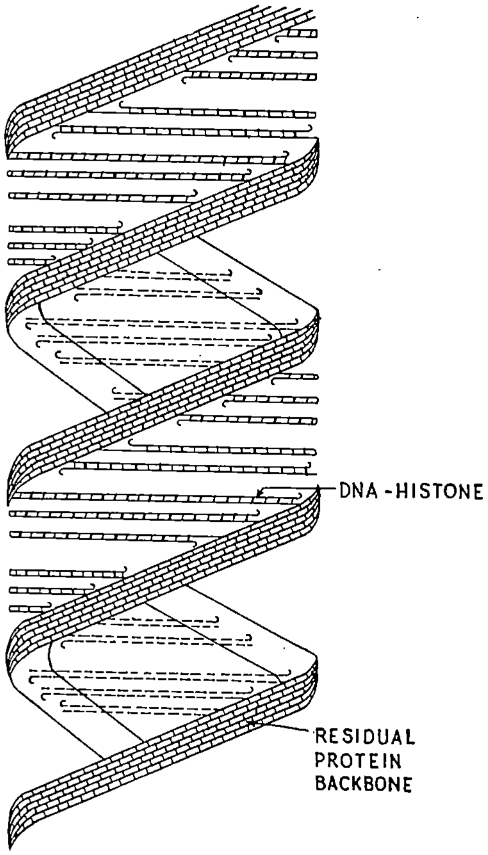

Fig. 2. Details of the model. DNA–histone segments are arranged like the rungs of a ladder and the protein backbone forms the two spines. Dark regions of the rungs represent the histone wrapped around DNA. Free ends of the DNA strands are exaggerated. The spacing between the consecutive DNA segments is not constant; nevertheless, there is a certain space between two consecutive segments. Two or more segments can form a group and act as a functional unit

The replication of the chromonema is like the semi-conservative replication mechanism of DNA molecule. The atomic identity of the sub-units of the chromonema is conserved, although effecting a permanent separation of the two sub-units from each other in course of replication. The replication begins with untwisting and gradual separation of the two sub-units from both ends of the

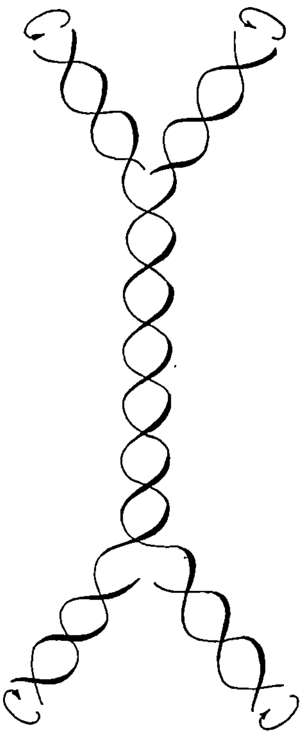

Fig. 3. A replicating chromonema, in which the upper arms rotate in the opposite direction of the lower arms. Replication and unwinding proceed *pari passu*

chromonema. The unwinding and replication proceed *pari passu*. Since the proposed model is supposed to replicate from both ends, the daughter chromonemata at one end will rotate clockwise and the other end anticlockwise (Fig. 3). The DNA segment also starts replication from both ends (Fig. 4). The free end of each strand of the DNA duplex untwists and separates and the complementary strand is progressively synthesized until it reaches its bound end. Finally, two hybrid molecules are produced. This is in perfect conformity with the results of Meselson and Stahl[21]. The histone chain may or may not separate from the parent DNA duplex, depending on whether the basic groups of a single histone chain simultaneously bind the phosphate groups of both the strands of the DNA duplex. Anyhow, after replication both the daughter DNA segments would be wrapped around with one old and one new histone protein. At this stage, small pieces of newly synthesized residual protein would be attached to the unbound end of the hybrid DNA. Joining together of these residual protein–DNA–histone complexes in 'zipper' fashion would progress from both ends of the chromonema until they meet each other at a place which may very well be the centromere as indicated by Taylor[22]. Thus the replication mechanism of the chromonema is in complete accord with the results of Taylor, Woods and Hughes[1].

Steffensen[5] has opined that any proposed mechanism of replication must satisfy at least the following restrictions: (1) labelled chromosome strands assort every other generation[1]; (2) DNA molecules are hybrid molecules[21]; (3) each fibril unit is organized in a progressive order of pairs of pairs[4]; (4) sister-chromatid exchanges are limited by the direction of the synthesis[23]; and (5) a mechanism of chromosome replication is compatible with DNA structure and synthesis[20]. The proposed model of chromonema structure and replication perfectly complies with all these restrictions.

The model is attractive in its simplicity and in its conformity with the general pattern of double-stranded coiled structure. It is likely that replication of the model would not need large mechanical activity. The model is expected to explain very easily almost all the cytological biochemical and genetical aspects of the chromosome.

The matrix is probably an artefact which is produced by extraneous material on the chromosome. The model fits with the observation that the end-view of a chromosome and fine fibres at the electron microscope level present a 'doughnut' or ring appearance, as has been shown by Kaufmann[24] and Kaufmann and De[4]. The euchromatin and heterochromatin regions of the chromosomes would probably have very little structural difference, but they probably differ in functional aspects. Optically observable chromomeres may be certain regions of the chromonema where DNA–histone segments are densely located and little more coiled. Cytologically observable centromeric granules are not different from chromomeres as electron microscopic studies have so far revealed no special structure. The centromeric granules could be visualized as a certain region of the chromonema where closely packed DNA segments synthesize a specific protein and/or where a special protein is attached to the residual protein, so that during spindle formation this protein couples up with the protein of the spindle.

The polytene structure of *Drosophila* salivary gland chromosomes are produced due to complete despiralization and increase in the length of the protein backbones of the numerous chromonemata without any loss in its integrity. One or more DNA segments of the chromonema give rise to band regions and the relatively wider spaces between the DNA segments to interband regions. This contention is supported by the fact that no DNA is observed in the interband regions as reported by Woods, Gay and Sengun[25] and Steffensen[5].

The induced and spontaneous chromosomal aberrations could be derived from breaking or loss of either one or both of the protein backbones of the proposed chromonema.

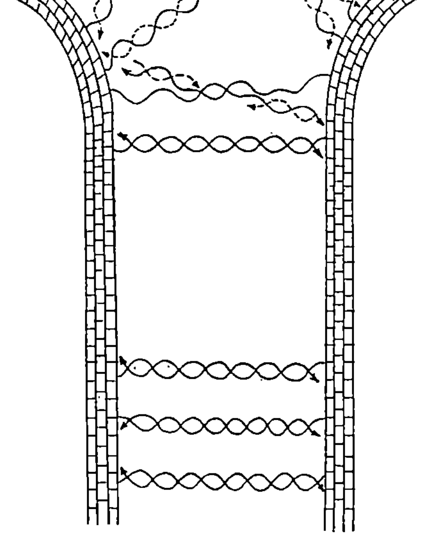

Fig. 4. Details of the replication mechanism. Histone round DNA segments are omitted for clarification. Arrows of the strands of DNA indicate free ends. Broken lines represent newly synthesized components. Each daughter chromonema is hybrid in both DNA and protein components

The breakage would not involve any damage to DNA segments. Wolff[26] has shown that protein synthesis is necessary for the rejoining of radiation-induced chromosome breaks. It has also been strongly suggested by Sharma and Sharma[27] that chemically induced chromosome breaks are chiefly due to hazards of protein synthesis. The mechanism of crossing-over could be easily explained by the proposed structure in which the protein backbone plays the vital part.

Mutation due to structural aberration is understood. The point mutations would involve change in the DNA segment. Any damage to DNA segment would cause intragenic mutation. According to Rhoades[28], in maize none of the mutations has been demonstrated by cytogenetic evidence to be an intragenic change. Nevertheless, position effect can be explained in the following way. A break of the protein backbone occurs between a particular sequence of DNA segments and the distal portion is rejoined to some chromonema in the chromosome complement. Even though the segments are not lost, the sequence is altered, causing alteration or blockade of gene function. The restoration of the segment-sequence restores normal gene function.

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OBITUARY

Dr. G. W. Monier-Williams, O.B.E.

WITH the death of Dr. G. W. Monier-Williams one of the last links, if not the last, with the control of food and drug administration by the old Local Government Board has been severed.

Dr. Monier-Williams was born on April 17, 1881, and came of a well-known legal family, the effects of which were often displayed in his approach both in his chemical work and also in his dealings with those with whom he came in contact in his official capacity. He was educated at University College, Oxford, where he took a first class in chemistry in 1903, having spent part of the summer vacation in 1902 at Leipzig studying physical chemistry. From Oxford he went to Freiburg (1903–05), where he undertook research and obtained his Ph.D., after which he spent some weeks at Cognac studying distillation. He spent short periods as research chemist at the tar works of the Gas Light and Coke Company at Becton, and with A. Chaston Chapman, before commencing work in January 1909 as an assistant inspector of foods at the Local Government Board under Sir William Power, medical officer, and Dr. G. S. Buchanan, who was in charge of the newly formed food section.

Monier-Williams's name was first mentioned in the eighth report on the application of formaldehyde to meat, when he received a mention for having examined a formaldehyde preservative; but after this a steady stream of reports began to appear over his own name and dealt with such diverse topics as the colouring matters of flour, the artificial bleaching of flour, and preservatives. His report on the freezing-point test for the determination of added water in milk appeared in 1914 and contains about 100 references to the scientific literature—surely almost a record at that time. This work was interrupted by service during the First World War, when he went to France with the Expeditionary Force and transferred to the Special Brigade, Royal Engineers, on its formation in 1915, being appointed adjutant at the depot at Helfaut, France, where he remained for the remainder of the War, being awarded the M.C. and the O.B.E. for his services. Owing to his retiring nature he gave the impression that he was reserved, but with his tall and rather handsome appearance combined with his government experience he made an ideal officer for the post.

With the formation of the Ministry of Health the series of reports on foods was discontinued, and Monier-Williams read occasional papers to the Society of Public Analysts, though a few important and authoritative reports issued by the Ministry were written by him, notably those on the solubility of glazes and enamels used in cooking vessels, antimony in enamelled hollow-ware, the determination of benzoic acid in foods, the determination of sulphur dioxide in foods, the effect on foods of fumigation with hydrogen cyanide, and lead in food. After his retirement he wrote his well-known book on *Trace Elements in Food*, a volume which will remain a standard for many years, although the methods used for analysis are now being superseded. It is probably safe to say that few analysts have issued so many reports, which, though few in total number, will remain for so many years as the outstanding works on their particular subjects.

Apart from his work as an analyst and inspector of foods, Monier-Williams was a recognized authority at the Ministry, to whom public analysts or manufacturers could appeal for advice not merely on what was believed to be the legal interpretation of various food orders, but, even more important, what had been the underlying intention of the Ministry, when a particular item had been introduced into a statutory order. Public analysts had cause to be grateful to Monier-Williams and his colleague Mr. R. Butcher, for the investigation of any complaints which they might have in their dealings with local authorities. Although he served for three periods on the Council of the Royal Institute of Chemistry, and published articles in the *Journal of the Chemical Society* and the *Biochemical Journal*, it was to the Society of Public Analysts, now the Society for Analytical Chemistry, that he gave his greatest support, serving for no less than seven periods as a member of council or vice-president and finally, during 1945–46, as president. Monier-Williams was no mean artist and exhibited his landscapes at the Royal Academy and elsewhere; but with increasing deafness he tended to withdraw gradually from public life, much to the regret of his colleagues and friends. He was twice married, his first wife dying young, and our sympathy is extended to his widow and children, who survive him.

T. McLACHLAN

NEWS and VIEWS

Chairman of the Medical Research Council's Clinical Research Board: Sir Robert Platt

Sir Robert Platt, professor of medicine at the University of Manchester, has been appointed chairman of the Medical Research Council's Clinical Research Board, from October 1, in succession to Prof. E. J. Wayne. Sir Robert was a member of both the Medical Research Council and the Clinical Research Board from 1953 to 1957, and has been chairman of the Council's Committee for Research in General Practice since its formation in 1957. He is a member of the Central Health Services Council, and was chairman of the Health Departments' Working Party on Medical Staffing Structure in the Hospital Service. The Clinical Research Board was set up in October 1953, with the task of assisting the Medical Research Council in the promotion of clinical research on a national scale, and of advising the Health Departments on their scheme for decentralized research.

National Physical Laboratory, Aerodynamics Division: Dr. W. P. Jones

Dr. W. P. Jones, superintendent of the Aerodynamics Division of the National Physical Laboratory, has been appointed director of the NATO Advisory Group for Aerospace Research and Development. Dr. Jones joined the staff of the National Physical Laboratory in 1935 after a distinguished academic career. He graduated with first-class honours in mathematics at St. David's College, Lampeter, where he was a mathematical scholar from 1927 until 1930. He then went up to Oxford, as a senior student, after winning the Jodrell Mathematical Scholarship at the Queen's College. In 1932 he again graduated with first-class honours in mathematics but, instead of taking the usual course of working for a D.Phil. degree, he decided to read physics and passed his final examination in 1934 after only two years of special study of this subject. At the National Physical Laboratory he was attached to a group that specialized in research on flutter and vibration of aircraft under the leadership of the late Dr. R. A. Frazer. In 1946 Dr. Jones became the leader of the group, and in 1953 he succeeded Mr. A. Page as superintendent. During the past decade Dr. Jones has been concerned with the planning and development of new facilities for aerodynamic research and with the general supervision of the Division's research programme. The Division is now particularly well equipped for research on high-speed flows, including hypersonic and low-density flows and gas dynamics. Dr. Jones's published work has been mainly concerned with flutter and the theory of airflow over oscillating surfaces. He was awarded a D.Sc. by the University of Oxford in 1953, and in the following year he was elected Fellow of the Royal Aeronautical Society and also of the American Institute of Aeronautics and Astronautics. Dr. Jones has served on the Aeronautical Research Council of the Ministry of Aviation and on many of its committees and sub-committees. He is also a member of the International Advisory Committee on Rarefied Aerodynamics. In 1960 he was elected by the Massachusetts Institute of Technology to the Jerome Clarke Hunsaker visiting professorship for that academic year, and gave the 1961 Minta Martin Lecture sponsored by the Institute of Aerospace Sciences. In 1962 he delivered the Lanchester Memorial Lecture to the Royal Aeronautical Society.

Dr. R. C. Pankhurst

Dr. R. C. Pankhurst has been appointed to succeed Dr. Jones. After graduating with honours in physics in 1937 at the Imperial College of Science and Technology, where he held a Royal Scholarship, Dr. Pankhurst spent two years on research in molecular spectroscopy, particularly the emission spectrum of sodium hydride, in the Astrophysics Department of the College. A by-product of this work was the discovery of a highly complex band-spectrum due in part to the triatomic molecule SiO_2: this was of particular interest as the spectra of very few polyatomic molecules had been obtained in emission. He became interested in aerodynamics through spending a summer vacation at the Royal Aircraft Establishment, and joined the Aerodynamics Division of the National Physical Laboratory in 1939. During the Second World War he was engaged in theoretical work on the design and performance of the propellers for high-speed aircraft, under the guidance of the late Mr. C. N. H. Lock, and also carried out extensive wind-tunnel experiments on the aerodynamic performance of contra-rotating propellers. After the War, Dr. Pankhurst worked on the characteristics of aerofoils in two-dimensional flow, and became an authority on boundary-layer control, including its application to the reduction of aerofoil drag and the increase of maximum lift. Other noteworthy contributions in this field were wind-tunnel experiments (with Dr. B. Thwaites) on the flow with circulation around a porous circular cylinder. He has also maintained particular interest in experimental methods in fluid dynamics. For several years he has been leading a group concerned with (1) aerodynamic problems of vertical take-off and landing and the interaction of jets with surrounding streams; (2) flow laminarization with application to slender-delta wings; and (3) the study of turbulence in jets and boundary-layers with special reference to the mechanism of the production of aerodynamic noise. Dr. Pankhurst was elected a Fellow of the Royal Aeronautical Society in 1957. He is a member of several committees of the Aeronautical Research Council and was formerly associated with the Commonwealth Advisory Aeronautical Research Council, to which he made valuable contributions during its formative years.

Applied Mathematics in the University of Reading: Prof. J. N. Hunt

Dr. J. N. Hunt has been appointed to the newly instituted chair of applied mathematics in the University of Reading. Dr. Hunt, who is thirty-six years of age, after serving for two years as a Royal Air Force instructor, joined the Imperial College of Science and Technology, London, where he obtained, in 1950, a first-class honours degree in mathematics and, in 1952, a Ph.D. degree working under Prof. A. T. Price on the mathematical theory of gravity waves of finite amplitude in deep and in shallow water. During 1952–55 he held the post of scientific officer at the Department of Scientific and Industrial Research Hydraulics Research Station, Wallingford, where he worked on problems in hydraulics, such as viscous wave damping, beach erosion, tidal currents and storm-surges in the Thames estuary. He then transferred to the Atomic Weapons Research Establishment, Aldermaston, where for three years he worked on compressible flow, atmospheric oscillations, and on numerical computations connected with various models of the Earth's atmosphere. He spent the session 1958–59 as associate professor at the Georgia Institute of Technology, Atlanta, Georgia, working on wave propagation over a permeable surface. In 1959 he was appointed lecturer in mathematics and later reader in physical oceanography at the Imperial College of Science and Technology. Most of Dr. Hunt's published work has been connected with waves, either in water or in air, and deals with damping, sedimentation and mass transport, turbulence, instability, wind or currents. He has published a text-book on incompressible fluid dynamics and is working on a book on gravity wave theory and its engineering applications. He will take up his duties on October 1.

U.S. National Science Foundation Division of Engineering

A DIVISION of Engineering has been established by the U.S. National Science Foundation. Dr. John M. Ide, formerly director of the SACLANT Antisubmarine Warfare Research Centre, La Spezia, Italy, has been appointed director of the new Division.

Engineering research supported by the National Science Foundation has brought all the traditional disciplines of science to bear on technical problems. Not limited to the physical sciences, although it has historically had most connexion with them, engineering research has involved biology or the social sciences. Hitherto, basic research in engineering has been supported within the Foundation by the Engineering Section of the Division of Mathematical, Physical and Engineering Sciences. Engineering now becomes the fourth research Division, the other three being the renamed Division of Mathematical and Physical Sciences, the Division of Biological and Medical Sciences and the Division of Social Sciences.

Engineering research eligible for support by the National Science Foundation will have to be of a true scientific nature and not routine engineering practice. Examples of the types of engineering research that may be supported are: the development of principles and techniques in systems engineering design; interdisciplinary research related to such matters as biomedical engineering, transportation, urban planning, fire prevention, etc.; the development of principles of generation and control of energy systems and information systems; the analysis and synthesis of processes and systems which contribute to mastery of the environment.

Research supported by the National Science Foundation in the engineering sciences for the fiscal year 1963 totalled nearly 12 million dollars, through approximately 290 grants; in 1962 it was 9 million dollars through about 240 grants. During the fiscal year ending June 30, 1964, the Foundation granted approximately 13 million dollars for basic research in engineering.

Universities of Manchester and Liverpool Joint Research Reactor

THE research reactor which was built for the universities of Manchester and Liverpool at Risley, near Warrington, with a grant of £350,000 from the Department of Scientific and Industrial Research, became critical on July 7. The reactor will be used for postgraduate study and research, in the fields of nuclear engineering, radiochemistry and neutron and solid-state physics, mainly by workers from the two universities, although facilities will be made available to other universities and technical colleges in the north-west. It will also provide valuable facilities for the production of short-lived isotopes which are not at present available in the north of England. The reactor is based on an American design which has been considerably modified in order to raise the power from 10 kW to 100 kW and to increase its flexibility for experimental purposes. The main contractors for the reactor were Pye, Ltd., acting under licence from the American Machine and Foundry Co. of New York. The main contractors for the building were Harry Fairclough, Ltd., Warrington. The United Kingdom Atomic Energy Authority, acting as agents for the universities and the Department of Scientific and Industrial Research, placed contracts for the manufacture and supervised construction of the reactor, associated buildings and services on the site purchased by the University of Manchester.

Employment of Graduates

THE eighteenth annual report of the Appointments Board of the Universities of Newcastle and Durham for the year 1962–63 (Pp. 18. Newcastle and Durham: The Universities, 1964) covers the last year of operation of the Durham University Appointments Board—the Appointments Board of the Federal University having been reconstituted under a new title, but for the time being continuing to be responsible for appointments work on behalf of students and graduates of the two new Universities. In reviewing the general employment situation of graduates in 1963, the report suggests that demand by the private sector of the economy was little, if anything, stronger than in 1962, although there appears to be no reason for thinking that an end is in sight to the shortage of mathematicians, physicists and engineers. The report, however, is very critical of the forecast in the report issued by the Advisory Council on Scientific Policy, *The Long-Term Demand for Scientific Manpower*, in 1961. It suggests that the forecast failed to foresee the imminent explosion of demand for teaching man-power and that it also took too much account of short-term recession in industrial demand. While taking note of explicit demand, it neglected inarticulate demand, and was too complacent about staffing schools with science specialists. It implied also that only a small, and perhaps comparatively unimportant, section of the scientific community needed a good mathematical background. The report insists that discussions of the problem of teaching man-power commonly neglect quality and deployment and concern themselves mainly with numbers. While opportunities for some science and engineering graduates became scarcer in 1962 and remained so until quite late in 1963, the difficulties largely arose because of inflexibility on the part of the graduates. There are signs of significant increase in industrial interest in the recruitment of biologists. The Board is watching with interest the progress of trends towards subject-specificity in employers' demands for graduates in non-technical subjects, and away from subject-specificity in fields where it is at present fairly uniformly assumed.

Political and Economic Affairs in France, 1959–64

A PAMPHLET issued by the Press and Information Service of the French Embassy gives a concise survey of political and economic affairs in France for the period 1959–64 (*A Survey of Political and Economic Affairs in France, 1959–1964*. Pp. 26. London: Ambassade de France, Service de Presse et d'Information, 1964). It covers first the political institutions and recent innovations, which have ended the instability of Government, the decolonization measures, including the Evian agreements and the measures taken in Black Africa and Madagascar, changes in economic and financial affairs, and in defence and foreign policy. The pamphlet is a distinct contribution to the understanding of the situation in France and the changes in French domestic and foreign policy.

Sound Radio in the Television Age

IN the sixth of the second series of Lunch-Time Lectures arranged by the British Broadcasting Corporation, "Sound Radio in the Television Age", which has recently been published, Mr. F. Gillard observes that while there are unmistakable signs that radio will continue to be a medium in wide and accepted currency, the form of survival is more important and significant than survival itself (Pp. 16. London: British Broadcasting Corporation, 1964). So long as sound radio continues to uphold the principles of public service, integrity, independence, fairness, impartiality, sense of taste and sense of purpose, it will continue to flourish and justly hold its head high among the mass media of our day. Nevertheless, there will be change, and flexibility is essential—broadcasting must be sensitive to new developments, particularly in education. Flexibility is also called for in the techniques of programme planning. As regards technical development, very high frequency, 'VHF', is undoubtedly the radio system of the future. The possibility of stereophonic broadcasting on some regular scheduled basis is just beginning to show above the horizon. Even to-day, some 6 million people who have no television depend entirely on sound for their broadcasting service, and the needs of this audience are as diverse as ever. Extensions which the British Broadcasting Corporation has in mind at present concern particularly the Light Programme and the Third Network. The Corporation also wishes to introduce a completely new service in a system of local broadcasting, but on this the Government has reserved its position. The Corporation has great faith in the potential of this new dimension of sound broadcasting. Administratively, the local stations would be part of its regional system.

Library Service in the Manchester Region

THE 111th annual report of the Libraries Committee of the City of Manchester covers the year ending March 31, 1963, in which issues from the lending libraries increased by 45,000, and from the reference libraries by some 120,000 (Pp. 36. Manchester: The Public Libraries, 1964). The Committee's plans for reorganizing the service have continued, but have fallen far behind the programme approved in January 1960. A feature of the year was the large number of visitors from educational establishments of all kinds and also from overseas. In the technical library, the number of readers increased to a new record of 830,000 and additions during the year brought the total stock to 101,000 volumes. The technical lending library's book stock now exceeds 19,000 volumes and more than 16,000 volumes were issued from the collection during the year, but the phenomenal issue from the patents library of the previous year decreased by some 5 per cent. The use made of the information services to firms and institutions throughout Britain continued to expand. The collection of technical translating dictionaries in all the major languages now exceeds 500 volumes.

A guide to some of the resources available in the technical library at the Central Library, Manchester, in addition to books has been published by the Libraries Committee (*What's Available in the Technical Library: a Guide to Some of the Resources in Addition to Books*. By F. R. Taylor. Second Edition. Pp. 29. Manchester: Libraries Committee. Available from the technical librarian, Central Library, 1964. 2*s*. 6*d*.). In it Mr. Taylor describes the whole range of resources in detail, and particularly the periodicals, periodical indexes and abstracts, periodical location guides, standards, atomic energy documents, trade catalogues, literature surveys, technical translating dictionaries, patents and micro-texts. Finally, Mr. Taylor explains the service that is offered to industry and the way in which the library forms an integral part of the national library network.

Termite Studies in Africa

A COLLOQUIUM on African termites was held at the Lovanium University, Leopoldville, during May 11–16 and was organized jointly by the University and Unesco. M. Michel Colin, Minister for Education, République du Congo, presided at the opening session. The attendance of delegates from abroad was facilitated by Unesco in arranging a meeting of the Termite Sub-committee of the Humid Tropics Research Programme in Leopoldville during the course of the colloquium. In the ten years which have passed since the founding of Lovanium, Prof. A. Bouillon has developed a large and active Department of Zoology, in which special attention has been directed to the investigation of the Isoptera, a group of insects well represented in the grounds of the University. In his opening address, Prof. Bouillon stressed the importance of termite investigations from both the scientific and the economic points of view, not only to the Congo but also to the Tropics as a whole. In the 22 papers presented by workers at Lovanium a wide range of interests was exhibited, covering taxonomy, morphology, social behaviour and ecology, with special reference to the large mound-building *Macrotermes natalensis*, a new species of *Cubitermes* making small mounds in grassland, and three species of the genus *Apicotermes*, the elegant subterranean nests of which are one of the marvels of insect architecture. Co-operation with workers in other departments of the University has resulted in interesting developments in techniques and apparatus for investigating termites; for example, the use of microanemometers to measure air-flow in mounds, the estimation of pterines and their possible role as regulators in the sexual cycle, and the application of pedological techniques to investigations of mounds. A number of papers were contributed by the visiting entomologists. The proceedings of the colloquium are in course of publication by the University in its series *Studia Universitas Lovanium*.

Stonehenge

STONEHENGE has always fascinated everyone who has visited the monument. Who made it and when and why was it erected ? The first two queries have now been answered by the archæologist with reasonable certainty, though to what extent, if any, Mycenæan architects helped in the erection remains doubtful. But the reasons why the building was put up are less certain; clearly it was not for fun. Years ago, Sir Norman Lockyer pointed out that the orientation of some of the stones made it clear that a line on the rising of the Sun at midsummer could be obtained, and that, therefore, the 'temple' was of the nature of a calendar. Such a purpose would, of course, be vital for an agricultural people, who would want to know when to sow their crops, etc. Articles on the subject by Prof. G. Hawkins have recently appeared in *Nature* (200, 306; 1963; and 202, 1258; 1964). At the same time, and seemingly independently, Mr. C. A. Newham has published a pamphlet on the same problem (*The Enigma of Stonehenge and its Astronomical and Geometrical Significance*. Pp. 42. Tadcaster: C. A. Newham, 5 Sedge Rise, 1964). Both agree that the 'priests' at Stonehenge were interested in the Moon as well as in the Sun at midsummer, but Mr. Newham goes further and brings the Pleiades into the picture. Many authorities, when writing about Stonehenge, refer to Diodorus, an author who was writing about 50 B.C. In one passage he is thought to be describing the monument, and he mentions the Metonic (19 year) cycle which does, of course, involve the Moon. Unfortunately, the exact original position of every stone cannot be determined with complete accuracy, and, as Mr. Newham points out, much of the work can be likened to trying to aim a gun when the back sights are defective. It is interesting to find two investigators working independently on this problem, and much information can be gained from both.

Applied Science and Technology in Japan

OF the *Memoirs* published periodically by the Faculty of Industrial Arts, Kyoto Technical University, Matsugasaki, Sakyoku, Kyoto, Japan, *Science and Technology* (12, December 1963) gives an impression of the versatility of the research work carried on in this important institution. This issue, entirely in English, contains six papers: measurement of fibre fineness by horizontal air-flow, by M. Uno, A. Shiomi and Y. Yanagawa; approximation theory of general thin elastic shells, by O. Matsuoka; autoxidation of alcohols and their reactivities, by S. Kato and F. Mashio; mineralogical study on the Brazilian aluminium phosphate ores, by T. Arimori and N. Yoshida; on the flow of flaming gases in the end-fired glass melting tank furnace, by K. Terada and M. Wakamatsu; and on the dynamic fatigue life and cracks of rubber vibration isolator. This particular *Memoir* is well printed, and the English translations are reasonably good, the papers, where appropriate, being illustrated with ample diagrams and reproductions of original photographs.

U.K. Central X-ray Library

The first central library of X-ray films in Britain is to be set up by the Faculty of Radiologists in London, with the aid of a grant from the Wellcome Trust. The object of the library is to gather in one place a wide range of X-ray studies of common and rare diseases, since recognition of an uncommon disease may depend on the knowledge gained from similar cases. Radiological techniques are of special importance in providing knowledge about the condition of inaccessible organs during life. A number of major advances in medical knowledge have already resulted from radiological investigations. In the past, clinicians and research workers investigating rare diseases have had to gather X-ray examples from different hospitals throughout Britain. The new central library will enable a quick comparison to be made of a cross-section of previous investigations of a particular disease. The Wellcome Trust was created by the will of the late Sir Henry S. Wellcome, who died in 1936. In the past six years it has made grants totalling more than £4 million for work in medical and allied sciences.

Nucleonic Instrument Modules

A new booklet which describes the Wells series of Nucleonic Instrument Modules is now available from EMI Electronics, Ltd. (*The Wells Series of Nucleonic Instrument Modules.* Pp. 12. Hayes, Middx.: EMI Electronics, Ltd., 1964). This series of all-transistor modular units can be assembled in any order to provide a wide variety of facilities in the nucleonic instrument field. Individual instruments which can be assembled from the modules include a simple or an automatic counting system, a γ-spectrometer, and a simple or a comprehensive ratemeter for Geiger–Müller and scintillation counter probes. They can be either mains or battery operated. Plug-in printed circuit boards facilitate servicing and a special panel can be supplied if required so that the modules can fit into a standard 19-in. rack. The range of modules at present available consists of a linear amplifier, a single channel pulse-height analyser, a linear rate-meter covering 0–5 kc/s in six linear ranges, a scanning recorder for use with the analyser and rate-meter, a five-decade scaler with a resolution time of 1 μsec, γ-probes and a γ-spectrometer. The range is to be extended.

The Geochemistry of the Rarer Elements

In 1956 the U.S.S.R. Academy of Sciences established a specialist institute for the investigation of the mineralogy and geochemistry of the rare elements (I.M.G.R.E.), from which an extensive flow of publications has since been maintained. Several of its works, including monographs on beryllium, selenium and tellurium, have been translated into English under the ægis of the Geochemical Society. The latest and most important publication of I.M.G.R.E., sponsored jointly by the Academy and by the State Geological Committee, comprises three encyclopædic volumes designed to summarize all aspects of the distribution of certain rarer elements in the lithosphere and hydrosphere (*Geokhimiya, Mineralogiya i Geneticheskie Tipy Mestorozhdenii Redkikh Elementov* (Geochemistry, Mineralogy and Genetic Types of Deposit of the Rare Elements), 1. Edited by K. A. Vlasov. Pp. 688. Moscow: "Nauka", 1964. Price 4*r*. 43*k*.). The first volume is concerned in successive chapters with the geochemistry of the elements Li, Rb, Cs, Be, Sr, Sc, Y, TR, Zr, Hf, Nb, Ta, Cd, Ga, In, Tl, Ge, Se, Te and Re (the actinides and the noble metals are outside the Institute's province). A great deal of new material has been incorporated and the work is likely to remain the most important reference text in its field for a good many years. A second volume, to be published in July 1964, will deal in detail with the mineralogy of these elements, describing the 415 relevant mineral species, of which 26 were first recognized in the laboratories of I.M.G.R.E. The third part of the work is concerned with field geology, paragenetic relationships, and classification of deposits, and will appear early in 1965. Altogether about 80 geochemists have been engaged in this large-scale co-operative enterprise, on the first results of which Dr. Vlasov and his colleagues are to be warmly congratulated.

Milk Iron in a Lactating Marsupial

I. Kaldor and Eric Ezekiel, of the Department of Physiology, University of Western Australia, have administered, in the lactating quokka (*Setonix brachyurus*), parenteral iron, as ferrous ammonium sulphate solution, in an attempt to raise the milk iron (*Austral. J. Exp. Biol.*, 42, Part 1; February 1964). Following a single large intraperitoneal dose, a rise in milk iron occurred within two hours. There was, however, no clear relationship between the rise and the amount of iron injected and there was reason to believe that the rise had been associated with unphysiologically high plasma iron concentrations. During continuous intravenous infusion of iron at controlled rates, plasma iron was elevated to maximal levels not exceeding plasma total iron-binding capacity and, under these conditions, no significant alteration in milk iron occurred within four hours. A progressive and statistically significant rise in total plasma iron binding capacity which occurred during intravenous infusion of iron was probably the manifestation of a redistribution of intra- and extra-vascular transferring in response to the need for more rapid removal of iron from the plasma.

Post-doctoral Fellowships in Neoplastic Growth

The Jane Coffin Childs Memorial Fund for Medical Research supports investigations in the fundamental aspects of neoplastic growth, and awards post-doctoral fellowships to candidates of outstanding potential in the same field. Applications for grants-in-aid are reviewed by the Board of Scientific Advisers and the Board of Managers of the Fund three times a year, in the autumn, winter and spring. Final dates for receipt of applications are September 1, November 1 and March 1. Fellowship applications are reviewed at the winter meeting of the Fund, the final date for applications being October 1 of each year. Grants-in-aid and fellowships are not restricted to United States citizens, but fellowship programmes should involve scientific interchange with the United States. Further information can be obtained from the Office of the Assistant Director, Jane Coffin Childs Memorial Fund for Medical Research. 333 Cedar Street, New Haven, Connecticut 06510.

National Amateur Astronomical Convention

The Rank Taylor Hobson and Ratcliffe College Astronomical Societies are holding their second national amateur astronomical convention at Ratcliffe College, Leicester, during September 4–6. Among the lectures which have been arranged are: "Polar Wander and Continental Drift", by Dr. H. C. King, curator of the London Planetarium; "Radio Telescopes and Radio Observation", by Mr. D. Barber, Royal Radar Establishment, Great Malvern; "Cosmology and Space Research", by Dr. R. C. Jennison, Jodrell Bank Experimental Station; "The Astrophysics of Visual Binaries", by Dr. D. Jones, Royal Greenwich Observatory; "Solar Activity", by Dr. J. Reed, acting director of Dunsink Observatory, Dublin; "Current Work at the Royal Observatory, Edinburgh", by Dr. P. Fellgett, Royal Observatory, Edinburgh; and

"Mechanical Design of Satellite Trackers and Radio Telescopes", by Mr. B. R. Tuppin. An exhibition has been arranged, which will include a satellite tracker model, an *Allouette* exhibit from Canada, and a colour film of the *Blue Streak* rocket. Further information can be obtained from the convention secretary, Mr. J. Maxwell, 81 Gresham Street, Belgrave, Leicester.

University News:

Aberdeen

Dr. R. G. Smith and Dr. P. A. Orkin have been appointed to senior lectureships in engineering and natural history, respectively. Mr. J. H. Savage Blaxter has been appointed to a lectureship in natural history (zoology). Dr. D. A. Archer and Mr. B. J. van der Kamp have been appointed to research fellowships in chemistry and forestry, respectively.

Cambridge

THE report of the head of the Department of Engineering, University of Cambridge, Sir John Baker, for the year 1962–63, records a total of 926 students, an increase of 8 on the previous year, although the undergraduate entry decreased by 5 to 272 (Pp. 14. Cambridge: The University, 1964). Research students in residence numbered 111, almost double the number in 1957 and 14 more than in 1961–62, while 12 postgraduate students took the Course of Advanced Study in Engineering. The main development was that the Electrical Optics in Part I of the Tripos was held for the first time. Some notes are included on research in progress in applied thermodynamics and gas dynamics, control engineering, electricity, fluid dynamics and structures, and there is a list of 95 publications.

Liverpool

THE following appointments to lectureships have been announced: D. W. Dickens (psychology); B. Selby (mathematical statistics); J. C. Wilkinson (numerical analysis); C. H. Taylor-Robinson (bacteriology); M. J. T. Peaston (clinical pharmacology); G. Hall (civil engineering); J. A. Rees (electrical engineering); J. W. Cleaver and Dr. R. P. N. Jones (mechanical engineering).

Sussex

DR. F. G. BAILEY, at present reader in Asian anthropology in the University of London, has been appointed professor of anthropology, with effect from October. Prof. J. F. Sutcliffe, at present professor of botany in the University of London, has been appointed professor of plant physiology. Dr. Marie Jahoda, at present head of the Department of Psychology and Social Science at Brunel College of Advanced Technology, has been appointed professor of social psychology. Dr. N. S. Sutherland, at present lecturer in experimental psychology in the University of Oxford, has been appointed professor of experimental psychology. Prof. J. C. West, at present professor of electrical engineering at Queen's University of Belfast, has been appointed professor of engineering and dean of the school of applied sciences. Prof. R. W. Cahn, at present professor of materials technology in University College, Bangor, has been appointed professor of materials science. The last-mentioned five appointments take effect from 1965. Other appointments have been made as follow: *Reader*, Dr. M. F. Lappert (chemistry); Dr. E. A. Barnard (biological sciences). *Senior Lecturer*, Dr. G. Martelli (experimental physics); Dr. H. R. Post (philosophy of science). *Lecturer*, Mr. R. W. Bott, Mr. J. R. Hanson, Dr. J. B. Pedley and Dr. P. Simmonds (chemistry); Dr. P. J. Bushel, Dr. J. A. Laurie, Dr. R. R. Laxton, Dr. W. Parry and Dr. Rutovitz (mathematics); Dr. M. Springford, Dr. J. Venables and Mr. A. J. Walton (experimental physics); Dr. N. Dombey and Dr. D. A. Goodings (theoretical physics); Dr. R. J. Cole (biological sciences).

St. Andrews

THE following appointments have been announced: *St. Salvator's College*, Dr. D. C. Weeks, senior lecturer in botany; *Queen's College*, Dr. J. McManus, lecturer in geology.

Announcements

DR. B. C. HEEZEN, of the Lamont Geological Observatory, Columbia University, has been awarded the third Henry Bryant Bigelow Medal by the Trustees of the Woods Hole Oceanographic Institution "in recognition of his physiographic studies of the deep ocean and of related marine sedimentary and tectonic processes". This medal, accompanied by a cash prize of 2,500 dollars, was established by the Trustees in 1960, "to be awarded to those making significant inquiries into the phenomena of the sea".

A SYMPOSIUM on "Chromatography", organized by the Belgian Society of Pharmaceutical Sciences, will be held in Brussels during September 14–15. Further information can be obtained from the Secretary, Belgian Society of Pharmaceutical Sciences, 11 rue Archimède, Brussels 4.

THE fourth international conference of the International Association for Analogue Computing will be held in Brighton during September 14–18. Further information can be obtained from the B.C.S./A.I.C.A. Honorary Secretariat, Ferranti, Ltd., Kern House, 36 Kingsway, London, W.C.2.

THE second congress of the Czechoslovak Society of Arts and Sciences, entitled "Czechoslovakia, Past and Present", will be held in Columbia University, New York, during September 11–13. Further information can be obtained from Dr. M. Rechcigl, jun., 1703 Mark Lane, Rockville, Maryland.

THE third International Measurement Conference will be held jointly with the sixth International Instruments and Measurements Conference in Stockholm during September 14–19. Further information can be obtained from the Secretary, Society of Instrument Technology, 20 Peel Street, London, W.8.

THE first Matscience summer school in "High Energy Physics", supported by the Council of Scientific and Industrial Research, will be held in Bangalore during August 24–September 14. Further information can be obtained from the Office of the Director, Matscience, Institute of Mathematical Sciences, Madras 20.

THE 1964 World Conference on Radio Meteorology, which will incorporate the eleventh Weather Radar Conference, will be held at Boulder, Colorado, during September 14–18. Further information can be obtained from Mr. J. W. Herbstreit, Central Radio Propagation Laboratory, National Bureau of Standards, Boulder, Colorado.

THE seventh International Embryological Conference will now be held in London, and not Edinburgh, as announced earlier (*Nature*, 202, 650; 1964), during September 6–10, 1965, at Bedford College, Regent's Park, London, N.W.1. Further information can be obtained from Dr. A. S. G. Curtis, Department of Zoology, University College, Gower Street, London, W.C.1.

THE second International Congress of Endocrinology will be held at the Imperial College of Science and Technology, London, during August 17–22. The programme will include symposia and lectures under the following general headings: general and comparative endocrinology; brain–endocrine relationships; reproductive physiology; clinical problems; tumours; steroid hormones; thyroid hormones; protein and polypeptide hormones. Further information can be obtained from Dr. D. A. Pyke, King's College Hospital, Denmark Hill, London, S.E.5.

INHALED RADIOACTIVE PARTICLES AND GASES

THE third Hanford Biology Symposium, held at Richland, Washington, U.S.A., during May 4–6, 1964, was devoted to inhaled radioactive particles and gases. It was organized by a committee of Hanford scientists under the chairmanship of Dr. W. J. Bair. The 200 participants included representatives of nine countries, who were welcomed by Dr. H. A. Kornberg, head of the Hanford Biology Laboratories.

Proceedings were opened by Dr. W. E. Lotz, head of the Division of Biology and Medicine of the U.S. Atomic Energy Commission. Consequent on the test ban treaty a proliferation of effort was in progress, and a glimpse was given of the novel and interesting outlets for atomic expertise which were being attempted.

Foremost among these were the systems for nuclear auxiliary power units; these are heat sources, operated by various nuclides, in the range 40–240 kilocuries, which generate electricity by thermoelectric inversion, using semiconductors. Lights for marine buoys with a life of 10 years produce 10–60 W; a 25-W generator for a navigational satellite was also described.

Nuclear-powered rocket engines carry a finite risk of re-entry after launching which can be faced by designing either for burn-up or by ensuring that the isotope container remains intact. At a height exceeding 100,000 ft. a fuel cone can disintegrate or burn-up into particles smaller than 10μ which dilute sufficiently in the biosphere to offer no risk; a ground contamination of 2,000 mc./mile^2 would not be exceeded.

Dr. T. T. Mercer (Lovelace Foundation, New Mexico) made a comparison of laboratory and field problems in handling aerosols of mixed fission products; there were hazards associated with natural sources of radon, with fuel melting in accidents and with fuel recovery operations.

Generation of laboratory clouds by aqueous sprays presented the possibility of the aerosol behaving anomalously owing to the particles carrying electric charges. These could arise from statistical fluctuations of ion concentration when liquid was fragmented from bulk. Natanson's theory had been verified at low numbers of particles, the mean charge being proportional to the 3/2 power of the diameter and the square root of the number per cm^3. The charge could amount to 100 electrons per particle. At higher concentrations the charge was independent of the number and proportional to the square root of the diameter.

Such charges can seriously distort deposition from laboratory aerosols and a tritium source was used to discharge them. Sampling had been most successfully carried out with the conifuge, which is a true aerosol spectrometer, in contrast with other so-called instruments, because the aerosol intake stream is narrow and confined between guard layers of pure air, which ensures that the site of deposition is sharply determined by Stokes's diameter. Mass parameters are needed for isotope inhalation investigations and their derivation from particle counts involves shape and density complications. The cascade impactor is useful for sampling because it yields mass parameters directly. Small jets, 0·015 cm diameter operating at 1/3 sonic velocity, had been useful for impacting very small particles.

Particles, particularly those containing plutonium which constituted an inhalation hazard when airborne, were discussed by K. Stewart (U.K. Atomic Energy Authority, Aldermaston). One problem was the large amount of extraneous material which accompanied the dangerous aerosol. For example, 4×10^{-5} μc./m^3 of plutonium was equivalent to only 100 particles/m^3 of 1μ diameter; this is only a minute fraction of the total adventitious aerosol in many circumstances.

High-altitude burn-up results in small particles because the entire mass is expanding; near the surface of the Earth there is massive and inhomogeneous entrainment. There are great difficulties in predicting the outcome of a quenched fireball in water.

Oxidation processes result in aerosols by break-away of particles of oxide from the surface; a higher temperature gives coarser particles. Some alloys had an adherent oxide film. In an ignition of plutonium only some 0·1 per cent of the material was likely to be found in the hazardous range of particle size. Whereas uranium aerosols contained large aggregates of spheres, particles of plutonium were usually individual fragments.

Investigations of the particle size distribution of airborne plutonium in work rooms, using filters, were described by B. V. Andersen, of the Hanford Laboratories. Membrane filters with 2μ pore size had been found useful and 5–50 α-tracks per particle were obtained by autoradiography. As a rough rule, doubling the particle size resulted in a ten-fold increase in radioactivity. $^{239}PuO_2$ was found in lognormal distributions with geometrical standard deviation (σ_g) of 1·8–3. 85 per cent of the activity was in the largest 1 per cent of sizes. Plutonium carbide melt operations yielded the finer clouds with a maximum diameter of $1{\cdot}7\mu$, GMD $0{\cdot}2$–$0{\cdot}48\mu$ and g = 1·4–1·8.

R. E. Albert *et al.* (Department of Industrial Medicine, New York University) have developed techniques for making monodisperse spheres of 'Lucite' and of iron oxide using a spinning disk. The sizes are uniform within 10 per cent standard deviation over a range of 1–8μ. The lucite solution which is fed to the disk contains a tagging isotope—either in carrier-free form or chelated with acetyl acetone. The presence of acetyl acetone in the xylene solution was found essential to the production of spheres with unwrinkled surfaces. These particles are not taken up by phagocytes. Good uniformity has also been achieved in the dispersal of sols of colloidal iron oxide. These particles are stable in water and are taken up by phagocytes. Various isotopes have been used for examining the rate of leaching out. In each case the particles require to be collected from the spinning disk generator and redispersed for experimental work.

Montmorillonite clay will absorb a number of radionuclides by ion exchange and can then be dispersed as an aerosol by spraying a suspension. The technique was described by S. Posner (Lovelace Foundation, Albuquerque). He also found that latex spheres would absorb iodine-131 from solution in ethyl alcohol. Data on isotope leach-out were given.

Two papers dealt with radioactive dust and gas from uranium mines. H. Fusamura *et al.* had sampled radon in Japanese mines with an ionization chamber and transistorized vibrating reed electrometer. The concentration ranged from 0·3 to $10{\cdot}0 \times 10^{-7}$ μc./c.c. and increased to 3×10^{-6} μc./c.c. when the mine ventilation was stopped. Daughter products were collected by filtration and the free ion concentration roughly estimated by placing a 2-m long diffusion tube in front of the filter; it was reckoned that free ions accounted for 20 per cent of the RaA. The dust on the filter contained 0·01–0·08 per cent U_3O_8 which corresponds to 1–45×10^{-3} μc./c.c.

Dr. Palmer (Hanford Biology Laboratories) had taken a whole-body counter to two Colorado uranium mines, one of which was disused and one in operation. Sampling was performed with membrane filters for daughter products and activated charcoal for radon. The counter was used very soon after exposure and the entire length of the subject's body could be scanned. Some 20 per cent of activity was found in the head, 10 per cent in the neck and 70 per cent in the chest. The maximum chest concentration was in the right side, 3 in. off centre. It seemed that all the activity inhaled by the subjects while in the mines was still in their bodies when measured by the counter.

Several papers dealt with the elimination of dust from the lungs, including an outline of the processes by S. Engel. The theory of fluid transport, rather than carriage by phagocytes, to the pulmonary lymph nodes was supported by P. Gross, who considered that insoluble particles which deposit in the alveoli are sequestered on the wall in nodules or plaques, although a few penetrate the wall directly. Sequestered particles may be freed in due course, and eliminated via the bronchial epithelium.

Three phases of clearance, at decreasing rates, were identified by L. J. Casarett and P. F. Milly (University of Rochester); these comprised bronchiolar clearance, phagocytosis from alveoli and transport to the lymphatics. The half-time for alveolar clearance ranges from 15 to 50 days and depends on the material and the animal. Much of the material deposited in the alveolar regions is found in macrophages after 2 h. Chemitaxis is a factor, but different substances do not have half-times in line with their solubilities. Protein binding favours phagocytosis. It was suggested, in the discussion on this paper, that the phagocytosis of radioactive particles may be delayed.

In formulating a theory of dust clearance account had to be taken of the long period during which some particles remained in the alveoli; a macrophage development sequence was visualized in which cells of the alveolar epithelium, which may have been penetrated by insoluble particles periodically detach themselves. According to the way in which the cell lies in the alveolar wall, detachment may proceed either into the air space or into the interstitium.

R. G. Thomas (Lovelace Foundation, Albuquerque) described experiments with aerosols of thorium, plutonium, niobium, uranium and polonium which were inhaled by dogs. All the data were grouped along a straight line when the proportion of lung content in the lymph nodes was plotted against time, using logarithmic scales. The proportion of the whole-body content eliminated in the fæces fell off exponentially much more rapidly than urinary excretion; this indicates the change-over from lung elimination as ciliary removal declines.

A biochemical examination of rabbit lung by J. M. Felts (University of California Medical Center) showed that 8–10 per cent of the oxygen uptake was used in lung tissue for the oxidation of long-chain fatty acids bound to serum albumin. Using carbon-14 it was shown that lung, in contrast to liver, converted fatty acids into phospholipids, mainly lecithin. Glucose probably acts as an acceptor intermediate. Lung phospholipids are a key component of the surfactant which stabilizes the alveoli by reducing surface tension to an extremely small value.

B. B. Boecker *et al.* (Lovelace Foundation) described a new whole-body plethysmograph in which a dog could be placed with its nose protruding through a rubber seal into an aerosol chamber. Air moving to and from the plethysmograph box was recorded with a pneumotachygraph inserted between the box and another sealed chamber. This system eliminates difficulties due to drift of pressure. The boxes were made of ⅜-in. 'Plexiglass' which responded to pressure changes in the room when strong winds blew outside.

An inhalation apparatus for small animals breathing $^{14}CO_2$ was described by D. L. Joftes (New England Deaconess Hospital, Boston). The increase in carbon-14 in the atmosphere is important since carbon dioxide may be a direct precursor of DNA. Assuming the animal fixes 0·5 per cent of the carbon dioxide available, a dose of 0·1 rad was calculated in 10 days. The exposure chamber was provided with constant environment and a metered supply of carbon dioxide up to 4 per cent. Estimates of individual testes by autoradiography and liquid scintillation counting were planned.

Experiments on the inhalation of cæsium-137 by rats had been performed at the Lovelace Foundation by Miss R. Lie. Aerosols of the chloride and nitrate in concentrations between 0·05 and 9·0 per cent were used and tissue and excreta samples analysed for 4 months.

C. E. Miller (Argonne National Laboratory) gave an account of the examination of two persons who had accidentally inhaled cæsium-137. Whole-body analysis was performed by the tilting chair technique and the distribution of cæsium-137 and potassium-40 was found in a low background room using a simulated 7-crystal array. No appreciable amount of cæsium-137 was found in the lungs after 9 days from exposure. There was no translocation over 185 days and a biological half-time of about 80 days was indicated. The nuclide was distributed throughout the body.

A case of human exposure to plutonium-239 was discussed by W. S. Snyder (Oak Ridge). On the model of the 'standard man', an elimination half-time of 120 days is assumed for insoluble lung deposits, most of this material being disposed of in the fæces. There is, however, some indication that plutonium-239, and other heavy metals, reach the blood in significant quantities, so that transfer to bone is possible. The bone may thus be the critical organ. Whereas the lung may carry a higher concentration for the first few years of exposure the bone content would eventually predominate and might become the limiting factor in permitting continuous exposure for a working life.

An investigation of the metabolism of radio-mercury by rats during chronic exposure for two months was performed by A. Rothstein and A. Hayes (University of Rochester). The body burden was determined by whole-body counting and fluctuated with the exposure régime. After three weeks, intake and excretion were in balance. On terminating exposure the body burden declined exponentially with a half-time of 15 days. Most mercury was found in the kidneys; there was an appreciable loss of mercury vapour via the lungs and the skin.

The production of test aerosols for lung clearance investigations in rabbits was the subject of a paper by A. Kajland *et al.* (Karolinska Institute, Stockholm). Octadecanol was dispersed by a spray-fed thermal generator with the nucleating substance dissolved in the octadecanol. The coefficient of variation in particle size was 5–10 per cent for diameters of 2μ–10μ and 20 per cent for 1μ–2μ. A spinning disk spray gave a variation coefficient of 10 per cent for spheres of 'Lucite' produced from a 0·2 per cent solution in 5 per cent acetone and 95 per cent acetamide. The use of a mixed solvent is necessary if smooth, unwrinkled spherical particles are to be obtained.

Lung clearance was measured by a profile-scanning method which allowed multiple examinations and re-exposure of a single animal. It had been observed that the phagocytosis of octadecanol was low.

A novel technique for measuring ciliary clearance from the lungs of rats was devised by A. A. Spritzer and J. A. Watson (Graduate School of Public Health, Pittsburgh). All material swallowed is collected through a polyethylene tube placed in the animal's œsophagus; the caudal end of the tube empties into a 100-c.c. bottle fastened to the flank. Rats were kept alive for 7 days and daily collections were made; there was no interference with swallowing. The pulmonary phlegm was flushed out by the water drunk. Following intratracheal injection of $^{133}BaSO_4$ particles it was found that 8 per cent, or about half the total pulmonary clearance, was by the ciliary mechanism.

Experiments on inhalation by man of aerosol particles were described by L. Dautrebande (Brussels) and W. Walkenhorst (Bergbauberufsgenossenschaft, Bochum, Western Germany); few particles were found in exhaled alveolar air, this being claimed to represent 90 per cent deposition. In contrast, C. N. Davies (Medical Research Council and London School of Hygiene and Tropical Medicine) emphasized, by comparing size distributions of lung dust and airborne dusts, that the access of particles to the alveoli of man during normal breathing, was more limited than was commonly supposed. Curves of alveolar deposition, derived by indirect arguments from total deposition experiments, considerably over-estimated alveolar deposition and could not be explained by phagocytosis from the alveoli.

Calculation of the cancer-related dose from the inhalation of radon and daughter products had been carried out by a synthetic approach by B. Altshuler *et al.* (Institute of Industrial Medicine, New York University). The dose was estimated from the surface activity on the bronchial mucosa, not from material lodged within pulmonary tissue. The atmosphere was supposed to contain 100 pc. (picocuries) of radon, 150 unattached daughter atoms and 200 pc. total daughter products per litre. The deposition of ions, nuclei and particles in the different regions of the respiratory tract was calculated on Landahl's model and the mucus transit time worked out for each region; the available α-activity on the surface of the bronchial mucus at all regions could thus be estimated. Allowing for the thickness of the mucus film and of the epithelial cells, an estimate was made of the dose to the basal cells of the bronchial epithelium throughout the regions. The thickness is a critical factor in the calculations. The basal cells of the segmental bronchi were found to receive the greatest dose, which amounted to 30 rads/working year of 2,000 h. In terms of animal experiments this indicates that the atmosphere assumed would deliver a carcinogenic dose in a working life-time.

A somewhat similar calculation was presented by W. Jacobi (Hahn–Meitner Institute of Nuclear Physics, West Berlin). He took 10^4 particles/cm^3 with an average diameter of 70 mμ for RaB and RaC and 100 mμ for ThB and ThC. The mean percentages of unattached atoms are 100 per cent ThA, 25 per cent RaA and 1 per cent RaB. Deposition and mucus transport in the various regions of the respiratory tract were estimated and the mean biological half-time in the lungs was measured as 8 h. He found, like Altshuler, that the dose from inhaled decay products was not negligible and that the basal cells of the lower bronchi received the highest rate. His overall figure was about 30 per cent below that of Altshuler. It is evident that current normal exposure runs near to the limit.

Observations on the contamination of Yugoslav miners of uranium were described by D. Djuric *et al.* (Institute of Occupational Health, Belgrade). Levels of radon up to 16 times the maximum allowable concentration had been recorded in the past, but improvement of the ventilation system had now been effected.

Assessment of polonium-210 in the urine correlated with radon exposure; the exposure to polonium-210 from dust was gauged by determining polonium, radium and uranium in the dust. There is an exposure to polonium-210 from cigarette smoke.

The pulmonary function of uranium miners had been measured by V. E. Archer *et al.* (U.S. Public Health Service, Salt Lake City). The data suggest that working for short periods in high levels of radon and daughter products improves ventilatory function, but long exposures are detrimental above the losses resulting from ageing and from cigarette smoking. The effects of silicious dust and of radiation could not be separated. The action of the latter comprises specific injury to the bronchial epithelium and diffuse injury to lung tissue. It has been suggested that the inhalation of positive ions causes the bronchi to contract while negative ions produce relaxation. A negative charge on dust particles has been claimed to increase phagocytosis.

G. Saccomanno (St. Mary's Hospital, Grand Junction, Colorado) reported an increase in the lung cancer of uranium miners. Most tumours were undifferentiated, in contrast to 71 per cent epidermoid for a non-uranium miner group. 79 per cent of the tumours were of the oat cell type with a preference for the hilar area.

A general review of the biological effects of radiation on the lung was made by M. Kuschner and N. Nelson (New York University). The syndrome of radiation pneumonitis was defined in 1920. Bronchial mucosal changes include the evolution of abnormal epithelial cells which are sloughed off; along with this an interstitial fibrosis develops. The dose response relationship is poor. Effects have been reported at 500 r and there is 20–50 per cent incidence at 2,000–6,000 r. The fibrosis is related to deposits of inhaled activity; alveolar capillary block may follow with a reduction of diffusing surface area.

Cancer is bronchogenic in origin. Long exposure is necessary to induce it and it was first demonstrated experimentally in 1957. 10^3–10^4 rads will induce tumours. 63 μc. of strontium-90 in a capsule will yield a squamous cell carcinoma of the lung when embedded for 3–15 months.

In calculating dose it is necessary to define a target area in an organ, because cancer does not begin in an organ but in a tissue. It is possible to over-interpret early metaplastic response; for example, the implantation of a 5-μc. ruthenium pellet produced denudation of epithelium followed by regeneration one or two cells deep; chemical irritants give a similar epithelial response. If a tumour is to appear it does so explosively after a steady induction period of metaplasia lasting, in rats, some 120 days; this follows the initial hyperplasia which increases for the first month. Work with S. Laskin showed that the tumour yield was proportional to the amount of radioisotope.

Peripheral tumours develop in areas of fibrosis which may play a part, as in scar cancer. Many Colorado miners have silicosis. Questioned on the nature of malignant change, the speaker said he regarded virus and radiation as co-carcinogens, each a tool which manipulated genetic material.

H. Cember (University of Cincinnati) had induced lung tumours in rats with the chloride and fluoride of cerium-144. Intratracheal doses of 0·5–50 μc. were injected. The mean radiation dose absorbed during survival time was calculated from lung clearance data and when plotted against the cumulative tumour frequency, extrapolated to zero at a value of 250 rad. This is interpreted as the threshold dose for lung tumour induction in rats by radioactive particles in the lungs.

The inhalation of $^{144}\mathrm{CeO}_2$ by dogs was the subject of a paper by B. O. Stuart, A. W. Casey and W. J. Bair (G. E. Hanford Laboratories). Aerosols of various particle sizes between 0·09μ and 1·40μ were administered and the amount deposited ranged from 50 to 2,200 μc. The effective half-life was similar to the radioactive one, 285 days, and the biological half-time was 1·5–5 years. Up to 900 μc. failed to produce significant change in blood gas or serum enzyme, but heavy doses up to 130 kilorads produced severe damage in lungs, mediastinal lymph nodes and liver. 22 per cent of the body burden at death was in the lungs, 44 per cent in the liver and 27 per cent in bone.

From the same laboratories a paper by J. W. Park, W. J. Clarke and W. J. Bair dealt with the chronic effects of inhaled $^{239}\mathrm{PuO}_2$ in dogs. The aerosol was generated by calcining in air at 300° C. The radiation dose to the lungs was 9–23 kilorads; the clinical symptoms were cardiopulmonary defect and lymphopenia. Bronchial and interstitial tumours were found, locally invasive and of multiple origin. No metastases were seen. For the period of exposure, up to 48 months, the lung was the critical organ causing death.

An original technique for the safe radiological visualization of the human lung was due to G. V. Taplin *et al.* (University of California).

Serum radioalbumin in a 1 per cent solution is readily aggregated by adjustment of *p*H and heating at 79° for 20 min. 10–20 mμ particles act like bacteria and are removed by the liver on first passage. By lowering the *p*H macro aggregates of 5–50μ are formed. These are too large to pass through the capillaries of the lung, where they are temporarily trapped for 1–2 h. The lung can be scanned photographically during this period, and on the resulting picture it is possible to detect pulmonary emboli, lung infarcts, pneumonia, atelectasis, certain tumours and cysts causing local ischæmia.

Work at the U.S. Public Health Service (Taft Laboratories, Cincinnati) on the intake of radiostrontium by infants was presented by B. Kahn *et al.* Levels in air, water and prepackaged baby foods were measured and the ingestion calculated. Retention was deduced by subtracting the activity in the excreta; strontium-89 and -90 are present in air in soluble forms.

The last papers read dealt with the removal of inhaled radioactive materials. I. Schmidtke (University Clinic, Freiburg-im-Breisgau) treated guinea pigs with diethylene triamine penta acetic acid after exposure to $^{91}YCl_3$ aerosol. A 95 per cent diminution of retention was found, in comparison with untreated animals, after 8 days. The action was mainly on the kidneys, and most of the activity was recovered from the urine. E. G. Tombropolous (Hanford Laboratories) considered that chelating agents were the most effective means, when applicable, of removing radioactive particles from the lungs. Drugs which decrease mucus secretion or increase phagocytosis are also worth testing. F. Gensicke and H. W. Nitschke (German Academy of Sciences, Berlin-Buch, G.D.R.) reported that sodium hexametaphosphate had a scavenging effect on inhaled yttrium-91 in mice.

Papers read in title dealt with a spinning disk atomizer, a routine technique for estimating plutonium in large biological specimens, fall-out from burnt-up uranium and plutonium, Zr-^{95}Nb in human lungs from medium life fission products in the atmosphere, atmospheric analysis for total β-activity and the monitoring of human subjects who had accidentally inhaled fission products.

The papers will be published in full in the December 1964 issue of *Health Physics*. C. N. DAVIES

BIOSYNTHETIC PATHWAYS IN HIGHER PLANTS

THE biosynthetic pathways leading to all the major groups of compounds present in higher plant tissues were discussed at the seventh annual general meeting of the Plant Phenolics Group which was held at Bodington Hall, University of Leeds, during April 13–15. The wide range of topics covered by the symposium coincided with a decision by members to change the name of the Group to the Phytochemical Group with the view of promoting the advancement of the chemistry, biochemistry and related sciences of all plant products.

The opening address was given by Prof. N. A. Burges (University of Liverpool), who discussed the biological aspects of biochemical reactions and the possible differences between reactions *in vivo* and *in vitro*. This is an important consideration which is still sometimes overlooked by chemists. Biochemical reactions *in vivo* are commonly studied by feeding in potential precursors labelled with 'heavy' or radioactive isotopes. Dr. T. Swain (Low Temperature Research Station, Cambridge) gave a critical account of these methods. In particular he noted that feeding experiments often led to an understanding of the starting and end points of a pathway but gave little information regarding the individual steps and the control mechanisms. He emphasized the need for investigating the enzymes which catalyse each step, and he brought to the attention of the meeting other conditions, such as environmental and genetical factors, which could affect biosynthesis.

The first paper on a particular group of plant constituents was presented by Prof. T. W. Goodwin (University of Aberystwyth), who gave an account of the formation of terpenoids, compounds which appear to play a key part in chloroplast development. When etiolated seedlings are illuminated there is a massive synthesis of terpenoids as the functional chloroplasts are produced. During the greening period, carbon dioxide but not mevalonate is incorporated into the chloroplast terpenoids, but the reverse is true for the related sterols which are also present. It is believed that the biosynthesis of terpenoids in developing seedlings is regulated by enzyme segregation and specific membrane permeability. A second paper concerning the detailed steps involved in the elaboration of carotenoids, starting from acetate, was also presented by Prof. Goodwin.

An account of the amino-acids found in plants was given by Dr. L. Fowden (University College, London). In his lecture he concentrated on the large number of non-proteinogenic derivatives of which at least two groups are recognized. First, those which are intermediates in the formation of the twenty protein amino-acids and also those which arise by the non-specific action of enzymes normally associated with the metabolism of the proteinogenic compounds. The role of most of them is unknown.

Studies with micro-organisms have provided evidence that DNA controls the synthesis of proteins. This evidence was reviewed together with the mechanism of amino-acid polymerization by Dr. D. Boulter (University of Liverpool). He also described the similar findings made with higher plants and commented on the work being carried out at the University of Liverpool on protein synthesis in developing seeds.

The vast field of carbohydrate biochemistry was also reviewed (Dr. J. S. D. Bacon, Macaulay Institute, Aberdeen) mainly with reference to simple sugar phosphates and the more complex nucleotides derived from them. The synthesis of insoluble polymeric carbohydrates still remains something of a mystery. For example, although cellulose is the most abundant plant polysaccharide, we know very little about the formation of this material. Recent investigations by Hassid *et al.*[1] at Berkeley have implicated guanosine diphosphate glucose, and Prof. R. D. Preston (University of Leeds) was able to tell the Group of other interesting observations, of a biophysical nature, which help in the general understanding of this problem. He discussed the arrangement of cellulose microfibrils in the cell-walls of seaweeds and showed electron micrographs of spherical granules which are apparently involved in the synthesis and orientation of the polymer strands.

An equally complex problem, which was summarized by Dr. F. A. Isherwood (Low Temperature Research Station, Cambridge), is the structure and biogenesis of lignin. Progress in this field has slowed down considerably during the past few years and the molecule still refuses to give up all its secrets. There is little doubt, however, that the aromatic rings in this polyphenol are derived from shikimic acid and it is probable that the

$C_6 : C_3$ units, of which it is composed, are condensed together in a random fashion. The development of methods for determining the sequence of these units in the polymer is, therefore, awaited with great interest. The shikimic acid pathway is also utilized by plants for the formation of flavonoids. Prof. H. Grisebach (University of Freiburg) described recent theories regarding the synthesis of these pigments. These involve the condensation of cinnamic acids with malonyl-CoA, but so far the formation of the necessary activated cinnamic acids has not been demonstrated using plant enzymes.

The early stages of hæm and chlorophyll biosynthesis follow a common pathway and this has been deduced mainly by investigations with preparations from animal tissues and photosynthetic bacteria. Dr. J. Lascelles (University of Oxford) outlined this chain of reactions and gave an account of the interesting investigations which are in progress, using *Chlorella* mutants and photosynthetic bacteria, on the final stages in chlorophyll synthesis.

The final paper in the symposium, by Prof. S. L. Ranson (University of Newcastle), was concerned with the formation of aliphatic carboxylic acids by plants. Some of these, such as malate, citrate and oxalate, commonly accumulate to a relatively high concentration in the cell. Others, such as fumarate and aconitate, accumulate infrequently. Why some cells accumulate one acid and others a different acid is a problem which remains to be settled.

The Group is indebted to the University of Leeds for the facilities which were placed at its disposal at Bodington Hall. Profs. F. C. Happold, I. Manton and R. D. Preston, all from the University, kindly acted as chairmen during three of the symposium sessions. J. B. PRIDHAM

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UNUSUAL FORMS AND ASPECTS OF CANCER IN MAN

EITHER too much or too little tends to be made of the rarities in medicine: purely chance phenomena are described *in extenso*, whereas sight is lost of significant observations in the flood of scientific literature. The conference held by the New York Academy of Sciences on "Unusual Forms and Aspects of Cancer in Man", in October 1963 (ref. 1), was an attempt to assess the importance of certain cancer rarities by grouping them together and discussing them in open forum. Many of the data and views presented at the conference had previously been put forward elsewhere. However, earlier communications were scattered through a variety of journals and several were not widely known. The publication of the proceedings of the conference therefore constitutes a valuable addition to the medical literature.

The comparative value of different types of cancer treatment can be assessed relatively simply, but it is less easy to assess the value of treatment *per se* during an era in which almost every case receives some form of therapy. From his survey of 250 female cases of untreated breast cancer included in the 1805–1933 records of the Middlesex Hospital Charity, Bloom[2] comes to essentially the same conclusions as Doland[3], Greenwood[4] and Nathanson and Welch[5]. He records a mean survival period of 3¼ years from the onset of symptoms, with almost 20 per cent of patients surviving for 5 years and 3 per cent for 10 years. Bloom is satisfied that all generally accepted methods of treatment both prolong life and reduce suffering from the effects of the disease. On the other hand, it appears that no measurable improvement in the results of treatment of breast cancer has occurred during the past few decades[6]. The possible value of radiography in the detection of early breast cancer has been under investigation for more than thirty years, but, mainly because of failures in techniques, it has not found general favour. It is now clear that, in the hands of the expert, mammography sometimes results in the detection of breast cancer before a positive diagnosis could be made clinically by inspection and palpation[6,7]. Nevertheless, in the opinion of Egan[6] it is too early to opine whether biannual mammography should be used to screen the female population for early breast cancer.

In an age of nuclear weapons, the carcinogenic effect of ionizing radiation is a political issue. It has therefore achieved more prominence than is justified by the facts. Investigations on the survivors of Hiroshima and Nagasaki[8] revealed the expected excesses of myeloid leukæmia[9] and thyroid cancer[10]. In addition, an excess of anæmia due to myelofibrosis has been observed. Many of the thyroid cancers were microscopic and did not contribute to the death of the victim. Moreover, the incidence of small foci of thyroid cancer has not been adequately investigated in comparable unirradiated populations. This raises another problem. One is apt to assume that there is little scope these days for changes in standards of diagnosis. In fact, attempts at earlier diagnosis[6,7], increasing autopsy rates, the classification of microscopic lesions as cancer[8,11,12], and a gathering interest in aged patients[13] are steadily altering the background against which other more closely investigated events are studied.

It is generally held that the majority of lung cancers arise in the large bronchi within a few inches of the bifurcation of the trachea. It is within the same areas that the particulate matter present in inhaled tobacco smoke and polluted air tends to be deposited[14]. Rigler presents a survey of patients in whom routine chest roentgenograms were taken at intervals prior to the diagnosis of lung cancer. The first radiological signs were usually present almost a year before the onset of significant symptoms. In some cases the interval between the first sign and the making of a definitive diagnosis was as long as 5–10 years. The finding that the majority of tumours arose in the lung periphery and were not symptomatic until they had spread centrally is surprising, since it suggests that the deposition of particulate matter is not closely related to cancer induction. One wonders how often centrally arising tumours would have been detected radiologically in their early stages, and to what extent Rigler's conclusions are influenced by the fact that they are based on selected material.

Successful treatment of cancer discovered during pregnancy may involve the loss of the fœtus. Most would regard this loss as an unfortunate necessity, but whether the discovery of cancer *per se* provides grounds for the deliberate termination of pregnancy is another matter. Rarely, cancer cells cross the placental barrier and grow metastatically in the fœtus[15,16]. The demonstration of so-called vertical transmission of murine leukæmia viruses[17,18] prompts the search for examples of similar transmission of tumour viruses in man. However, as yet no human tumour viruses have been positively identified. A few forms of cancer have an undoubted familial tendency. It is exceptionally rare for one of these types of cancer to be diagnosed for the first time during pregnancy. Cancer during childhood is rare, and the occurrence of two cases in one family suggests a transmitted genetic or viral factor. Dargeon[15] records six examples of malignant disease arising in two children of the same family: in no case was it possible to say whether these arose as the result of coincidence, transmission of a genetic aberration, or transmission of a tumour virus, and in no case was malignant disease recorded in the mother during or before either of the pregnancies. The remaining consideration is whether the continuance of the pregnant state adversely affects the progress of the cancer. This is a complicated question without any clear answer. Most would agree that it is necessary to judge each case separately on its merits.

If cancerous foci of microscopic dimensions are taken into consideration, the incidence of multiple primary malignant neoplasms is relatively high. Moertel[19] writes, "The traditional concept of carcinoma as a single neoplasm intruding in an otherwise normal tissue is not always tenable. In a significant number of cases the initial neoplasm is only the first and most obvious manifestation of a carcinogenic process that may involve a large portion, or all, of a given tissue". Mersheimar, Ringel and Eisenberg's[20] results suggest that second cancers arise in the same tissue or system more frequently than would be expected by chance where the first cancer was in the breast, skin, colon, rectum or genito-urinary tract. According to Moertel[19], these findings should be taken into account in deciding the best treatment for cancers of these sites.

A series of contributions on the occurrence and treatment of certain uncommon or rare neoplasms are valuable for reference purposes. Of more general interest are papers on the relation between immune mechanisms and cancer and on the spontaneous regression of cancer. According to Grace[21], the immunological impairment not infrequently observed in cancer patients[22,23] is often related to disease directly affecting the lymphatic system. On the other hand, there are several examples of patients reacting immunologically to components or products of their own tumours; and the failure of advanced cancer patients to accept autografts of their own tumours is a puzzling phenomenon which needs further investigation. Everson's[24] survey of examples of the spontaneous regression of cancer is superb and it would be fitting to conclude this article with his assessment of the significance of this phenomenon: "(1) The existence of spontaneous regression of cancer, in at least some cases, supports the concept of biologic control of cancer and reinforces the hope that a more satisfactory method of treating cancer than surgery and/or radiation, may be found in future years.

"(2) The occurrence of spontaneous regression of cancer demonstrates the need for caution in the assessment of the value of chemotherapeutic and unorthodox therapeutic measures in isolated 'cures' of cancer.

"(3) The possibility of spontaneous regression of cancer must be considered in the evaluation of the prognosis of certain cancers. The remote possibility of spontaneous regression of cancer may be of some psychotherapeutic value in the patient with cancer which is not amenable to surgical and/or radiation treatment." F. J. C. Roe

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BRITISH TECHNICAL CO-OPERATION OVERSEAS

In replying to a series of questions in the House of Commons on June 25, the Secretary for Technical Co-operation, Mr. R. Carr, said that he was charged to give the sort of help to the developing countries which they themselves said they most needed. British Embassies and High Commissions kept under review the progress and results of his Department's work, and specialist advisers and other staff from his Department made frequent tours overseas. The £175 million spent on overseas aid this year was a record figure and illustrated the rapid rate of expenditure over recent years. Consultation with the Agency for International Development in the United States was close and continuous. The 2,900 recruited under the auspices of the British Government in 1963 for service in the developing countries included a very small number not of British nationality; the 1962 figure was 2,300. Questioned as to alleged difficulties experienced by professional people in finding suitable employment on return to Britain from service overseas, Mr. Carr said that the Overseas Services Resettlement Bureau has been set up specially to assist members of the Overseas Civil Service retiring prematurely, and was doing so with outstanding success. His Department did all it could to encourage employers to release staff on terms which provide for their re-absorption and also to encourage general recognition that overseas service is of positive value in a professional career. He would welcome assistance in making the arrangements better known.

On Latin America, he said that more than £500,000 had been allocated from his Department's funds in this financial year compared with £90,000 in 1963, and this represented about 2 per cent of Britain's total bilateral technical assistance expenditure and about 20 per cent of that allocated to foreign countries. Much of the £400,000 allocated by the British Council to Latin America was also for technical assistance, and he hoped there would be 70 experts in Latin America by the end of the year. Britain could probably help most in agriculture and natural resources work and in technical education.

During the past 12 months, Mr. Carr said he had had requests for training in the United Kingdom of 20 Indians for maintaining and operating new plant to be installed at the Durgapur Steel Works; for 4 engineering experts to assist the chief construction engineer; and for continuing the services of the British general manager until September 1965. More than 400 Indian steel workers had been trained in Britain, and Britain had also supplied substantial managerial and technical staff to the Durgapur Steel Works. Mr. Carr estimated that Britain's expenditure on technical assistance in Malaysia, excluding the Overseas Aid Service, would be about 50 per cent higher in 1964–65 than in 1962–63.

On locust control, he said that the work of the Anti-Locust Research Centre had become the basis for the international strategy of locust control. Two of the major types of plague locust had now been under control for more than 10 years and a third was at its lowest ebb for 24 years. The cost of killing a swarm of locusts had been reduced by 98 per cent compared with 20 years ago. The Centre had recently moved into new premises, which gave it greater scope. On the whole, research, including much field work, was done in Britain. Mr. Carr also referred to a startling and satisfactory increase in recruitment of teachers, which had risen by 300 per cent since his Department was established, compared with an average increase in recruitment of 80 per cent. Doctors still constituted a difficult field. Expenditure on bilateral assistance to foreign countries had risen by 170 per cent and was now 10 per cent of the total.

On research institutions in East Africa and the Caribbean, Mr. Carr said that the 27 such institutions in these regions employed about 250 senior and 350 supporting scientific staff and were doing most valuable work in medicine, agriculture and the development of natural resources. He had no specific proposals for increased grants before him at the moment, and his Department's research expenditure was now on a triennial basis. He estimated that his Department's expenditure on technical assistance to Pakistan was about £650,000 in 1962–63 and it would be about 20–30 per cent higher in 1964. He was somewhat disappointed at the initial response to the scheme of Commonwealth awards to university teachers. His Department employed or supported financially more than 1,200 research staff in research on problems affecting developing countries at a cost of about £2 million a year: just more than a quarter were in home-based units. Under the Low-priced Books Scheme, the university text-books series was available to 32 African countries and included technical and other text-books suitable for use in technical colleges. Preliminary investigations were being made of problems involved in extending the scheme to Latin America, but no decision had been taken.

In a written answer on June 25, Mr. Carr gave the number of overseas students in full-time education in Britain in 1950 as about 12,500, compared with 64,320 in 1963, of whom 50,100 were from the developing countries. In January 1963, 2,600 teachers were serving in developing countries who had been appointed under the auspices of the British Government; just more than 1,300 were appointed in 1963 and more than 400 were sent out by other bodies, in addition to an unknown number recruited direct.

Also in a written answer on June 25, Mr. Carr gave the distribution of the 915 research workers overseas supplied by his Department, 643 of whom were in Africa, 142 in the West Indies and 108 in the Far East and the Pacific. Of these at December 31, 1963, 343 were senior staff, 36 being directly employed, 18 supported 100 per cent, 251 between 50 and 100 per cent, and 38 less than 50 per cent supported. For the supporting staff, the figures were: 100 per cent, 10; 50–100 per cent, 485; and less than 50 per cent, 77. Twenty of those directly employed were in agriculture, including forestry; 2 in veterinary science; 5 in medicine; and 9 miscellaneous. Twenty-seven of those receiving 100 per cent support were in medicine. Of those receiving 50–100 per cent support, 383 were in agriculture, 41 in fisheries, 53 in pest control, 118 veterinary and trypanosomiasis, 99 medical, and 12 social and economic sciences. Of those receiving less than 50 per cent support, 30 were in agriculture, 49 in pest control, 4 veterinary science, 18 medical, and 8 social and economic sciences.

DEVELOPMENT AND SUPPORT OF SYSTEMATICS IN BRITAIN

At present many universities are re-considering the teaching of biology and proposing a unified syllabus. A recent committee of the Royal Society has made certain recommendations on the place that taxonomy should take in the development of biology[1]. The Systematics Association (c/o British Museum (Natural History), Cromwell Road, London, S.W.7) has appointed a small committee to consider the part the universities should play in this development. Members of the committee are: Prof. P. C. Sylvester-Bradley, Department of Geology, University of Leicester; Dr. V. H. Heywood, Hartley Botanical Laboratories, University of Liverpool; Dr. P. H. A. Sneath, National Institute for Medical Research, London, N.W.7. The committee felt that in any consideration of university policy on systematics it is important to distinguish between the broader field of systematics, which is concerned with the scientific study of the diversity and differentiation of organisms and the relationships which exist between them, and taxonomy, which is that part of systematics which deals with the study of classification, including its bases, principles, procedures and rules. Their respective roles in general biological courses and in the specialized training of taxonomists are different. The following aspects, although they interact with each other, should be considered separately: (1) the teaching of systematics at the undergraduate level; (2) the training of taxonomists; (3) the promotion of research in systematics.

The role that systematics should occupy in undergraduate courses in biology has been hotly debated in recent years. Some university biology departments hold that everything that is not molecular biology or biochemical physiology is 'systematics'. Thus such diverse fields as anatomy, morphology, palynology, ecology, cryptogamic botany, palæontology, populations genetics, etc., have been added to the responsibilities of the systematist. Partly as a consequence of these views, and partly as a result of the rapid expansion of the frontiers of biology into chemistry and physics, the expansion of systematics, not only to serve biology by the production and interpretation of classifications, but also to assume responsibility for the furtherance of several of its basic fields, has led to a situation that needs to be pondered by all biologists. Some experimental biologists feel that systematics has an insufficiently rigorous scientific basis, and have thus accorded to the subject a limited portion of the timetable. All too often the resources and staff allocated are quite inadequate for systematics to be carried out effectively. As a consequence many of the basic biological disciplines on which we depend for the consolidation of our factual information are in a state of neglect. The result of these attitudes is that it is becoming difficult to obtain suitably qualified staff in many fields, and this in turn leads to failure to apply modern techniques to these fields.

The point of view that these subjects have been bypassed by modern developments in biology is untenable. Although anatomical investigations may lack the glamour of molecular biology, it is research in systematics which contributes to the advance in our knowledge of whole organisms and on which we ultimately rely for a full comprehension of the significance of biological science.

The importance of systematics in biological courses was considered at a meeting of the Systematics Association in Birmingham, on the "Place of Taxonomy in the Teaching of Biology"[2]. There the role of systematics as a focus for the integration of knowledge on biological diversity was stressed, and this, it must be pointed out, is quite independent of the special interests of students. In other words, it is important for all biological students, not just for potential taxonomists.

Little attempt has been made in Britain, largely due to the departmental system, to teach systematics as a unified subject. Thus it is possible for the same topics (classification, phylogeny, species concepts, evolution, etc.) to be taught separately and in widely diverse (sometimes even conflicting) ways in different departments within the same biological school. Wherever possible, taxonomy should be taught as a unified subject and not compartmentalized between botany, palæontology, zoology, etc.

The teaching of taxonomy in Great Britain could often be greatly improved. In many universities it is not taught at all in a recognizable form. Undergraduate teaching in taxonomy should enable the student to negotiate with reasonable success almost any group of organisms without being a specialist in them, and with a minimum of help from specialists. To achieve this it is necessary to instil the basic principles of taxonomy which are applicable, with some modification, to all groups of organisms. At the same time, teaching should not be restricted to narrowly taxonomic subjects, but should include the wider aspects of systematics and be integrated with evolutionary, cytological, genetical and physiological work. On the other hand, systematics without taxonomy will lead to an equally unbalanced point of view.

The specialized training of students in taxonomy must of necessity follow the undergraduate course. The kind and duration of the training will depend to a large extent on the future occupation which is envisaged for the candidates. One approach is to establish one-year M.Sc. or diploma courses in taxonomy, like those recently initiated at Edinburgh and Liverpool. These are directed at providing adequate preparation in taxonomic methods and procedures for those who are unable to spend 2–3 years on a Ph.D. course, or who do not require such extensive training (it should be remembered that a short taxonomic training may be required for people working in related fields). For the successful running of such 1-year training courses it will be necessary to establish postgraduate schools of taxonomy, each staffed by several taxonomists. Two or three universities should set up such schools as part of their forthcoming expansion plans. Wherever possible they should be based on existing nuclei in universities where the existing staff and facilities could be quickly increased. There is also much to be said for such a school to be formed at one of the new universities. The appointment of additional taxonomists scattered over the country is not a satisfactory alternative to the establishment of a few postgraduate schools of taxonomy, since lone taxonomists can no more be expected to make a satisfactory contribution to teaching and research in a large biological school than can any other lone scientists.

After a training course for one year in taxonomic methods, as suggested here, the best experience for a future taxonomist is a period of creative research leading to a Ph.D degree. This stage will require special herbarium, museum or library facilities. It is for this reason that the number of schools of taxonomy should be limited. Adequate provision for the financing of fieldwork, both at home and abroad, should be made, and this will be assisted by the proposal to set up "Wallace" studentships made in the Royal Society Report[1]. It is also essential that financial provision be made for travel to consult material in other institutions, in this country and abroad.

Research in taxonomy and systematics is closely linked with teaching and training in these fields. Thriving research schools cost money, and it should be the aim of the schools of taxonomy which are recommended here to provide facilities which are comparable with the best found in the United States and on the Continent. In some of the fields of systematics which are listed below as appropriate for further development, little satisfactory progress can be made if the basic resources are not available. There is a need for intimate practical knowledge of traditional approaches if radical new developments are to be expected. A large number of such developments of interest both to systematics and taxonomy are being initiated even with the limited support that is at present available. But there are many aspects of taxonomy which have remained essentially unchanged for decades or even centuries, and up-to-date methods are neglected. This is in part a consequence of the concentration of Britain's taxonomic resources in the national museums which are largely obliged—by their nature and finance—to restrict the amount of pure research they can carry out without prejudice to their heavy practical obligations. Their divorce from the universities and academic research institutions further aggravates this situation. It is urgent that, in addition to the build-up of basic resources and facilities in a few selected universities, substantial support should be given to setting up research units in such fields as comparative biochemistry, comparative physiology and ethology, comparative serology, data-processing methods and machines, developmental physiology, environmental control, epigenetics, population cytology, DNA comparison, numerical taxonomy, palæobiochemistry, protein-sequence taxonomy and theoretical taxonomy.

Systematics occupies to-day a dual role in biology. Systematists have the function of evaluating, interpreting and co-ordinating the greatly increased flow of data from many fields so as to serve biology as helpfully as possible. They are also having to assume, increasingly, responsibility for developing many of those aspects of biology that are discarded by its advancing spearheads. If other biologists wish systematists to occupy these roles effectively, they must be prepared to accept that adequate support be made available to them for teaching and research.

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[1] *Taxonomy:* Rep. Committee appointed by the Council of the Royal Society (Royal Society, London, 1963).

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B.B.C. ENGINEERING MONOGRAPHS

SEVERAL times a year the Engineering Division of the British Broadcasting Corporation publishes a monograph either describing some novel technical equipment used in the development and operation of the Corporation's sound and television services, or making an appraisal of some aspect of its engineering activities. For example, *Monograph* No. 46 was published in February 1963, under the title *The Application of Transistors to Sound Broadcasting*, by S. D. Berry[*]. This describes the circuit arrangements and mechanical construction of a number of transistor audio-frequency amplifiers and associated apparatus designed for general use in the sound broadcasting services of the B.B.C.

Of a somewhat different type is *Monograph* No. 48 (June 1963) entitled *The Development of B.B.C. Internal Telecommunications*, by J. M. Chorley and J. S. Norwell[†]. In order to support the sound and television broadcasting services a comprehensive network of telecommunications is essential. While the British Post Office provides many of the required facilities over its country-wide network, the Corporation is concerned with special circuits and, particularly, with their termination at various centres throughout the United Kingdom. This *Monograph* traces the development of such B.B.C. telecommunications and describes some of the equipment used. It does not, however, deal with the distribution of television and sound programmes.

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[*] British Broadcasting Corporation. *BBC Engineering Monograph*, No. 46: *The Application of Transistors to Sound Broadcasting*. By S. D. Berry. Pp. 20. (London: British Broadcasting Corporation, 1963.) 5s.

[†] British Broadcasting Corporation. *BBC Engineering Monograph*, No. 48: *The Development of BBC Internal Telecommunications*. By J. M. Chorley and J. S. Norwell. Pp. 23. (London: British Broadcasting Corporation, 1963.) 5s.

The most recent of the published *Monographs* (No. 52: March 1964) is entitled *Stereophony: The Effect of Cross-Talk Between Left and Right Channels*, by H. D. Harwood and D. E. L. Shorter‡. As the short list of references appended to this publication shows, the B.B.C. has been exploring the practical possibilities of stereophonic broadcasting for several years. One of the technical defects which may appear in a stereophonic sound system is the unintentional transfer of a portion of the signal in one channel to the other channel, a phenomenon known as 'cross-talk' in telephone engineering. This results in a displacement or blurring of the reproduced sound images. The experiments described have covered the investigation of certain occurrences of cross-talk increasing at high or low frequencies, as well as cross-talk being independent of frequency. It was concluded that the minimum perceptible degree of cross-talk is influenced to some extent by the position of the observer in the listening room, but is largely independent of the acoustics of the room and of the bandwidth of the system.

Other *Monographs* published recently deal with such differing subjects as the measurement of the non-linear distortion generated by a loud-speaker system, and the methods of testing and measuring the properties of lenses used in the photoelectric components of television cameras. All these publications serve to demonstrate the extent to which the operation of the British Broadcasting Corporation's services are well supported by the comprehensive work of the Research, Designs and Lines Departments of the Corporation's Engineering Division.

R. L. Smith-Rose

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‡ British Broadcasting Corporation. *BBC Engineering Monograph*, No. 52: *Stereophony—The Effect of Cross-Talk Between Left and Right Channels*. By H. D. Harwood and D. E. L. Shorter. Pp. 23. (London: British Broadcasting Corporation, 1964.) 5*s*.

ISOLATION OF SEROLOGICALLY ACTIVE FUCOSE-CONTAINING OLIGOSACCHARIDES FROM HUMAN BLOOD-GROUP H SUBSTANCE

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THE results of hæmagglutination inhibition experiments demonstrated more than ten years ago that L-fucose, or more exactly an *O*-α-L-fucopyranosyl structure, is an important part of the serologically specific grouping in the H-specific blood-group substance[1]. Since that time other evidence, involving the inhibition by L-fucose of the enzymatic decomposition of H substance[2] and the demonstration that L-fucose is the sugar most readily liberated from H substance by the action of specific H-decomposing enzymes from *Trichomonas foetus*[3] and from *Bacillus fulminans*[4], has supported these early conclusions. More recently, results of a further serological analysis[5] with the eel serum anti-H reagent suggested that the L-fucose concerned in H specificity may be joined to the next sugar by an α-(1→2) linkage. Thus, 2-*O*-α-L-fucopyranosyl-L-fucose and 2-*O*-α-L-fucopyranosyl-D-galactose are more powerful inhibitors than either 3-*O*-α-L-fucopyranosyl-L-fucose or 4-*O*-α-L-fucopyranosyl-L-fucose.

Difficulties in drawing clear-cut inferences from these results arise from the fact that methyl-α-L-fucopyranoside inhibits the eel reagent as strongly as the disaccharides containing the α-(1→2)-linkages and that other oligosaccharides, such as lacto-N-fucopentaose I (ref. 6), are poor inhibitors of hæmagglutination although they contain α-L-fucopyranosyl-(1→2)-galactose units as terminal non-reducing end-groups. The anti-H reagent extracted from *Lotus tetragonolobus* seeds shows similar anomalies[5], and it was pointed out that the failure to obtain greater inhibition with α-L-fucosyl-(1→2) compounds than with the simple methyl α-L-fucoside might be due to the limitation imposed by the heterologous nature of the eel and plant seed anti-H agglutinins, the receptor sites of which may be largely saturated by a simple α-L-fucopyranosyl grouping. Nevertheless, further support for the belief that L-fucose is linked α-(1→2) in the H determinant structure was gained from experiments with partially purified enzymes from *T. foetus*[7]; preparations which destroy the serological activity of H substance split fucose from oligosaccharides in which the sugar is joined by α-(1→2) linkage to galactose but not from an oligosaccharide in which L-fucose is joined by a (1→4) linkage to *N*-acetyl-D-glucosamine.

That more than one terminal non-reducing sugar residue may be important for H specificity was indicated by inhibition experiments with anti-H reagents from seeds of the plants *Cytisus sessilifolius* and *Laburnum alpinum* These reagents are strongly inhibited by human H substances but are not inhibited by L-fucose[8]. The disaccharide 4-*O*-(*N*-acetyl-β-D-glucosaminopyranosyl)-*N*-acetyl-D-glucosamine (*N*,*N'*-diacetyl-chitobiose), however, inhibits both *Cytisus* and *Laburnum* reagents[8], indicating that an *N*-acetyl-β-D-glucosaminopyranosyl structure may be a serological determinant unit in H-substance in addition to an α-L-fucopyranosyl residue.

Valuable as the results of inhibition and enzymatic experiments are as indirect pointers to the structures of the serologically active groupings in the blood-group macromolecules, the final proof of structure must rest on the chemical characterization of active fragments isolated from the substances themselves. Examination of the four di- and the three tri-saccharides obtained from the partial acid hydrolysis products of a human blood-group H specific substance[8,9] revealed two serologically active fragments, the disaccharide 3-*O*-(*N*-acetyl-β-D-glucosaminopyranosyl)-D-galactose, and the trisaccharide *O*-(*N*-acetyl-β-D-glucosaminopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-*N*-acetyl-D-galactosamine. These compounds, however, inhibited only the *Cytisus* anti-H reagent and failed to inhibit human, rabbit or eel anti-H agglutinins. This is not surprising, as none of the oligosaccharides described contained L-fucose owing to the rapid liberation of this sugar under the conditions of hydrolysis used. An investigation of the isolated fragments indicated the presence of two main types of carbohydrate chain in H substance for which the following partial structures were proposed[9]:

(1) β-Gal-(1→3)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc-

(2) β-Gal-(1→4)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc-

(Gal = D-galactopyranose; GNAc = *N*-acetyl-D-glucosaminopyranose; GalNAc = *N*-acetyl-D-galactosaminopyranose.)

The isolation and identification of three H-active fucose-containing oligosaccharides from H-specific substances are now reported. Alkaline degradation was previously observed to give good yields of fucose-containing oligosaccharides from blood-group substances[10], and two of those now described were obtained from the products of partial alkaline hydrolysis. The third was isolated in low yield from a partial acid hydrolysate[8,9] of H-substance.

Alkaline degradation of blood-group H substance (obtained from human ovarian cyst fluid[11]) was accomplished by heating 1 g portions at 60° for 18 h in 50 ml. 50 per cent aqueous methanol containing 2·5 per cent v/v triethylamine. The volatile base was then removed by distillation under diminished pressure, and the residual solution was dialysed against water in a 'Cellophane' sac. After six further treatments of the indiffusible residue in the same way, 70 per cent of the starting material was converted into diffusible fragments.

The diffusible material (2·1 g) from 3 g of H substance was adsorbed on a column (15 × 3·6 cm) of charcoal/'Celite' (1 : 1 w/w). Stepwise elution of the column with water (3·5 l.) and aqueous ethanol (3·5 l. of 1·5 per cent v/v, followed by 3 l. of 2·5 per cent v/v) displaced a complex mixture of monosaccharides, chromogens[12], acidic degradation-products of sugars and other unidentified components. Aqueous ethanol (5 per cent v/v; 6 l.) then displaced a fraction (300 mg) which, after further resolution by preparative paper chromatography, yielded 85 mg of a sugar (I) having $R_{\mathrm{lactose}} = 1{\cdot}55$ in ethyl acetate/pyridine/water (10 : 4 : 3 v/v; solvent *a*), and $M_G = 0{\cdot}47$ in borate buffer at *p*H 10 (ref. 13). It reacted rather slowly on paper with the silver nitrate–sodium hydroxide reagent[14], and with the benzidine–trichloroacetic acid reagent[15] gave a dark brown colour similar to that given by D-galactose.

The sugar (I) was obtained as a hygroscopic glassy solid, having $[\alpha]_D^{23} - 53°$ ($c = 0{\cdot}8$ in water). Chromatography of its acid hydrolysis products indicated the presence of fucose and galactose, and when the glycitol of the sugar, prepared by reduction with sodium borohydride, was hydrolysed and examined, fucose and galactitol were the only detectable products. Analysis of the sugar by the phenol–sulphuric acid method[16] and estimation of its fucose content[17] showed that fucose and galactose were present in equimolar proportions.

That the disaccharide was (1→2)-linked was suggested by its failure to give a red colour with alkaline triphenyl tetrazolium chloride[18], and by its resistance to degradation by alkali[19]. Confirmation of this, together with evidence that the fucose residue was in the pyranose form, was obtained by periodate oxidation. In 0·01 M sodium metaperiodate at 22°, the disaccharide consumed 3·1 mol. of oxidant within the first 4 h and liberated 1·0 mol. of formic acid; further oxidation took place more slowly and after 24 h gave 1·0 mol. of formaldehyde; over-oxidation was observed. Oxidation under the same conditions after borohydride reduction[20] resulted in the rapid (4 h) consumption of 4·9 mol. of periodate and liberation of 0·98 mol. of formaldehyde; there was no over-oxidation.

Disaccharide (I) was therefore identified as 2-*O*-α-L-fucopyranosyl-D-galactose. Comparison of the crystalline 1-benzyl-1-phenylhydrazones (m.p. and mixed m.p. 164°) showed this disaccharide to be identical with a specimen of 2-*O*-α-L-fucopyranosyl-D-galactose kindly supplied by Prof. R. Kuhn[21].

After the displacement of disaccharide (I), the charcoal column was eluted with more aqueous ethanol (2 l. of 5 per cent v/v; 5 l. of 7·5 per cent v/v; and 5 l. of 12·5 per cent v/v). The 12·5 per cent aqueous ethanolic effluent contained 140 mg of carbohydrate material which, after fractionation on paper chromatograms, yielded a component (II; 12 mg) having $R_{\mathrm{lactose}} = 1{\cdot}11$ in solvent *a* and $M_G = 0{\cdot}39$ in borate buffer at *p*H 10. With the benzidine–trichloroacetic acid reagent[15], it gave a golden-yellow colour similar to that given[22] by 4-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine (*N*-acetyl-lactosamine).

The compound (II) had $[\alpha]_D^{22} - 45°$ ($c = 0{\cdot}5$ in water). Chromatography of its acid hydrolysis products indicated the presence of fucose, galactose and glucosamine, and analysis[16,17,23] showed that these monosaccharides were present in approximately equimolar amounts. However, after reduction of (II) with sodium borohydride, its hexosamine content, determined by the Elson–Morgan method[23] after acid hydrolysis, was negligible whereas the content of galactose and fucose was unchanged. The trisaccharide gave a negative indirect Ehrlich reaction[24], indicating[25] that the reducing terminal *N*-acetyl-glucosamine residue was substituted at position 4.

When trisaccharide (II) was heated in 0·02 N-polystyrene-sulphonic acid[26] at 80°, fucose was rapidly split from the molecule, with the formation of a disaccharide which was chromatographically and ionophoretically indistinguishable from *N*-acetyl-lactosamine and which gave, with the benzidine–trichloroacetic acid reagents, the golden-yellow colour characteristic of that sugar[22]. The disaccharide also gave a negative indirect Ehrlich reaction and slowly underwent further hydrolysis to give galactose and *N*-acetylglucosamine.

In aqueous methanolic triethylamine[9] at 80°, trisaccharide (II) was very slowly degraded, at a rate similar to that of *N*-acetyl-lactosamine, with the liberation of a disaccharide which was completely resistant to further degradation. This disaccharide was chromatographically and ionophoretically indistinguishable from 2-*O*-α-L-fucopyranosyl-D-galactose (disaccharide I), and also resembled that sugar in its behaviour with the benzidine–trichloroacetic acid, silver nitrate–sodium hydroxide, and alkaline triphenyltetrazolium chloride spray reagents.

In 0·01 M sodium metaperiodate at 22°, trisaccharide (II) consumed 3·07 mol. of oxidant within 4 h and liberated 0·96 mol. of formic acid and 0·14 mol. of formaldehyde. Further oxidation under these conditions was very slow. The glycitol of the trisaccharide obtained by reduction with borohydride[20] consumed, under the same conditions, 3·96 mol. of periodate in 4 h and liberated 0·99 mol. of formaldehyde.

On the basis of this evidence, trisaccharide (II) is believed to be *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-*N*-acetyl-D-glucosamine.

In addition to disaccharide (I) and trisaccharide (II), at least five other fucose-containing compounds were present in the alkaline degradation products of H substance. None of these has yet been fully identified, but one of them (IV), believed to be either a tetra- or penta-saccharide, was obtained sufficiently pure for serological examination (Table 1). Oligosaccharide (IV) was isolated from the 20 per cent v/v aqueous ethanolic effluent of the charcoal column and yielded fucose, galactose and glucosamine on acid hydrolysis; it had $R_{\mathrm{lactose}} = 0{\cdot}73$ in solvent (*a*), and $[\alpha]_D^{22} - 54°$ in water.

The third chemically characterized fucose-containing oligosaccharide (III) was obtained from a partial acid hydrolysate[8,9] of 2·7 g of H substance, and appeared in the 7·5 per cent v/v and 10 per cent v/v aqueous ethanolic effluents from the charcoal–'Celite' column used for fractionation. After purification on paper chromatograms, 5 mg of the sugar was obtained as a syrup. It had $[\alpha]_D^{21} - 36°$ ($c = 1{\cdot}0$ in water) and $R_{\mathrm{lactose}} = 0{\cdot}83$ in solvent *a*. It gave a strong indirect Ehrlich reaction and with the benzidine-trichloroacetic acid reagent gave a greenish-brown colour similar to that given[22] by 3-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine (lacto-*N*-biose 1) (ref. 27).

Table 1. H Activity of Fragments isolated from Alkaline and Acid Hydrolysis Products of Human H Substance

	Minimum amount of substance giving inhibition (μg/0·1 ml.)				
	Human anti-H	Rabbit anti-H	Eel serum	*Cytisus sessilifolius* reagent	*Ulex europaeus* reagent
Human H substance	0·03	0·07	0·03	0·1	0·07
Alkaline degradation products					
Disaccharide (I) (2-*O*-α-L-fucosyl-D-galactose)	500	1,000	4	2,000	> 2,000
Trisaccharide (II) (*O*-α-L-fucosyl-(1→2)-*O*-β-D-galactosyl-(1→4)-*N*-acetyl-D-glucosamine)	125	63	250	16	32
Oligosaccharide (IV)	—	16	—	8	16
Acid degradation product					
Trisaccharide (III) (*O*-α-L-fucosyl-(1→2)-*O*-β-D-galactosyl-(1→3)-*N*-acetyl-D-glucosamine)	—	32	—	16	16
Control sugars					
4-*O*-β-D-Galactosyl-*N*-acetyl-D-glucosamine	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000
3-*O*-β-D-Galactosyl-*N*-acetyl-D-glucosamine	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000
4-*O*-(*N*-Acetyl-β-D-glucosaminoyl)-*N*-acetyl-D-glucosamine	> 2,000	> 2,000	> 2,000	63	2,000
L-Fucose	> 2,000	> 2,000	16	> 2,000	> 2,000
D-Fucose	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000

(All non-reducing sugar residues are in pyranose form.)

Chromatography of the acid hydrolysis products of (III) indicated the presence of fucose, galactose and glucosamine in approximately equal amounts. However, after reduction of (III) with sodium borohydride, its hexosamine content was negligible, whereas the amounts of fucose and galactose present were unchanged. On mild acid hydrolysis as described for trisaccharide (II), fucose was rapidly split from the molecule with the liberation of a disaccharide which, on further hydrolysis, slowly gave galactose and *N*-acetylglucosamine. The disaccharide was chromatographically indistinguishable from lacto-*N*-biose 1, and gave a strong indirect Ehrlich reaction.

Trisaccharide (III) was extremely sensitive to alkali and on heating at 65° in 0·1 N aqueous pyridine[28] was completely degraded within 30 min with the liberation of free chromogen[12,28] and a disaccharide which was chromatographically and ionophoretically indistinguishable from 2-*O*-α-L-fucopyranosyl-D-galactose (disaccharide I). The liberated disaccharide was identical with disaccharide (I) in its behaviour with the spray reagents named earlier and in its resistance to alkaline degradation.

Trisaccharide (III) is therefore considered to be *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→3)-*N*-acetyl-D-glucosamine.

The structures of trisaccharides (II) and (III) suggest that, in the native blood-group H substance, the two type of chain (1) and (2), depicted here, have α-L-fucopyranose residues attached to position 2 of their non-reducing terminal galactose residues. Support for this idea arises from a consideration of the probable mechanism by which disaccharide (I) and trisaccharide (II) are formed by alkaline degradation of the specific substance. The chains must presumably be joined to the rest of the molecule by alkali-labile linkages and, once liberated, should undergo stepwise degradation from their reducing ends according to the accepted mechanisms[29] until a relatively alkali-stable linkage is reached. Chains of the first type (1) consist entirely of alkali-labile (1→3)-linkages, and therefore disaccharide (I) probably originated mainly from chains of this type although a small amount could be formed by further degradation of trisaccharide (II). Trisaccharide (II) must originate from chains of the second type (2)—the stepwise degradation in this case being largely arrested by the relatively resistant *N*-acetyl-lactosamine structure. The very alkali-labile trisaccharide (III) does not survive alkaline degradation of H-substance.

The fucose contents (20–25 per cent) of some preparations of H substance are higher than can be accounted for on the assumption that each of the two types of chain carries only one fucose residue. It is likely, therefore, that some of the chains carry additional fucose residues attached to sugar units other than the non-reducing terminal D-galactose residue. The assignment of locations to these additional fucose residues must await the identification of larger oligosaccharides from the alkaline degradation products of H substances.

Anti-H agglutinins of human, animal or plant origin are all, by definition, inhibited in their action on group O red cells by human H substance; they differ, however, in the ease with which they are inhibited by low molecular weight compounds, in the nature of the small units which cause inhibition and in the extent and nature of the changes which can be brought about in H substances before specificity is lost[30]. It is, therefore, important to test isolated fragments with a number of different anti-H reagents. Disaccharide (I) and trisaccharide (II) were tested for inhibition with the following anti-H agglutinins: (1) a human serum from a donor of the 'Bombay phenotype'[31]; (2) an immune rabbit serum prepared by injection of human H substance coupled with the conjugated protein from *Shigella shigae*[32]; (3) a natural eel serum[33]; (4) an extract of seeds of *Cytisus sessilifolius*[34]; (5) an extract of seeds of *Ulex europaeus*[35]. Owing to the limited quantities available for test, trisaccharide (III) and the unidentified oligosaccharide (IV) were examined only with rabbit and plant seed reagents. The results (Table 1) show for the first time inhibition of human and rabbit anti-H sera by low molecular weight fragments. Disaccharide (I) was only slightly inhibitory with the human, rabbit or plant seed reagents, but the trisaccharides were considerably better inhibitors. When both trisaccharides were tested with any one reagent the inhibition titres were the same or differed by only one two-fold dilution factor. The total absence of inhibition with any of the anti-H agglutinins noted for 4- and 3-*O*-β-D-galactosyl-*N*-acetylglucosamine, which are derived from trisaccharides (II) and (III), respectively, by the removal of fucose, once more points to the dominant role of L-fucose in H specificity. The unidentified oligosaccharide (IV) was slightly more active than any of the identified fragments, indicating that a trisaccharide unit does not represent the complete H determinant structure for the reagents examined. The results with the eel serum differed from those of the other anti-H agglutinins in that disaccharide (I), as had been shown previously[5], was a good inhibitor, whereas trisaccharide (II) had considerably less inhibiting potency. The reason for this anomalous behaviour is not clear; but the decrease in inhibiting power with increasing size of fragment, even when the terminal non-reducing units are identical, is consistent with the earlier results obtained with eel serum[5]. The inhibition of the *Cytisus* anti-H reagent by the fucose-containing trisaccharides which did not have terminal non-reducing β-*N*-acetylglucosaminoyl groupings introduces a new aspect into the specificity requirements of this plant seed reagent, and an evaluation of these and the earlier results must await further work. Human anti-Le^{a}, anti-Le^{b}, anti-A or anti-B sera were not inhibited by disaccharide (I) or trisaccharide (II).

Disaccharide (I) and trisaccharide (II) have been detected in the products of alkaline degradation of A and B substances that had H activity, but have not been detected in the products of an Le^{a} substance that showed no H activity (unpublished results). These observations, together with the results of the hæmagglutination inhibition tests, strongly suggest that oligosaccharides (I), (II) and (III) represent integral parts of blood-group H specific determinant structures.

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HÆMOGLOBIN $\mathrm{G}_{\mathrm{ACCRA}}$

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IN 1954 the major hæmoglobin types, A, M, S, C, D, E and F, had been described, and the observation of a new variant, hæmoglobin G (ref. 1), was the first of a series of reports on rarer human hæmoglobins which is still continuing. The hæmoglobin then described—and differentiated from other hæmoglobins with identical or similar electrophoretic properties as hæmoglobin $\mathrm{G}_{\mathrm{Accra}}$—has never been found again, except in the family in which it was originally observed. Hæmoglobin $\mathrm{G}_{\mathrm{Accra}}$ is abnormal in the β-chain[2]. The hæmoglobins with identical electrophoretic and chromatographic properties hitherto examined were α-chain abnormal pigments. There are three: (1) hæmoglobin $\mathrm{G}_{\mathrm{Philadelphia}}$ with which numerous other hæmoglobins observed in West Africa, or in persons of West African origin, are identical[3]; (2) hæmoglobin $\mathrm{G}_{\mathrm{Chinese}}$, which has only been found in persons of Chinese extraction[4]; (3) a hæmoglobin found in a British family, hæmoglobin $\mathrm{G}_{\mathrm{Norfolk}}$[5]. There is one other hæmoglobin named G which also carries its substituted amino-acid residue in the β-chain-hæmoglobin $\mathrm{G}_{\mathrm{San\ José}}$[6], but this pigment differs from hæmoglobin $\mathrm{G}_{\mathrm{Accra}}$ in its electrophoretic properties, and should perhaps have been described under a different letter.

Hæmoglobin $\mathrm{G}_{\mathrm{Accra}}$ is remarkable for the fact that it is the only rare hæmoglobin for which an individual has been described the homozygous state of which could be established on the basis of family investigation and of laboratory findings[7]. This homozygote was found to be healthy without an enlarged spleen, without anæmia, and his red cells were morphologically indistinguishable from those containing only normal hæmoglobin. This was, and still is, in contrast with what is seen in the hæmoglobin S, C, D and E diseases, the only other homozygous conditions for adult hæmoglobin variants so far described.

There was, therefore, a special interest in establishing the precise nature of the amino-acid substitution in hæmoglobin $\mathrm{G}_{\mathrm{Accra}}$. Whatever its nature, it does not have a similar effect on the morphology and, presumably, the life span of the red cell which is produced by the known substitutions in hæmoglobins S, C, D and E.

Amino-acid Substitution in Hæmoglobin $\mathrm{G}_{\mathrm{Accra}}$. Hæmoglobin G was purified from the blood of the homozygote and a peptide-chromatogram (fingerprint) was prepared according to Ingram[8] and Baglioni[9]. It was noted that peptide 6 according to the old numbering system, or tryptic peptide β*Tp*IX according to the new nomenclature[10], was missing and another new peptide was seen instead which had moved nearer towards the cathode (Fig. 1). The new peptide gave a positive colour reaction

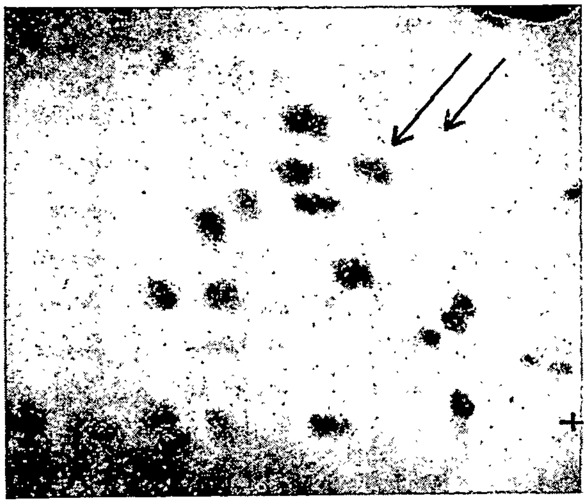

Fig. 1. Fingerprint of the tryptic digest of hæmoglobin G_{Accra}. The right arrow points to the area where β^A*Tp*IX is missing, and the left arrow to the peptide which has taken its place—β^G*Tp*IX. +, Point of application

for histidine[11], as does the β*Tp*IX of hæmoglobin A (β^A*Tp*IX). This peptide contains in hæmoglobin A 16 amino-acid residues[12,13] (Table 1).

Table 1. AMINO-ACID SEQUENCE OF TRYPTIC PEPTIDE IX OF THE β-CHAIN OF HÆMOGLOBIN A

Val–	leu–	gly–	ala–	phe–	ser–	asp–	gly–	leu–	ala–	his–	leu–	asp–	asn–	leu–	lys
67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82

The NH_2-terminal group[14], was valyl in both peptides, and leucine amino peptidase[4] which splits off amino-acids from the NH_2-terminal part of a peptide chain released valine and leucine in both. As the peptides had arisen from tryptic digestion which implies lysyl or arginyl as the COOH-terminal, and as the colour reaction and amino-acid analysis failed to show arginine[15], lysyl had to be the COOH-terminal group.

Table 2 shows a comparison of the results of quantitative amino-acid analysis on an EEL automatic analyser[16], after the acid hydrolysis of the two peptides from hæmoglobins A and G. It will be seen that the results were the same in both, although the electrophoretic mobility of the G-peptide had indicated that it had a greater positive charge than the corresponding A-peptide. The only explanation was that one of the two aspartic acid residues of β^A*Tp*IX had been replaced by an asparaginyl in β^G*Tp*IX, and that this had been converted into aspartic acid on acid hydrolysis.

Table 2. MOLAR RATIO OF AMINO-ACID RESIDUES AFTER ACID HYDROLYSIS OF TRYPTIC PEPTIDES IX FROM HÆMOGLOBIN A AND HÆMOGLOBIN G_{Accra}

Residues	β^A*Tp*IX		β^G*Tp*IX	
Asp	2·8	2·7	2·6	2·6
Thr	Trace	Trace	Trace	Trace
Ser	1·0	1·2	1·1	1·3
Glu	Trace	Trace	Trace	Trace
Pro	Trace	Trace	Trace	Trace
Gly	2·1	2·0	1·9	2·2
Ala	2·5	2·4	2·3	2·1
Val	1·3	1·1	1·0	0·9
Leu	3·6	3·7	3·7	3·7
Phe	1·0	1·0	1·1	1·2
Lys	0·9	0·9	1·1	1·1
His	0·9	1·1	0·9	1·2

Although on electrophoresis at *p*H 6·4 the mobility of the A peptide is 0·0 and that of the G peptide +0·17 (lysine = +1·0) there seems to be no difference in amino-acid composition after acid hydrolysis.

To investigate this possibility, both peptides β*Tp*IX were eluted with 10 per cent (v/v) pyridine to prevent hydrolysis of asparaginyl, and then digested with pronase for 4 h using the same arrangement as had been used for trypsin, and the resulting smaller peptides and amino-acids were separated by high voltage electrophoresis at *p*H 6·4. Pronase attacks virtually all peptide linkages, although it is relatively inactive towards those which involve aspartic acid residues. Table 3 shows the electrophoretic mobility of these breakdown products, and their amino-acid composition found after acid hydrolysis. This latter method of course converts asparaginyl to aspartic acid. It will be seen that the number of acidic breakdown products in which aspartic acid could be demonstrated differed in the two peptides—four from hæmoglobin A, and three from hæmoglobin G. Three seemed to be the same in both hæmoglobins. Free aspartic acid was obtained from both, and there were two peptides which had nearly the same electrophoretic mobility, and the same amino-acid composition in both. One peptide with the mobilities of −0·77 and −0·78 respectively was hydrolysed into aspartic acid and glycine with aspartyl as the NH_2-terminal residue. The other peptide with the mobilities of −0·64 and −0·62 respectively showed on hydrolysis only aspartic acid, glycine, and leucine with NH_2-terminal aspartyl and COOH-terminal leucyl[14,17]. The sequence 73–75 was therefore the same in both β^A and β^G, namely, asp-gly-leu, and the substitution of aspartyl in hæmoglobin G had to be in position 79 of the β chain.

Table 3. PRODUCTS OF PRONASE HYDROLYSIS OF β^A*Tp*IX AND β^G*Tp*IX

	Mobility*	Amino acids on acid hydrolysis	NH_2-terminal DNP	COOH-terminal
	−1·00	Asp		
	−0·77	Asp, gly	Asp	
	−0·68	Asp		
	−0·64	Asp, gly, leu	Asp	Leu
β^A*Tp*IX	−0·52			
	0·00	Val, gly, leu, asp Ala, phe, ser		
	+0·53	His		
	+0·67	Leu, lys	Leu	
	+1·00	Lys		
	−1·00	Asp		
	−0·78	Asp, gly	Asp	
	−0·62	Asp, gly, leu	Asp	Leu
	−0·54			
β^G*Tp*IX	0·00	Val, gly, leu, asp Ala, ser, phe		
	+0·51	His		
	+0·67	Leu, lys	Leu	
	+1·00	Lys		

* Electrophoretic mobility is measured at *p*H 6·4 using as markers aspartic acid (−1·00) and lysine (+1·00).

From β^A*Tp*IX one other peptide yielding aspartic acid was obtained. It had a mobility of −0·68 but produced aspartic acid only. However, aspartic acid or an asp-asp peptide would have had a mobility of −1·00, hence this peptide had to consist of aspartyl and asparaginyl, and it represented the 79–80 sequence of the β^A-chain, namely, asp-asn.

This peptide was not found in β^G*Tp*IX. However, free asparagine could be demonstrated in the neutral band before hydrolysis. No free asparagine was found in the neutral band of β^A*Tp*IX. From this it could be concluded that the residue next to leucyl in position 79 of the β^G-chain was asparaginyl and hæmoglobin G_{Accra} could be defined as $\alpha_2^A\,\beta_2^{79\ Asn}$.

The sequence asn-asn-leu in hæmoglobin G would explain why pronase digestion produced the peptide asp-asn from β^A*Tp*IX preferably to the free asparagine as obtained from β^G*Tp*IX. In β^G*Tp*IX due to the aspartyl→ asparaginyl replacement the 79–80 linkage was more easily broken because of the absence of an acidic aspartyl residue.

Homozygote. The homozygote is now sixty-four years old, or even a little older, and is physically fit with the exception of a mild hypertension of 160/100 mm mercury.

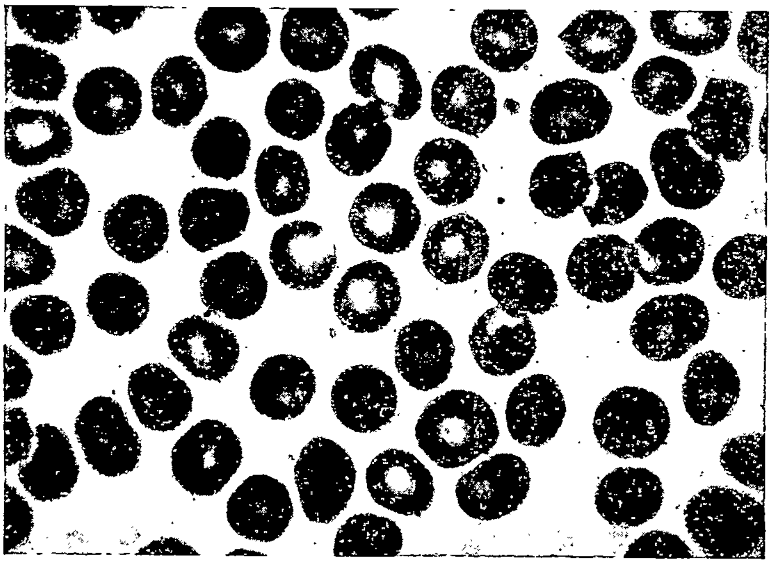

Fig. 2. Stained blood smear of the hæmoglobin-G homozygote. The cells are not hypochromic and the target cells and other abnormal forms associated with the blood picture of hæmoglobin S, C, D and E homozygotes are absent

From 1923 until his retirement in 1963 he worked continually as a printer in the Accra Government Printing Department. Of his twenty children by two wives, all but one is alive and well. The age of the oldest offspring is thirty-six years and the youngest is eleven years old.

His blood shows the following values:

hæmoglobin	12·6 g/100 ml.
red cells	4,500,000/mm^3
packed cell volume	40 per cent

The mean corpuscular values are:

cell volume	89μ^3
hæmoglobin	28 $\gamma\gamma$
hæmoglobin concentration	32 per cent

The red cell fragility showed an almost normal distribution: initial hæmolysis at 0·5 per cent sodium chloride, 50 per cent hæmolysis at 0·415 and 90 per cent hæmolysis at 0·3 per cent sodium chloride. The only unusual feature was that 10 per cent of the cells were still not hæmolysed at 0·3 per cent sodium chloride, and that complete hæmolysis was only achieved in distilled water. There was no enlarged spleen, the red cells were normal in appearance, and there were no target cells. Hæmoglobin A_2 was within normal limits, and no hæmoglobin F was demonstrable. These findings contrast with those in hæmoglobinopathies of the homozygotes for the genes controlling hæmoglobins S, C, D and E. Hæmoglobin $\alpha_2^A\ \beta_2^{79\ \mathrm{Asn}}$ seems to cause no hæmoglobinopathy and the substitution of an asparaginyl residue for an aspartyl in position 79 of the β-chain seems to have no deleterious effect.

Differences in the Morbidity of Hæmoglobin Variants. Of the rarer hæmoglobins only hæmoglobin $\mathrm{G}_{\mathrm{Accra}}$ has so far been seen in the homozygous state. It is probable that many of the other rare hæmoglobins may be similarly harmless, and that there is a relation between the effect a hæmoglobin has on the red cell morphology in the homozygote and the advantage it confers on the heterozygote in natural selection which causes its high frequency in a population.

In the heterozygote for the genes for hæmoglobin A, and for hæmoglobins S, C, D and E respectively, the proportion of the abnormal variant is less than 50 per cent. This has usually been interpreted as the outcome of a difference in the rate of production of normal and abnormal polypeptide chains within the cell[18]. An alternative explanation has recently been proposed by Levere, Lichtman and Levine[19]. From iron incorporation studies in anæmic sickle-cell trait (A + S) carriers they concluded that, in the case of hæmoglobin A + S at least, cells with a wide range of A : S proportions are produced—some with A < S and some with A > S. The first are rapidly eliminated and as a result the final over-all proportion of A : S becomes greater than 1.

In the case of hæmoglobin A + $\mathrm{G}_{\mathrm{Accra}}$ heterozygotes, the proportion of the two hæmoglobins is exactly 1. We have established this by a number of investigations because by ordinary paper electrophoresis at alkaline *p*H the leading (A) band contains about 53 per cent of the total hæmoglobin This proportion is also obtained when an artificial mixture of 50 per cent A and 50 per cent G is submitted to electrophoresis at alkaline *p*H. Furthermore, when open boundary electrophoresis of hæmoglobin from the A + G heterozygote was performed at *p*H 6·5 (ref. 7) and the G fraction was the leading component, the G fraction then amounted to from 50 to 55 per cent of the total pigment.

This equal proportion of A and G in the heterozygote would indicate that there is no elimination of cells with a higher proportion of G than A, and that the whole range of cells survives equally, those with A < G, and those with A > G.

One might predict that other hæmoglobins which show an equal proportion of A and abnormal variants in the hæmolysates from heterozygotes are similarly harmless as hæmoglobin $\mathrm{G}_{\mathrm{Accra}}$. There would, therefore, be a series of hæmoglobin types: (1) Harmless in homozygotes and heterozygotes (heterozygotes show 50 per cent of the abnormal variant): example, hæmoglobin $\mathrm{G}_{\mathrm{Accra}}$. (2) Harmful in homozygotes, harmless in heterozygotes (abnormal variant < 50 > 20 per cent). Hæmoglobins S, C, D and E would belong to this group. (3) Harmful in heterozygotes (abnormal variant < 20 per cent). To this would belong the hæmoglobins which are associated with hæmolysis even in the heterozygote, such as hæmoglobin Köln[20].

The correlation between the morbidity of a hæmoglobin and its molecular structure might throw an important light on the function of hæmoglobin as a whole.

We thank Mr. D. Irvine for valuable assistance, also Dr. G. Braunitzer and his colleagues of the Max Planck Institute für Biochemie, Munich, Germany, for advice and discussion.

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INHIBITION OF INTERFERON ACTION BY *p*-FLUOROPHENYLALANINE

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THE mechanism of action of the antiviral protein interferon[1] is unknown. Incubation for some hours at 37° C is necessary for interferon action since cells treated with interferon and kept at 2° C showed much less resistance to virus infection than cells similarly treated but kept at 37° C (ref. 2), suggesting that an active metabolic process is necessary for development of full antiviral activity. The action of interferon also requires DNA-dependent RNA synthesis on the part of the cell since interferon action was blocked in cells incubated with actinomycin *D* before the addition of interferon[3].

Since RNA synthesis is required for interferon action, it was of interest to determine whether protein synthesis is also necessary. The results of the work recorded here indicate that protein synthesis is required, as interferon action was inhibited in cells incubated with both interferon and the amino-acid analogue *p*-fluorophenylalanine (FPA).

The origin and preparation of the Semliki Forest virus (SFV) pools used in these investigations and the preparation of chick embryo fibroblast (CEF) cultures have been previously described[3]. The virus growth medium consisted of *tris* (0·002 M) buffered Gey's salts with 0·25 per cent lactalbumin hydrolysate, 0·1 per cent peptone, and 2·5 per cent calf serum. During treatment with FPA, buffered Gey's salts only was used for both experimental and control plates. In experiments using FPA the virus growth medium was supplemented with DL-phenylalanine, 100 μg/ml. SFV was assayed and interferon titrated by previously described methods[4]. DL-FPA was obtained from Calbiochem. In all experiments it was used at a concentration of 100 μg/ml.

In order to estimate interferon action in the experiments to be described, CEF's were infected with SFV at a virus plaque-forming unit to cell multiplicity of 20 : 1. This multiplicity of infection was shown in preliminary examinations to yield maximum virus growth at 8 h after infection; in addition, endogenous interferon production had not yet started at that time. After 1 h at 37° for virus adsorption the cells were washed, and the virus growth medium added. After an additional 7 h at 37° the plates were frozen and thawed and the fluids assayed for SFV.

CEF plates were incubated with buffered Gey's salts, or FPA, or interferon, or FPA with interferon. After 5 h, the cells were washed three times and infected with SFV. The results of assays for SFV growth are shown in Table 1. In this experiment pretreatment with FPA only slightly inhibited the growth of SFV. Interferon 1·2 or 0·6 units†, respectively, inhibited virus growth to 5 and 11 per cent of virus controls. On plates incubated with both FPA and interferon, growth of the virus was inhibited, respectively, to 35 and 49 per cent of virus controls. The figures in parentheses in Table 1 and the other tables are the percentage of virus growth in the plates treated with FPA and interferon if the plates treated with FPA alone are taken as controls.

At both concentrations used in this and other similarly conducted experiments, the antiviral action of interferon was inhibited by FPA. Preliminary investigations utilizing tritiated adenosine or leucine labelled with carbon-14 showed that chick cells treated with 100 μg/ml. of FPA had rates of RNA and protein synthesis equal to those of untreated controls confirming the findings of other workers[5].

Table 1. INHIBITION OF INTERFERON ACTION BY *p*-FLUOROPHENYLALANINE (FPA)

Pretreatment of cells*	Yield of SFV at 8 h (PFU × 10^6/ml.)	Virus growth as % of virus control
None (virus control)	215	100
FPA only	210	98 (100)†
Interferon (1·2 units)	11	5
FPA + Interferon (1·2 units)	70	33 (33)
Interferon (0·6 units)	23	11
FPA + Interferon (0·6 units)	105	49 (50)

* Cells were pretreated with buffer, interferon, FPA (100 μg/ml.) or both interferon and FPA (100 μg/ml.) for 5 h, then washed 3 × and infected with Semliki Forest virus (SFV).

† Figures in parentheses represent virus growth in plates treated with FPA and interferon as a percentage of the growth of virus in plates treated with only FPA.

It was of interest to determine whether the inhibition of interferon action by FPA waned with time. CEF's were therefore treated in the same manner as in the previously described experiment. Immediately after the FPA and interferon had been washed off, one set of plates was infected with SFV (Table 2*A*). Another set was allowed to incubate at 37° C for 20 h with virus growth medium and then infected with SFV (Table 2*A*). On the plates infected immediately after the removal of FPA and interferon the inhibitory effect of the FPA was not completely reversed by washing and addition of phenylalanine. The virus growth in plates treated with interferon was 5 per cent of that of the virus control. In plates treated with both interferon and FPA, the virus growth was 13 per cent of the controls, 25 per cent if the inhibition due to FPA was considered.

On plates kept for 20 h before being infected, the antiviral action of the FPA had been almost completely reversed. The effect of the interferon was somewhat less than in the plates infected immediately. The action of FPA to inhibit the antiviral activity of interferon was not reversed by the prolonged incubation between the removal of FPA and interferon and the infection with SFV, since virus growth was increased to 34 per cent of controls in these samples. These results indicated that FPA inhibition of interferon action was persistent.

The preceding experiments suggested that protein synthesis was required for full interferon action. The following experiment was performed to test whether FPA could inhibit the antiviral activity of interferon after protein synthesis had been allowed to proceed for some time following the addition of interferon. CEF's were treated with interferon for 5 h and then with FPA for 5 h. The results (Table 2*B*) showed again that interferon and FPA together had less inhibitory action than interferon alone. When cells were first incubated with interferon and then with FPA, however, the inhibitory action of interferon was the same as in cells treated with interferon and then with *tris* buffered Gey's salts. These results indicated that once protein synthesis had been allowed to proceed after the

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* U.S. Public Health Service, at present attached to the National Institute for Medical Research.

† One unit of interferon reduced by 50 per cent the number of vaccinia virus plaques formed on CEF.

Table 2. FPA INHIBITION OF INTERFERON ACTION

Pretreatment of cells*	Yield of SFV at 8 h (PFU × 10^6/ml.)	Virus growth as % of virus control
(A) Persistence of FPA inhibition of interferon action		
SFV infection immediately after removing FPA and interferon		
None (virus control)	39	100
FPA only	20	50 (100)†
Interferon (0·6 units)	2·1	5
Interferon (0·6 units) + FPA	5·0	13 (25)
SFV infection 20 h after removing FPA and interferon		
None (virus control)	32	100
FPA only	29	90 (100)
Interferon (0·6 units)	3·9	12
Interferon (0·6 units) + FPA	11	34 (38)
(B) Lack of inhibition of interferon action by FPA added after interferon		
FPA and interferon added simultaneously		
None (virus control)	52	100
FPA only	45	87 (100)
Interferon (0·6 units)	8	15
Interferon (0·6 units) + FPA	16	32 (36)
Interferon added before FPA		
None (virus control)‡	54	100
FPA, then buffer	33	61 (100)
Interferon, then buffer	6·5	12
Interferon, then FPA	4·3	8 (13)

*, †, See footnotes for Table 1.

‡ Cells were pretreated with FPA (100 μg/ml.) or interferon (0·6 units) for 5 h, FPA or interferon washed off, then buffer or FPA (100 μg/ml.) added for 5 h and washed off. Plates were then infected with SFV.

addition of interferon, the antiviral activity of interferon could not be inhibited by FPA.

FPA is incorporated into proteins which in some instances investigated have reduced biological activity[6]. Since no inhibition of RNA synthesis was found at the concentration of FPA used in this work, the inhibition of interferon action observed indicates that synthesis of protein is required for interferon action.

The findings that interferon did not directly inactivate viruses[2], that a period of incubation at 37° was necessary for interferon action[2], and that protein and RNA synthesis[3] are required for interferon action all indicate that metabolic activity is necessary for interferon action. This suggests that either interferon itself is altered intracellularly to express its antiviral action, or the antiviral action is due to an induced change within the cell. As protein synthesis is required for interferon action, the latter would appear to be the simpler explanation of interferon action, though the former is possible. Interferon could be an inducer of a specific RNA which in turn acts as a messenger for the production of an antiviral protein. Interferon does not stimulate interferon production[7] so that interferon itself could not be the antiviral protein. Since interferon inhibits both RNA and DNA viruses, the proposed antiviral protein might act on RNA synthesis. There is indeed some indication that both crude and purified interferon inhibit total cellular RNA synthesis[8].

After the experiments described in this work had been completed, a paper by Lockart was published with conclusions generally in agreement with those reached by us[9]. The experimental evidence presented by Lockart was, however, open to other interpretations than that protein synthesis was necessary for interferon action.

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PROTEIN SYNTHESIS AND TURN-OVER IN CULTURED PLANT TISSUE: SOURCES OF CARBON FOR SYNTHESIS AND THE FATE OF THE PROTEIN BREAKDOWN PRODUCTS*

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A PREVIOUS investigation of metabolism and protein synthesis in highly organized plant cells directed particular attention to the contrast between actively growing carrot cells and those in a more quiescent state[1,2]. It was then found necessary to recognize the complexity of the cells, that is, the existence of distinct compartments in which the same metabolite might undergo different reactions. It was also necessary to distinguish between different sources of carbon for protein synthesis and to involve the carbon of protein after turn-over in the general metabolism of the cell to a greater extent than is usually done. A diagrammatic scheme to express these general ideas was formulated (ref. 1, *loc. cit.* Fig. 1).

Further experiments along these general lines have been made from several points of view. (*a*) To bring new evidence to bear on the sources of carbon for protein synthesis; (*b*) to provide new evidence on protein turn-over in these cells and to show the metabolic fate of the carbon so released; (*c*) to contrast the metabolism of asparagine and glutamine in cultured plant cells.

Because *in toto* the experimental data are extensive, they will be presented here in summary form only. The purpose of this summary is to bring the results obtained into the context of present-day thinking on cellular metabolism. This is of particular interest because of present views on the mechanism of protein synthesis, its genetic determination, and the mechanisms which are at present invoked to link the action of the genes—through amino-acid activation, messenger-RNA, soluble RNA complexes, and the template surface—to the actual process of synthesis. It is also necessary to bring these results into harmony with present-day ideas on the path of carbon in the breakdown of sugar through such events as glycolysis, the Krebs's cycle in its various forms, the pentose pathway, etc. To demonstrate these various metabolic pathways they are best investigated separately, whereas in the cell they occur concurrently; but, although all the enzymes for these different systems may be present concurrently, the machinery of the cell may emphasize one part or another. Therefore, these experiments represent a contribution to the understanding of the metabolism of the whole growing cell regarded as an integrated mechanism.

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The new experiments are of three types which will be described in general terms before the results are summarized. Experiments were performed with explanted tissue from the storage root of the carrot under aseptic conditions. The explants were either in the actively growing state (achieved by the use of coconut milk) or in the virtually non-growing state (using the basal medium without coconut milk). These experiments were performed after the explants had become adjusted to these conditions for a period of 8–10 days. In addition, some experiments have been made with freely-suspended, cultured, cells derived from carrot root, potato tuber, tobacco stem, peanut cotyledon and *Haplopappus* shoot. These cells were grown and cultivated by methods which simulate those commonly in use for micro-organisms[3,4].

Summarized Description of the Experiments and Methods

(*A*) *Experiments which bear on the sources of carbon for protein synthesis and its entry into protein from the soluble compounds of the cell. Exp. A*1. The plan of this experiment was simple and direct. The carrot explants were supplied with radioactive sucrose as the major carbon source, either with or without a general non-radioactive source of amino-acids in the form of casein hydrolysate. The objective was to ascertain whether the casein hydrolysate would interfere with the movement of carbon from sugar into the protein, perhaps by furnishing, pre-formed, the obvious amino-acid precursors of protein.

*Exp. A*2. In this experiment 8 parallel samples of tissue explants were exposed to a medium containing sucrose and 7 different amino-acids, but in any one treatment only one of these compounds was radioactive. This design permits one to detect the accessibility of each of these exogenous compounds to the stored soluble compounds of the cell and to the protein.

*Exp. A*3. In this experiment the tissue was furnished with specifically labelled substrates (amino-acids or sugars) to see whether the carbon entered into the amino-acids of the protein by incorporating certain molecules intact or only after their prior metabolism in the soluble phases of the cell.

(*B*) *Experiments which relate to the turn-over of protein and to the fate of the carbon so released. Exp. B*1. In this experiment the carrot tissue was pre-grown for seven days and then treated with ^{14}C-sucrose for another seven days, in order to label the protein heavily. The ^{14}C-substrate was then replaced in the external medium with ^{12}C-sugar, and the subsequent fate of the ^{14}C-labelled soluble compounds and protein amino-acids was determined at five successive intervals during a further period of one week. The thought here was that the soluble pools would rapidly lose their radioactivity, and the fate of any carbon-14 from the radioactive proteins should be detectable. In this way it should be possible to determine the respective contribution to respiration of carbon drawn from the soluble compounds and from the proteins.

(*C*) *Experiments on the respective roles of sucrose, glutamine and asparagine in protein synthesis and respiration. Exps. C*1 *and C*2. In both these experiments ^{14}C-glucose, ^{14}C-glutamine or ^{14}C-asparagine were separately furnished to the tissue. In experiment *C*1 a short pulse of very high specific activity substrate was supplied, and the subsequent progress of the carbon-14 was followed during the metabolism of the cells. The carbon-14 labelled sucrose and glutamine of especially high specific activity were prepared by one of us (R. G. S. B.) by methods developed for the purpose. In experiment *C*2 the carbon-14-labelled source was continuously available to the tissue but at a much lower specific activity. In these experiments, rapidly growing tissue explants, slowly or non-growing tissue explants, and rapidly growing but freely suspended cells from various plants were used.

Methods and Analytical Techniques

In these experiments the compounds to be analysed, whether in the alcohol-soluble fractions of the cells or in the hydrolysates of protein, were first separated by two-directional paper chromatography and their radioactivity was detected by autoradiography. The important compounds were then isolated from the paper, analysed, and their radioactivity determined for the calculation of the specific activity of the carbon. Where necessary, results obtained by the quantitative paper chromatographic methods were compared with, and checked by, data obtained with an automatic Beckman/Spinco amino-acid analyser in series with an automatic recording scintillation counter. The respired carbon dioxide was collected, and its radioactivity determined, after acidification, in an electrometer. All these techniques have been described[2,5-8] and shown to be sufficiently accurate for the purposes of these experiments. It would not be appropriate, however, to present the full details here.

All these experiments were carried out in the dark, in order to avoid the complications of light fixation of

Table 1. Exp. *A*2. Amounts of radioactive carbon (as μg of carbon per 20 explants) acquired by the free (*F*) or protein (*P*) amino-acids during 23 h from various substrates supplied to rapidly growing carrot explants

Compound analysed free (*F*) or protein (*P*) amino-acid	Component of the medium which was radioactive									
	Sucrose		Glutamic acid		Serine		Alanine		Valine	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Aspartic acid	6·7	2·9	1·9	0·46	2·2	0	0	3·1	0	0
Glutamic acid	17·3	Some	11	2·7	0	0	0	11	0	0
Serine	27	9·3	0	0	30	8·0	20	0	0	0
Threonine	0	1·3	0	0	0	0	0	1·6	0	0
Alanine	66	2·6	0	0	0	0	134	11	0	0
Valine	0	4·0	0	0	3·2	0	0	1·8	60	1·0

Interpretation: Note the lack of relationship between the amount of carbon from various sources which entered the free, as compared with the protein, amino-acids. For example, serine supplied carbon for free but not for protein aspartic acid. Alanine, on the other hand, labelled protein aspartic acid but not the free substance. Other similar examples may be seen. Sucrose frequently supplied more carbon to the protein amino-acids than did the corresponding free amino-acids. Also note that alanine tended to supply carbon to a number of the amino-acids and preferentially entered the protein.

Table 2. Exp. *A*3. Radioactivity (mμc. per 20 explants) in free (*F*) and protein (*P*) amino-acids and in the respired carbon dioxide of rapidly growing (plus coconut milk) or slowly growing (no coconut milk) carrot explants, after the supply of ^{14}C-labelled substrates for 24 h

Compound analysed free (*F*) or protein (*P*) amino-acid or CO_2	^{14}C-Substrate supplied and specific activity of the labelled carbon atoms											
	Glutamic acid-1-^{14}C 258 μc./mg C				Glutamic acid-3,4-^{14}C 129 μc./mg C				Glucose-UL-^{14}C 12·5 μc./mg C			
	Fast growing		Slow growing		Fast growing		Slow growing		Fast growing		Slow growing	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Glutamic acid	159·4	13·2	24·3	0	358	89·4	47·3	11·4	30·0	13·1	45·8	7·6
Aspartic acid	0	0	0	0	542	13·6	3·8	9·4	21·4	6·6	6·8	6·9
Serine	0	0	0	0	0	0	2·3	0	24·6	11·6	12·0	4·9
Alanine	0	0	0	0	29·8	0	8·3	0	75·0	13·9	31·5	4·1
CO_2	1,164		930		100				936		890	

Interpretation: Note the greater radioactivity of protein amino-acids and the decreased radioactivity of the CO_2 when glutamic acid-3,4-^{14}C was supplied compared with glutamic acid-1-^{14}C. This shows that a major portion of supplied glutamic acid only enters protein after decarboxylation and reconstitution. Also note that glucose-^{14}C generously labelled the protein amino-acids (although its specific radioactivity was very much lower than that of the glutamic acid). Little difference was observed between the results from glucose-UL-^{14}C, as here recorded, and those for glucose labelled in the 1, 2 or 6 position; this shows that all these carbons of glucose are equally accessible for protein synthesis. It is noteworthy that free aspartic acid acquired about 25 times as much radioactivity from glutamic acid 3,4-^{14}C as from glucose-^{14}C yet the resultant protein aspartic acid was only twice as radioactive.

Table 3. Exp. *A*1. Specific activities of carbon in the soluble and the insoluble compounds and the CO_2 of rapidly growing (plus coconut milk) and slowly growing (no coconut milk) explants after 24 h of contact with sucrose-UL-^{14}C (sp. act. of carbon, 337 mμc./mμmol) with or without the addition of 2 per cent casein hydrolysate to the medium. Results as μc. per millimol of carbon

	Rapidly growing explants (plus coconut milk)				Slowly growing explants (no coconut milk)			
	– Casein		+ Casein		– Casein		+ Casein	
Analysed compound	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.
Sucrose	6·73	–	6·66	–	5·38	–	+	–
Asparagine	14·2	–	7·6	–	12·56	–	+	–
Glutamine	13·8	–	11·4	–	9·02	–	7·58	–
Aspartic acid	+	6·62	+	8·29	+	6·47	+	12·2
Glutamic acid	31·9	6·12	28·0	6·97	23·8	5·51	+	11·8
Serine	0	5·88	0	5·99	0	0	0	7·58
Glycine	0	6·64	–	8·88	0	0	0	10·7
Alanine	3·76	8·1	2·70	7·40	5·83	4·79	+	7·91
Valine	0	0	0	5·82	0	5·17	0	14·3
Leucine	0	2·88	0	3·15	0	0	0	2·96
Phenylalanine	–	4·97	–	5·55	0	0	–	15·4
CO_2 collected 0–6 h	7·45	–	9·09	–	7·33	–	7·88	–
CO_2 collected 18–24 h	50·1	–	51·8	–	44·3	–	49·6	–

Interpretation: The casein hydrolysate caused no change in the specific activity of the soluble sugar in the cells, but it depressed that of the free amino-acids in the tissue; in spite of this it caused an increase in the specific activity of protein amino-acids and respired CO_2. This effect was more noticeable in the slowly growing tissue because of the much smaller amounts of carbon present in these explants. The casein hydrolysate supplied nitrogen and thereby caused a greater conversion of carbon from sugar into protein amino-acids. Note that alanine in the fast-growing tissue is the only exception to this rule.

$^{14}CO_2$. Experiments showed that dark fixation of carbon dioxide, although it occurred, was very low, and the products did not interfere with the interpretations which have been drawn.

In these experiments all the identifiable sugars and ninhydrin reactive compounds were analysed in the manner described. Records were made of their total carbon and their total radioactivity; the specific activity of their carbon was then calculated. The radioactivity of other carbon compounds (organic acids, peptides, phosphorylated compounds, etc.) was also measured. The movement of carbon to and fro within the soluble pool, and into and out of the protein, was calculated from data obtained on tissue samples taken in time sequence. The data selected to illustrate this article are, therefore, part of a large body of consistent information which is not presented here in full.

Experimental Results and their Interpretation

(*A*) *Evidence on the various sources of carbon and its entry into protein.* The selected results from experiments *A*1–3 are presented in Tables 1–3, and the special points to be noted are enumerated in their legends. The cumulative conclusions to be drawn from these three types of experiments (*A*1–3) are the following:

(i) Sugar from an exogenous source proved to be a more direct source of carbon for the amino-acids in protein than either exogenously supplied amino-acids or the internal pools of stored amino-acids in the cells (Tables 1 and 2).

(ii) Externally supplied amino-acids (that is, in the form of casein hydrolysate) tended to increase the use of ^{14}C-carbon from sugar in protein synthesis. This is because the amino-acids provided a source of nitrogen, but not necessarily of carbon, which increased the overall pace of metabolism and the demand for carbon for protein synthesis (Table 3).

(iii) Although carbon from free glutamic acid can appear in protein, both as glutamic and as other amino-acids, only a small fraction enters the protein directly; a large part of its carbon framework needs to be re-worked in metabolism. The α-carboxyl of glutamic acid largely comes off as carbon dioxide, and the remaining carbon enters the metabolic pool to form carbon acceptors for nitrogen prior to protein synthesis (Table 2).

(iv) The results in Tables 1–3 and the conclusions drawn from the aforementioned (i–iii) require that there are isolated and protected pools of amino-acids in the cell. These are very closely connected with sites of protein synthesis, but they are separate and remote from the pools of the stored amino-acids.

These concepts can be illustrated in the form of a diagram (Fig. 1) the salient features of which are:

(1) The main source of the carbon for protein synthesis is sugar.

(2) The amino-acids which are *en route* to protein and which derive their carbon from sugar are protected or isolated from the storage pools of amino-acids.

(3) Of the amino-acids investigated only alanine is able to bridge these two pools with ease.

(4) Whereas the nitrogen for protein synthesis can be drawn either from external sources or the stored amino-acids the carbon of the stored amino-acids is not so readily used.

(5) From evidence previously reported[1,2] and to be summarized in sections (*B*) and (*C*) which follow it is necessary to postulate some protein breakdown and turnover and that the amino-acids of breakdown contribute to the storage pool and supply carbon for respiration and the evolution of carbon dioxide.

Thus the conclusions *A* i–iv summarized in Fig. 1 are compatible with concepts which emerged from earlier experiments[1,2], with other experiments on leaves[8,9,10], and also with the results from the other experiments, now to be described, in sections (*B*) and (*C*).

(*B*) *Evidence for protein turn-over and the fate of the carbon so released.* The broad conclusions from these experiments are as follows:

(i) The data require that most of the amino-acids of protein must, of necessity, be subject to turn-over. The following type situations which were encountered and which demand this conclusion are:

(*a*) Where the total protein remained approximately constant but its radioactivity changed (Table 4); (*b*) where, during growth, the total protein increased but its radioactivity concurrently decreased (Table 5); (*c*) where the protein remained constant, or increased only slightly, although there was a large increase in its radioactivity which could not be accounted for by a net gain of protein

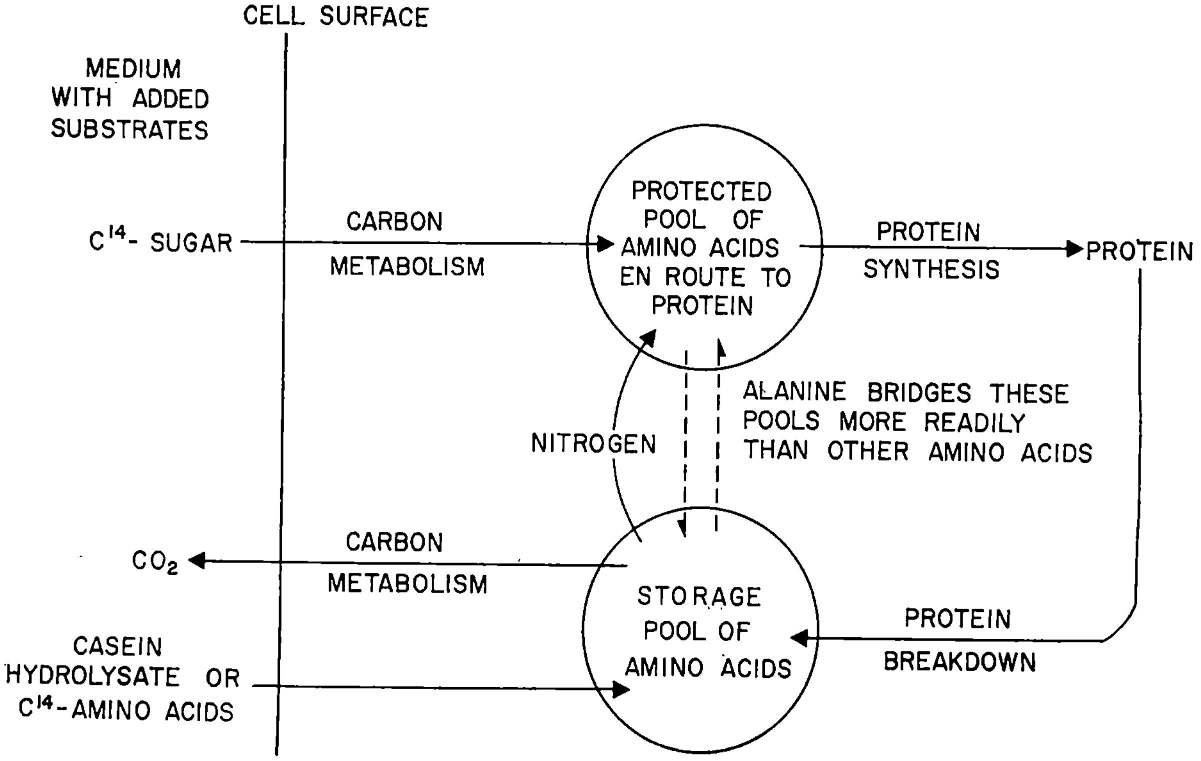
CELL SURFACE
MEDIUM WITH ADDED SUBSTRATES
C14- SUGAR
CARBON
METABOLISM
PROTECTED POOL OF AMINO ACIDS EN ROUTE TO PROTEIN
PROTEIN
SYNTHESIS
PROTEIN
ALANINE BRIDGES THESE POOLS MORE READILY THAN OTHER AMINO ACIDS
NITROGEN
CO2
CARBON
METABOLISM
STORAGE POOL OF AMINO ACIDS
PROTEIN
BREAKDOWN
CASEIN HYDROLYSATE OR C14-AMINO ACIDS

Fig. 1. The relationships of exogenous sugars and amino-acids to soluble pools in the cell and to protein synthesis

from an obvious radioactive source (because no source of a sufficiently high specific activity was available). It is necessary, in these circumstances, to assume that a protein moiety of low radioactivity was replaced by new protein containing more radioactivity than that which was degraded. This situation (Table 4) is, therefore, a special case of that shown at (*a*).

(ii) It is possible to calculate minimum turn-over rates of protein amino-acids. These are of the general order of 0·2–2·0 per cent per h of the total carbon in the protein; Table 6, however, measures the turn-over of protein carbon in absolute units as affected by the addition of coconut milk. Clearly the coconut milk growth-promoting stimulus also promotes the turn-over.

Table 4. Exp. *B*1. Total carbon (μmols) and total radioactivity (mμc.) and specific activity (mμc./μmol) of carbon in protein glutamic acid of slowly growing carrot explants (basal medium without coconut milk). ^{14}C-sugar was supplied for one week previous to Time 1, ^{12}C-sugar after Time 1. All the data refer to 20 explants

	Time 1 (0 h)	Time 3 (36 h)	Change, Time 1–3
Total carbon	2·94	2·95	+0·01
Total radioactivity	82	97	+15
Specific activity	27·9	32·9	

Interpretation: The specific activity of soluble sugar during Time 1–3 was 29·8 mμc./μmols carbon. The total activity which could have been introduced into protein glutamic acid from sugars by the observed increase of carbon was 0·3 mμc. (0·01 × 29·8) compared with the observed increase of 15 mμc. Therefore, there must have been a loss by turn-over of non-radioactive carbon from the protein to cause the observed increase in specific activity. This kind of result is characteristic of most of the protein amino-acids of slowly growing explants over the several time-intervals during which measurements were made.

Table 5. Exp. *B*1. Total carbon (μmols), total radioactivity (mμc.) and specific activity of the carbon (mμc./μmols) in protein aspartic acid of rapidly growing explants (basal medium plus coconut milk). ^{14}C-sugar was supplied for one week before Time 1, ^{12}C-sugar after Time 1. All the data refer to 20 explants

	Time 1 (0 h)	Time 2 (12 h)	Change, Time 1–2
Total carbon	14·8	17·3	+2·5
Total radioactivity	231	195	−36
Specific activity	15·6	11·3	

Interpretation: Since the total radioactivity in the form of protein aspartic acid decreased by 36 mμc. during the period of observation while the total amount of protein aspartic acid increased by 2·5 μmol of carbon, it is obvious that radioactive carbon in this protein amino-acid must have been replaced as a result of turn-over by carbon of lower specific radioactivity. This kind of result is characteristic of most of the protein amino-acids in rapidly growing explants over the several time-intervals during which measurements were made.

Table 6. Exp. *B*1. Minimum turn-over as μg of rates carbon per 100 explants per day, of protein amino-acids in rapidly growing (plus coconut milk) or slowly growing (no coconut milk) carrot explants, calculated from data of the type shown in Tables 4 and 5

Amino-acid analysed	Turn-over rates of rapidly growing explants between times specified (h)				Turn-over rates of slowly growing explants between times specified (h)			
	0–12	12–36	36–96	96–128	0–12	12–36	36–96	96–128
Aspartic acid	26·7	7·1	10·5	0	–	1·4*	0·85	3·1
Glutamic acid	4·1	0·50	30·1	17·4	1·6*		0·92	–
Alanine	0	3·3	9·0	8·7	0·76*	0·50*	2·1	0·08
Serine	–	0	5·0	0	0	0·39*	0·19	–
Glycine	–	33·4	5·6	2·6*	1·3*	0·60*	0·37	–
Threonine	9·3	11·9	5·5	3·3	0·94*	1·06	0·38	–
Valine	0	28·3	0·98	5·1	5·4*	1·01	0	0·26
Leucine	–	9·6	7·6	2·8	3·4*	0	0·40	–
Isoleucine	–	31·9	0	7·7	3·2*	0·73	0	–
Tyrosine	–	5·5†		1·2	–	–	–	–
Phenylalanine	–	21·6	1·7*	6·5	2·1*	1·04*	0·44	–
Proline	–	3·5	0	0	1·7*	3·2*	0·17	–
Lysine	4·9	–	4·0	7·1	0	1·1*	0	1·0
Arginine	0		9·2		3·2*	0	0·14	0·33

* Figures derived using equation (2) below. All others derived using equation (1) below.

† Figures between columns were derived from data extending over two time intervals.

Interpretation: These rates were calculated by the method of Hellebust and Bidwell (ref. 6) derived from that of the earlier work (ref. 2) as follows: When turn-over of a protein amino-acid occurs, the observed change in total activity, ΔA, will differ from what would be expected if no turn-over took place, $\Delta C.SA_s$ (change in carbon times specific activity of source carbon), by the amount of activity added or removed by turn-over, $CT\ (SA_s - SA_p)$, where CT is the amount of carbon turned over and SA_p is the specific activity of carbon in the protein amino-acid and SA_s that of the free substance. Thus:

$$\Delta A - \Delta C\,.\,SA_s = CT(SA_s - SA_p) \text{ and}$$

$$CT = \frac{\Delta A - \Delta C\,.\,SA_s}{SA_s - SA_p} \qquad (1)$$

In situations where negative values would be obtained from this formula, as when $\Delta C.SA_s$ was less than ΔA, and SA_p was greater than SA_s, the data require that some protein of low radioactivity was breaking down and being replaced by more strongly radioactive protein. In these circumstances, SA_p approaches zero, and the formula becomes:

$$CT = \frac{\Delta A - C\,.\,SA_s}{SA_s} \qquad (2)$$

These expressions determine the amount of carbon turn-over necessary to account for the observed changes in carbon and activity which occurred. Various values for SA_s are possible; so the value used is the one which gave a minimum figure for turn-over. Equation 2 was used (*) if the data required that carbon leaving proteins did so from a fraction of lower specific activity than the whole. In this case SA_p was assumed to have a value of 0 to give minimum turn-over rates.

It should be stressed that these calculated values for turn-over are minimal; the data permit much higher rates. The turn-over rates recorded here represent values of 0·02–2·0 per cent of the total protein amino-acid per hour, or 0·5–50 per cent per day. Note the greatly increased turn-over rates in rapidly growing as compared with slowly growing explants.

(iii) It is concluded that the breakdown products that result from protein turn-over contribute directly to respiration (Tables 7 and 8) and may account for as much as 27 per cent of the total carbon dioxide which is produced (Table 9).

Table 7. Exp. *B*1. Specific activities (mμc./μmol) of carbon in soluble sugars, protein amino-acids (average), and respired CO_2 of rapidly growing (plus coconut milk) carrot explants at various times following the replacement of ^{14}C-sugar by ^{12}C-sugar

Specific activity of:	Time after removal from ^{14}C sugar 0–12 h	12–36 h	36–96 h	96 h	168 h
Soluble sugars	16·1	10·7	7·8	6·2	3·2
Protein amino-acids	15·2	10·7	7·4	6·9	5·2
Respired CO_2	15·8	11·2	9·1	7·4	5·6

Interpretation: The specific activity of CO_2 was initially below that of the soluble sugars in the tissue, but progressively it rose above this value. This could be brought about by the utilization of a source of carbon for respiration, in addition to the sugars, which in the later time periods had a higher specific activity than sugars, that is, the proteins. The average specific activity of the protein amino-acids was probably slightly below the true value for those protein amino-acids which turned over rapidly because of the presence of some more stable protein of lower specific activity. Another effect of protein turn-over, that is, the decrease in specific radioactivity of proteins with time, is clearly to be seen.

Table 8. Exp. *B*1. Total carbon (μmols per 20 explants) and specific activity of carbon (mμc./μmol) of certain free and protein-bound amino-acids in rapidly growing (plus coconut milk) carrot explants. All data refer to 20 explants

Compound analysed	Time after removal of ^{14}C-sugars 0 h	12 h	36 h	96 h	168 h
Free glutamic acid,					
Carbon	6·75	8·13	7·46	5·10	6·65
Sp. act.	27	33	30	26	19
Free glutamine,					
Carbon	7·87	10·1	–	10·1	9·3
Sp. act.	23·0	30·2	–	30·2	21·0
Protein glutamic acid,					
Sp. act.	15·0	11·9	9·0	5·0	3·3
Free alanine,					
Carbon	26·7	30·6	31·4	32·9	44·3
Sp. act.	19·4	15·5	10·6	8·5	6·6
Protein alanine,					
Sp. act.	17·8	16·4	11·5	8·0	5·3

Interpretation: Note that the total quantity and specific activity of both glutamic acid and glutamine did not change much, while the specific activity of protein glutamic acid decreased greatly with time, largely due to turn-over. Thus the glutamic acid released from proteins by turn-over (having a lower specific activity) did not enter the soluble pool of glutamic acid and glutamine, but must have been converted to CO_2. The specific activity of the soluble alanine pool, on the other hand, closely followed that of protein alanine, showing that they were in equilibrium. The alanine which left the protein could not, however, be freely available for re-synthesis of protein, otherwise turn-over values of 0 would be obtained. Since this is not so (see Table 6), there must have been some preferential conversion of alanine from protein into CO_2.

Table 9. Exp. *B*1. The contribution of carbon derived from protein turn-over to respired CO_2 in rapidly growing (plus coconut milk) or slowly growing (no coconut milk) carrot explants during 12–36 h after replacing ^{14}C-sugar by ^{12}C-sugar in the medium. All results on the basis of 20 explants

	Fast growing	Slow growing
Total protein turn-over*, μg C/h	1·37	0·093
Total respired CO_2, μg C/h	5·0	1·3
Percentage of respired carbon derived from protein turn-over	27	7·2

* Derived from results of the type shown in Table 6.

Interpretation: A substantial percentage of the carbon of respired carbon dioxide was derived from the products of protein turn-over and this was increased by treatment with coconut milk.

(iv) The fates of alanine and glutamic acid after they are released by protein breakdown are different. The alanine passes through a homogeneous soluble pool *en route* to carbon dioxide without any evidence of its protracted storage. The behaviour of the carbon of alanine thus resembles that of sugar much more closely than that of other amino-acids. This alanine pool may well be replenished from sugar and, from the results of experiments *A*1–3 (Tables 1–3), the cellular pools of alanine may well be in dynamic equilibrium with exogenous alanine. By contrast, however, it is necessary to assume that the glutamic acid from protein by breakdown yields carbon dioxide directly, without passing through the main storage pool of glutamic acid which is in equilibrium with glutamine (Table 8).

These conclusions (i–iv) may be illustrated in a diagram (Fig. 2*a* and *b*) and its salient features are as follows:

(1) The amino-acids of the protein are in a state of turn-over.

(2) Carbon released from protein by turn-over contributes to respiration and is evolved as carbon dioxide.

(3) Carbon for the re-synthesis of protein in turn-over is supplied by sugar, the nitrogen being re-cycled.

(4) Glutamic acid released from protein enters a distinct and isolated pool *en route* to carbon dioxide and does not mix with storage glutamic acid or glutamine. By contrast, alanine released from protein mixes freely with the main soluble pool of alanine and also contributes its carbon to a variety of other compounds including carbon dioxide.

(*C*) *Evidence on the respective roles of sucrose, glutamine and asparagine in metabolism, protein synthesis and respiration.* The main conclusions which emerged from these experiments are summarized as follows:

(i) As shown previously, ^{14}C-sugar again labelled the amino-acids of the protein both quickly and generally (Table 10).

(ii) The carbon of exogenously supplied glutamine behaved in the same general way as sugar, and was much more effective than glutamic acid in furnishing carbon to the amino-acids of protein (Table 11; cf. Tables 1 and 2). In this way exogenous glutamine behaved very differently from the storage pool of glutamine which resulted from protein breakdown and to which previous reference has been made (Section *B*, Table 8) and which is less prone to act as a source of carbon for re-synthesis (cf. ref. 2).

Table 10. Exp. *C*1. Amounts of radioactivity (mμc. per sample) in protein amino-acids of slowly growing explants (no coconut milk), rapidly growing explants (plus coconut milk) and cultured cells of carrot, following 4 h of exposure to ^{14}C-sucrose of very high specific activity. (Specific activity of supplied sucrose, 450 mc./millimol). (Samples consisted of 10 slowly growing explants, 5 fast growing explants or 100 mg of free cells)

Compound analysed	Tissue type	Time in hours, after removal of ^{14}C-substrate 0	8	20	44
Aspartic acid	Slow growing	0	3·1	3·9	5·0
	Fast growing	–	6·8	11·4	10·0
	Free cells	2·03	5·25	3·48	2·90
Glutamic acid	Slow growing	1·5	5·0	9·7	17·0
	Fast growing	–	16·8	22·2	16·0
	Free cells	5·51	1·75	0·58	0
Alanine	Slow growing	0	0	0	2·5
	Fast growing	–	1·1	15·6	11·2
	Free cells	0	+	4·64	0
Valine	Slow growing	0	0	0	0
	Fast growing	–	19·4	21·0	13·0
	Free cells	0	+	7·25	2·90

Interpretation: The proteins of the slowly growing explants acquired radioactivity slowly and continuously throughout the experiment. The proteins of fast growing explants first acquired radioactivity, then lost it, showing that rapid turn-over of the proteins was taking place. The behaviour of cultured cells was similar to that of rapidly growing explants, but was greatly accentuated.

(iii) Asparagine behaved very differently from glutamine. Although it reached high concentrations in the soluble fraction of the cells, it contributed its carbon almost exclusively to aspartic acid in the protein. Other protein amino-acids acquired small amounts of carbon-14 from asparagine, but only after long exposure. Therefore, it is very clear that asparagine and glutamine stand in quite different relationships to protein synthesis in these cells (cf. Table 11).

(iv) These experiments also furnish supplementary evidence for protein turn-over in growing tissue explants and especially so in free cells, whereas turn-over in the non-growing explants took place only slowly, if at all (Table 10).

Concluding Remarks

The major general points to be emphasized are as follows.

Protein turn-over has again been demonstrated to be a general feature of the intermediary metabolism of plant cells during their growth, but it is not confined to cells in active proliferation and growth, for it also occurs in cells in a more quiescent, non-growing state. The evidence for protein turn-over, at minimum rates of the order of 0·2–2·0 per cent per h, seems unequivocal. An alternative interpretation that might have been raised can be dismissed. This is that the protein breakdown occurs in different cells from the protein synthesis. This would

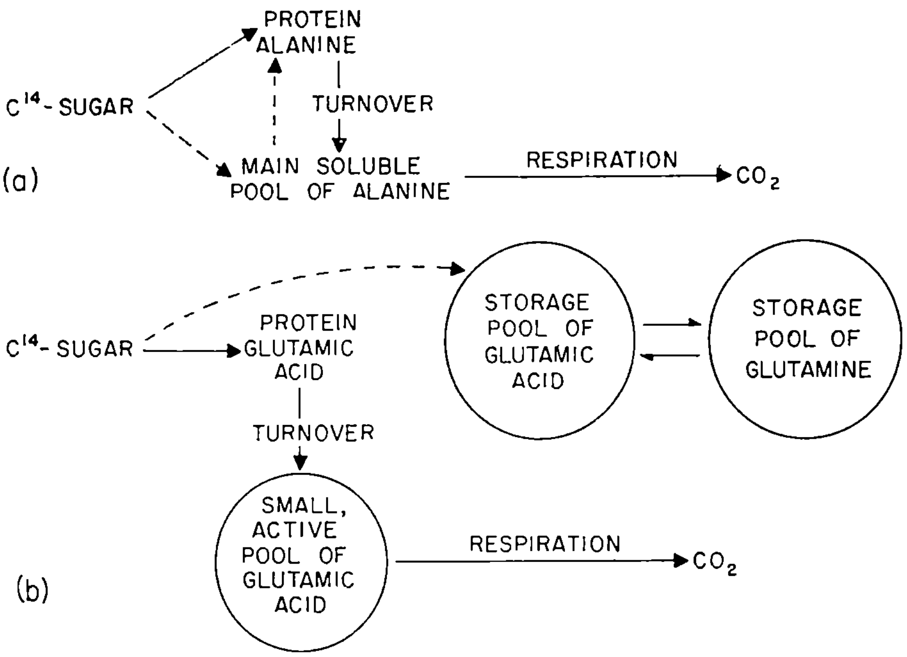

Fig. 2. The relationships of endogenous alanine (*a*) and endogenous glutamic acid and glutamine (*b*) to soluble pools and to protein turn-over and respiration. Distinct and immiscible pools are shown encircled. Dotted lines indicate leakage or minor pathways

constitute protein turn-over within the tissue mass though not in individual cells. However, this would imply the irreversible change, senescence, or death of cells at a minimum rate of 0·2–2·0 per cent per h. This does not occur in the slowly growing explants on basal medium, which do exhibit turn-over, and certainly not in the rapidly growing explants in the exponential phase of their growth. Therefore, neither simple accretion of new protein during growth, nor irreversible loss of old protein during senescence and death of some cells, can account for the results. Cyclical protein turn-over, that is, breakdown and re-synthesis, is the only possible explanation.

It is again found that the carbon of protein, via turn-over, becomes much more involved in the processes of respiration than is usually considered. Sharp distinctions are again made between sugar as the most effective source of carbon for protein synthesis in the intact cells and the stored, free amino-acids as the most obvious source.

Sugar, which either enters the cells exogenously or is locally produced, is metabolized and thereby produces carbon skeletons for the formation of the amino-acids out of which protein is built. The nitrogen either comes from nitrate reduction or ammonia or is donated by nitrogen-rich compounds. The amino-acids used to build protein are, however, strictly localized and do not mix with other pools in the cell. Protein synthesis, as it is at present conceived, proceeds from these protected pools of amino-acids which are derived from sugar in this way. The breakdown products from protein turn-over, some of which are re-worked to form the nitrogen-rich storage products, enter separate pools or phases from the amino-acids which are *en route* to protein. Much of the carbon of these stored amino-acids (which do not build up conspicuously during growth) is eventually converted to carbon dioxide during respiration and may account for more than 25 per cent of the total carbon dioxide, whereas their nitrogen becomes re-available for protein synthesis (ref. 1, *loc. cit.* Fig. 1). Within this general scheme different behavioural patterns of individual amino-acids have been encountered, such as the contrasted behaviour of alanine and glutamic acid and its amide glutamine as shown in Fig. 2 and the sharply contrasted roles of asparagine and glutamine as shown in Table 11.

In all these respects, therefore, it is necessary to add to conventional ideas of the paths of carbon in respiration and the mechanisms of protein synthesis some further concepts which involve the organization of the cells. The localization of given intermediates in special compartments in the cells accounts for their distinctive roles in metabolism. The many interactions between distinct parts of the cells here postulated and between different metabolic processes now help us to account for the difficulties which have so often been encountered in reproducing some of the major synthetic attributes of intact cells in cell-free systems. This has been especially notable in the case of protein synthesis. Cell-free systems by their very nature can include only a part of the machinery of the intact cell, so that any essential features which depend on interactions between its parts are obviously lacking. The localization of function and of segregated pools of metabolites in distinct compartments or organelles obviously provides for 'division of labour' within the cell; it also provides for the degree of complexity required of the organized growing cell, but it also involves a need to bring these diverse activities under co-ordinated and regulatory control.

It is, therefore, now necessary to relate modern concepts of metabolism, as conceived in terms of gene-controlled enzymes, to the organization of the cells within which the reactions occur and by which they are modulated. Present views on molecular biology may go far to reveal the individual reactions of cells and the way these capacities are inherited. Much more than this is, however, necessary to understand how cells work as integrated metabolic

Table 11. Exp. *C*2. Amounts of radioactivity, as mμc. per 5 explants, which entered the protein amino-acids of rapidly growing (plus coconut milk) carrot explants during 48 h from exogenous ^{14}C-labelled sucrose, glutamine or asparagine. (Specific activity of carbon in sources, 0·66 *μ*c./*μ*mol)

Compound analysed	^{14}C-substrate supplied Sucrose	Glutamine	Asparagine
Aspartic acid	40	32·4	40·8
Glutamic acid	144	33·6	4·0
Serine	40	12·0	2·0
Glycine	8	24·0	2·4
Alanine	56	0	2·8
Valine	56	0	0
Leucine	84	16·0	0
Phenylalanine	60	0	0
Proline	40	22·8	0
Threonine	0	13·8	0

Interpretation: Note the relative ease with which sucrose and glutamine label a wide range of protein amino-acids in comparison with the weak labelling from asparagine, except in aspartic acid. Similar results were obtained for different time intervals and a variety of other tissues and cultured cells. Whereas alanine and phenylalanine in the protein were readily labelled from sugar, they were not labelled by glutamine.

machines, and to show how, during active growth or steady states, their metabolism is brought and kept under regulatory control. These major problems of cellular organization need to be solved to bridge the gap between our knowledge of molecules and reactions in homogeneous systems and of the heterogeneity which is necessary for life. In doing this, a place must be found for the regulatory role of stable substances, not of nuclear origin, which may even be supplied exogenously to the plant cells within which they can control the pace of growth and protein synthesis.

Though the very recent article on "What is Heredity?" by Dr. Dean and Sir Cyril Hinshelwood[11] can only be cited, it contains many penetrating observations on the role of structural organization and cytoplasmic architecture that seem relevant even to the very different kind of system which is here discussed.

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A LEUCINE AMINOPEPTIDASE POLYMORPHISM IN *Drosophila buskii*

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THROUGH the use of the starch-gel zymogram technique[1,2] a wide variety of electrophoretic enzyme variations have been described in recent years. In *Drosophila melanogaster* electrophoretic variations in the esterases have been found by Wright[3] and by Beckman and Johnson[4]. These variations were found to be controlled by two independent loci on the third chromosome. Three different phenotypes can be observed for each of the esterase systems: *F/F* (a fast-moving component), *S/S* (a slow-moving component) and *F/S* (the heterozygous type with both fast- and slow-moving components). Crossing experiments suggest that about 20 recombination units separate the two esterase loci.

Another biochemical variation in *Drosophila melanogaster* is the leucine aminopeptidase (Lap) system described by Beckman and Johnson[5]. Leucine aminopeptidase is operationally defined as an enzyme that hydrolyses L-leucyl-β-naphthylamide. In the work done by Beckman and Johnson, two independent Lap systems were found and were observed to be controlled by two closely linked genes. Not a single case of recombination was found in their investigation.

This preliminary report is also concerned with the enzyme leucine aminopeptidase but in a relative of *Drosophila melanogaster*, *Drosophila buskii*.

The stocks of *D. buskii* used in this investigation were obtained from Dr. M. Wheeler of the University of Texas, who is at present engaged with the *Drosophila* research group in the University of Hawaii. The flies were cultured on a medium containing the following ingredients: water, 1,000 ml.; agar, 10 g; banana, 300 g or 400 ml.; yeast, 50 g; cornmeal, 30 g; propionic acid, 5 ml.; ethanol, 5 ml.

These flies were originally collected from the Tantalus area in Honolulu, Hawaii, during July–August 1963. The cultures were started from relatively small numbers of individuals, but were mass transferred when fresh medium was required. Approximately 22 generations had elapsed between collection and examination for leucine aminopeptidase.

Single *Drosophila* individuals (larvæ, pupæ or adults) were placed on a microscope slide and homogenized with a lancet in a small drop (approximately 0·02 ml.) of distilled water. The homogenate was absorbed into a 4 mm × 6 mm piece of filter paper and inserted into a vertical slot in the starch gel. In order to prevent streaking from cell fragments in the electrophoresis, a small piece of thin paper (single layer of 'Kleenex Celluwipes', Kimberley-Clark Corp.) was first placed over the drop of homogenate to act as a filter.

The discontinuous buffer system of Poulik[6] was used and the electrophoresis was carried out for 2·5 h or until the brown borate front zone had migrated 7 cm past the sample slot. A voltage gradient of 6–8 V/cm at room temperature was used.

On completion of the electrophoresis the gels were sliced horizontally and one-half stained for 2 h in a solution consisting of 40 mg L-leucyl-β-naphthylamide, 50 mg Black K salt and 100 ml. 0·2 *tris*maleate buffer, *p*H 5·2. The other half of the gel was stained with a non-specific protein dye, amido black. After staining, the gels were fixed and preserved in a solution of acetic acid, methanol and water (1 : 5 : 5).

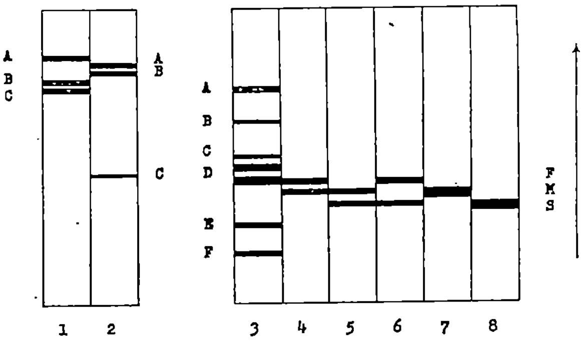

Fig. 1. Diagrammatic comparison of isozyme variations in *D. melanogaster* and *D. buskii*: Sample (1), major protein zones of *D. melanogaster* as revealed by amido black staining; (2), major protein zones of *D. buskii*; (3), lap zones of *D. melanogaster* (a heterozygote for the D zone is shown); (4–8), some different lap types of *D. buskii*. The arrow shows the direction of migration

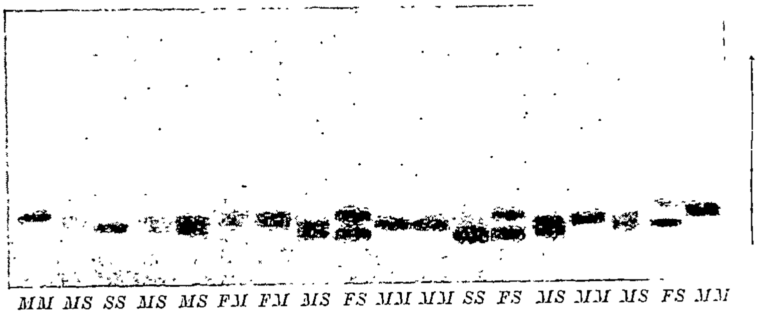

Fig. 2. Photograph of typical electrophoretic run showing some of the various Lap types in *D. buskii*. Genotypes are below each sample. The arrow indicates the direction of migration

When single individuals are run in the electrophoresis, a number of different[1] Lap patterns are observed (Figs. 1 and 2). Either a single band or two bands may be present in any of three different positions in the starch gel. These three positions are designated *F* for the fastest band, *S* for the slowest band, and *M* for the band of intermediate mobility. Preliminary experiments indicate that individuals with only a slow band are homozygous (Lap^S/Lap^S). Similarly, Lap^M/Lap^M is thought to produce an enzyme of intermediate mobility and Lap^M/Lap^S both an intermediate and a slow band. In like manner it is suggested that the other patterns, where a single zone is present, is due to a homozygous Lap gene, and where two zones are present, the pattern is due to heterozygosity at the Lap locus. Thus the basis of determination of the Lap type is apparently three co-dominant alleles. Preliminary examination of our cultures indicates that the gene frequencies of *F*, *M* and *S* are 0·130, 0·538 and 0·332 respectively. All the patterns described for *D. buskii* have been observed in the larval, pupal and adult stages whereas in *D. melanogaster* strong Lap activity is found only in pupæ[6].

Leucine aminopeptidase is a proteolytic enzyme that is probably of considerable importance in protein degradation during metamorphosis. The fact that one species produces the enzyme in all developmental stages while in another enzyme production is limited to the pupal stage suggests that different evolutionary pathways have been followed. It seems in general, though this is but a preliminary finding, that continued investigation of enzyme variations, through the use of starch-gel electrophoresis, promises to be a powerful tool toward greater understanding of phylogeny especially when it involves such a genetically well-known group as the *Drosophila* species. Furthermore, the presence of an apparently tri-allelic polymorphic system in *D. buskii*, in which the three homozygous types as well as the heterozygotes are easily recognized, can be of considerable importance in the investigation of certain population genetic models.

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BOUND WATER, METABOLITES AND GENETIC CONTINUITY

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PREVIOUS work has shown that there is a parallelism between the mechanism by which desiccation[1–3], ultraviolet light[4] and X-ray[5] damage both bacterial cells and viruses. These experiments suggested that the removal of water bound to cell macromolecules causes a change in the structure of the latter which leads to death of the cell. This hypothesis was supported further when it was found that the protection of a cell by an added chemical relied on the presence of strategically placed $—NH_2$ or —OH groups in the structure of the added molecule with inositol the most efficient all-round stabilizer[1–5]. Using this latter compound, the only function of the cell protected appeared to be its ability to synthesize new proteins[6,7] and it was assumed that inositol reacted in some way with the nucleoproteins. This assumption was rendered more plausible when Rous sarcoma virus (RSV) was found to be protected by inositol both against desiccation[8] and ultra-violet radiations[9] and that this compound appeared able to displace nucleoproteins from a dried cell[10]. During the course of these investigations it was noticed that colour mutant cells appeared in greater numbers when cells were damaged in the dry state at certain levels of relative humidity[4], which suggested that desiccation alone could bring about mutation. Since inositol appeared able to prevent this particular mutation, and in the light of its protective ability against ultra-violet and X-rays, it was assumed that death and mutation were part of one process and that inositol and other small molecules were responsible for maintaining the biological integrity of large conjugate molecules. It was of interest then to examine further the mutagenic action of desiccation, and it is the purpose of this article to describe some rather remarkable findings which seem to shed some light on the mechanism of enzyme induction, protein and DNA replication, and the method by which the DNA code functions.

Cultures of a *Lac*$^+$ strain of *Escherichia coli* were grown on either (*a*) a minimal medium containing 0·25 per cent ammonium sulphate plus 2·0 per cent glucose in 0·01 M phosphate buffer *p*H 7, or (*b*) a full medium containing 2·0 per cent glucose, 1·0 per cent casamino acids, and 0·05 per cent yeast extract. After growth for 24 h at 37° C on a rotary shaker the cells from 20-ml. aliquots either aerosolized directly (slurry), washed once in 0·85 per cent NaCl and once in distilled water and resuspended in the mother liquor (supernatant) or in 20 ml. of a solution of inositol or lactose. In the case of the adaptation experiment, film drying was used as previously described[6,7]. Aerosol samples were collected in saline and plated on: (*a*) the minimal medium, (*b*) the full medium and (*c*) lactose–eosine methylene blue agar. After growth for 18 h, large plates of (*a*) containing approximately 1,000 colonies were photographed and overlaid with agar containing a mixture of 16 amino-acids.

The medium in which the cells were grown had a significant effect on their stability in aerosols. Those grown in minimal medium were far more resistant to desiccation than those grown on the full medium, and apparently the addition of the minimal medium spent culture fluid offered protection to both kinds of cells. The interesting point seemed to be the considerable stability of the minimal medium grown cells aerosolized as a slurry; they displayed almost the same stability as those cells to which inositol had been added (Table 1). It was evident, also, that the added protection afforded cells by growth in minimal medium resulted from the extra metabolites produced. However, since washing removed some of the protection their position in the cell as a result of physiological processes was assumed to be important.

It was noticed that the recovery of cells grown in the full medium after aerosolization was always slightly larger if the cells were plated on a full medium, and since desiccation damage appeared to result in the inability of cells to

Table 1. EFFECT OF GROWTH AND PLATING MEDIUM ON THE DEATH-RATE OF AIR-DRIED *E. coli*

Aerosolization conditions	Death-rate (*K*)* *MM* (plating)	Death-rate (*K*)* *FM* (plating)
FM cells in water	0·048	0·042
MM cells in water	0·026	0·021
FM cells in slurry	0·033	0·028
MM cells in slurry	0·009	0·009
FM cells in *MM* spent culture fluid	0·010	0·012
MM cells in *FM* spent culture fluid	0·021	0·018
FM cells in 5 per cent inositol	0·006	0·007
FM cells in 2 per cent arginine	0·018	0·017

FM, full medium; *MM*, minimal medium

* Death rate calculated from $N_t = N_0e^{-Kt}$.

synthesize protein it seemed likely that some specific blocks might have developed. This was found to be true when overlays of amino-acid agar allowed the development of a few more cells which on sub-culture proved to have a genetic block in amino-acid synthesis. The specific nature of these blockages was not determined as it had little bearing on the objective of the investigations. When this phenomenon was investigated further, the number of these mutant cells was found to be dependent on the relative humidity at which the airborne cells were held (Fig. 1). Therefore, since relative humidity is known to control the bound-water content of the cells[1,3] and as these mutants did not appear in minimal medium slurries or when inositol was added, both bound water and metabolites appeared to be involved, not only in preserving the viability of the cells, but also in preventing mutation. In an attempt to clarify the situation the ability of cells to produce adaptive β-galactosidase was studied using the spectrophotometric method with *O*-nitrophenol-βD-galacto-pyranoside. As had been reported earlier[6,7], cells dried at 40 per cent relative humidity from water showed reduced ability to manufacture the enzyme. However, if inositol, lactose or glucose was added to the cells prior to aerosolization this loss of inducibility was prevented, even though the latter two compounds only reduced the death-rate of the full medium grown cells from 0·042 to 0·021 and 0·028 respectively. Similarly, cells grown in minimal medium and aerosolized as a slurry retained their inducibility. It now seemed clear that inositol and cell metabolites prevented damage to those molecules concerned in protein synthesis, but why lactose and glucose should perform this function also was not immediately clear. Since it is well known that glucose will inhibit β-galactosidase syntheses, its ability to preserve the adaptive powers of the cell had to reside in a competitive association with another molecule and presumably this association also led to the preservation of the integrity of this associated molecule. In the light of the previous findings with the occurrence of amino-acid deficient mutants it was considered possible that a combination between the sugars or inositol and DNA might be responsible, hence it appeared logical to look for sugar-deficient mutants in the form of lactose negative (*Lac*⁻) cells. The initial experiments with cells grown on minimal or full media and plated on 'EMB–lactose' medium failed to demonstrate mutants in any significant numbers. Then it was realized that glucose was, in fact, a starting point of metabolism and that some glycolytic enzymes might be adaptive. The full medium was changed, therefore, and, instead of glucose, sodium acetate or succinate was added. After several sub-cultures in the above medium the cells produced *Lac*⁻ mutants in relatively large numbers on drying, and since glucose, lactose or inositol appeared able to prevent their occurrence (Fig. 2) it could only be assumed that a combination between these additives and DNA prevented this particular mutation.

In a later series of experiments the airborne cells, aerosolized in minimal medium or distilled water, were irradiated for 5 min with a G.E.R.S. sunlamp, and while all the phenomena described here for unirradiated cells were observed the relative humidity range at which the mutants appeared was much sharper (Fig. 1).

Since 'early' protein synthesis is required for bacteriophage DNA replication, and these proteins are considered to be the DNA polymerase and enzymes concerned in the syntheses of hydroxy-methyl-cytosine, it appeared likely that drying might interfere with a similar system in uninfected cells. Cells of a thymine-deficient *E. coli* were grown in a medium containing a mixture of amino-acids 1·0 per cent, glucose 1·0 per cent and a mixture of the five nucleic acid bases. 10 ml. aliquot were washed in phosphate buffer and allowed to starve in buffer for 1 h. 1 ml. of these cells was then placed in the same medium as above, except that each cold compound was replaced by the carbon-14-labelled metabolite under investigation.

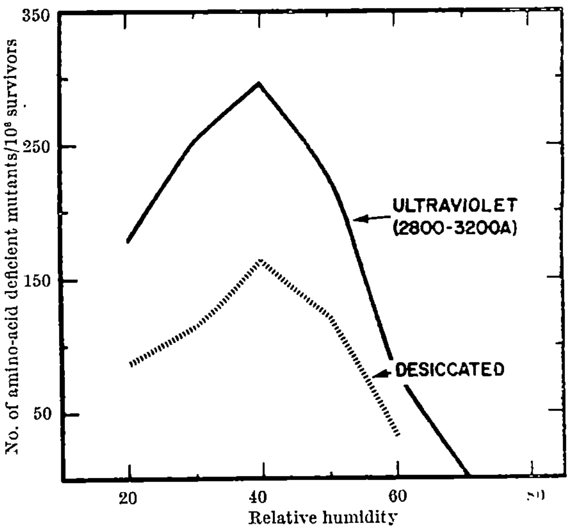

Fig. 1. Effect of relative humidity on the production of amino-acid dependent mutants of *Escherichia coli* by desiccation and ultra-violet light

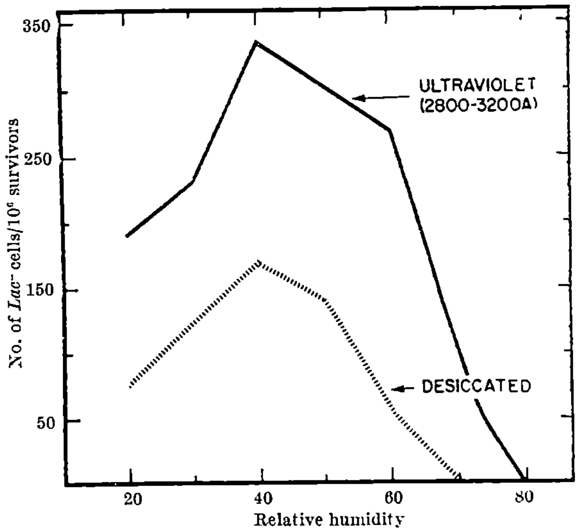

Fig. 2. Effect of relative humidity on the production of lactose negative mutants of *Escherichia coli* by desiccation and ultra-violet light

From these experiments it was clear that there was an immediate uptake of uracil, amino-acids and lactose which lasted for about 10 min during which time there was no thymine uptake. β-Galactosidase activity occurred, however, along with the 'early' proteins. In the next 10–20 min the uptake of the former three components almost ceased, but DNA synthesis occurred very rapidly, then, as this slowed the uptake of amino-acids, uracil and β-galactosidase activity increased again.

This behaviour strongly suggested that early protein synthesis was required for DNA replication in a normal cell even when there was no change in medium. Dried cells protected with inositol or minimal medium supernatant behaved as the controls, but the pattern of events with unprotected cells was somewhat confusing. The initial uptake of uracil by these cells was far greater than the controls, yet there was no speed-up of either amino-acid uptake or β-galactosidase activity; in fact the latter two were considerably delayed. This simulated earlier findings[10] where this large uracil uptake was found to be correlated not with survival but with death. Apparently the delay in enzyme synthesis and DNA replication is connected with the survival mechanism, and it seems reasonably clear that this delay is evident only during the first 30 min; from then onwards the cells behave

Table 2. Syntheses of Protein and Nucleic Acids by Air-dried and Normal Cells of *E. coli*

Compound examined		Time (min) 5	10	15	30	45	60
Thymine-^{14}C	*A*	Nil*	14	242	231	839	1,821
	B	Nil	Nil	18	275	272	863
Uracil-^{14}C	*A*	455	637	651	1,192	1,363	1,923
	B	683	984	1,392	2,106	2,505	4,217
Amino-acids-^{14}C	*A*	68	213	233	1,052	1,884	2,649
	B	34	85	196	241	1,194	2,103
Lactose-^{14}C	*A*	21	139	132	105	—	—
	B	15	21	138	142	—	—
β-Galactosidase	*A*	Nil†	0·15	0·08	0·19	0·26	0·41
	B	Nil	Nil	0·10	0·08	0·18	0·28

A, controls; *B*, unprotected dried cells

* Counts/min per 10^7-viable cells.

† *O.D.* of *o*-nitrophenol in sonic filtrates of cells.

normally. The most significant finding would seem to be the fact that a definite amount of amino-acid uptake is necessary before DNA replication occurs as well as the small lactose uptake during this time.

Because of the apparent role of bound water in maintaining the biological integrity of macromolecules, and since most chemical carcinogens as well as ultra-violet and X-rays could be considered as molecular desiccants they would compete with or remove bound water from, Swiss mice were inoculated with normal spleen and thymus cells obtained from young mice. Some of the animals were given undried cells washed in basic salt solution, and others cells dried for 30 min at 40 per cent relative humidity. After 12 months, surviving animals were killed, autopsied and the malignant nature of each tumour confirmed by histological examination. All the leukæmias could be classified as lymphoblastic lymphomas with thymus, lymph nodes, liver, spleen and kidney generally involved.

The results given in Table 3 show clearly that in those groups given dried cells the number of animals with tumours jumped from 11·0 to 42 per cent, and the total tumours from 12·6 to 56 per cent. Most of the increase, however, was in the incidence of leukæmia.

Table 3. Incidence of Tumours in Swiss Mice inoculated with Dried or Non-dried Normal Cells

Group	No. of animals	Animals with tumours (per cent)	Total No. of tumours	Total tumours (per cent)
Controls	72 (4)†	11·0	5 (*L*)* 4 (*MC*)	12·6
Dried	50 (4)	42·0	22 (*L*) 6 (*MC*)	56·0

* (*L*) = leukæmias; (*MC*) = mammary carcinomas.

† No. of separate groups set up.

Previous work over many years has demonstrated that the aerosol is an excellent and unique way of investigating the role of bound water and chemicals in the maintenance of molecular structure and integrity of metabolic processes[1-7]. The results presented in this article tend to support the hypothesis that bound water and some small molecules, with strategically placed groups, maintain biological integrity. One could argue that an association between an enzyme and its substrate, or the attachment of a substrate to RNA, leads to a more stable cell. However, if DNA is the master molecule, damage to either a protein or RNA should be reparable. This is perhaps why protein synthesis in the damaged cells is delayed since new messenger RNA will be required. There seems to be no doubt that both RNA and DNA can be protected by inositol, and the latter by metabolites also. It is possible, therefore, that some of the proteins synthesized during the first 10 min are manufactured by the existing RNA, and the preservation of its structure may help to prevent death of the cell, provided no drastic change has occurred in the DNA.

Whether the observed increase in incidence of tumours represents an acceleration of tumour development, or an actual induction, either by virus release or mutation, remains to be determined. However, the latter hypothesis is favoured in view of past experience with desiccated bacterial and mammalian cells and because the observed increases go well beyond the normal incidence found in these mice. Unless one assumes that enzymes and RNA are responsible for genetic continuity, the foregoing arguments do not explain the observed mutation in bacteria and their prevention by inositol or metabolites. A more plausible explanation seems to be that mutation is one step towards death, or, in other words, damage to DNA leads to mutation, then death. If one makes this assumption, then it becomes necessary to explain why small molecules preserve the integrity of a gene. Proteins are known to be bound to the 'outside' of the DNA molecule, and from time to time there have been reports of sugars and even inositol being associated with this molecule; water certainly is. Also, cells grown on minimal medium are known to be independent of the amino-acids usually necessary for the production of back mutant prototrophs[11], and an explanation of these facts now appears possible from the data presented.

The types of investigations we have carried out in our laboratories are perhaps an unusual approach to the problem of genetic continuity; but the implications from stability studies nevertheless seem quite valid. In order to explain the results, however, one must view the whole picture of experimental facts. (*a*) Desiccation at certain relative humidity-levels, ultra-violet irradiation and X-ray are all lethal and mutagenic. (*b*) The efficiency of the latter two agents on semi-dried cells is also dependent on relative humidity. (*c*) Protection appears to rely on the right compound in the right place or on strategically placed —OH groups such as in inositol. (*d*) Protection against all three agents is afforded cells by the same compounds. (*e*) The action of inositol seems to be to replace water and some metabolites. (*f*) The gene or genes controlling certain physiological functions can be protected by the metabolites themselves or inositol. (*g*) The genes for many metabolic pathways appear to be in linear array. (*h*) The replication of DNA requires 'early' protein syntheses. (*i*) The addition of such compounds as chloramphenicol prevents the synthesis of biologically active protein, stops the production of mutants and protects cells against desiccation[12].

If all the experimental facts are considered it would appear that the biologically active code of the DNA molecule is not only the base-pair H-bonds of the Watson and Crick model, but also the display of groups on the 'outside' of the molecule. It is suggested, therefore, that the 'operational' gene for enzyme production is a particular configuration on the outside of the DNA plus a given metabolite, and that in the absence of the metabolite the structural configuration is preserved by hydrogen-bonded water molecules which can be replaced by inositol or like compounds. If this particular DNA configuration is damaged by loss of water on desiccation or irradiation it will obviously affect the inner code when the two halves of the helix separate and deformed daughter DNA will result. The idea is shown schematically in Fig. 3.

A_1 and A_2 are meant to represent the deep and narrow grooves, respectively, on the outside of the intact double-helix DNA molecule. In the various parts of the shallow groove certain configurations exist which allow the attachment of specific small hydrated molecules or water. The exposed configuration of the DNA surface is altered by their attachment and protein formed on this will automatically be coded for the attached substrate. The formation of the protein will result, also, in the production of a specific RNA coded for the enzyme. The attachment of the substrates, proteins and RNA will seriously affect the strength of the base-pair hydrogen-bonds and the two halves of the helix will separate exposing the 'inner' code. Because of the presence of protein (P_1) in the deep groove of the single strand it will attract the necessary complementary bases and form a new double helix with a narrow groove ready for action. Protein (P_1) is, therefore, considered to be a DNA polymerase the internal energy of which is sufficient to 'activate' an existing single strand of DNA and naturally it cannot function unless some DNA is present. The happenings on the other half

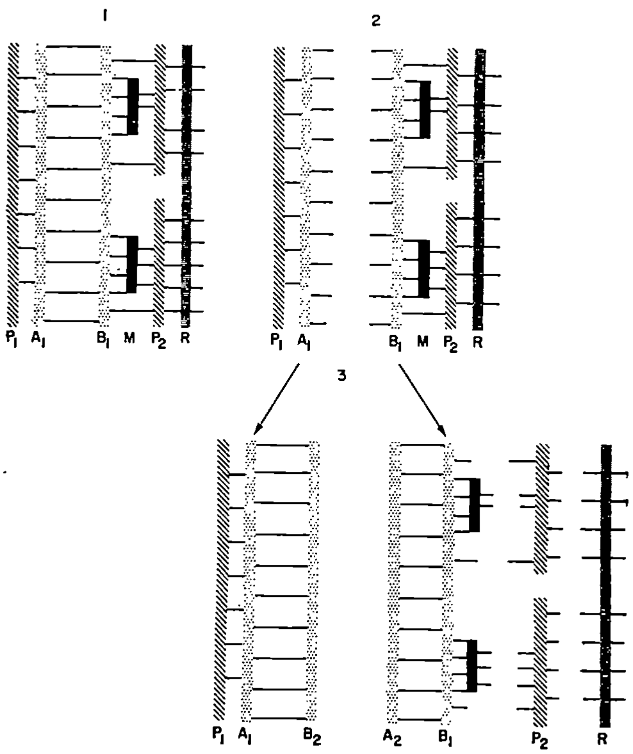
1
2
3
P_1 A_1 B_1 M P_2 R
P_1 A_1 B_1 M P_2 R
P_1 A_1 B_2 A_2 B_1 P_2 R

Fig. 3. A suggested scheme showing why protein synthesis is necessary for DNA replication

A and *B* represent the two single strands of DNA; *P* represents proteins; *R* represents RNA; *M* represents metabolites

of the original DNA molecule must be somewhat different, and it is suggested that the action of the formed enzyme changes the original substrate and forms a new intermediary compound; this results in the liberation of the coded RNA and the enzyme and the re-orientation of the new metabolite in the same site or its positioning in the next site along the DNA molecule (or more probably along a given chromosome). The latter process will allow a second daughter molecule to form plus new P_1 protein. The process will now repeat itself using the newly formed substrate. Since RNA coded for these various enzymes is being made and shed as the protein functions, it follows that on reaching the cytoplasm it will manufacture more enzyme molecules and protein synthesis as such will go on regardless of what is happening to the DNA. Moreover, if the RNA molecule is a double helix it will duplicate itself in the same fashion as proposed for DNA; the only difference between it and DNA will be that the duplication of the latter takes place in orderly sequence along the chromosomes so that the continuity of the linear arrays, so essential to the life process, is kept intact. There can be no doubt that the RNA carries out of the nucleus parts of these arrays intact, and it can only be assumed that the RNA viruses have reached a stage where their duplication is independent of DNA.

The foregoing hypothesis fits the facts known about gene expression and protein synthesis. It follows that DNA duplication will rely on active protein synthesis, yet once started the latter process can occur without DNA. The necessity of linear array of genes governing the synthesis of a compound, such as an amino-acid, immediately becomes apparent as well as the mechanism by which DNA synthesis takes place in linear fashion on the chromosome. If such assumptions are made, the induction of enzymes becomes the normal method of protein synthesis rather than the abnormal one and our present-day ideas as to what is an adaptive enzyme are largely a matter of the kinetics of the reactions.

Apart from these physiological pointers the stability of a gene towards ultra-violet, X-rays and desiccation in terms of water content, growth medium and phase of growth is readily explained. If metabolites do act in combination with the gene, it follows that in the presence of the metabolite the gene and cell may be more stable, and this is exactly what is found. Presumably the inactive genes are merely hydrated, and hence they are damaged more easily and it can only be assumed that their replication requires the correctly orientated water or attachment of a non-specific molecule such as inositol. This could lead to the production of non-enzymatic histones at biologically inactive sequences of gene sites. These sites, however, would be freed when DNA replication occurred.

If the assembly of amino-acids on the DNA is necessary for its replication, it follows that some cells, as is demonstrated here, may develop multiple blocks in amino-acid syntheses on irradiation, and, hence, only when an external supply of amino-acids is supplied can the DNA replicate and the cells divide. If, as is usual, complementary molecules exist in the cells, some of the progeny will receive DNA in which only the back mutation is apparent. On the other hand, cells grown in minimal medium will possess pre-coded RNA capable of manufacturing the amino-acids and these cells will not require an external supply.

Finally, as is generally accepted, a compound able to bind with the inner code may stimulate the incorporation of an incorrect base. However, irreversible binding of small molecules to the outer codes, or the loss of structural water, could well lead to the distortion of the DNA molecule when it attempts to replicate. Also, compounds able to bind with the outer codes of RNA or DNA should be capable of inducing the synthesis of specific enzymes. In the work recorded here the fact that the cells in minimal medium slurries were so stable would suggest that the metabolites are not removed as a result of enzyme formation.

However, it is well known that DNA synthesis goes on during about 80 per cent of the lifetime of a bacterial cell, so presumably most of the metabolites remain in position. The cell is small, and there is little room to house all the formed enzymes and supplied metabolites; perhaps this is why it has such a short mean generation time. Alternatively, it is possible that the formed enzymatic proteins do not separate from the DNA and, hence, only one of the daughter DNA molecules with the open narrow groove will actually function. In any event, the concept that the 'outer' grooves of the DNA molecule have separate integrities and have definite separate biological functions in the coding of protein and RNA seems to fit not only the present data but also most of the facts known concerning protein syntheses and gene expression. Considered from the point of view of energetics and the shifts in hydrogen-bond strengths that will occur when metabolites are attached or freed from the DNA molecule, the foregoing hypothesis seems not unreasonable and further experimentation designed to test it is being conducted.

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RELEVANCE OF BIOCHEMICAL TAXONOMY TO THE PROBLEM OF LATHYRISM

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VARIOUS species of *Lathyrus* are cultivated throughout the world, and it has been known from earliest times that the seeds of some of these species are toxic when ingested by man and domestic animals, giving rise to a 'disease' known as lathyrism. In man, lathyrism is characterized by a loss of muscular control, paralysis of the legs and in extreme cases death. These symptoms have usually been associated with the ingestion of seeds of *L. sativus*, although seeds of *L. cicera* and *L. clymenum* have also been implicated. Lathyrism is endemic in certain parts of Asia, particularly India, where outbreaks of exceptional severity have been reported during periods of famine when excessive quantities of *Lathyrus* meal have been eaten for want of other food[1,2]. Isolated outbreaks have also been reported from time to time from Europe and the Mediterranean countries.

In seeking the cause of lathyrism some confusion has arisen in the past from conflicting reports as to which experimental animals are affected by the ingestion of *Lathyrus* seeds and from equally conflicting reports as to the symptoms manifested by those which are. I believe that this confusion has resulted from a failure to appreciate sufficiently the important differences which exist between the plant species themselves, and I suggest that the key to the problem lies in the biochemical taxonomy of the genus.

The fact that there are at least two toxic syndromes associated with the ingestion of *Lathyrus* seeds was recognized by Selye[1], who proposed that the term 'neurolathyrism' should be used to describe the classical symptoms caused by those species previously mentioned, and the term 'osteolathyrism' to describe the effects, which include skeletal changes, seen in rats and other animals following the ingestion of *L. odoratus* seeds.

The first toxic compound to be isolated from this genus was the 'osteolathyrogen' obtained from seeds of *L. pusillus* by Dupuy and Lee[3], and from seeds of *L. odoratus* by McKay, Lalich, Schilling and Strong[4]. This compound was later shown to be β-(γ-glutamylamino)propionitrile[5], the toxic portion of the molecule being the β-aminopropionitrile[6]. Some while later, Ressler *et al.* isolated a second toxic compound, showing neurological properties, from seeds of *L. latifolius*. This they identified as α,γ-diaminobutyric acid[7].

When these findings were related to the survey made by me[8] of all the amino-acids and related compounds occurring in relatively high concentration in the seeds of forty-nine species of *Lathyrus*, it was apparent that those species responsible for 'neurolathyrism' in man contained neither β-(γ-glutamylamino) propionitrile nor αγ-diaminobutyric acid. These three species, however, all fell into one of the sub-genera into which *Lathyrus* may be divided on the basis of associations of amino-acids and related compounds in its seeds. The sub-genus concerned was characterized by the presence of high concentrations of two unidentified ninhydrin-reacting compounds. One of these has since been isolated from seeds of *L. cicera*[9] and *L. sativus*[10] and shown to be L-homoarginine. While the possibility that this amino-acid may be toxic to man cannot be completely discounted on evidence obtained from experiments with animals, L-homoarginine does not produce neurological symptoms in chicks[11], which are affected by extracts of the whole seed[12].

The second ninhydrin-reacting compound (designated A_1 by me) is acidic in character and has recently been isolated by Adiga, Rao and Sarma[11], and also independently by Murti, Seshadri and Venkitasubramanian[13]. This compound has been identified as α-amino-β-oxalylaminopropionic acid[14] and, like the seed extracts of *L. sativus*, produces characteristic neurological symptoms when injected into chicks. There can be little doubt that α-amino-β-oxalylaminopropionic acid is the compound responsible for classical 'neurolathyrism' in man.

In my survey it was found, however, that of the twenty species which contain α-amino-β-oxalylaminopropionic acid (A_1), nine (to which a tenth has since been added) also contain two other unidentified acidic ninhydrin-reacting compounds which were designated A_2 and A_3.

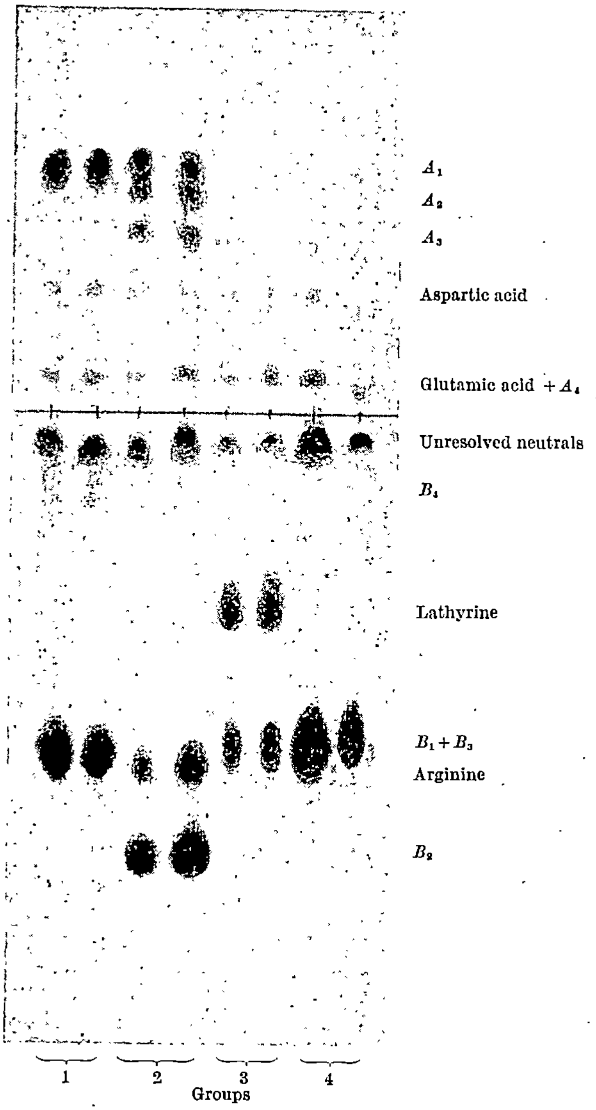

Fig. 1. Extracts of the seeds of two species from each of groups 1–4 after ionophoresis

Table 1. AMINO-ACIDS AND RELATED COMPOUNDS IN SEEDS OF *Lathyrus*

Group	No.	Name	B_1	B_2	B_3	B_4	N_1	N_2	A_1	A_2	A_3	A_4	Lat.	Arg.
1	1	*L. alatus*	+ +						+ +					T
	2	*L. articulatus*	+ +						+ +					T
	3	*L. arvense*	+ +					T	+ +					+
	4	*L. setifolius*	+ +						+ + +					T
	5	*L. pannonicus*	+ +						+ +					T
	6	*L. ochrus*	+ +						+ +					T
	7	*L. clymenum*	+ +						+ +					T
	8	*L. sativus*	+ +						T					T
	9	*L. megallanicus*	+ +						T					T
	10	*L. quadrimarginatus*	+ +						T					T
	11	*L. cicera*	+ +						T					T
2	12	*L. sylvestris*		+ +				T	+	+	T			+ + +
	13	*L. latifolius*		+ +					+	+	T			+ +
	14	*L. heterophyllus*		+ +					+	+	T			+ +
	15	*L. gorgoni*		+ +					+	+	T			T
	16	*L. grandiflorus*		+ +					+	+	T			T
	17	*L. cirrhosus*		+ +					+	+	T			+
	18	*L. rotundifolius*		+ +				T	+	+	T			+
	19	*L. tuberosus*		+ +					+	+	T			+
	20	*L. multiflora*		+ +				T	T	+	T			+
	21	*L. undulatus*		+ +				+	T	+ +	T			+
	22	*L. aurantius*		+ +										T
	23	*L. luteus*		+ +										T
	24	*L. laevigatus* sp. aureus		+ +										T
3	25	*L. pratensis*	+ +										T	
	26	*L. laevigatus* sp. occidentalis	T										T	+
	27	*L. varius*	+										+	T
	28	*L. niger*	+										+	T
	29	*L. machrostachys*	+										+	T
	30	*L. maritimus*	+									+	+ +	T
	31	*L. aphaca*	+	+								+	+	T
	32	*L. sphaericus*	+	+									+ +	T
	33	*L. tingitanus*	+	+ + +									+ + +	T
	34	*L. cyanus*	+	+ +									+ +	T
	35	*L. alpestris*	+			+		+ +				T	+ +	
	36	*L. variegatus*	+			+		+ +				T	+ + +	
	37	*L. venetus*	+			+		+ +				T	+ +	
	38	*L. inconspicuous*	+									+ +	T	
	39	*L. incurvus*	+ +									+ +	T	+
4	40	*L. vernus*	+ + +					+ + +					T	+
	41	*L. montanus*	+ + +					+ +						+
	42	*L. palustris*	+ +					+						
	43	*L. aureus*	+ + +					+ +						T
	44	*L. neurobolus*	+ +					+						T
	45	*L. nissolia*	+ +					+						T
5	46	*L. roseus*					T							T
	47	*L. hirsutus*					+							T
	48	*L. odoratus*	+				+							T
	49	*L. pisiformis*	T											T
	50	*L. annuus*	T											T
	51	*L. angulatus*	T									+		T
	52	*L. laetiflorus*	T										+ + +	
	53	*L. venosus*	+											T

T = trace, B_1 = homoarginine, B_2 = α,γ-diaminobutyric acid, B_3 = γ-hydroxyhomoarginine, B_4 = unidentified, N_1 = β-(γ-glutamylamino)propionitrile, N_2 = unidentified, A_1 = α-amino-β-oxalylaminopropionic acid, A_2 = α-amino-γ-oxalylaminobutyric acid (tentative), A_3 = second acidic derivative of α,γ-diaminobutyric acid, A_4 = unidentified, Lat. = lathyrine, Arg. = arginine. Of the five last species listed, three (Nos. 49, 50, 51) contained low concentrations of amino-acids and were not assigned to the previous groups; *L. laetiflorus* contained a neutral compound and *L. venosus* a basic compound not seen in other species. All species contained traces of glutamic acid and aspartic acid.

Using seed of *L. latifolius* the isolation and identification of A_1, A_2 and A_3 were undertaken in this laboratory prior to the isolation of α-amino-β-oxalylaminopropionic acid from seed of *L. sativus*. While this work is not yet complete, it may be of interest to report that in addition to confirming the presence of α-amino-β-oxalylaminopropionic acid in seeds of *L. latifolius*, it has been found that A_2 is an oxalyl derivative of αγ-diaminobutyric acid (the toxic amino-acid previously isolated from the same species). It has been tentatively identified as α-amino-γ-oxalylaminobutyric acid[15], the higher homologue of the 'neurolathyrogen' first isolated from *L. sativus*. Preliminary experiments have also shown that A_3 is yet another acidic derivative of the same amino-acid.

The distribution of these compounds and of other amino-acids found in seeds of *Lathyrus* is summarized in Table 1, from which it will be seen that the seeds of all species in group 1 contain α-amino-β-oxalylaminopropionic acid and their ingestion may be expected to produce symptoms of classical 'neurolathyrism' in man. The seeds of the last three species in group 2 contain αγ-diaminobutyric acid, and their ingestion would induce the neurological symptoms associated with that amino-acid. The seeds of the remaining species of group 2 contain both α-amino-β-oxalylaminopropionic acid and αγ-diaminobutyric acid, the last-named being present both in the free form and combined in two different acidic derivatives. The 'osteolathyrogen', β-(γ-glutamylamino)propionitrile, is found in the seeds of species in group 5 (and also in *L. pusillus*, a species which has not been examined in this laboratory).

When considering methods for the prevention of lathyrism in man, Murti *et al.*[13] suggested that the seeds of *L. sativus* might be rendered non-toxic by boiling in water to remove the α-amino-β-oxalylaminopropionic acid. While such a measure might be used effectively as a temporary expedient it would seem that the permanent eradication of the disease can only be achieved by providing seed and encouraging the cultivation of alternative legumes in the areas concerned. If the choice of alternatives is to be made from species of the same genus, agricultural evaluation may be limited, on the basis of the present account, to species other than those of groups 1, 2 and 5. While some, or all, of the remaining species may yet prove to be toxic, lathyrine (β-(2-aminopyrimidin-4-yl) alanine[16]), which occurs in high concentration in many of them, has been found (like homoarginine, which it accompanies) to be without toxic effects in the mouse and chick[17]. The toxicity of γ-hydroxyhomoarginine[18–20], which also occurs in varying concentrations in certain of these species, has not yet been determined, however, nor have the compounds designated A_4, N_2 and B_4 been isolated in the pure state.

To illustrate the sub-division of the genus and the resolution of most of the compounds discussed, extracts of the seeds of two species from each of the first four groups of Table 1 have been subjected to ionophoresis (5 kV for 15 min) on paper at *p*H 3·6 (Fig. 1). β-(γ-Glutamylamino)propionitrile being uncharged at *p*H 3·6 is not resolved from the neutral amino-acids by this method, but its presence may be readily detected chromatographically on paper using lutidine–water (65 : 35 by vol.) which separates it from the other ninhydrin-reacting compounds found in comparable concentrations in the extracts examined (R_F 31).

It is appreciated that much work on the biosynthesis of these compounds, their phylogenetic significance and in some instances their toxicity remains to be done. Nevertheless, it is hoped that this account may explain some of the unexpected and apparently contradictory results reported in the literature on lathyrism and lead to a clearer understanding of the problems involved.

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CONTINGENT NEGATIVE VARIATION: AN ELECTRIC SIGN OF SENSORI-MOTOR ASSOCIATION AND EXPECTANCY IN THE HUMAN BRAIN

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THE amplitude of the electric responses evoked in the human brain by sensory stimulation is usually rather small compared with that of the intrinsic background activity. However, recently developed methods of electronic averaging and cross-correlation[1] have disclosed consistent patterns of response, particularly in the extensive non-specific regions of the frontal lobes[2]. Records from scalp electrodes (which inevitably introduce considerable spatial diffusion) have been compared with those from multiple electrodes implanted in frontal cortex for therapeutic purposes[3]; these latter can be left in position for several months and demonstrate convergent responses to auditory, visual and tactile stimuli in most regions of medial, lateral and orbital frontal cortex. The scalp responses are seen as compounded of these widespread synchronized discharges which, however, involve only a very small proportion of the neural tissue in any one zone.

The non-specific responses (in contrast with those in specific primary sensory areas) showed marked 'habituation' when stimuli in any modality are repeated frequently and monotonously, but their amplitude is restored when stimuli are associated. The rule is that the electro-negative components of the responses to the first (that is conditional or warning) of two stimuli are progressively augmented while those to the second stimuli (considered as unconditional or 'indicative') are progressively attenuated. These effects are generally emphasized and accelerated if the subject is instructed to perform an operant response to the indicative stimuli, which thereby become 'imperative'.

A series of experiments has been performed to investigate the effects on these components of varying the probability of association between the conditional and imperative stimuli, and also the influence of variations in effector participation and mental attitude. The effects of direct suggestion under hypnosis have already been described[4].

The non-specific responses to conditional stimuli usually consist of three main components: a brief surface positive wave, a brief surface negative wave superimposed on this, and a much more prolonged surface negative component which may last several seconds, particularly in children. The details of the first two components depend somewhat on the modality, the responses to auditory stimuli being usually larger, simpler and more consistent than those to visual or tactile stimuli. The features which seem most closely related to the contingency of the situation and to the attitude of the subject are the prolonged secondary negative waves and their interaction with the subsequent responses to imperative stimuli.

Because of its dependence on the statistical relationship between the conditional and imperative stimuli this protracted component of the conditional response is referred to here as the 'contingent negative variation' (CNV). This effect can be recorded accurately only with equipotential non-polarizable electrodes and long time-constants or directly coupled amplifiers. For these experiments, specially prepared and selected silver–silver chloride electrodes were used, connected to an Offner type *TC* 16-channel recorder, fitted with a two-channel automatic wave analyser and a two-channel barrier-grid averager[5]. In order to permit comparison of responses in a variety of situations during a session of reasonable duration with intervals of several seconds between stimuli, the results from sets of only six or twelve presentations were stored and averaged, and the outputs from the averagers were written out directly on the recorder with increased gain and paper speed at the end of each set of presentations, which lasted about 90 sec.

The stimuli, singly or in pairs, were presented automatically at irregular intervals of 3–10 sec. When paired, the interval between associated stimuli was usually one-half or one second, but longer intervals have also been used. The conditional stimuli were either single flashes (*F*) or single clicks (*C*); the indicative or imperative stimuli were repetitive clicks or repetitive flashes. The operant response was provided by a button which the subject was asked to press when the repetitive imperative stimuli were given; this could be connected so that it terminated the imperative stimuli, but the circuit was so arranged that it was ineffective if pressed before the onset of the imperative stimuli.

Observations have also been made during the establishment of involuntary conditioned blink responses to clicks preceding puffs of air to the cornea. Records were obtained from electrodes on several parts of the head, but those illustrated here were all from a vertico-mastoid pair, an upward deflexion indicating negativity of the vertical electrode with respect to the mastoid. Simultaneous records were also obtained of the electro-myogram of the operant muscles, the stimuli, variations in palmar skin resistance, pulse rate and respiration.

The development of the CNV and its interaction with the imperative responses are illustrated in Fig. 1, which is from a normal adult subject. The averages of 12 responses to isolated clicks (Fig. 1*A*) consist of a small positive wave almost obscured by a much larger negative component. The responses to isolated trains of flashes (Fig. 1*B*) show several brief negative components, also superimposed on a small positive deflexion. When the clicks are paired with the flicker about 1 sec later (Fig. 1*C*) both responses are reproduced with little alteration. In these conditions both responses dwindle to noise-level after about 50 presentations. The addition of an operant response by the subject (Fig. 1*D*) is accompanied by a pronounced change in the conditional response to the clicks; a large CNV appears, rising to the peak amplitude of the imperative response during the interval between them. The imperative response itself is now seen merely as a sharp drop in potential difference to the base line. This pattern is maintained indefinitely as long as the subject is attentive and presses the button promptly. It is the same whatever the modalities of the first and second stimuli (Fig. 2*A*). However, when the imperative stimulus is withdrawn, the responses to the unreinforced conditional stimuli show a progressive subsidence of the CNV which disappears almost entirely after 30 unreinforced trials (Fig. 2*A*–*E*). Restoration of the imperative auditory stimulus immediately re-established the CNV (Fig. 2*F*). The dependence of the CNV on the contingency of the association between the two stimuli is shown most clearly when their relation is made equivocal. After a long series of regular associations of flashes with clicks (Fig. 3*A*) the probability of association was 'diluted' by presentation of 27 unreinforced flashes at random between 21 associated pairs, providing only 21 reinforcements out of 48 presentations. Averages taken during this series of equivocal presentations show that the CNV is progressively reduced to about half its previous amplitude, while the imperative response reappears as a negative wave (Fig. 3*B*, *C*, *D*). In most normal adult subjects the CNV shows signs of attenuation when the probability of occurrence of the association drops below 0·7 during 12 or more trials; but the total number of trials, their distribution in time and the critical proportion of reinforcements needed for suppression of the CNV vary considerably from subject to subject. Unequivocal restoration of the conditional stimulus once again brings about a rapid re-establishment of the CNV with submergence of the imperative negative wave (Fig. 3*E*).

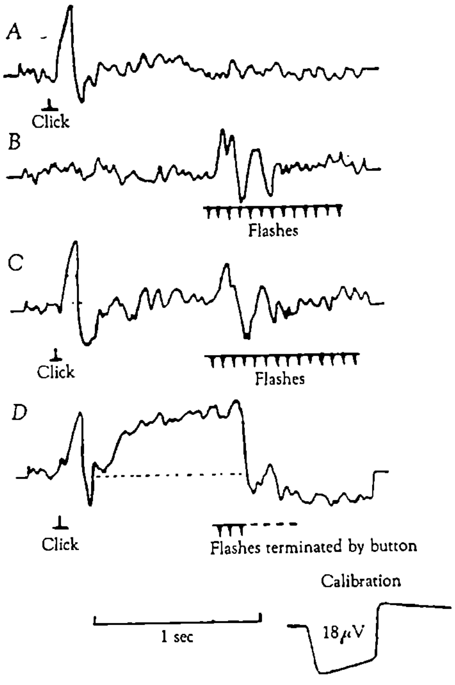

Fig. 1. Averages of responses to 12 presentations. *A*, response in fronto-vertical region to clicks; *B*, flicker; *C*, clicks followed by flicker; *D*, clicks followed by flicker terminated by the subject pressing a button as instructed. The contingent negative variation (CNV) appears following the conditional response and submerges the negative component of the imperative response

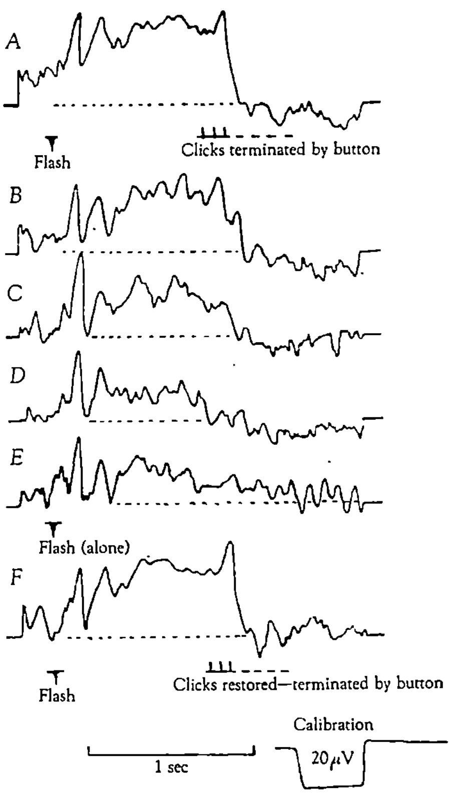

Fig. 2. Averages of 6 presentations. *A*, responses to flicker followed by clicks terminated by subject's operant response. The CNV is fully developed; *B*, average of first six presentations after withdrawal of the imperative stimuli; the CNV is slightly attenuated and there is a 'conditional' negative wave at the phase where the clicks would have been expected; *C*, *D*, *E*: progressive diminution of the CNV as the expectancy of reinforcement subsides. *F*, restoration of the imperative stimulus quickly re-establishes the CNV

Another factor which influences the form of the CNV is the attitude of the subject. During one experiment the subject was told that during a long series of unequivocal presentations of clicks followed by flashes he could decide

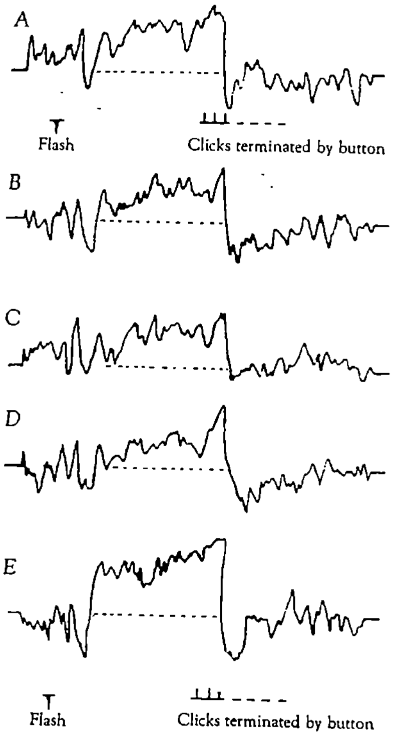

Fig. 3. Averages of six presentations. *A*, responses evoked by regular, repeated association of flashes with clicks terminated by subject; *B*, eight unreinforced flashes interspersed at random between the six associated stimuli reduce the CNV by equivocation; *C*, *D*, continued equivocation progressively attenuates the CNV and the negative component of the imperative response emerges; *E*, unequivocal association again restores the CNV

not to press the button for the operant response. The average of six of these trials showed almost complete absence of a CNV (Fig. 4*A*), but this reappeared immediately when the subject decided to resume the operant response. The CNV is also susceptible to social stimuli in the form of verbal statements. When the imperative stimulus was withdrawn without notice or warning (Fig. 2*B*–*E*) the change in the pattern of physiological stimuli ultimately suppressed the CNV, but only after about 30 trials. In contrast, when the subject was told: 'There will be no more flashes' the CNV disappeared immediately (Fig. 4*B*). In this objective sense, therefore, progressive changes in subjective expectancy produced by repetitive physiological experience can be matched against those established by a single phrase. There may be a qualitative as well as a quantitative difference between the effects of physiological and social suppression. In Fig. 2*B*, the first average after unexpected withdrawal of the visual imperative stimuli, the CNV is slightly smaller than during reinforcement, but there is a small negative wave followed by an abrupt fall in potential difference exactly 1 sec after the conditional stimulus, that is at the instant when the imperative stimuli would have been expected. Bearing in mind that this is the average of six trials and that the second, third and fourth sets of unreinforced trials show steadily diminishing signs of this effect, it may be regarded as a true conditioned brain response induced by the long experience of unequivocal presentations but extinguished as the probability of association declined when the imperative stimuli were withheld. There was no sign of this conditioned response when the subject was told beforehand that there would be no reinforcement (Fig. 4*B*); in fact, with this 'social extinction' there was a slight slow positive variation following the conditional response. Before the next series the subject was told that the flashes would be restored, but this was not done; the first three trials showed an appreciable CNV but the second three showed none, and the average consequently contained only a small CNV (Fig. 4*C*). These features reflected accurately the subjective reports of fluctuations in expectancy induced by the conflict between the verbal and physiological experiences.

When a subject has become accustomed to the presentation of conditional and imperative stimuli separated by 1 or 2 sec the CNV can be sustained even when the imperative stimulus is omitted if he is instructed to estimate the time-interval and perform some action at the moment when the imperative stimulus would have occurred (Fig. 4*D* and *E*). The termination of the CNV is therefore not an 'off-effect'; furthermore, a purely mental judgment of a time-interval, without operant response, is often accompanied by a prominent CNV in trained subjects.

In most adult subjects the development of a consistent CNV with an abrupt termination is associated with a marked reduction in reaction-time to the imperative stimulus. This is not due to the establishment of a time-reflex, as was at first supposed, since the subjects seldom make a single false response when the imperative stimulus is withdrawn, as in the extinction or equivocation trials. Electromyograms of the operant muscles sometimes show a slight change in their tonic balance following the first few unreinforced conditional stimuli, suggesting a persistent anticipatory bias.

The close correlation between attentiveness, expectancy, contingent significance, operant response and the ampli-

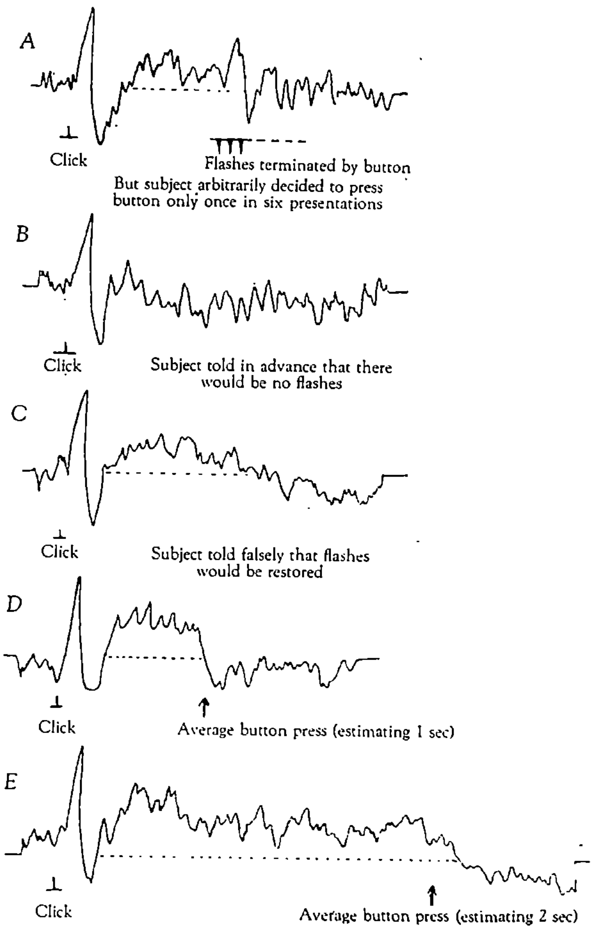

Fig. 4. Averages of six presentations. *A*, the subject decided arbitrarily not to press the button in response to the clicks; the CNV disappears (compare Fig. 1*D*); *B*, when told in advance that the imperative stimuli would be withdrawn the CNV vanishes at once (compare Fig. 2 *B*–*E*); *C*, subject told falsely that flashes would be restored; CNV reappears for first three presentations and then subsides; *D*, without imperative stimulus, subject is asked to press button at estimated one second after click; CNV develops as for associated stimuli; *E*, the same, with subject estimating 2 sec for button press

tude of the CNV suggests that this may be the electric sign of cortical 'priming' whereby responses to associated stimuli are economically accelerated and synchronized. The final discharge, whether in voluntary action or mental decision, is indicated by the abrupt decline in the CNV about 120 msec after the imperative stimuli, corresponding with the positive phase of the non-specific response to isolated stimuli.

The mechanisms underlying this remarkable effect can be inferred from other sources. Caspers[6] has described similar variations in cortical potential in rats during sensory stimulation and arousal which are attributable to depolarization of the apical dendritic feltwork of the cortex. Rowland and Goldstone[7] observed slow shifts of voltage in cats with implanted cortical electrodes during conditioning; they interpret these as "extension of an electrical arousal effect in terms of activation of cortical structures based on relation to drive". Rusinov[8] has also described slow negative waves recorded by Shvets[9], which appeared in the cortex of rabbits with the first appearance of conditioned movements; these were more generalized in the early stages than when the responses were 'concentrated' and vanished during extinction.

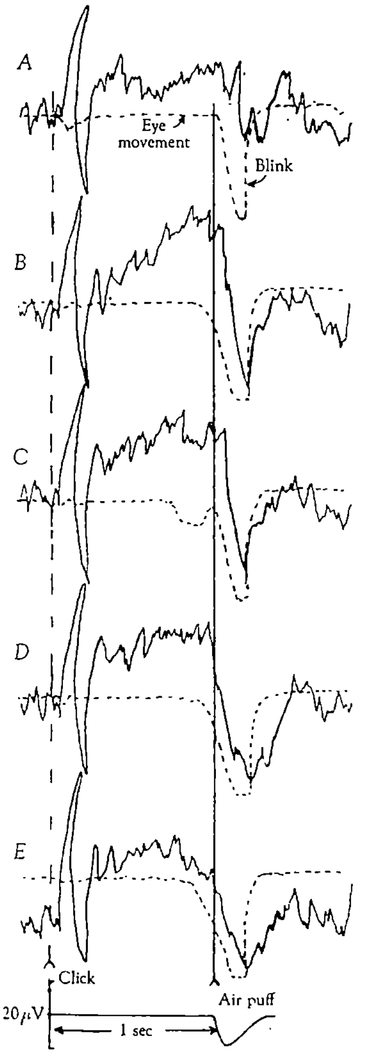

Fig. 5. Averages of 12 presentations; 'classical' conditioning of involuntary blink. Conditional stimulus click, unconditional stimulus air-puff to eye. *A*, first 12 trials show brain responses to both clicks and puffs; blink starts just after puff; *B*, second 12 trials: CNV appears, with conditioned blink just before puff which evokes no brain responses since eyelid is shut before corneal stimulus; *C*, *D*, *E*, progressive decline in CNV as conditioned response is consolidated

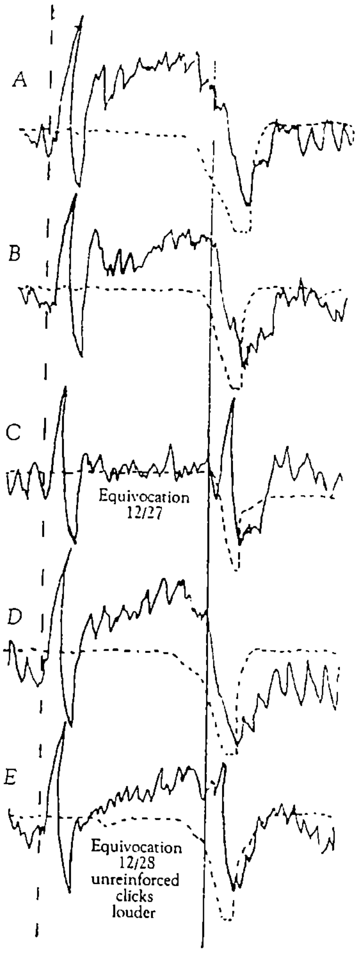

Fig. 6. Averages of 12 presentations of click and puff to eye (same series as Fig. 5). *A*, first 12 presentations after interval; CNV is larger than in Fig. 5*E* but conditional response is still present; *B*, second 12, CNV is smaller; *C*, equivocal association of clicks (only 12 of 27 reinforced) abolishes CNV and conditioned blink; brain response reappears since eye is open to corneal stimulus; *D*, regular association of clicks and puffs restores CNV and conditioned blink; *E*, equivocation as in *C*, but subject detected slight difference in loudness of reinforced and unreinforced clicks

In patients with chronic electrodes implanted over frontal cortex the CNV can be recorded from widely separated areas and remains generalized indefinitely during operant conditioning as in scalp records. During the establishment of a 'classical' defensive reflex, however, the CNV is more prominent in the early stages. This is shown in Fig. 5, taken during the acquisition of a conditioned corneal blink reflex elicited by clicks followed by puffs of air to the eye. In Fig. 5*A* the blink follows the air puff, but in Fig 5*B*, the average of the second dozen presentations, there is a prominent CNV, the blink precedes the air puff and the brain response to the corneal stimulus, which was prominent at first, has disappeared. In subsequent trials (Fig. 5*C*, *D*, *E*) the CNV shrinks progressively, until after about 60 trials it is scarcely above the base-line and the eye is closed well before the air-puff. The effects of 'probability dilution' or equivocation are particularly clear in this situation (Fig. 6). When the significance of the association between the auditory and corneal stimuli is diminished by partial reinforcement both the CNV and the conditioned response vanish entirely and the brain response reappears (Fig. 6*C*). However, when the subject discovered a slight difference between the reinforced and unreinforced conditional stimuli the CNV and conditioned response return, Fig. 6*E*.

Although the electric field of the CNV is generally limited to the anterior regions, there is some indication that variations in its spatial extent may be related to the degree and pattern of contingent response interaction, particularly in children. The application of these methods to the investigation of neuropsychiatric disorders has already shown a surprisingly close correspondence between the objective signs of cerebral expectancy and the mental state of disturbed patients.

This work was supported in part by the Mental Health Research Fund and the Parapsychology Foundation of New York.

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RECEPTORS IN THE SUCKER OF THE CUTTLEFISH

By PROF. P. GRAZIADEI

Department of Anatomy, University College, London

CERTAIN proprioceptors of crustaceans have proved to be useful in experimental investigations because both the cell body and dendrites lie at the periphery[1,2]. This situation has been found in the mantle of cephalopods[3,4]. The work recorded here shows that such multipolar nerve cells with the characteristics of receptors are also found in the suckers of the cuttlefish.

The general arrangement of the tentacular arm of *Sepia officinalis* has been described by Guérin[5] and my previous papers[6–8]. The sucker is cup-like, joined to the arm by means of a short peduncle (Figs. 1 and 2). The cup contains three principal zones where primary receptors can be demonstrated. These are: (1) the external side of the infundibulum (*A*, Fig. 1); (2) the flat rim of the cup (*B*, Fig. 1); (3) the bottom of the acetabulum (*C*, Fig. 1). These three zones contain several sorts of sensory cells, differing in shape and presumably in their capacity of recording information. Zone *A* is covered with columnar epithelium 30–40μ thick (all the numerical data given in this paper are related to suckers of approximately 3 mm diameter, from adult animals, 10 cm mantle-length). There are two types of receptors, probably touch- and chemo-receptors, in zone *A*. The touch-receptors have round or flask-like cell bodies lying in the inferior third of the epithelial layer and their axons go to one of the twelve nerve bundles that radiate from the peduncle into the wall of the sucker (Figs. 1 and 2). From the intra-epithelial course of the axon may arise some fine collaterals branching between the epithelial cells and intermingling with each other in a fine plexus at the base of the epithelial layer. The other type of cell is probably a chemo-receptor, because similar cells have been found in the lip of *Sepia officinalis*[9]. These cells are tapered and end at the free surface of the epithelium with a tuft of short cilia (2–3μ long). It is very difficult to state the number of these receptors because they stain only sporadically with the methods used (Bielschowsky, Cajal), but an approximate estimate is not less (probably much more) than 600 and 100 for touch- and chemo-receptors respectively in each sucker.

The flat rim of the sucker (zone *B*) is covered by a single layer of epithelium, unusual in that each cell is covered by a tooth-like cuticular apparatus (Fig. 3). The size of the epithelial cells is 30–40μ long and the cuticle is not less than 40–50μ thick. The cuticle provides a ring-shaped surface by means of which the sucker holds the objects grasped. There are multipolar nerve cells between the epithelial cells (approximately 300 in each sucker) which send branching processes ('dendrites') to the base of the stratum (Figs. 4, 5 and 6). These cells are presumably tension receptors. The size of these irregular nerve cells varies from 20 to 60μ (the size is taken as the average of the two largest diameters of the cell) and the processes of each cell spread over an area of 100μ diameter, each dendritic field overlapping the neighbouring ones. The axon arises from the hillock of the cell but light microscopy shows no changes in structure (Nissl and Cajal methods). The diameter of the axon remains considerable (8–12μ) along its entire course, and it may run without collaterals for more than 50μ in the epithelium (Fig. 4), where it turns into the muscular wall of the sucker (Figs. 1 and 6). Here it intermingles with the fibres forming the nerves of the sucker running to and from the arm ganglion. In this epithelium some very fine fibres of unknown nature (probably coming from the sucker ganglion) branch and intermingle with the dendritic processes of the nerve cells previously described.

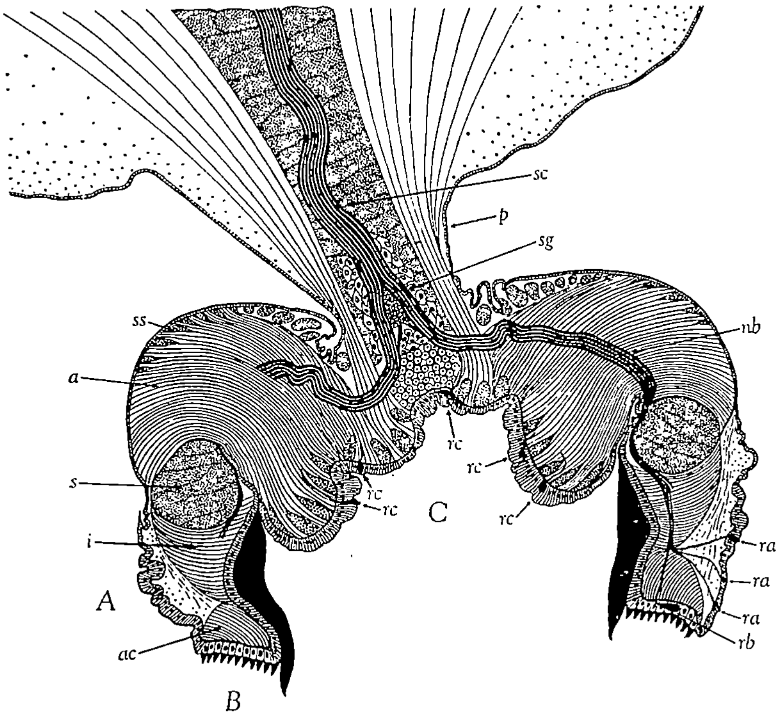

Fig. 1. Diagram of a sucker of *Sepia officinalis*, showing the general arrangement of the organ and its nerve supplies. *A*, *B*, *C*, zones of the sucker provided with different types of receptors. *a*, acetabular muscle; *ac*, acetabulo-cutaneous muscle; *i*, infundibular muscle; *nb*, nerve bundles radiating from the ganglion into the sucker: *p*, peduncle of the sucker; *ra*, receptors of the zone *A*; *rb*, receptors of the zone *B*; *rc*, receptors of the zone *C*; *s*, principal sphincter of the sucker; *sc*, sucker's nerve; *sg*, sucker ganglion; *ss*, secondary sphincters of the acetabulum

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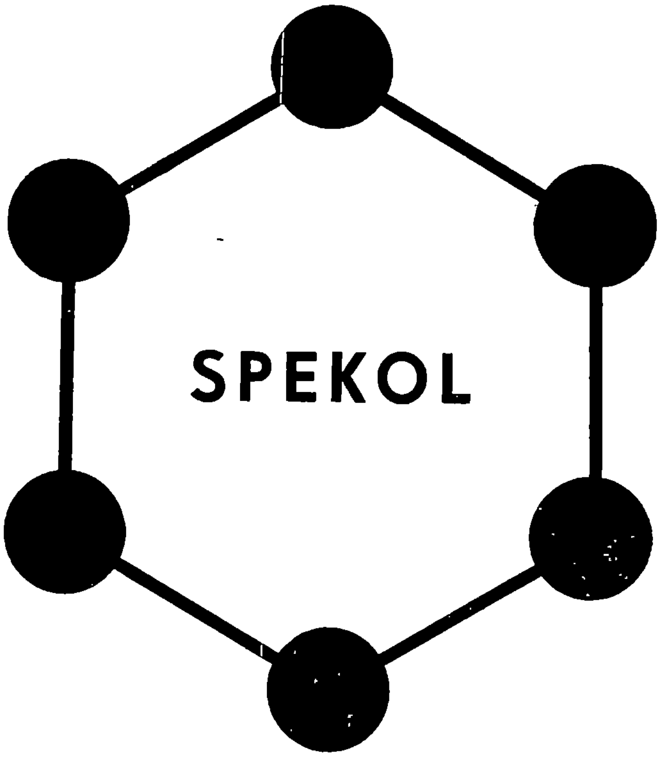

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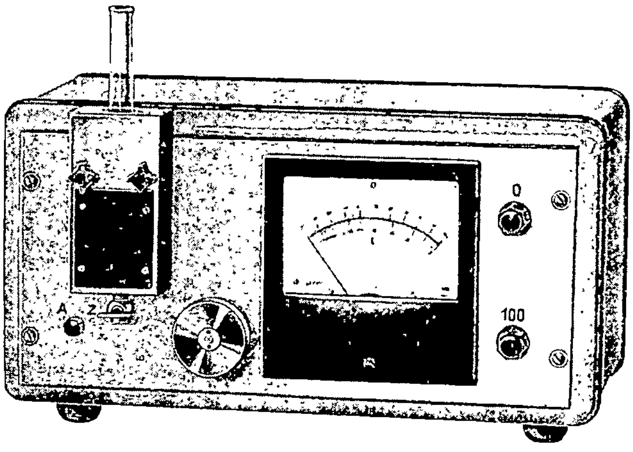

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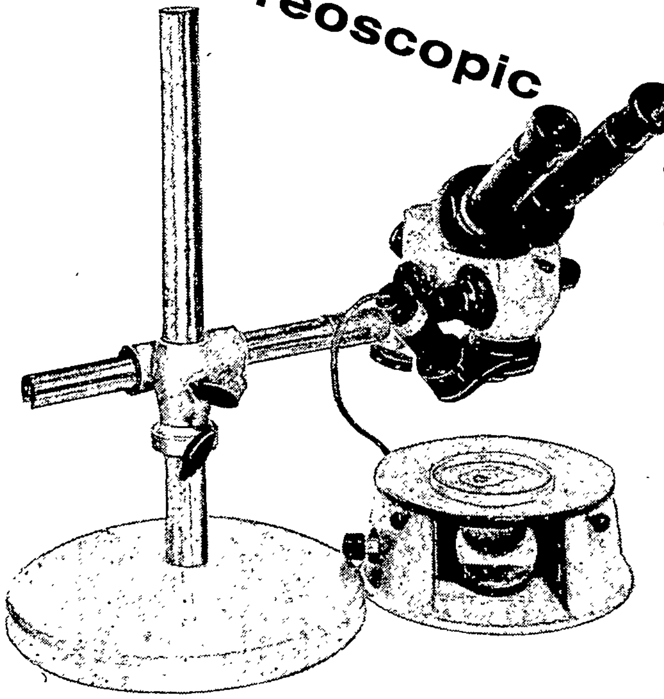

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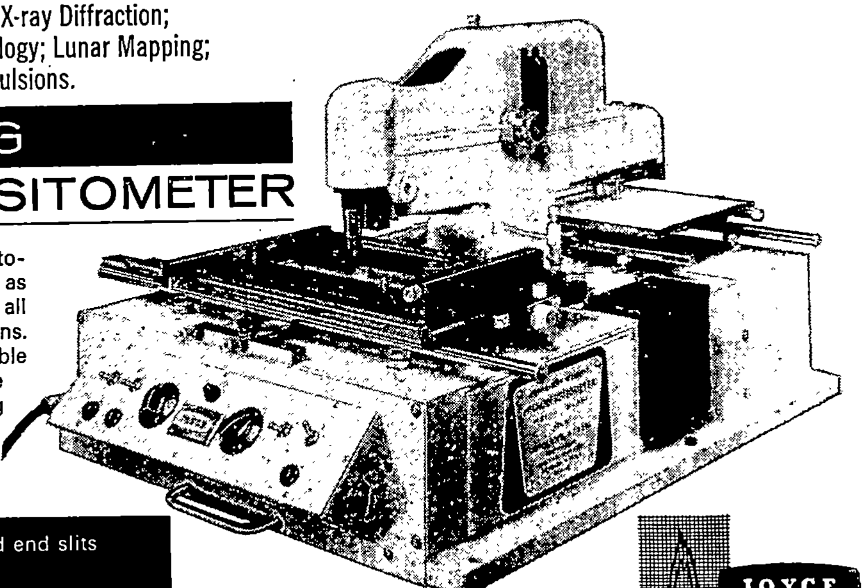

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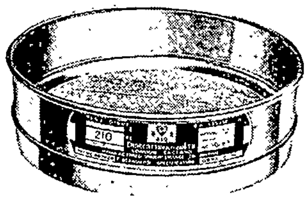

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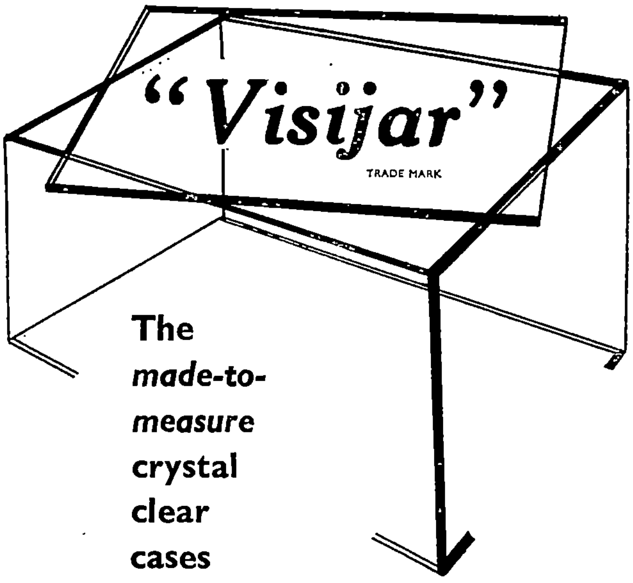

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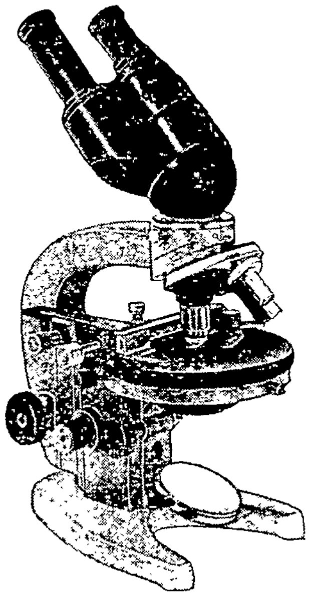

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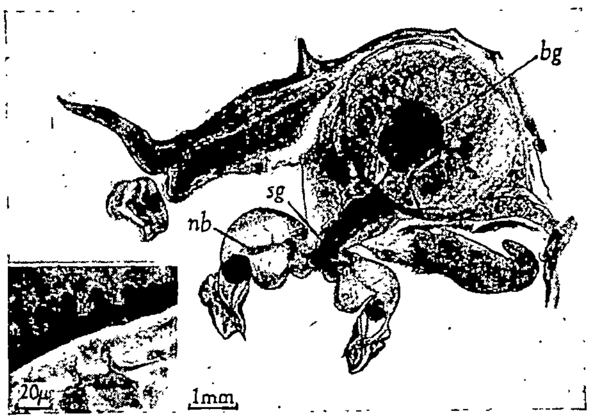

Fig. 3 Fig. 2

Fig. 2. Transverse section of an arm of *Sepia officinalis*, showing two suckers (Bielschowsky–Gros). The brachial ganglion (*bg*) and the sucker ganglion (*sg*) are joined by means of a nerve trunk, the nerve of the sucker. Nerve bundles (*nb*) radiating from the peduncle into the sucker carry sensory as well as motor fibres

Fig. 3. Epithelium of the zone *B*, with its tooth-like cuticular apparatus. Method: hæmatoxylin–eosin

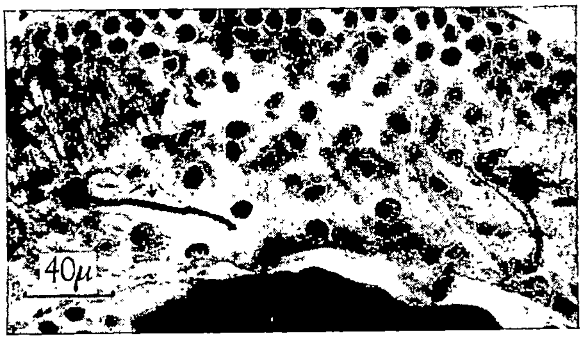

Fig. 4. Tangential section of the epithelium of zone *B*, with two multipolar nerve cells. The arrow shows the axon (Cajal–Young)

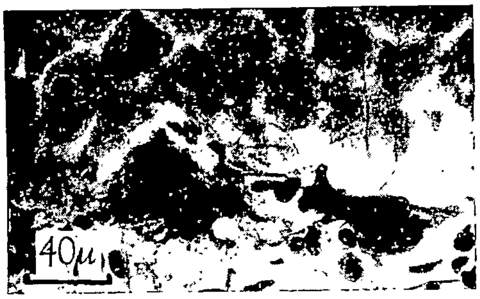

Fig. 5. Same epithelium as in Fig. 4, with two multipolar nerve cells (Cajal–Young)

The bottom of the sucker (zone *C*, Fig. 1) also contains primary receptors, of the three types already mentioned. Two of these closely resemble those previously described in zone *A*. There are very few, perhaps not more than 50, tapered sensory cells with a tuft of cilia, and there are about 200 flask-shaped cells at the base of the epithelium. The third category of receptors consists of a small number of multipolar nerve cells (Fig. 7), perhaps not more than 20, found only in a limited area of the epithelium above the ganglion that lies in the peduncle. These cells are triangular or polygonal in shape. Three or four dendritic processes leave the cell body. Each has a large base and maintains a large diameter (10μ or more) between the epithelial cells, in spite of giving off frequent collaterals. The dendrites of each cell form a dense arborization at the base of the epithelium, each one covering an area 100–200μ in diameter and intermingling with the neighbouring ones. There are, however, some differences between these cells and those previously described of zone *B* (Figs. 4 and 5). The cell body of zone *B* (Figs. 4 and 5) is large, with an irregular mass of cytoplasm round the nucleus and the processes arise from short, irregular laminar expansions of the cell body, forming a complex hair-like ramification round the cell. In zone *C* the cells have a small, pyramidal body, with little cytoplasm, and the dendrites arising are few and large (Fig. 7). The common feature of these two types of receptors is that their dendrites expand only in one plane, parallel to the epithelial surface and near the basal membrane so that some tangential sections show the entire complexity of every cell covering with its processes an area of 100μ, or more, diameter.

In the peduncle of the sucker (Figs. 1 and 8) there is a small, pear-shaped ganglion containing about 200 nerve cells. The larger cells (30–50μ) are motor ones sending axons to the muscles of the sucker. The ganglion is in the path of the bundles running from the arm ganglion to the sucker (Fig. 2) and these bundles carry approximately 1,000 fibres of various types (motor, sensory and interconnecting fibres).

The three types of receptors presumably send inputs of different kinds from the sucker of *Sepia* to the arm ganglia. The tapered ciliated cells may be chemo-receptors as comparative observations with light- and electron-microscope seem to confirm[10], and the flask-shaped receptors are perhaps touch-receptors. The multipolar nerve cells found in zones *B* and *C* seem to belong to a particular category of sensory elements which can now be called 'tension receptors'. In fact, these cells lie under a deep stratum of cuticle (Figs. 3 and 6) or at the bottom of the sucker, where tactile stimuli can scarcely be recorded (if at

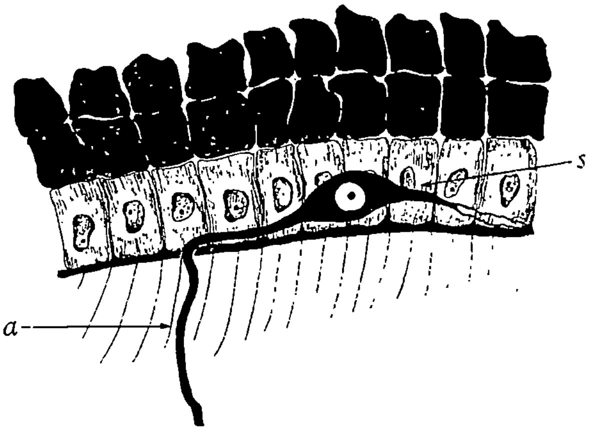

Fig. 6. Schematic diagram of the epithelium of the zone *B*, covered by a deep stratum of cuticle (in black). The sensory cell (*s*) lies between the epithelial cells, above the basal membrane, to which it sends fine, hair-like dendritic processes. (*a*) Axon of the sensory cell

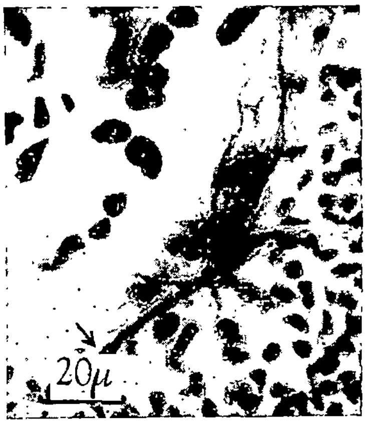

Fig. 7. Multipolar nerve cell of the zone *C*. The arrow shows the axon (Cajal–Young)

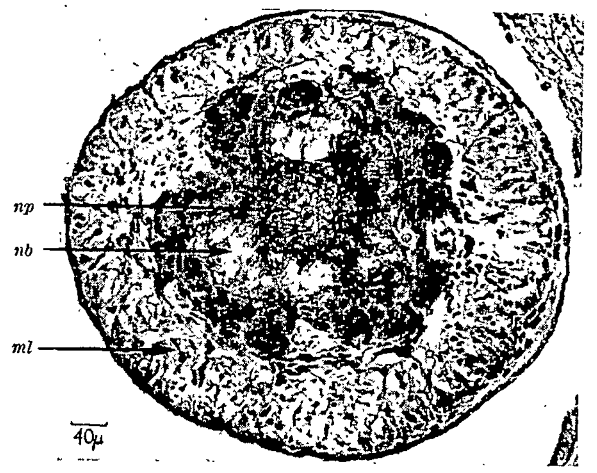

Fig. 8. Transverse section of the peduncle of the sucker (Cajal–Young). The ganglion of the sucker is surrounded by the peduncular muscles (*ml*). A fine network of nerve fibres in the core of the ganglion constitutes the neuropil (*np*). Between the neuropil and the nerve cell bodies lie bundles of fibres (*nb*) connecting the sucker to the arm ganglion (see also Figs. 1 and 2)

all). The extension of the dendritic field and the close relationship of the dendrites with the epithelial basal membrane suggest a comparison with the stretch-receptors previously described in the muscles of these animals by some authors[3,11]. When the animal grasps an irregular object or prey, the rim of the sucker bends and the deformation of the cup cannot be detected by the other receptors. There seem to be no muscle stretch-receptors in these organs and the third type may be the morphological structure underlying the recording of stretch events.

I thank Prof. J. Z. Young for his helpful advice. Mr. A. J. Aldrich took the photographs and drew Fig. 6, and Mrs. J. I. Astafiev drew Fig. 1.

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COOLING TIME OF SILICA FIBRES

R. G. C. ARRIDGE and KATHLEEN PRIOR
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IN many discussions of the strength of glassy materials reference is made to the rapid cooling rate achieved when fibres are drawn from a melt. This rate is held to be so high that relaxation processes in the glass do not have time to occur and therefore the filamentary glass retains the structure belonging to a higher temperature.

During the exploratory stage before the development of a silica fibre drawing-coating process, we decided to measure the temperature of a silica fibre by a direct method since the fibres appeared to be much hotter than expected on Anderson's theory[1].

The method used was an adaptation to high temperatures of the Culpin and Madoc Jones[2] heated thermocouple method. The thermocouple was welded to a thin strip of nickel–chromium alloy resistance element bent into a V shape. The temperature attained by the strip on heating electrically was recorded by the thermocouple potentiometer. When this device was dabbed momentarily on to the running silica thread a kick of the potentiometer needle was obtained in one direction or another accordingly as the thread conveyed heat to, or away from, the thermocouple. When a position on the thread was found where the temperature corresponded to that of the thermocouple no kick in either direction was registered.

Surface temperatures of threads could therefore be measured either by selecting a particular temperature and moving up or down the thread until no deflexion was registered on dabbing, or by selecting one place and adjusting the thermocouple temperature to give no deflexion. Both methods gave the same result to within experimental error.

The thermocouple device was calibrated by inserting it into a uniform temperature enclosure.

The effect of frictional heating was shown to be negligible by tests on cold running threads.

Fig. 1 shows the cooling curves for fibres of various diameters (28–68μ) drawn from 4-mm rod at speeds of 178–508 cm/sec. For comparison, the calculated (Anderson) cooling curve for a 48μ fibre drawn at 178 cm/sec is shown in the same diagram.

Fig. 2 shows the cooling curve for the 48μ fibre replotted on a log temperature–time basis which should yield a straight line if Newton's law of cooling were followed. The curve shows a turning point at about 150° C, the departure from Newton's law becoming excessive below this temperature.

Comparison of the measured times with Anderson's predicted values shows that the measured value is about 30 times greater than that predicted in the logarithmic cooling region and becomes even greater below the critical temperature, 150° C.

On Fig. 3 we have plotted the measured times for cooling to 200° C against the fibre radius on a log–log plot.

The fit is reasonably linear for all speeds and gives a slope of 1·8.

Thus the time for cooling to 200° C is proportional to $(\text{radius})^{1\cdot8}$ as against Anderson's predicted figure $(\text{radius})^{2\cdot5}$. There are too few results really to comment, but it would

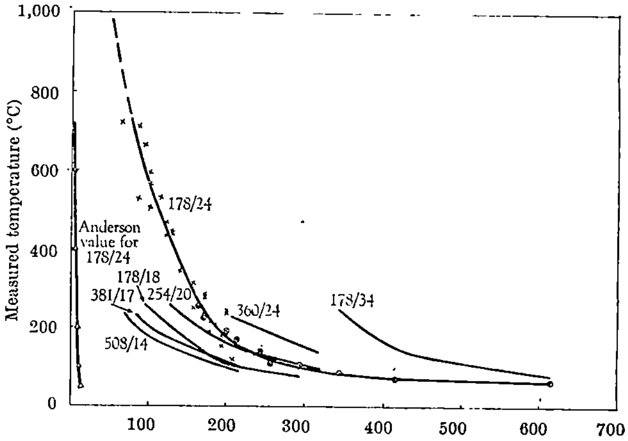

Fig. 1. Comparison of theoretical (Anderson) and experimental cooling rates for silica fibres drawn from 4-mm rod at 1750° C at various speeds

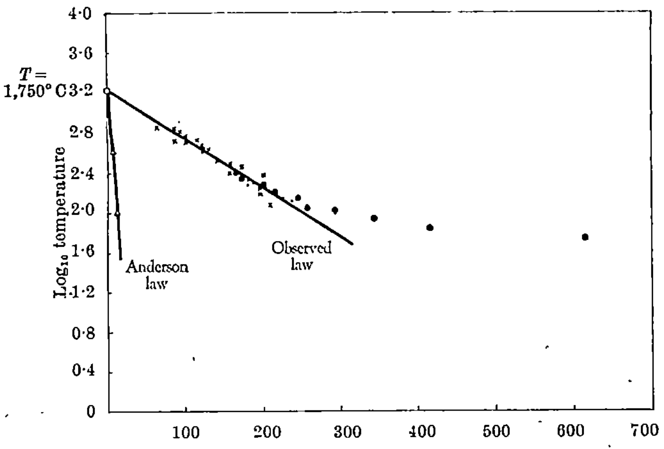

Fig. 2. Log temp.-time plot

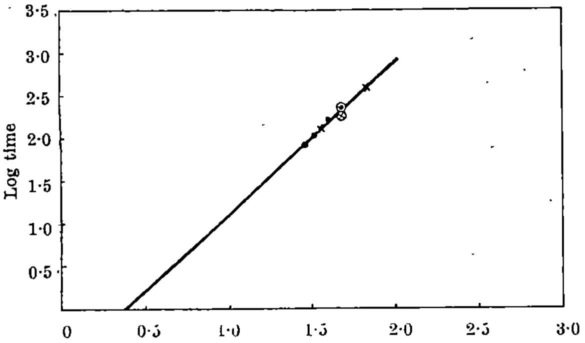

Fig. 3. ●, Constant Q; ×, constant V; ○, constant R

appear that Anderson's law of radius dependence is not followed.

In the (one) case of a change of speed where the radius is kept constant the faster thread, in fact, cools more slowly than the slower, whereas Anderson's theory predicts a $v^{-\frac{1}{2}}$ relation.

Anderson's theory predicts a relation:

$$t = \frac{B\,R^{2-n}}{v^n}\ln(1/y)$$

$B \sim 400$ for silica (using a value 0·007 for the thermal diffusivity)

$n = \frac{1}{2}$

$y = (T - T_0)/(T_i - T_0)$

R is the radius of the thread, v the velocity.

We assume $T_i = 1{,}750°$ C, $T_0 = 20°$ C.

Using this relation we have calculated the cooling times predicted by the Anderson theory for our fibres. for comparison with the observed values.

Table 1

Process (speed/radius)	Time to cool to 400° C (msec)		Time to cool to 200° C (msec)		Time to cool to 100° C (msec)	
	Theor.	Obs.	Theor.	Obs.	Theor.	Obs.
508/14			2·1	85	2·9	200
381/17			3·1	100	4·2	220
254/20			5·1	165	6·9	310
178/24	5·4	130	8·0	190	10·8	300
178/34			13·5	375	18·3	540
178/18			5·2	130	7·0	220
360/24			5·6	240	7·6	—

We offer no explanation of the large time difference between Anderson's predicted and our measured values, but a possible explanation of the departure from Newton's law of cooling below about 150° C is as follows.

When a silica fibre is being drawn it is nearly invisible, yet on touching it a bright spot of light is seen where the internally reflected radiation from the hot molten zone can emerge at the point of damage.

The magnitude of the radiative heat transferred assuming a black body radiator of area equal to that of the fibre is 5×10^{-4} cal/sec. On Newton's law of cooling, the heat lost per second by the fibre at, say, 100° C is of the order of $1{\cdot}37 \times 10^{-2}$ cal/sec. Allowing for the possibility that a larger area may be involved in radiation at the point of draw than the area of the fibre cross-section (for example, during draw down) then the radiative term may affect a measuring instrument more than the Newton term and, being of constant value, contribute increasingly at the lower temperatures.

The fact that the times of cooling are considerably longer than are predicted on the Anderson theory calls into question some of the ideas about structure which require rapid cooling times. Either these ideas are erroneous (for example, the frozen-in high temperature structure) or the relaxation time in silica is several orders of magnitude longer than it was thought to be.

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ELECTRON MICROSCOPE OBSERVATION OF NUCLEAR REACTIONS

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It has been shown that it is possible to observe by electron microscopy the tracks formed in various crystals due to the passage of heavy charged particles such as fission fragments[1–3], and that the appearance of these tracks is sensitive to the energy and mass of the bombarding particle and to the thickness of the crystal[4]. In an attempt to observe directly the damage caused by lightly ionizing particles, thin crystals of molybdenum trioxide were irradiated with helium-3 ions of energy 29 MeV.

Molybdenum trioxide was prepared by condensation from the vapour directly on to a carbon support film on an electron microscope grid: grown by this method the crystals ranged in thickness from less than 50 Å to several thousand Å. The grids were mounted on brass backing plates and bombarded for varying periods of time with 29-MeV helium-3 particles accelerated by the Nuffield cyclotron of the University of Birmingham, the total doses ranging from 10^{10} to 10^{16} ions/cm^2. The irradiated crystals were investigated using the A.E.I. *E.M.*6 electron microscope operating at 75 keV.

Observation of crystals bombarded by 10^{15}–10^{16} ions/cm^2 revealed a number of particle tracks, which were occasionally darker than the surrounding crystal due to the operation of diffraction contrast but were more often light tracks due to the removal of material of the crystal (Fig. 1). The track thickness was generally considerably less than that of fission-fragment tracks, varying from ~ 25 Å to ~ 100 Å. A comparison with an electron micrograph of fission-fragment tracks in MoO_3 is given in Fig. 2. The density of the tracks, which was estimated to be $\sim 10^6$/cm^2 in a grid bombarded by 10^{16} helium-3

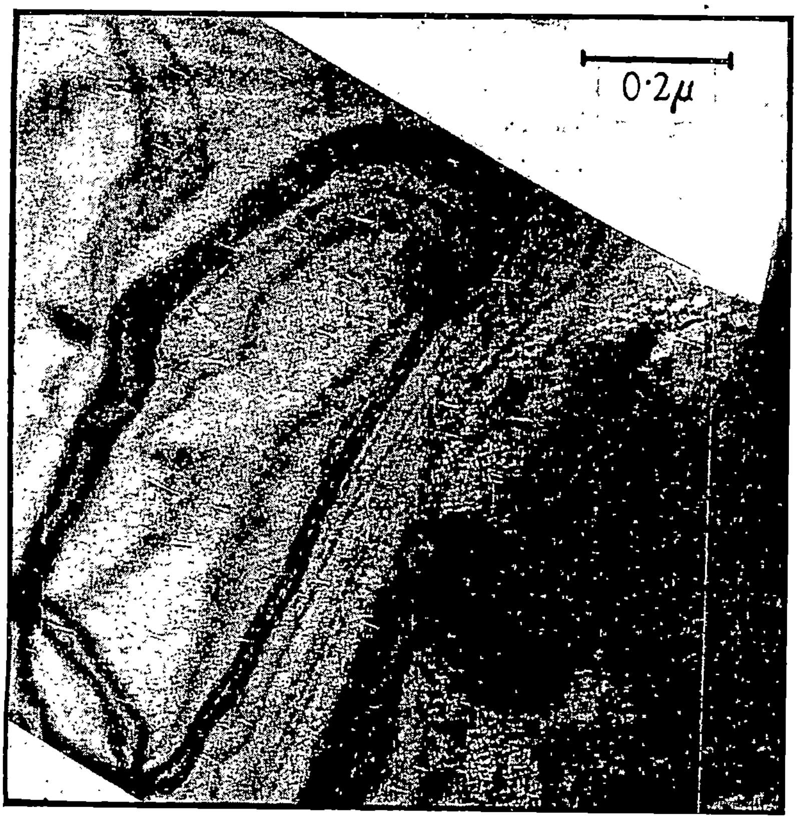

Fig. 1. Tracks in a ³He-irradiated crystal of MoO_3. Often, as in this micrograph, they appear to come from a thick crystal. It is suggested that they are formed by nuclei recoiling from nuclear reactions in the thick crystal

ions/cm², was extremely low in comparison with the beam intensity. It varied considerably over any one grid square, in that the tracks tended to occur in clusters where the density was up to a thousand times the average density, and there were large areas containing no visible tracks. Similarly, the directional variation of the tracks was quite uniform when averaged over a complete grid, but they were usually well collimated (to within 20°) in any one crystal or group of crystals, though not in any particular crystallographic direction. In this respect it is significant that they were not visible in all crystals, and were more often observed where a thin crystal was close to a cluster of thick crystals, aligned approximately in the direction of the cluster.

Mechanism responsible for track formation. The evidence presented here suggests that what we are observing is some form of secondary effect caused by the interaction of the incident helium-3 ions with the atoms of the crystal or with the copper of the electron microscope grid. The bombardment was performed with the plane of the grid perpendicular to the ion beam, and so the tracks in thin crystals must make an angle of ~ 90° with the incident particles. Consideration of the types of secondary effects possible shows that they must be created by one or more of the following effects: (1) the helium-3 particles themselves, having undergone large angle collisions; (2) primary knock-ons due to small impact parameter collisions of the incident ions with the molybdenum or oxygen atoms of the crystals, or with copper atoms of the grid; (3) ions resulting from nuclear transmutations initiated by the helium-3 particles in the nuclei of the crystals or grid.

It is unlikely that the tracks are formed directly by the incident ions after being scattered through approximately 90°. The rate of loss of energy by ionization and excitation of a heavy energetic charged particle passing through matter can be calculated using equations derived by Bohr[5] and by Bethe and Bloch[6]. The Bohr equation applies to a particle the effective charge of which decreases as it slows down in the medium, and is therefore mainly used in calculations involving very energetic heavy ions. The Bethe–Bloch equation can be stated:

$$-\frac{dE}{dx}=\frac{4\pi N Z_1^{*2} e^4}{m_e v^2} Z_2 \log_e\left(\frac{2 m_e v^2}{K Z_2}\right)$$

where v is the velocity of the charged particle, m_e and e the electronic mass and charge, N the atomic density of the matter (No. of atoms/c.c.), K is a constant of the order of 11·5 eV, Z_1 and Z_2 the atomic numbers of the particle and material through which it is travelling, and Z_1^* is the effective charge of the energetic particle, which depends on its degree of ionization. By considering Z_2 as a weighted average of the atomic numbers of molybdenum and oxygen, the energy loss rate for a helium-3 ion travelling in MoO_3 can be calculated by the Bethe–Bloch equation to be:

$$\left(-\frac{dE}{dx}\right)_{^3\mathrm{He}} = 0{\cdot}00247 \text{ erg/cm}$$

This can be compared with the energy loss for a median-light fission fragment (at the lower mass peak in the fragment/distribution curve[7]) where $Z_1 = 39$, $E = 95$ MeV:

$$\left(-\frac{dE}{dx}\right)_{\substack{\text{fission}\\ \text{fragment}}} = 0{\cdot}654 \text{ erg/cm}$$

which is very much larger than that for a helium-3 ion.

Since the energy transferred to the crystal in this way dissipates mainly by ionization and localized heating and hence track formation, we should expect that the helium ion will form a very narrow track which will under most conditions be invisible in the electron microscope. This hypothesis is supported by the fact that no helium-3 tracks have so far been observed in similar specimens of MoO_3 bombarded at low angle (10°) with a radiation dose of 10^{10}–10^{13} particles/cm². At these doses individual particle tracks, if visible, could be resolved. Thus it is fairly certain that the tracks observed here must be due to a heavier particle.

In addition, we can eliminate primary knock-ons as a possible cause by considering the mechanics of a close collision between a helium-3 ion (E = 29 MeV) and an atom of the crystal or grid. In the classical approximation, which is sufficient for our purposes, the differential cross-section for Rutherford scattering of an energetic ion may be expressed in terms of the energy transfer T:

$$d\sigma\,(E, T) = \frac{\pi b^2}{4}\, T_m \,\frac{dT}{T^2}$$

where E is the incident particle energy, b is the collision diameter, and T_m is the maximum energy transfer which can occur in the collision. In a collision between particles of masses M_1 and M_2, and charges Z_1e and Z_2e:

$$T_m = \frac{4\, M_1\, M_2}{(M_1 + M_2)^2}\, E$$

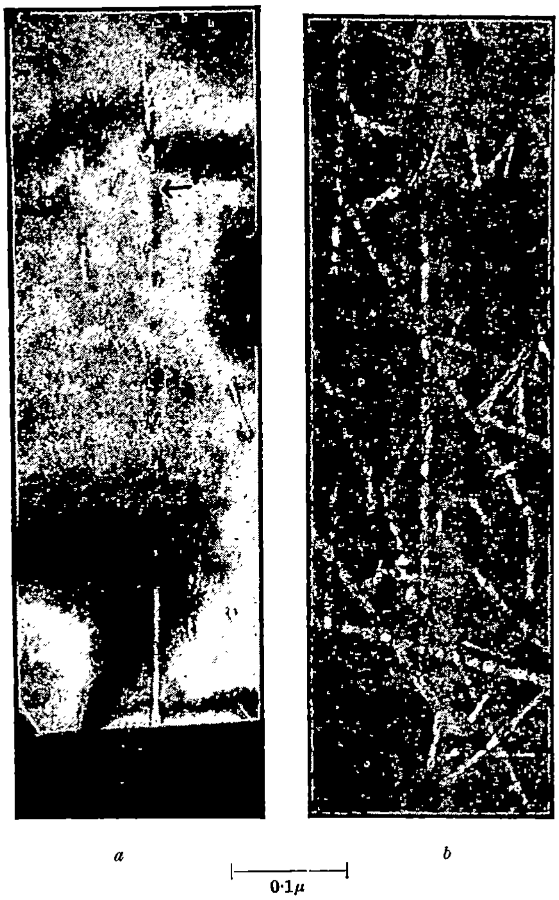

Fig. 2. *a*, Track produced by a recoiling nucleus showing diffraction contrast and a large angle collision (arrowed). The width is much less than in tracks formed by fission fragments (*b*)

and:

$$b = \frac{Z_1 Z_2 e^2 (M_1 + M_2)}{M_2 E}$$

Consideration of the variation of $d\sigma$ with T reveals that the scattering process favours low energy knock-ons. These inevitably carry off much less energy than would be transferred in a head-on impact ($T_m \simeq 4$ MeV), and their rate of loss of energy is therefore not sufficient for track formation.

The removal of this possibility leaves us with the third and most probable cause, namely, the heavy ion end-products of nuclear reactions. The cross-section for a reaction of the form:

$$X + {}^3\text{He} \rightarrow Y + z$$

(where X and Y are heavy ions and z represents one or more light particles) in crystals of the thickness normally used in transmission electron microscopy is negligibly small, even with as high a dose of radiation as has been used in these experiments. This would explain qualitatively why the tracks were extremely rare in isolated thin crystals. The cross-section for reactions in a cluster of crystals thick enough to be opaque to the electron beam is greatly increased, both by the much greater distance travelled by the helium-3 ions in the crystal, and by the fact that the energetic heavy ion products can be emitted at a smaller angle than 90° with the incident beam direction and still form visible tracks in adjacent thin crystals. The probability of heavy ion emission from the nuclear reaction is obviously greatest in a forward direction with reference to the incident beam, and it is quite possible that some of the tracks observed here could result from the large-angle scatter of these ions into the plane of the thin crystals.

Reactions most likely to give rise to the observed tracks are those yielding heavy energetic radioactive ions of isotopes with mass number in the region of that of the crystal atom struck by the helium-3 ion. For example, the following reactions with oxygen atoms in oxides and nuclear emulsions have been observed[8,9]: ${}^{16}\text{O}({}^3\text{He}, d){}^{17}\text{F}$; ${}^{16}\text{O}({}^3\text{He}, p){}^{18}\text{F}$; ${}^{16}\text{O}({}^3\text{He}, n){}^{18}\text{Ne}$; ${}^{16}\text{O}({}^3\text{He}, {}^4\text{He}){}^{15}\text{O}$. The products of reactions such as these are generally unstable, tending to decay with half-lives varying from a few minutes up to several days. In this connexion it is interesting to note that the specimens and backing plates were observed to be radioactive immediately after irradiation. Similar reactions in copper and molybdenum trioxide have been detected, with helium-3 and helium-4 as the incident ions[10–12]. In a reaction of this nature, the incident particle energy and the Q-value of the reaction are shared by the end-products, and thus it is probable that the heavy ion will have energy 10–20 MeV, which is quite sufficient to form a visible track.

Conclusion. The results so far imply that the tracks observed in these crystals are formed as a consequence of collisions between helium-3 ions and nuclei of the crystal or grid atoms, in which penetration of the nuclear potential barrier occurs, leading to transmutation and a high-energy transfer to the product nuclei. If this is the case, we not only have an efficient solid-state detector, but a possible method of investigating the mechanism of track formation. An accurate quantitative treatment of fission-fragment tracks is normally impossible since little is known about the statistics of the particle. The energy and mass can lie at any point on the twin-peaked curve of the fragment distribution, and the energy is further influenced by the depth of uranium foil traversed by the fragment between its point of creation and the surface of the foil[7]. In the case of the ion tracks observed after ^{3}He-irradiation, although the energy can lie within a fairly broad range, the mass may be reasonably estimated to within a few atomic mass units if, as seems possible from differences in track thickness, we can differentiate between ions resulting from a ${}^3\text{He}$–${}^{16}\text{O}$ reaction and those from a ${}^3\text{He}$–${}^{96}\text{Mo}$ reaction. In the reaction with a molybdenum atom, the product heavy ion could have a mass number considerably less than 96, if, for example, 3 α-particles were emitted, but the cross-section of this type of reaction is relatively small compared with those in which the mass change is three or less. Thus it may be possible to compare track parameters such as thickness and frequency of large angle collisions with theoretical predictions in an attempt to discover more about the mechanism of track formation in a binary crystal such as MoO_3.

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HENBURY IMPACT GLASS: PARENT MATERIAL AND BEHAVIOUR OF VOLATILE ELEMENTS DURING MELTING

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THE nature of the parent material of tektites has been discussed for many years. A basic question is whether the present composition reflects that of the source material, or whether it has been modified during melting, with loss of more volatile constituents. We are concerned here only with elements which will be lost at temperatures in excess of 1,000° C, and exclude the material, such as water, lost at lower temperatures, commonly reported in chemical analyses as 'lost on ignition'. Elements which have been thought to be volatile during the processes of tektite formation include the alkali elements[1,2], zinc, copper[3] and germanium[4]. Other possibilities are lead, thallium and mercury.

In most potential source materials, sodium and potassium will be present at concentration-levels of a few per cent, and the others in trace quantities. The selective loss of these elements would raise some difficulties in identification of the parent material, but would not modify the concentrations of the other elements by more than a few per cent. If there has been no appreciable loss of the volatile elements, the present concentrations can be used to identify possible parent materials. Losses of up to 10 or 20 per cent of the concentrations of the volatile elements will not seriously hinder the search. Some workers[1-3] have attempted to show that tektites may be derived from granitic-type igneous rocks by loss of such elements.

Friedman *et al.*[5] found no evidence for selective loss during fusion of rocks at 2,500° C in air and concluded that "the observed composition of tektites probably reflects the composition of the material from which they came". In extensive investigations of the composition of australites[6,7] no effective depletion of volatile elements such as cæsium and rubidium has been observed. It has not been necessary to invoke such mechanisms to account for the chemical composition of these tektites, which may be matched with that of terrestrial sedimentary rocks. Geochemical examinations of the enigmatic Darwin glass have led to similar conclusions[8].

A useful new approach is to examine the composition of undoubted terrestrial impact glasses and their source material. The extrapolation to the unknown conditions of tektite formation is uncertain, but may be distinctly possible because glass formation probably occurs under a similar range of conditions whether the event is large or small. A further reason for this investigation has been the lack of comprehensive trace element data for impact glasses which has handicapped workers on tektites and other glasses of debatable origin.

Suitable areas to compare impact glasses and their parent materials are rare. At Henbury, Northern Territory, Australia (24° 35′ S.; 133° 09′ E.), the impact of an iron meteorite shower has produced twelve craters and minor amounts of glass associated with the largest crater. This occurrence is well described by Alderman[9] and Spencer[10]. The rock outcropping at the surface is sandstone, dipping south at a low angle. Blocks of an underlying subgreywacke are exposed in the main crater. Glass samples from two areas, one on the crater rim and the other about 100 m north, were collected. The glass occurs as small brown cellular masses. Samples for analysis were obtained by gentle crushing without metallic contact, followed by ultrasonic cleaning and hand-picking of the washed material under a binocular microscope. Eight samples of the sediments were also analysed. Full details of sample localities, descriptions, sample preparation and analytical methods will be reported elsewhere.

A brief comment on the analytical methods is necessary. The major elements were determined by Dr. H. B. Wiik using wet chemical methods. The trace elements were determined by emission spectrochemical methods utilizing standard methods previously developed[11] and used for the analysis of tektites[6,7] and Darwin glass[8]. Instrumental calibration was carried out using interlaboratory standard samples[12]. The high content of iron and nickel in the glasses made it necessary to check commonly used spectral lines for interference by lines of these two elements. A correction for titanium interference by the line at 8518 Å was made for the cæsium analytical line at 8521 Å. This line was resolved from the iron line at 8514 Å. Work is proceeding on the determination of gallium. The line at 2944 Å is subject to nickel interference at the concentrations observed in the glasses, the lines at 2874 Å and 4172 Å are subject to interference by iron lines and the line at 4033 Å is unusable because of the presence of a strong manganese line.

Table 1. MAJOR ELEMENT DETERMINATIONS ON HENBURY GLASS AND SUBGREYWACKE

	Glass No. 1		Glass No. 2		Sediment No. 5	
	A	*B*	*C*	*D*	*E*	*F*
SiO_2	70·84	75·09	68·11	75·60	75·01	77·26
Al_2O_3	11·62	12·32	11·12	12·34	10·54	10·85
Fe_2O_3	3·91	—	2·39	—	2·89	—
FeO	5·83	—	11·43	—	1·00	—
FeO*	(9·34)	3·71	(13·57)	3·71	(3·60)	3·71
MgO	2·25	2·39	2·10	2·33	1·95	2·01
CaO	0·70	0·74	0·53	0·59	0·81	0·83
Na_2O	0·87	0·92	0·78	0·87	0·97	1·00
K_2O	3·01	3·19	2·95	3·27	2·86	2·95
TiO_2	0·83	0·88	0·80	0·89	0·80	0·82
H_2O^+	0·00	—	0·00	—	2·08	—
H_2O^-	0·05	—	0·08	—	0·45	—
P_2O_5	0·03	—	0·03	—	0·02	—
CO_2	0·00	—	0·00	—	0·20	—
	100·28	99·20	100·83	99·60	99·65	99·43

Columns *B* and *D*, meteoritic iron content removed.
Column *F*, water and carbon dioxide removed.
* Total iron expressed as FeO.

The analytical results obtained answer three questions: the nature of the parent material; the extent of addition of iron, cobalt and nickel from the meteorite; and the effect of melting on the chemical composition of the source material.

Preliminary qualitative spectrochemical analysis showed a close correspondence in composition between the glass and the subgreywackes, except for iron, cobalt and nickel, which were present in much higher concentrations in the glass. The composition of the sandstones was not similar to that of the glass. These conclusions have been confirmed by the quantitative data. A comparison of the major elements, expressed as oxides, is given in Table 1 for the two glass samples and the sediment closest in composition. The high iron content of the glasses is noteworthy. Columns *B* and *D* give the glass composition with the excess iron removed. Results for the non-volatile trace elements are given in Table 2, which also includes data for the major-constituents expressed as elements. There is a close correspondence in composition between glass and sediment except for iron, cobalt and nickel. Using the

Table 2. ANALYTICAL RESULTS FOR SOME NON-VOLATILE ELEMENTS IN HENBURY IMPACT GLASS AND SUBGREYWACKE

Element		Glass No. 1		Glass No. 2		Sediment No. 5
		A	*B*	*C*	*D*	*E*
B	p.p.m.	140	148	130	144	135
Ba	p.p.m.	600	640	700	780	620
Ca	%	0·50	0·53	0·38	0·42	0·60
Cr	p.p.m.	78	83	81	89	56
La	p.p.m.	53	56	56	62	51
Mg	%	1·36	1·44	1·27	1·41	1·22
Mn	p.p.m.	1,050	1,110	980	1,090	840
Sc	p.p.m.	11	12	10	11	11
Sr	p.p.m.	80	85	90	100	90
Ti	p.p.m.	4,980	5,280	4,790	5,320	4,930
V	p.p.m.	77	82	77	85	65
Y	p.p.m.	59	63	65	72	90
Zr	p.p.m.	415	440	430	480	630
Fe	%	7·26		10·55		2·88
Co	p.p.m.	190		370		11
Ni	p.p.m.	2,500		4,000		26

Columns *B* and *D* corrected for meteoritic iron contribution.
Column *E* corrected for 2·73% loss on ignition.

Table 3. 'VOLATILE' ELEMENT CONTENT IN HENBURY IMPACT GLASS AND SUBGREYWACKE

		Glass No. 1		Glass No. 2		Sediment No. 5
		A	*B*	*C*	*D*	*E*
Li	p.p.m.	38	40	32	36	35
Na	%	0·65	0·69	0·58	0·64	0·74
K	%	2·50	2·65	2·45	2·72	2·44
Rb	p.p.m.	120	127	105	117	110
Cs	p.p.m.	4	4·2	3·5	3·8	3
Cu	p.p.m.	37	39	47	52	18
Pb	p.p.m.	20	21	23	26	24

Columns *B* and *D* corrected for excess iron.
Column *E* corrected for 2·73% loss on ignition.

sediment data as representing the original composition, the iron/nickel and nickel/cobalt ratios derived from contamination by the impacting meteorite average 18·4 and 12·5. These compare with iron/nickel ratios of 12·8 and 12·1 and nickel/cobalt ratios of 33 and 20 from published analyses of the Henbury iron[10]. The match for the other trace elements is close, with chromium and vanadium higher and titanium, zirconium and yttrium lower in the glass compared with the sediment. Probably the parent material of the glass was slightly richer in clay and poorer in resistant minerals such as zircon than the sediment listed.

Results for the 'volatile' elements are given in Table 3. The composition of the two glasses and the sediment is very similar. Sodium is about 20 per cent higher in the sediment. Lithium, potassium and rubidium are close in concentration, and the volatile element, cæsium, is higher in the glasses than in the sediment. The higher concentrations of cæsium parallel the higher copper, vanadium, chromium and magnesium contents, which may be interpreted to indicate that the source material of the glass was slightly higher in clay content than the sediment listed. The volatile element, lead, is present at similar concentrations in both glasses and sediment. It seems clear from an examination of Table 3 that no significant depletion of the 'volatile' elements has occurred during glass formation. The slight differences observed may be readily attributed to minor differences in composition of the source rock. The results do not exclude losses of a few per cent, but limit selective distillation to a minor role, not materially affecting the composition. The changes in composition are limited to the addition of iron, cobalt and nickel from the impacting meteorite.

The results considered here strengthen the interpretation based on internal compositional evidence that the compositions of australites[6,7] and Darwin glass[8] have not been seriously modified by loss of the more volatile constituents, so that their present compositions reflect that of the parent materials.

We thank Dr. H. B. Wiik for analyses of the major element and Mrs. A. Halsey for carrying out the spectrographic determinations.

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LAYERED INTRUSIONS OF THE GILES COMPLEX, CENTRAL AUSTRALIA

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THE Giles Complex[1] consists of a series of basic–ultrabasic layered intrusions of Archæan age, which are situated in Western Australia and the north-west corner of South Australia. Basic igneous rocks were first recorded from the area by Basedow[2], and later examination of this material[3] showed their ultrabasic nature. The investigation recorded here deals with those intrusions found in the South Australian part of the complex, with particular emphasis on the largest of these at Mt. Davies (Fig. 1).

The Mt. Davies intrusion is an east-west trending elongate body about 10 miles long and 3 miles wide. The intrusion is marked by spectacular compositional banding which in the main body of the intrusion dips north at 70°–80°. The rocks are almost unweathered, making them ideal for petrological and geochemical investigation.

Contacts with the country rocks are often faulted, but the common presence of biotite in the igneous rocks at some of the contacts, suggests that these represent the true margins of the body, which have been contaminated by the incorporation of acid granulites.

Petrological and geochemical investigations of the intrusion are at an early stage of development, and although there has been a major specialized investigation of the intrusion by a commercial organization and by the South Australian Department of Mines, no systematic petrological description has been published.

Some chemical analyses on gabbros and norites have already been made, and olivine and feldspar compositions determined. The olivine compositions were determined using the method of Yoder and Sahama[4] with the modification that zinc oxide instead of silicon has been used as an internal standard. The results indicate a progressive enrichment in the iron molecule fayalite from 5 per cent in the ultrabasic zone to 30 per cent in the olivine norites on the south side of the intrusion.

It is considered that if the layering in the body is gravitational in origin (that is, originally close to the horizontal) then the mineralogy indicates that the rocks are younger to the south and so are overturned. This leads to a tentative subdivision of the intrusion into: (1) ultrabasic zone; (2) olivine-enstatite-gabbros grading

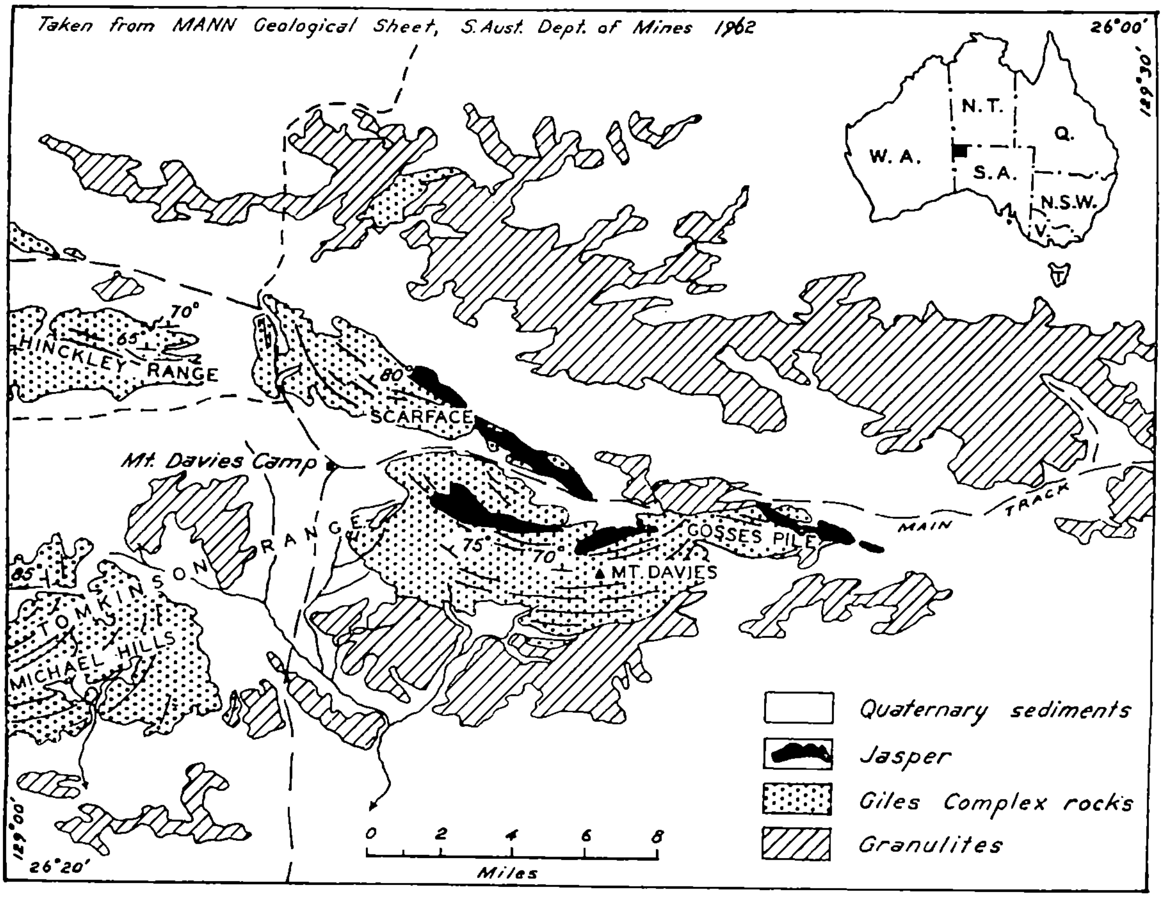
Taken from MANN Geological Sheet, S.Aust. Dept. of Mines 1962

Fig. 1

up into (3) olivine-norites with prominent anorthosite bands, the total thickness being approximately 15,000 ft. The ultrabasic zone consists of repeated layers of green clinopyroxenites, olivine clinopyroxenites, black anorthosites and more normal olivine gabbros. The whole sequence of ultrabasics and olivine-enstatite-gabbros is remarkably free of opaque material, and magnetite does not appear until the olivine-norites of the upper part of the intrusion.

Optical investigations on the plagioclases indicate that the composition remains fairly constant in the ultrabasic and olivine-enstatite-gabbro zones (An_{86-80}) but changes in the olivine-norites, becoming more albitic (An_{70}), especially toward the southern contact.

Immediately north of the ultrabasic zone there is a zone of jasper which separates the main body of the intrusion from about 6,000 ft. of layered olivine-norites and picrites, again dipping north at about 80°. The jasper occupies a major fault area and the rocks on either side of this fault are remarkably dissimilar. This northern sector is characterized by fine-scale banding, slump structures and high concentrations of magnetite and ilmenite, and it is thought that these rocks are not part of the Mt. Davies intrusion, but are the sheared portion of another body. Olivine determinations show a range of Fa_{25-35}.

A programme of chemical analyses has been commenced and initial results are presented in Fig. 2. The specimens so far analysed are all olivine gabbros or olivine-norites from the main part of the intrusion and from the layered gabbros of the ultrabasic zone. The overall iron enrichment observed in the olivines toward the south margin of the body is substantiated by analysis of five specimens taken from varying parts of the intrusion. The values of MgO, total iron as FeO, and total alkalis are plotted in Fig. 2 to indicate the trend in specimens from the northern margin (excluding the zone north of the jasper) through to a rock which occurs about 5,000 ft. from the southern contact. The increase in total iron is from 2·1 to 4·3 per cent and there is little doubt that subsequent analyses of material higher in the intrusion (that is, toward the south) will continue to show this trend.

Fig. 2 is also of significance in that it indicates the unusual deficiency in iron exhibited by the rocks of the Mt. Davies intrusion. As yet we have been unable to find any described examples of gravity stratified intrusions in which the ratio of total iron to magnesium is so low. For comparison, the trends of Stillwater[5], Skaergaard[6,7],

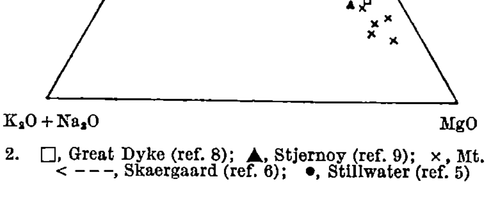

Fig. 2. □, Great Dyke (ref. 8); ▲, Stjernoy (ref. 9); ×, Mt. Davies; < - - -, Skaergaard (ref. 6); ●, Stillwater (ref. 5)

the Great Dyke of Southern Rhodesia[8], and Stjernoy in Norway[9] are plotted.

The importance of the Mt. Davies body appears to lie in its possible contribution to the relationships of the alpine-type and stratiform complexes[10,11]. Thus its affinities to the stratiform bodies may be listed as: (1) regular layering which is continuous over several miles; (2) cryptic layering especially exhibited by the olivines; (3) the absence of any large quantities of dunite or peridotite; (4) the preponderance of pyroxene over olivine in the ultramafic zone; (5) lack of stress effects in olivines except at the margins; (6) presence of abundant anorthosites. On the other hand, it also bears relationships to the 'alpine-type' complexes: (1) very high MgO/FeO ratio, not only in the ultramafic rocks but also in the gabbros; (2) forsterite-rich olivines; (3) almost vertical layering and sheared contacts; (4) the absence of rocks more acidic than bytownite gabbro.

Further work in the area is planned to elucidate the relations between the various intrusions and their relationship to the country rocks and a major petrological programme is envisaged.

We thank the Nuffield Foundation for financial support and the Department of Aboriginal Affairs and the Weapons Research Establishment, South Australia, for their cooperation. Chemical analyses were carried out by the Australian Mineral Development Laboratories.

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RATE OF ACCUMULATION OF SNOW AT THE SOUTH POLE AS DETERMINED BY RADIOACTIVE MEASUREMENTS

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THE importance and the difficulties of estimating the annual rate of accumulation of snow on the Antarctic polar plateau have been stressed by various authors[1,2]. These difficulties are basically due to the very low annual rate of accumulation which could give rise to complete disappearance of an annual or seasonal layer by wind erosion or by some other mechanism. Thus all methods based on identification of annual layers would tend to under-estimate the number of years, whether these methods be stratigraphic or isotopic.

In order to overcome this difficulty two methods based on radioactive measurements have recently been proposed; these methods avoid the subjective interpretations inherent in glaciological and isotopic methods.

The first method[2] is based on radioactive fall-out from the Castle thermonuclear bomb test which is used to identify a reference-level corresponding to the 1954–55 austral summer. This method could be used to measure the mean accumulation over the past nine years. The second method[3,4] is based on the radioactive decay of the lead-210 contained in the precipitation. This method would normally allow the dating of the snow layers for up to one hundred years in the past.

During the course of a summer campaign supported by the U.S. Antarctic Research Program (U.S. National Science Foundation), two of us (E. P. and W. D. B.) were able, in December 1962, to collect a series of samples of snow and firn to be investigated by both methods.

A number of samples, at depths of up to 26 m, were collected in the 'Snow-Mine'[5] of the Amundsen–Scott South Pole Station and were brought frozen to Belgium.

Estimations of the rate of accumulation of snow at the South Pole published so far are based on three groups of results:

(1) *Stratigraphic observations.* The most extensive are those of Giovinetto[5], which deal with the 26-m section of the 'Snow-Mine' representing a period of more than two hundred years. He reports a mean annual accumulation of 6·6 cm of water during 1760–1947 with, however, an annual rate of 7·4 cm over the past hundred years.

More recent observations[6] confirm the figure of 7·0 cm of water as the annual rate over the past few years.

(2) *Direct measurements carried out, since 1957, on stakes placed in the vicinity of the station.* The mean value of these measurements kindly communicated by L. Aldaz, indicates an accumulation of 20 cm/yr. in the relative level, which, taking into account a density of 0·35 for the surface snow, confirms the value of 7·0 cm of water.

(3) *Variations in the oxygen isotope ratio.* Sharp and Epstein[1] note a systematic disagreement between the values of the annual accumulation deduced from their measurements of the O–18/O–16 ratio and those obtained by direct measurement or deduced stratigraphically at various locations in West Antarctica. They suggest the possibility that the accepted rate of accumulation for the whole West Antarctica may have been under-estimated by a factor of 2.

They put forward an annual rate of accumulation of water at the South Pole of the order of 15 cm. This figure results from the analysis of an 80-cm ice section, showing only 3 isotopic alternations.

This article presents the results obtained by our stratigraphic observations, and by the measurements on the fission products and on lead-210.

(*a*) *Stratigraphy.* Fig. 1 presents the stratigraphic profile of a glaciological pit dug in the north-east quadrant, about 500 m from the Amundsen–Scott South Pole Station.

The stratigraphic features of the snow cover in this area have been previously described[5-7]. The identified summer layers are shown in Fig. 1. The average annual accumulation deduced for the period 1951–62 inclusive is 7·5 cm of water. One or more year-level having probably been missed, this value must be considered an upper limit. For the period 1955–62 inclusive, for which the stratigraphy is more clearly marked, the average annual increase is 6·3 cm.

(*b*) *Fission products.* Fig. 1 also shows the values of the gross β-activity of the snow as a function of depth in the same pit (cf. ref. 2 for experimental details and interpretation). According to the results obtained at the King Baudouin Base, the sudden increase in the β-activity noted above the − 145-cm level should correspond to early 1955. The results here reported confirm this con-

Table 1. Independent Estimations of the Annual Accumulation at the South Pole

Annual interval (estimated)	Method of measurement	Snow accumulation (cm)	Water equivalent (cm)	Ref.
Feb.– Nov. 1962	Direct ('New Field', 140 stakes)	20	7·0	L. Aldaz (private communication)
Feb. 1958– Nov. 1962	Direct ('Old Field', 36 stakes)	18·5	6·6	,,
(1947–1850)	Stratigraphy ('Snow-Mine')		7·4	Giovinetto (ref. 5)
(1958–1954)	0–18/0–16		15	Sharp and Epstein (ref. 1)
(1963–1951)	Stratigraphy	20	7·5	Present work
(1963–1955)	Fall-out	17·5	6·5 ± 0·5	,, ,,
(1963–1850)	Lead-210		6 ± 1	,, ,,

clusion and are in good agreement with the stratigraphic interpretation of the upper 140 cm. They indicate a mean annual accumulation of 6·5 ± 0·5 cm of water between 1955 and 1962.

(*c*) *Lead*-210. We have measured the lead-210 activity on a 2-m deep section covering the 1962–54 interval and on 60-cm thick specimens collected in the 'Snow-Mine' at intervals of about 4 m up to a depth of 26 m. The experimental details are described elsewhere[4]. The results are shown in Fig. 2 in which the logarithm of the lead-210 activity is plotted with respect to the depth, expressed in cm of water.

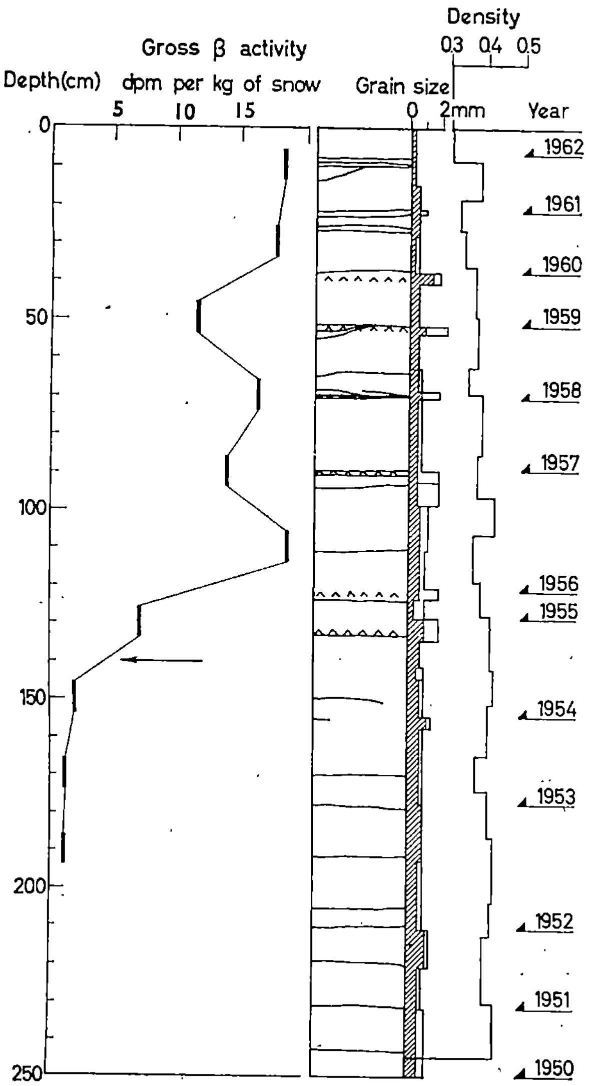

Fig. 1. South Pole: 'Pit 1'. Stratigraphic profile and gross β-activity. The interpretation of the stratigraphic profile in terms of annual layers is given on the right side of the figure

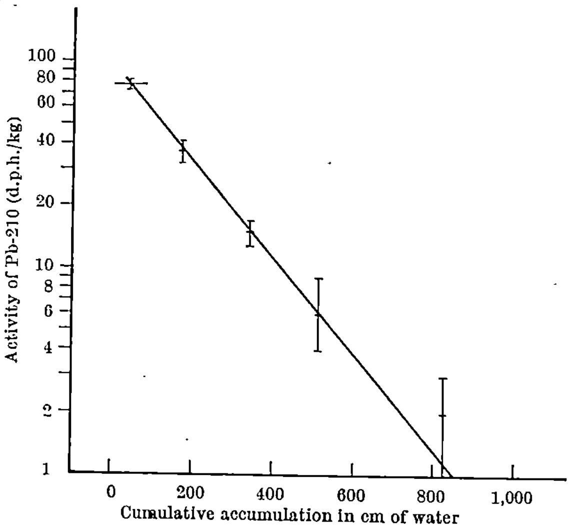

Fig. 2. South Pole: 'Snow-Mine'. Variation of the lead-210 activity in the firn as a function of depth. The slope of the line, in semi-logarithmic co-ordinates, corresponds to an annual accumulation of (6 ± 1) cm of water

It will be noticed that, within experimental error, the lead-210 activity decreases exponentially with the depth, the most plausible interpretation[3,4] being that the rate of accumulation of the water and the initial activity of lead-210 in the snow have remained constant over the past hundred years. Taking the half-life of lead-210 as 21·4 years[8], the mean annual accumulation over the past century is found to be 6 ± 1 cm of water.

This value is slightly lower than that deduced by Giovinetto from the 'Snow-Mine' stratigraphy for the same period, but confirms, however, the excellent quality of his observations carried out under particularly difficult conditions. The results are summarized in Table 1.

In conclusion, three completely independent methods (stratigraphy, fission products and lead-210) lead to a mean value of the annual rate of accumulation between 6 and 7 cm of water at the South Pole. The two former methods lead to an average over the past nine years, whereas the lead-210 activity decay (in agreement with the stratigraphic observations of Giovinetto) indicates that this average has not appreciably varied over the past hundred years.

The value of 15 cm of water put forward by Sharp and Epstein does not appear to be acceptable. Possible reasons for this disagreement have been already discussed[9].

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We thank Mr. J. Deschamps for help in carrying out the radioactive measurements.

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LETTERS TO THE EDITOR

PHYSICS

Bearing of Recent Experiments on the Special and General Theories of Relativity

In a recent communication Dr. L. Essen[1] concluded that special relativity cannot be applied to a rotating system. Jennison[2] and Synge[3] have previously treated the problem considered by Essen and have shown it to be consistent with special relativity. As the application of special relativity in this context is of considerable importance and in order to clarify its correct application the following points should be noted.

(1) Any point in the system may be considered to exist instantaneously in a Lorentz frame. This frame only applies momentarily to the elementary locality or neighbourhood around the point but is sufficient to establish local time and angular measure.

(2) If a signal is transmitted from any point participating in steady-state rotation and received by another point in the same rotating system, then, over any distance exceeding the elementary size of a Lorentz locality and within the range of velocities $\omega r \leqslant c$, the Lorentz frame associated with the neighbourhood of transmission always differs from the Lorentz frame associated with the neighbourhood of reception.

(3) The ray angle associated with the locality of transmission is related to the ray angle in the locality of reception by the usual relativistic aberration for the angles in the Lorentz frames appropriate to the velocities of the localities concerned.

(4) When the appropriate aberration is introduced the Doppler shift that is computed is precisely that observed in the Mössbauer experiments and predicted by general relativity. No Doppler shift occurs for transmission and reception between any two points equidistant from the centre, for the angles of transmission and reception are precisely those which are necessary for the transmission from one Lorentz frame to be received in another at the identical frequency.

(5) One should exercise care in considering distances in the Lorentz frame associated instantaneously with a point in the system, for the Lorentz frame embraces the conditions in only a vanishingly small locality of the rotating system. Only the identical instantaneous velocity and not the radius is required for the computation of the Doppler shift and local ray angles by special relativity.

It is in this respect that special relativity may be misleading, but not incorrect.

Consider the velocity of an inertially radial pulse of light relative to a rotating system of radius r and angular velocity ω. In a frame of reference containing the centre let the group velocity of the pulse relative to the system (and the system relative to the pulse) be c. Then the tangential velocity of the pulse in the system is ωr and the radial velocity $= c\left(1 - \frac{\omega^2 r^2}{c^2}\right)^{\frac{1}{2}}$. This defines the light path in the rotating system as a circular arc of length $s = \frac{c}{\omega} \sin^{-1} \frac{\omega r}{c}$.

It is now helpful to use brackets and subscripts to indicate where in the rotating system the measurements shall be made. Thus the rotating radius measured from the centre:

$$(r)_0 = \frac{c}{(\omega)_0} \sin \frac{\omega s}{c} = r$$

But the rotating radius measured from a rotating point at inertial radius r is given by:

$$(r)_r = \frac{c\left(1 - \frac{\omega^2 r^2}{c^2}\right)^{\frac{1}{2}}}{\omega} \sin \frac{\omega s}{c}$$

$$= \frac{c}{(\omega)_r} \sin \frac{\omega s}{c} = r\left(1 - \frac{\omega^2 r^2}{c^2}\right)^{\frac{1}{2}}$$

$(\omega)_r = \dfrac{\omega}{\left(1 - \frac{\omega^2 r^2}{c^2}\right)^{\frac{1}{2}}}$ is the rate at which the universe appears to rotate to a rotating observer at the rotating radius $(r)_r$. The unit of time is taken as the ticking of his own (standard) atomic clock and the unit of distance as the wave-length that he associates with the same clock. The relationships for $(\omega)_r$ and $(r)_r$ may also be obtained by considering radar signals transmitted and received by a single observer having an appropriate local time and restrained at constant distance from the centre. In individual inertial systems there appears no transverse relativistic contraction despite the dilatation of time, for when the observer is considered to be in motion relative to the target, the ray geometry introduced by the finite transit time cancels the contraction that might be expected by radar. The rotating system, however, is constrained so that the observer is maintained at the same distance from the target at all times and the result follows from the removal of the geometric factor. The radial contraction may also be demonstrated by considering signals continuously propagated between two radar transponders, one at the centre and the other in the rth ring, each triggering the other so that they set up a regular series of pulses. The pulse repetition frequency may be measured at each end in terms of local atomic clocks and thus gives the radar range; as the frequency of the clocks may also be transmitted for comparison it will be observed that the radar range contracts in the manner predicted. Similarly, if a signal is made each time that a point in the rth ring passes over a fixed point in the inertial system of the centre and this information is transmitted to the centre together with the ticking of a local clock, the increase in angular velocity is immediately apparent.

It should be noted that the product ωr is always equal to the associated product $(\omega)_r\ (r)_r$ for a given point or ring in the steady-state system, but:

$$(\omega)_r^2\ (r)_r = \frac{\omega^2 r}{\left(1 - \frac{\omega^2 r^2}{c^2}\right)^{\frac{1}{2}}}$$

Whence, multiplying both sides by m_0:

$$m_0(\omega)^2_r\ (r)_r = \frac{m_0}{\left(1 - \frac{\omega^2 r^2}{c^2}\right)^{\frac{1}{2}}}\ \omega^2 r$$

The left-hand side of this equation is the correct expression for the centrifugal force at the point where it is considered to act, and where the proper angular velocity and radius are $(\omega)_r$ and $(r)_r$ respectively.

It is of interest to consider the ray curvature and limiting radius (ref. 2, Fig. 3 of which requires slight amendment), in the plane normal to the spin axis, of a particle which has an independent spin parallel to the axis of rotation in a system which itself has a very high value of ω at the centre and therefore a much higher value at the particle. The theory predicts the possibility of completely isolating the particle in this plane, while maintaining the steady-state rotation, by virtue of the combined spin at the particle. The application of these principles to the understanding of the quantization of angular momentum deserves some attention.

Finally it is interesting to note that the observed geometry is such that a disk remains a disk under steady state rotation.

Note added in proof. Essen has courteously shown me his reply; the Lorentz frames in my treatment are permanent, only the co-existence (or coincidence) is fleeting.

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[1] Essen, L., *Nature*, **202**, 787 (1964).

[2] Jennison, R. C., *Nature*, **199**, 739 (1963).

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Dr. Jennison quotes only a part of my conclusion and it seems to me that he does not answer any of the points I raised.

In paragraph (1) of his communication it is stated that any point in the system can be considered to exist instantaneously in a Lorentz frame. In the experiment, however, the absorber is continuously accelerated and Jennison's assumption is therefore not in accord with the experimental conditions.

His definitions of 'instantaneously' and 'elementary locality' are both strange and variable. In his paragraph (1) the duration and extent are sufficient for local time to be established. For this the procedure described by Einstein must be adopted. A light signal is sent from the local clock to the distant clock and back again. The time is thus that of the double journey—which is double the time occupied in the actual experiment under discussion—and the 'elementary locality' includes the absorber and emitter. In (2), on the other hand, it is stated that for a distance exceeding the 'elementary size of a Lorentz locality' the absorber and emitter are in different frames. In (5) the elementary locality is called 'vanishingly small'. The assumptions in (2) and (5) thus contradict those in (1). Those in (1) cause the acceleration to be neglected while those in (2) and (5) enable the effects to be attributed to the acceleration. This is the very weakness in Einstein's argument to which I directed attention.

Later in his communication Jennison uses the practice so popular in discussions on relativity of propounding an imaginary experiment and of stating the result obtained. Although this artifice may occasionally have some illustrative value, it is in general useless and misleading. It is useless because an imaginary experiment cannot yield any information, and misleading because it appears to provide experimental support for what are mere assumptions. Jennison, for example, discusses the results observed in his experiment. In imaginary experiments there are neither observers nor observations and the use of such expressions is simply a misuse of language. Scientific theories, if they are to be of value, must be based on the results of actual experiments.

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Scattering and Attenuation due to Snow at Optical Wave-lengths

The narrow beam-widths obtainable with coherent light permit one to examine forward scattering caused by atmospheric effects in some detail. For example, it is found that inhomogeneities of refractive index in the otherwise clear atmosphere give rise to considerable broadening of a narrow beam. However, when heavy snow is falling, scattering and the ensuing attenuation of the optical wave are caused predominantly by the flakes; this also holds true in propagation through droplets of fog and rain.

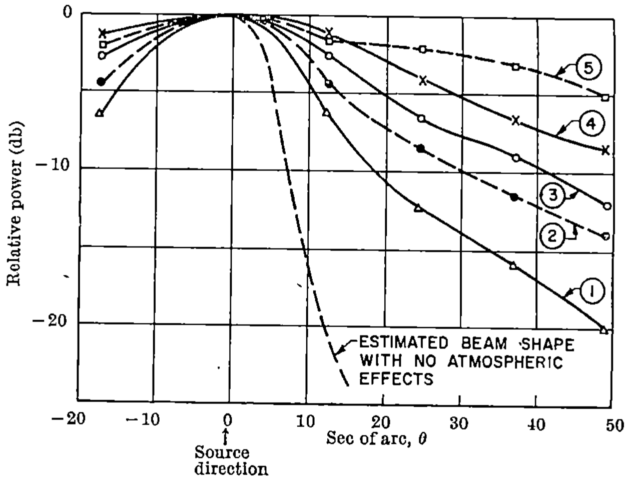

Fig. 1. Beam broadening due to snow: λ, 0·63μ; path-length, 2·6 km. Data of 2/10 and 2/11/64. Excess attenuations in curves 1–5 are 3·5, 5·0, 13·6, 15·6 and 17·9 db/km respectively, corresponding to increasingly heavy snowfalls. The attenuations are measured at dead reckoning, that is, with the receiving aperture on the maximum of the beam pattern ($\theta = 0$)

Fig. 1 shows beam patterns measured at a wave-length of 0·63μ over a path 2·6 km in length. The source consists of a helium–neon optical maser operating in several modes, and a cassegrainian telescope of 3·5 in. in diameter; the receiver is a 2-in. telescope with a photomultiplier. If the intervening path were vacuum, this combination would produce a half beam-width at the −5 db points of about 5 sec of arc as indicated by the narrow dashed curve. The broadening shown in curve 1, where the snowfall and corresponding attenuation are quite light, is due mainly to refractive effects, that is to say, it does not differ much from beam patterns measured during clear weather. However, as the rate of snowfall and attenuation increase, the beam-width does likewise, as indicated by curves 1–5. The patterns are normalized at $\theta = 0$. In curve 5, corresponding to an excess attenuation of about 18 db/km, the −5 db point of the pattern has increased to almost 50 sec of arc and the beam appears quite flat.

Although beam-broadening due to snow is a readily measurable effect, its onset occurs when the attenuation is fairly high. On the other hand, in the case of rain, broadening effects are observable at much lower attenuations; this is because raindrops are more efficient than snowflakes in forward scattering, the latter suffering from poor geometry and, more often than not, random orientation. Unfortunately, consistent data on beam-broadening due to rain are not obtained as readily as those of snow because of the time variability and inhomogeneity in rainfall along a given path.

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Effect of Precompression on the Critical Tension of Liquids

A prior application of pressure may alter the properties of a liquid when subjected to tension. Harvey[1] found that precompression of water up to a pressure of 1,000 atm. increased its ability to withstand tension. In his experiments the tension was generated by means of a hammer blow, that is, the liquid was subjected to stressing under 'dynamic' conditions. In our work we have applied tension under 'static' conditions and have investigated the effect of precompression using the Berthelot tube method described by Temperley and Chambers[2].

Briefly, a Berthelot tube consists of a cylinder, closed at both ends, and is almost completely filled with the liquid under investigation. When this tube and its contents are heated the liquid expands at a greater rate than the tube and fills the tube completely at a certain temperature t_f, the 'filling' temperature. When the tube is later allowed to cool the liquid adheres to the walls and continues to fill the tube at temperatures below t_f. Thus as the temperature falls below t_f a gradually increasing tension is set up in the liquid until it eventually ruptures at a temperature t_b, the 'breaking' temperature. At the temperature t_b the sudden release of tension is accompanied by a sudden increase ΔV in the external volume of the Berthelot tube. The value of the critical tension T in the liquid just before the break can be estimated if ΔV can be measured. Furthermore, the value of T will depend on t_b; for a given Berthelot tube, the lower t_b the greater the critical tension set up and vice versa.

To investigate the effect of precompression on the value of t_b for water, a sealed steel Berthelot tube was chosen the t_f of which was previously determined as 59·5°C. The tube was then heated slowly to a temperature above t_f. As the temperature rose above t_f the water in the tube became subjected to a pressure which increased as the temperature rose. It can easily be shown that the internal pressure P at any temperature t' ($> t_f$) is given in atm. by:

$$P = (\beta - \alpha)\,\Delta t/K \qquad (1)$$

where $\Delta t = (t' - t_f)$, α is the coefficient of cubical expansion of steel, β that of water and K the compressibility of water in atm.$^{-1}$ over that range of temperatures. If such a precompression resulted in a change in the critical tension this should be accompanied by a corresponding change in the breaking temperature t_b. The results of one set of such experiments are shown in Table 1.

Table 1

Δt (° C)	P (atm.)	t_b (° C, mean of four readings)
5·6	80	51·7
10·8	146	51·6
15·3	204	51·6
21·1	286	52·0
25·8	350	51·7
30·5	414	51·9

The values of P in the second column were calculated from equation (1). From the last column it is seen that a precompression of the water of up to 414 atm. had no appreciable effect on the breaking temperature.

Again the length of time for which this maximum pressure of 414 atm. was applied had no significant effect on t_b. This was seen in another series of experiments in which the temperature t' was kept constant at 90° C for varying periods of time τ; the results are summarized in Table 2.

Table 2

τ (h)	t_b (° C, mean of four readings)
0·25	51·8
1·0	52·0
2·0	51·9

We conclude that a precompression of water to pressures of the order of 400 atm. had no discernible effect on the value of the critical tension set up in it. It is possible that the explanation for the difference between these results and those of Harvey lies in the fact that the tension in the water is built up far more slowly in our experiments than in those of Harvey. The matter is by no means straightforward and requires further investigation.

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Preparation of Spherical Caps for Use on Terrestrial Globes in Investigations of Palæomagnetism and Continental Drift

In many different fields of geophysics and geology it would be convenient to be able to plot data on a terrestrial globe and then move the plotted data over the globe. For example, anyone interested in seeing how good the fit between the east coast of South America and the west coast of Africa really is would like to be able to trace an outline of South America on a transparent spherical cap and then move this outline against Africa.

Carey[1] used a terrestrial globe and a mould with the same radius of curvature turned from Huon pine. He stretched a suitable tracing plastic across this mould, fixed the film in place by a press and moulded the plastic to the shape of the globe by using boiling water and a drumstick swab. The moulded plastic film could be used for tracing directly from the globe.

I use the 12-in. terrestrial globes of the National Geographic Society. These globes are handy in use because there is no fixed axis which can get in the way and because they show the bathymetry of the oceans. They are large and accurate enough for rough work in continental drift and palæomagnetism.

A mould of plaster of paris was made from this globe. First, however, the globe was given two coats of clear plastic varnish which ensured that the entire globe was absolutely waterproof. A 16 in. × 16 in. box, 5 in. high, was made of 0·5 in. plywood, and this was filled with best quality plaster of paris. The globe was pressed into the plaster and held there for some time, so that a spherical cap mould with a semi-angle of about 50° resulted, which is large enough to accommodate every continent except Eurasia. After the plaster had set the undamaged globe could be removed, and the top surface of the block was scraped flat.

An arrangement was then made so that the space between the plaster mould and a plastic film lying flat on top of the mould could be partly evacuated by means of a vacuum pump. In an earlier arrangement, four horizontal tubes were used about 0·75 in. below the rim of the mould. In a newer model, there is only one tube which comes up vertically through the plaster and ends flush with the surface of the mould at its deepest point.

For the actual moulding two different processes are available—a wet process and a dry process. For the wet process, which is that of Carey[1] with vacuum suction added, a 16 in. × 16 in. piece of tracing plastic is stretched across the mould with its matt surface down and clamped in place by a circular wooden ring which has a sponge rubber gasket on its lower surface. Near-boiling water is poured on, vacuum applied and, without much rubbing, a perfect spherical cap can be moulded. The hot water is then gradually replaced by cold water with the vacuum still applied so that the plastic becomes firm again and preserves its shape.

One difficulty encountered in the wet process is that it is not always easy to obtain locally types of tracing plastic which become sufficiently plastic at temperatures less than 100° C as the present trend is towards dimensionally extremely stable tracing plastics. For this reason I now use a dry vacuum moulding process. The tracing film with its matt side down is stretched across the mould and firmly clamped in place as described before. The tracing film is then heated gently by means of heat lamps or any other source of radiant heat (two 250-W brooder lamps were used), vacuum applied and a perfect mould is made in a few minutes.

Though these spherical caps, on which one can trace and erase, are useful for many purposes, they are particularly useful for the fitting of land forms and geology in investigations of continental drift, and for plotting palæomagnetic results. For example, one may plot on one or several combined spherical caps the outline of a continent and the appropriate palæomagnetic pole position for a given period, and repeat this for all continents for the same geological period. One may then attempt a reconstruction of the ancient position of the continents by making the pole positions coincide (for example, on an inverted thumb tack fixed at the present geographic north pole of the globe). Each continent is then in its correct latitude and is oriented correctly. By rotating the continents around the common pole, the uncertainty in relative longitude can be fully explored. This may be repeated for every suitably documented geological period.

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CRYSTALLOGRAPHY

X-Ray Scattering by Irradiated Single Crystals of Potassium Permanganate

HIGH-ENERGY radiation interacts with crystalline solids in a variety of ways, but in general the result is the creation of damage in the solids. Among the effects which may be produced is the creation of localized static lattice defects, that is, defects which are small in three dimensions, namely, interstitials, vacancies, or agglomerates of only several atoms at the most. Theoretical calculations of the X-ray diffraction effects to be expected when lattice atoms are given small displacements from their lattice sites have been carried out by Zachariasen[1], Ekstein[2] and Matsubara[3]. All show that such defects lead to the production of diffuse maxima surrounding the sharp Bragg reflexions at the reciprocal lattice points (relp).

Huang[4] has given a general theory of displacement disorders and their X-ray effects. He has considered the case of spherically symmetric elastic singularities in a face-centred cubic lattice and has predicted the following X-ray scattering effects: (1) an isotropic expansion of the lattice; (2) an artificial temperature factor; (3) no broadening of the Bragg reflexions; (4) diffuse scatter surrounding the reciprocal lattice points; the region of scatter taking the form of a lemniscate.

Konazaki[5] has also calculated the X-ray scattering effects due to lattice defects. Several examples were treated in the case of a face-centred cubic lattice, and it was found that there are two possible types of diffuse maxima around a reciprocal lattice point. One is the lemniscate type and the other is the ellipsoid type, showing a closed surface surrounding the reciprocal lattice point. In general, in cubic crystals isolated defects (vacancies or interstitials) show the lemniscate, and pairs of defects the ellipsoid. Expansion of the lattice was also predicted due to distortion around the defects.

Effects of the type predicted here have been observed to varying degrees in neutron-irradiated boron carbide, diamond, silicon carbide and magnesium oxide crystals[6]. The artificial temperature factor was not very strong with magnesium oxide. Lattice expansions of the order of 1 per cent were observed in irradiated diamond and silicon carbide while that in magnesium oxide was about 0·1 per cent. Pronounced diffuse scattering with the diffuse reflexions being roughly ellipsoid in shape was also found. Rochelle salt irradiated with cobalt-60 γ-rays (dose $3{\cdot}1 \times 10^7$ r.) also showed pronounced diffuse reflexions[7]. The diffuse reflexions are not diminished in intensity at liquid air temperature.

Pre-irradiation of crystals of potassium permanganate by cobalt-60 γ-rays produces crystal damage, which has a profound effect on the subsequent isothermal decomposition[8]. In an attempt to determine the nature of the radiation damage X-ray diffraction investigations have been made of heavily irradiated (500 Mrads) single crystals of potassium permanganate. The following lattice expansions were found: *a*, 0·21 per cent; *b*, 0·31 per cent; *c*, 0·45 per cent. No observable artificial temperature factor is apparent, but pronounced diffuse scattering is present. Laue photographs were taken with the crystal mounted with the *b* axis (needle axis) perpendicular to the X-ray beam (unfiltered copper radiation, 40 kV, 20 m.amp). A flat-plate camera loaded with Kodak 'Industrex' film was exposed for 4 h at a distance of 4 cm from the crystal, which was rotated 5° between successive exposures. The resulting photographs showed a large number of pronounced diffuse maxima. In Fig. 1, a similar set of photographs, but with smaller angular rotations, indicate the nature of the diffuse reflexions associated with the 210 relp. Fig. 1(*a*) shows the appearance of the 210α diffuse spot. Fig. 1(*b*) shows the 210β diffuse reflexion, and the Laue reflexion (*L*) approaching the 210α diffuse spot. Coincidence of the latter two spots occurs in Fig. 1(*c*), and of 210β diffuse and Laue spots in Fig. 1(*d*). Oscillation and stationary crystal photographs of irradiated and unirradiated crystals using filtered (Ni) copper radiation confirmed the location of the diffuse maximum as being at the 210 relp. The full three-dimensional form of the diffuse maxima around the relps is being investigated. It is suggested that the observed effects are due to the breaking of the Mn–O bonds in MnO_4 with the production of oxygen vacancies and oxygen interstitials. Difference Fourier syntheses of irradiated and unirradiated crystals are being computed to test this possibility.

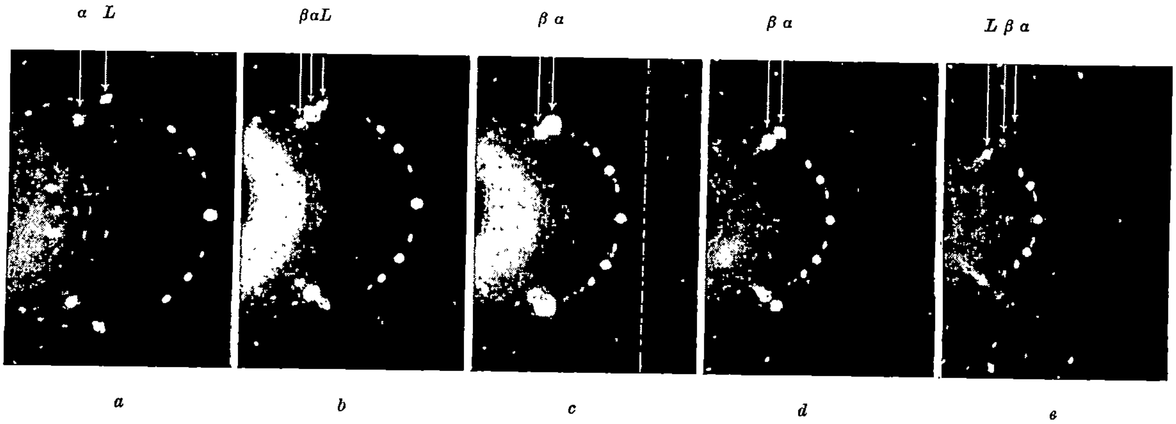

Fig. 1. Laue photographs of a crystal of potassium permanganate mounted with the *b* axis perpendicular to the X-ray beam. The angles between the *a* axis and the X-ray beam were: (*a*) 70°, (*b*) 72°, (*c*) 74°, (*d*) 76°, (*e*) 78°. The Bragg positions corresponding to the reflexions for copper K_α, copper K_β, and the Laue reflexion (*L*), from (210) are indicated

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Co-crystallization of Two Tautomers: Crystal Structure of Isocytosine

DURING the course of investigations on the crystal structures of purines, pyrimidines and related compounds, we have determined the crystal structure of isocytosine and, in so doing, have uncovered an example of two different tautomeric forms of the same molecule co-crystallizing in a 1 : 1 ratio. The two tautomeric molecules of isocytosine are hydrogen-bonded to one another in a manner analogous to the pairing proposed by Watson and Crick for DNA[1].

Isocytosine crystallizes in the monoclinic space group $P2_1/n$, with unit-cell dimensions $a = 8{\cdot}745$ Å, $b = 11{\cdot}412$ Å, $c = 10{\cdot}441$ Å and $\beta = 94{\cdot}79°$. There are eight molecules in the unit cell and, accordingly, two crystallographically distinct molecules in the asymmetric unit. Complete three-dimensional intensity data for copper X-radiation were obtained from Weissenberg photographs about the *a* and *c* axes; approximately 1,750 non-zero intensities were measured. A trial structure was deduced from a sharpened three-dimensional Patterson map and was confirmed and improved by Fourier projections on to the (010) plane. Refinement was by three-dimensional least-squares methods, including anisotropic temperature factors for the heavier atoms but a single, fixed temperature factor for the hydrogen atoms. The disagreement factor *R* between observed and calculated structure factors is now 6·8 per cent. In order to obtain direct

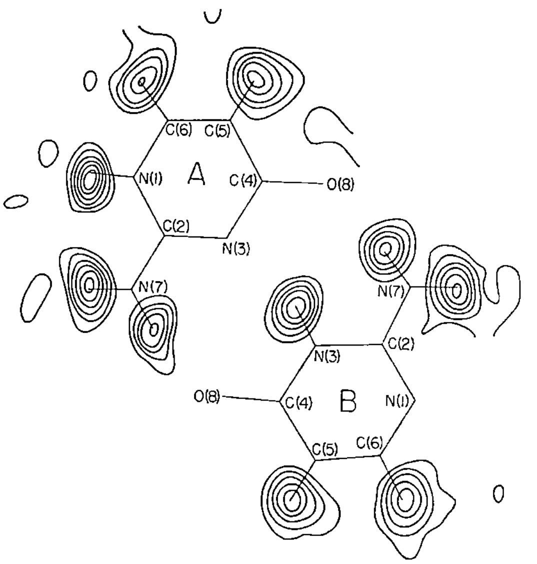

Fig. 1. The 'difference' electron density in the planes of the two tautomeric molecules of isocytosine, showing the positions of the hydrogen atoms. The coefficients used in the Fourier summations were the differences between the observed structure factors and the structure factors calculated on the basis of the carbon, nitrogen and oxygen atoms

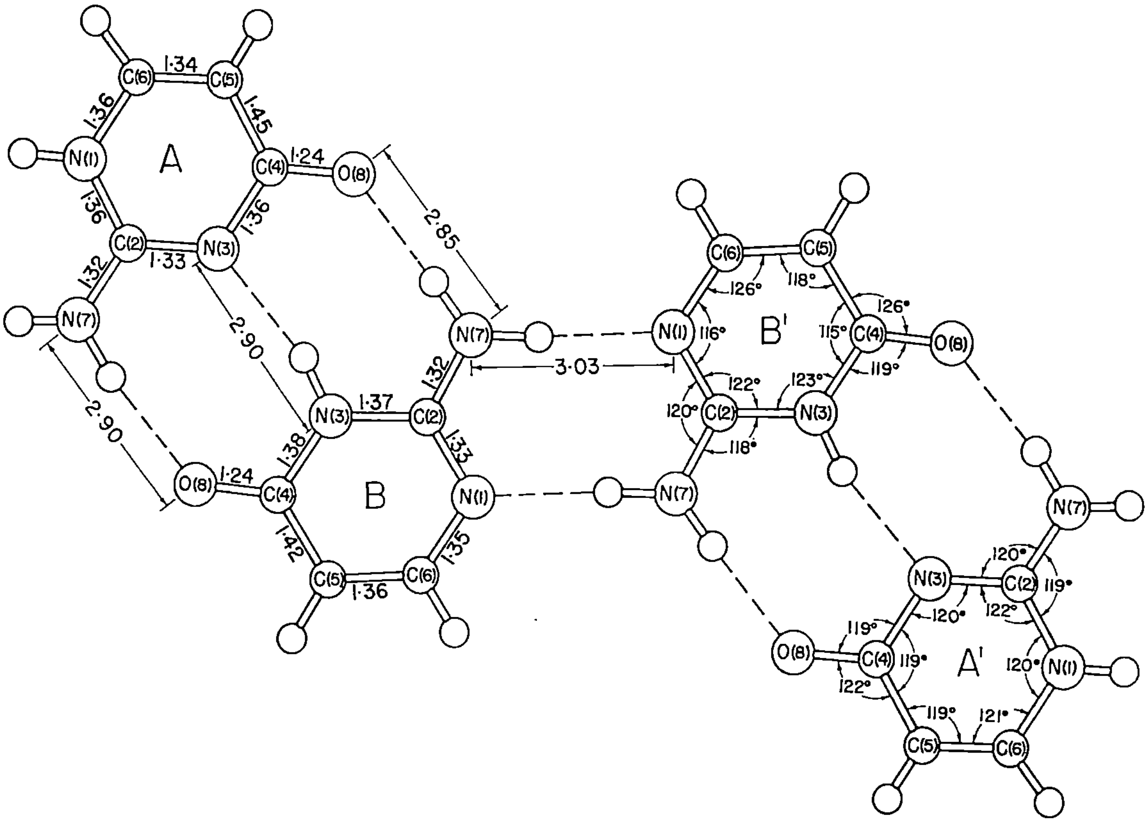

Fig. 2. Interatomic distances and angles and the hydrogen-bond arrangement in crystals of isocytosine

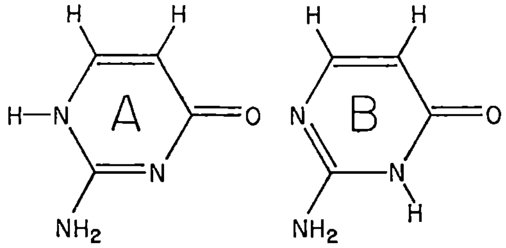

Fig. 3. The two tautomeric forms of isocytosine

evidence for the locations of the hydrogen atoms, difference Fourier syntheses were calculated in the planes of the two non-equivalent molecules, which are inclined at an angle of about 9° to one another. A composite drawing of these difference syntheses is shown in Fig. 1. The values for the bond distances and angles at the present stage of refinement are shown in Fig. 2.

The two tautomeric forms of isocytosine are shown in Fig. 3. In the crystal, a molecule of form *A* is linked to a molecule of form *B* through three hydrogen bonds, forming a hydrogen-bonded pair analogous to the purine–pyrimidine pairs proposed by Watson and Crick for the structure of DNA[1]. Each of these hydrogen-bonded pairs is, in turn, linked to another pair through two N–H · · · N hydrogen bonds arranged around a centre of symmetry, as shown in Fig. 2. Finally, the two protons at N(1) and N(7) of molecule *A* form hydrogen bonds to oxygen atoms of adjacent molecules (not shown). All the hydrogen bond lengths are typical of relatively strong bonds; with the exception of the N(1)–H · · · O and N(7)—H · · · O bonds, where the acceptor oxygen atoms are pushed apart by intermolecular van der Waals forces, the hydrogen bonds are approximately linear.

The existence of these two tautomers in the same crystal strongly suggests that they are of about equal stability, and points out the necessity of considering *p*H and the distribution of protons in formulating structures for the nucleic acids and their derivatives.

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RADIATION CHEMISTRY

Synthesis of Deoxyadenosine under Simulated Primitive Earth Conditions

Recent experiments have demonstrated that many organic compounds of biological significance can be produced in a wide variety of experiments simulating primitive Earth conditions. The synthesis of the purines, adenine[1,2] and guanine[3], and the sugars, ribose[4] and deoxyribose[4,5], have been reported. It was therefore of interest to find out whether the nucleosides and nucleotides present in RNA and DNA could be synthesized under similar conditions.

Previously we found that adenosine, AMP, ADP, ATP and A4P were formed by the ultra-violet activation of adenine in the presence of ribose and ethyl metaphosphate[6]. The investigation reported here shows that deoxyadenosine in appreciable yield may be synthesized by the use of conditions which appear to be far more likely to have occurred on the primitive Earth. The dehydration reaction needed to link the purine adenine and the sugar deoxyribose can be effected by the use of hydrogen cyanide. This reaction appears to be activated by ultra-violet light. The deoxyadenosine is, however, formed even in the absence of ultra-violet light. The function of the ultra-violet light appears to enhance the yield.

100 µl. each of solutions of adenine-8-^{14}C (specific activity: 100 µc./1·7 mg), deoxyribose, $NH_4H_2PO_4$ and NaCN, were used in a series of experiments as outlined in Table 1. The concentration of the solutions used was approximately 10^{-3} M. The glass tubes containing these mixtures were frozen in liquid nitrogen, evacuated and sealed. Two sets of tubes were prepared. The first ('Vycor' glass) was exposed for 2 h to a total ultra-violet flux of approximately 10^8 ergs/cm^2 from four General Electric ultra-violet germicidal lamps, type 782*H*-10, which emit 95 per cent of their light in the mercury resonance line at 2537 Å. The 'Vycor' tubes were maintained at room temperature during this reaction. The second was allowed to stand at room temperature for 3 days.

Table 1

	Ultra-violet (per cent)	Room temperature (per cent)
(1) Adenine + deoxyribose	—	—
(2) Adenine + deoxyribose + $NH_4H_2PO_4$	5	—
(3) Adenine + deoxyribose + $NH_4H_2PO_4$ + CN^-	1	1
(4) Adenine + deoxyribose + CN^-	7	1

100 µl. of the solution from each of these tubes was spotted on a thin layer plate using 'Ecteola' cellulose as a supporting medium, together with 25 µg of non-radioactive deoxyadenosine as carrier. The thin-layer chromatogram was developed in two dimensions using water in the first dimension and butanol/acetone/acetic acid/ammonium hydroxide (35 : 25 : 15 : 25 v/v) in the other. The position of the non-radioactive carrier deoxyadenosine, which performed the function of a marker, was located by its ultra-violet absorption. Autoradiographs of the thin-layer chromatography plates on X-ray film showed coincidence of radioactivity with the location of the carrier deoxyadenosine. This area was then scraped off the thin-layer plate, eluted with N/100 ammonium hydroxide and re-chromatographed on Whatman No. 4 paper using tertiary butanol/methyl ethyl ketone/formic acid/water as solvent (40 : 30 : 15 : 15 v/v). Once again there was coincidence between the ultra-violet absorbing area on the shadowgram[7] and the radioactive spot on the autoradiograph. This coincidence, perfect in location and precise in shape, clearly established that radioactive deoxyadenosine was being synthesized from carbon-14-labelled adenine and deoxyribose. Fig. 1 illustrates one such example of coincidence. The bright spot on the shadowgram represents the carrier deoxyadenosine. The dark area on the autoradiograph shows the radioactive deoxyadenosine synthesized when a mixture of adenine, deoxyribose, and $NH_4H_2PO_4$ is exposed to ultra-violet light in the presence of CN^- The identity of the deoxyadenosine synthesized was further confirmed by ion exchange chromatography. In a control experiment where adenine and deoxyribose were subjected to the entire procedure, except treatment with CN^- and ultra-violet light or ambient heat, no detectable amount of deoxyadenosine was produced. This control experiment also established that any synthesis due to microbial action was minimal.

The results are summarized in Table 1. No deoxyadenosine was detected in the absence of CN^- or $NH_4H_2PO_4$. The maximum yield was in the experiment where CN^- alone was used with ultra-violet light. The quantitative aspects are being further investigated.

The synthesis of nucleosides and nucleotides involves a dehydration reaction. In our previous work this was effected by the use of phosphoric acid or ethyl metaphosphate in the case of adenosine, and by ethyl meta-

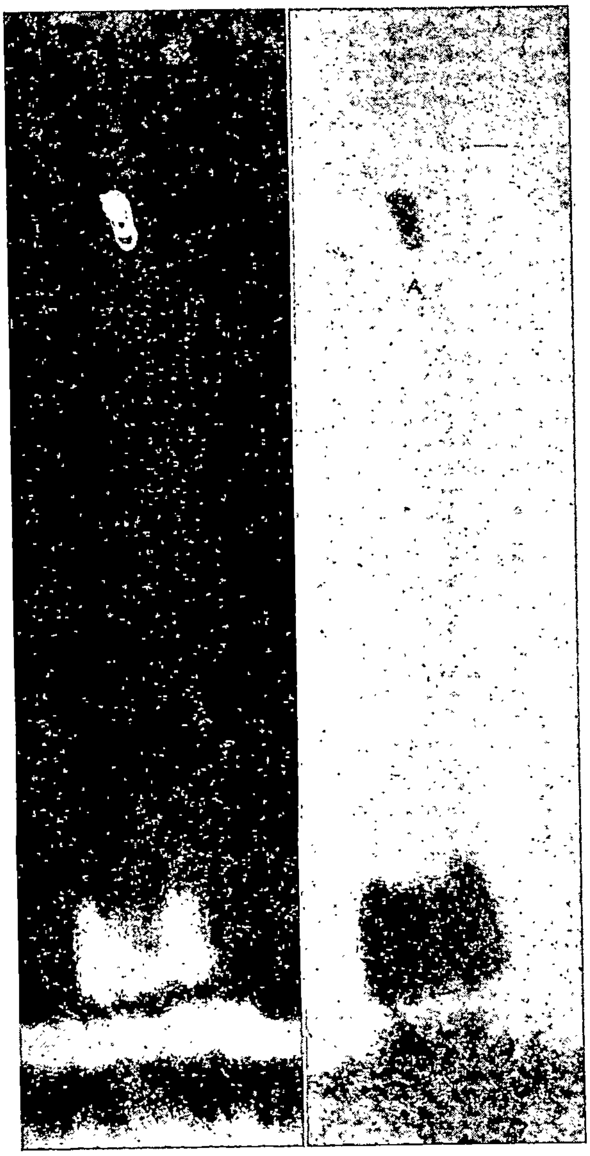

Fig. 1. Chromatogram illustrating formation of deoxyadenosine by the action of ultra-violet light on a solution of adenine and deoxyribose in the presence of CN^-. Shadowgram is on the left and the autoradiograph on the right

phosphate in the case of the nucleotides of adenosine. We now find that the same result can be obtained by the use of materials which could have occurred under primordial conditions. Hydrogen cyanide is one of the primary products of the electric discharge on mixtures of methane, ammonia and water[8], and presumably was readily available on the primitive Earth. It can react with a molecule of water to give formamide, which in turn can be hydrolysed to ammonium formate. While this report establishes the formation of deoxyadenosine by the use of CN^-, the preliminary evidence indicates that the synthesis of nucleotides and peptides can be effected by this means.

There is also evidence to indicate that cyanamide or Mg^{++} can replace CN^- to produce deoxyadenosine[10] in this reaction. The action of cyanamide may be analogous to that of di*cyclo*hexylcarbodiimide[9], which has been so extensively used by Khorana *et al.* for the synthesis of nucleotides. The presence of cyanamide, however, has not yet been detected in simulation experiments; but urea, which is formed by the addition of a molecule of water to a molecule of cyanamide, has been identified in them[8]. The chemical role of Mg^{++} in this experiment is obscure, and it is perhaps not altogether irrelevant to speculate on the relationship of this reaction to the extensive involvement of magnesium in biological processes. These results are under further investigation.

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CHEMISTRY

Synthesis of Glucopeptides

THE provision of model compounds[1-5] containing amino-acids attached to sugars is of importance in evaluating the stability of the various types of carbohydrate–peptide linkages which may occur in glycopeptides. Hitherto, investigations[1,2,6-8] with hexosamines (usually D-glucosamine) have been confined largely to the synthesis of compounds (the so-called glucopeptides) involving single amino-acid residues, and instances of the condensation of hexosamines with dipeptides and higher peptides are rare. Bertho and Maier[9], for example, synthesized *N*-(dialanyl)-D-glucosamine by treatment of 1,3,4,6-tetra-*O*-acetyl-*N*-alanyl-D-glucosamine with 2-azidopropionylchloride followed by reduction of the azide group to an amine. Such syntheses involve several stages and are difficult to accomplish.

The selectivity of amide bond formation in the presence of di*cyclo*hexylcarbodiimide[8] provides a simple method for direct, selective *N*-acylation of unprotected amino-sugars. The exclusive formation of *N*-substituted derivatives in aqueous pyridine has been attributed[8] to the specific action of di*cyclo*hexylcarbodiimide and to the instability of *O*-acyl derivatives under these conditions. This reagent has been used recently in the synthesis[10] of several *N*-aminoacyl derivatives of D-glucosamine and the amino-acid moiety extended in length to di- and tri-peptides. Thus, reaction of *N*-glycyl-D-glucosamine with *N*-carbobenzoxyglycine afforded *N*-(*N*-carbobenzoxydiglycyl)-D-glucosamine from which the protecting group was later removed by catalytic hydrogenation. We have used the alternative approach in which D-glucosamine has been condensed directly with suitably protected dipeptides by the following general procedure.

To a cooled (0°) and stirred solution of D-glucosamine hydrochloride (1·2 g) in water (5 ml.) was added 2 N sodium hydroxide solution (2·8 ml.) and thereafter a solution of *N*-carbobenzoxyglycylglycine (1·5 g) in pyridine (25 ml.). To this was added a solution of di*cyclo*hexylcarbodiimide (1·75 g) in pyridine (25 ml.) and stirring was continued for 24 h at room temperature. The precipitated di*cyclo*hexylurea was filtered off and unreacted di*cyclo*hexylcarbodiimide was extracted with ether (2 × 50 ml.). The aqueous layer was concentrated under reduced pressure to an amorphous residue which was crystallized from methanol to give pure *N*-(*N*-

carbobenzoxydiglycyl)-D-glucosamine (0·74 g), m.p. 185°–187° (decomp.), $[\alpha]_{Hg}^{20}$ + 77° (equil., *c*, 0·37 in pyridine) (lit.[10] m.p. 190°–192° (decomp.), $[\alpha]_{D}^{20}$ + 56·7°→ + 58·4°) (found: C, 50·7; H, 5·7; N, 9·6. $C_{18}H_{25}N_3O_9$ requires C, 50·6; H, 5·9; N, 9·8 per cent). The product gave a positive response on paper chromatograms with aniline hydrogen phthalate[11] and potassium iodide-starch[12] sprays but no response with ninhydrin[13].

The foregoing procedure has been used in the synthesis of *N*-(*N*-phthalyldiglycyl)-D-glucosamine, m.p. 220°–222° (decomp.) (from aqueous ethanol), $[\alpha]_{Hg}^{21}$ + 71° (*c*, 0·93 in pyridine) (found: C, 51·2; H, 5·2; N, 10·0. $C_{18}H_{21}N_3O_9$ requires C, 51·0; H, 5·0; N, 10·0 per cent). The products resulting from a similar condensation of D-glucosamine with *N*-phthalylglycyl-DL-leucine are diastereoisomers. Fractional recrystallization from methanol gave a pure isomer, m.p. 238°–242° (decomp.), $[\alpha]_{Hg}^{23}$ + 138° (*c*, 0·02 in pyridine) (found: C, 54·9; H, 6·3; N, 9·0. $C_{22}H_{29}N_3O_9$ requires C, 55·1; H, 6·1; N, 8·8 per cent). Attempts to separate the diastereoisomers following condensation of D-glucosamine with *N*-phthalylglycyl-DL-alanine were unsuccessful.

The commercial availability of a number of di- and tri-peptides renders this procedure of potential, general application in the synthesis of glucopeptides incorporating these units. The stability of the amide linkages in the foregoing compounds to acidic hydrolysis will be discussed in a subsequent publication.

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Optical Investigations of Film Formation and Removal on Gold Anodes in Acidic Oxalate Solutions

RECENT investigations of the oxidation of oxalate ions in acidic solutions using gold anodes have provided electrochemical evidence for the formation of a film on this anode during steady-state electrolysis at potentials well below that for Au_2O_3 formation, 1·36 V normal hydrogen electrode[1]. The determination of the nature of the film on gold at potentials below 1·36 V using electrochemical techniques has introduced uncertainties as to whether the film is a 'three dimensional' oxide film or a 'two dimensional' chemisorbed oxygen film. Some investigators[2–5] using charging curves as their basis conclude that the formation of an oxide occurs only above 1·36 V, the formation of a chemisorbed oxygen film being the process occurring below this potential. The formation of this chemisorbed oxygen film begins at potentials as low as 1·15 V (ref. 6). Other work, however, also based on electrochemical evidence, indicates that the formation of an oxide film does occur below 1·36 V (ref. 7).

This communication describes work that is directed at resolving these uncertainties and supplementing the evidence for concurrent film formation with oxalate oxidation[1] by applying an optical technique, ellipsometry[8]. This technique enables one to study the formation and decay of films on anodically polarized metal surfaces. With ellipsometry it is possible to determine in a more direct way the nature and extent of formation of the film found on gold below 1·36 V than by the purely electrochemical methods used in the previous studies described here.

All observations were made in a cell similar to that previously described[9] with the exception that no attempt was made to exclude air. The solutions were prepared directly from reagent grade sodium oxalate and c.p. sulphuric acid. All experiments were performed at room temperature. The gold electrodes (both foil and rod were used) were degreased in spectroscopic grade acetone and methanol, then flamed in a burner briefly before use. They were then placed in the cell and the potential of the gold brought to selected potentials by means of a potentiostat. The gold surface was either cleaned by evolving hydrogen vigorously or by reduction at 0·0 V. Either treatment was equally satisfactory. Ellipsometer readings were then made at various anodic potentials in two different concentrations of oxalate—4 g/l. and 20 g/l. in 1 M sulphuric acid or in a 1 M sulphuric acid solution containing no oxalate.

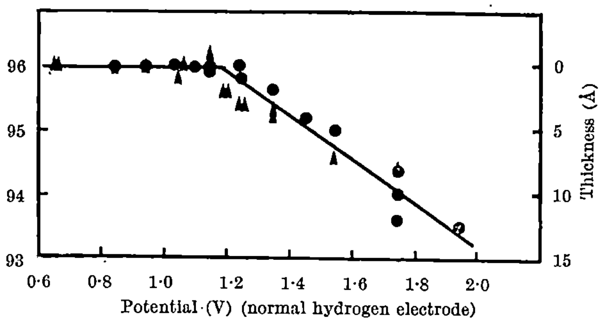

Fig. 1. ▲, 1 M sulphuric acid; ●, 1 M sulphuric acid and 4 g/l. $Na_2C_2O_4$

A film formed in the 1 M sulphuric acid solution with and without 4 g/l. $Na_2C_2O_4$ as indicated by the observation that relative phase retardation, Δ, varied linearly with the anode potential above 1·1 V (Fig. 1). The relative amplitude reduction (tan Ψ) was essentially constant in this potential region. The fact that Δ decreased while tan Ψ was essentially constant with increasing potential (increasing film thickness) indicates that a film having a refractive index greater than that of the solution (1·33) was forming[9]. This would strongly indicate that an oxide of gold, which would be expected to have a refractive index higher than the solution, would be formed rather than an oxygen film, the optical constants of solid oxygen films on gold being 1·25 (ref. 8). For such a film, calculations using the equations connecting Δ and tan Ψ (ref. 8) show that, if a film is thin (< 30 Å), a reasonable estimate of the film's thickness can be made without knowing the exact value of its refractive index; namely, a 2° decrease in Δ corresponds roughly to a 10 Å increase in film thickness. This is the basis for the estimated thickness values shown in Fig. 1. This figure also shows that the growth of a film on gold (above 1·1 V) occurs in a continuous manner, and not at discrete potentials, in general agreement with the type of mechanism proposed by Laitinen and Chao[10].

The time-interval between successive values of potential was several minutes. If, however, the potential was kept constant, the film continued to grow very slowly with a logarithmic dependence on time. Thus after 1,000 min at 1·74 V the film thickness was observed to be $\sim$ 20 Å. The fact that optical changes occurred while the potential was constant indicates that these changes were due to film growth rather than to changes in optical parameters occurring in the solution at the metal-solution interface produced by a change in the potential of the metal surface.

Once the film had begun to form in the solution containing 4 g/l. of $Na_2C_2O_4$ the rate of oxalate oxidation decreased. Lowering the potential from 1·74 V to 1·24 V resulted in a fairly slow first-order decay of the film. The same was true on open circuit. The film was easily reduced by setting the potential to values below 1·1 V with the potentiostat. Essentially the same results were obtained in 1 M sulphuric acid alone, but the film was not readily removed by the acid either on lowering the potential to about 1·2 V or on open circuit. With a sodium oxalate concentration of 20 g/l. the rate of reaction of oxalate with the film was so rapid that no film formation was observed up to 1·94 V, within the limit of detection of the ellipsometer.

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Infra-red Spectra of Aqueous Solutions by the Attenuated Total Reflectance Technique

IT has recently been suggested[1,2] that the attenuated total reflectance (ATR) technique is superior to the conventional transmission technique for obtaining the infra-red spectra of aqueous solutions, with the implication that the intense absorption bands of liquid water appear to be relatively weaker in ATR spectra. We have examined the spectra of a number of aqueous solutions by both methods and have in no case found the ATR spectra to be superior.

It is generally accepted that in the ATR system the radiation beam interacts only with the surface layer of the sample although the exact depth of penetration is a function of the complex refractive index, angle of incidence and wave-length. Parker[2] reports an apparent transmission of about 50 per cent for pure water by ATR methods at the 6·3 μm absorption peak. The transmission spectra of Ackermann[3] show that a path length of the order of 5 μm does in fact allow 50 per cent transmission at this wave-length.

It seems, therefore, that the ATR technique possesses no inherent advantage over the transmission technique for the examination of aqueous solutions. For concentrated solutions in regions of intense water absorption, the ATR technique circumvents the practical difficulties of filling and cleaning very short path length transmission cells. Where adsorption on the ATR prism surface allows higher solute concentrations to be established, the multi-reflexion ATR technique could probably be used with advantage, although the spectrum of the adsorbed material will not necessarily be the same as that of the material in true solution. These observations in no way detract from the value of the ATR technique for obtaining the spectra of intractable water containing samples which would be difficult or impossible to examine by transmission methods.

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Electronic Structures of Kryptates and Xenates

THE recent preparation of kryptates[1] and xenates[2] by Grosse *et al.* opens a new chapter in rare-gas chemistry. Up to now the compounds that have been made have presented no new problems in chemical bonding; no problems that were not already present in the chemistry of iodine and tellurium, etc. Most of the fluorine and oxygen compounds of xenon that have been prepared have isoelectronic analogues in the chemistry of iodine, for example, $XeF_6(IF_6^-)$, $XeF_4(IF_4^-)$, $XeF_2(ICl_2^-)$, XeO_3 (IO_3^-) and $XeO_6^{4-}(IO_6^{5-})$. Xenates based on XeO_6^{6-} are therefore a new type of compound. Similarly krypton forms $KrO_3(BrO_3^-)$, but kryptates (KrO_4^{--}, KrO_6^{6-}?) have no isoelectronic counterparts.

Rubidium and cæsium follow krypton and xenon in the periodic sequence, and in these elements the circum-rare-gas electron is in a 5*s* or a 6*s* orbital. While these electrons are loosely bound (Rb, 4·2 eV; Cs, 3·9 eV) it seems reasonable to suppose that such orbitals may play some part in bonding in rare-gas compounds. Calculations by Craig and Zauli[3] have shown that many ligands and especially electron attracting ligands can, by polarizing the valency-shell electrons of a non-transitional element, confer on the diffuse and loosely bound *d* orbitals an enhanced bonding potential. The empty 5*s* and 6*s* orbitals that surround krypton and xenon should be similarly affected by ligands such as oxygen and fluorine. These ligands will also enable the krypton 4*d* and the xenon 5*d* and 4*f* orbitals to play some part in bonding. Oxygen's 2*p* orbital is less tightly bound than fluorine's. It is, therefore, nearer in energy to the 5*s* (Kr) or 6*s* (Xe) orbitals and, being more diffuse than the fluorine orbital, it will overlap better with the loosely bound rare-gas orbitals. These two effects combine[4] in making the outer orbitals of krypton and xenon more important in bonding to oxygen than to fluorine.

In the tetrahedral oxy-anions, germanate, arsenate, etc., the four oxygen ligands may be thought of as each having one *p* orbital directed towards the central atom. These four *p* orbitals will belong to irreducible representations a_1 and t_2 of the point group T_d. The a_1 oxygen orbital will, therefore, interact with the valency 4*s* orbital and the empty 5*s* orbital in these anions. Ignoring the possible effect of the 5*s* orbitals it has been argued[5] that since the 4*s* orbital becomes more tightly bound in going from germanium to bromine the efficiency of a_1-type bonding will decrease. In BrO_4^- only the bromine 4*p* orbitals contribute effectively to the bromine–oxygen bonds. Thus BrO_3^- is stable, but, in the absence of other effects, BrO_4^- should be unknown. (In isoelectronic ClO_4^-, 3*d* orbitals form π-bonds with oxygen enhancing the anion's stability. Because of the presence of a spherical node $4d$ orbitals do not π-bond well.)

The effect, of including the $5s$ orbital, on bonding in these tetrahedral oxygen complexes is to change the 'antibonding' molecular orbital associated with the a_1 oxygen orbital into a 'bonding' orbital for krypton and possibly for bromine as well. In these elements the $4s$ orbital is very tightly bound (Br, 23·8 eV; Kr, 27·5 eV)[6] and so will play little part in bonding with the oxygen orbitals. Thus $5s$–a_1 is the more important interaction and a molecular orbital, bonding relative to the oxygen $2p$ atomic orbital, is generated.

When electrons are put into this orbital (a_1) framework the first two will contribute little or nothing to the bonding and KrO$_4$, like BrO$_4^-$, should be unknown. But the next two electrons can enter a bonding molecular orbital so that KrO$_4^{--}$ should be stable and perhaps BrO$_4^{---}$ as well. A further possibility is RbO$_4^-$, but the rubidium $4p$ orbitals are quite tightly bound (20·7 eV) and t_2 bonding to oxygen might be too weak for the anion to exist.

Octahedral co-ordination of the third row elements is rare. While this may be due to the radius–ratio effect[7], it may also be ascribed to the absence, if $4s$, $4p$, $4d_{z^2}$ and $4d_{x^2-y^2}$ are all used for σ bonding, of enough suitable orbitals with which oxygen might form π bonds. In the fourth-row elements a small effective nuclear charge (~ 3) is sufficient to bring the radial maximum of $4f$ hydrogen like orbitals[8] into the bonding region. Elements such as tin, iodine and xenon can enjoy octahedral co-ordination by oxygen and also the formation of π bonds. Kryptates are, therefore, expected to be KrO$_4^{--}$ but xenates XeO$_6^{6-}$. Cæsates based on CsO$_6^{5-}$ might also be formed, and since the $5p$ orbitals of cæsium are less tightly bound than the $4p$ orbital of rubidium, they might be more stable than rubidiates.

As with krypton, xenon's outer $6s$ orbital may well interact strongly with the 'antibonding' a_{1g} molecular orbital derived from $5s$–a_{1g} (oxygen) interaction (weak with xenon because the $5s$ orbital is tightly bound, 23·4 eV), making it bonding relative to the oxygen $2p$ atomic orbital. In per-xenates (XeO$_6^{4-}$) this bonding pair is absent but π bonding strengthens the bonding in both anions. Tetrahedral co-ordination of tellurium and iodine by oxygen is also known (TeO$_4^{--}$, IO$_4^-$). In these anions π bonding probably strengthens weak σ bonds as in ClO$_4^-$. Xenon might, therefore, form isoelectronic XeO$_4$ and xenates based on XeO$_4^{--}$ may also be formed.

The models for xenates and kryptates considered here have been based on regular octahedra and tetrahedra. This is reasonable only if the $4s$ (Kr) and $5s$ (Xe) orbitals are so tightly bound that they have no stereochemical effect. Since the formation of these anions depends on the s orbitals being tightly bound this does not seem too extreme an assumption.

Note added in proof. Since this communication was submitted the preparation of XeO$_4$ has been reported[9].

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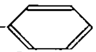
Free Radicals from Selenium and Sulphur Antioxidants

Research on the biochemical function of trace selenium in animal nutrition indicates it probably functions in lipid antioxidant and free radical acceptor reactions. Selenoprotein fractions from selenate-fed animals have up to 500 times the antioxidant activity of vitamin E (ref. 1). Selenoamino-acids were very effective in reducing free-radical damage to enzymes and amino-acids when aqueous solutions were exposed to ionizing radiation[2]. Woodbridge[3] found organic selenium compounds to be powerful antioxidant agents. These unusual properties of selenium compounds in reacting with free radicals suggested that an electron paramagnetic resonance investigation of selenium amino-acids and antioxidants and their sulphur analogues would be of interest.

Sulphides and selenides were irradiated at 2537 Å at liquid nitrogen temperature in quartz tubes for periods up to several hours. Spectra were immediately taken on an X-band electron paramagnetic resonance spectrometer with the samples maintained at liquid nitrogen temperature. The electron paramagnetic resonance spectra fall into four categories characteristic of the radicals formed by irradiation. These are: alkyl radicals, benzyl-type radicals, sulphur radicals and selenium radicals.

Alkyl radical, —CH$_2\dot{\text{C}}$HCH$_2$—, spectra were obtained from di-n-dodecyl and di-n-octadecyl sulphide and selenide. These consist of eight-line spectra centred at $g = 2{\cdot}002$.

Dibenzyl sulphide, dibenzyl selenide and dibenzyl diselenide each gave a simple spectrum centred at $g = 2$ and 30 gauss wide. Since no sulphur or selenium spectra are observed we interpret the results as the spectrum of a benzyl radical, —X—ĊH—. A similar spectrum from selenocystine suggests that it may form the radical HOOCCH(NH$_2$)ĊHSe - - -.

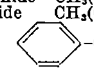
Electron paramagnetic resonance spectra of the sulphur radicals gave a derivative pattern with seven points of inflexion indicating three unequal g values (Table 1).

Table 1. g Values of Sulphur Radicals

Irradiated compound	g_1	g_2	g_3
Cystine	2·001	2·022	2·062
Cysteine	2·000	2·025	2·052
Dibenzyl disulphide	1·995	2·024	2·053
Di-n-butyl disulphide	1·998	2·025	2·058
Di-n-dodecyl sulphide	1·998	2·022	2·057

Spectra that we assign to selenium radicals have derivative shapes characteristic of axially symmetric g factors. The g factors are designated as $g_{\parallel}$ and $g_{\perp}$ for directions parallel and perpendicular to the symmetry axis. As a check on this interpretation, an average g value was calculated, $\langle g \rangle_{\text{calc}} = 1/3\ g_{\parallel} + 2/3\ g_{\perp}$, and compared with the experimental $\langle g \rangle$ value. The g values for selenium are given in Table 2. The agreement between $\langle g \rangle_{\text{calc}}$ and $\langle g \rangle_{\text{exp}}$ is within experimental error. The g values for selenium are higher than those for sulphur, as would be expected since the g-value shifts depend on the degree of spin-orbit coupling which increases rapidly with atomic number.

Table 2. g Values for Selenium Radicals

Substance	Radical	$g_{\parallel}$	$g_{\perp}$	$\langle g \rangle$	$\langle g \rangle_{\text{calc}}$
Di-n-octadecyl selenide	CH$_3$(CH$_2$)$_{17}$Se·	2·115	2·083	2·095	2·094
Di-n-dodecyl selenide	CH$_3$(CH$_2$)$_{11}$Se·	2·122	2·088	2·098	2·099
Benzyl selenomercaptan	–CH$_2$Se·	2·124	2·091	2·099	2·102

General results of ultra-violet irradiation and electron paramagnetic resonance examination of these sulphur and selenium antioxidants are: (1) in disulphide compounds the disulphide bonds always rupture; (2) in diselenide compounds the diselenide bond does not rupture; but a radical may be located elsewhere, usually on a methylene carbon; (3) in thiol and —SeH compounds the proton on sulphur or selenium is labile; (4) in di-aliphatic sulphides and selenides the C—S or C—Se bond usually ruptures; (5) in dibenzyl sulphide and selenide compounds the C—S or C—Se bonds do not rupture; instead, a proton is removed from the methylene group.

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BIOPHYSICS

Light-induced Absorption-change Transients in Some Blue-green Algae

As reported recently[1,2], in aged chloroplasts of spinach the decay of the light-induced absorption decrease at 430 mμ becomes biphasic when the system is coupled with ascorbic acid and an appropriate amount of the redox dye phenazine methosulphate. It was further found that the slowly decaying portion becomes saturated at a lower actinic intensity and the rapidly decaying portion starts to appear only when the actinic intensity exceeds the threshold-level. The biphasic decay was attributed to the re-reduction of two separate photo-oxidized species, possibly the chlorophyll complex *P*700 and cytochrome *f*.

In this communication is reported the finding of a decrease in light absorption at 430 mμ with a similar complex decay kinetics in whole cells of several blue-green algae. *Plectonema boryanum, Phormidium luridum, Glœocapsa alpicola*, and *Anacystis nidulans* have been examined, and all show more or less the same characteristics. Fig. 1 shows a typical transient absorption change at 430 mμ obtained from *Plectonema*, which gave the largest signal among the species studied. The total optical density change shown in Fig. 1 was about 0·006. Broad-band red (645–745 mμ) flashes of 20-μsec duration spaced at 2-sec intervals were used for excitation. The signal shown in Fig. 1 was obtained by integration of 30 flashing cycles through the *CAT*-computer. Detailed experimental procedures have been described previously[3].

On the average, the decay constants of the rapid and slow portions are 10 and 300 ± 50 msec, respectively. In most fresh blue-green algal cells, the ratio of the two signal heights is nearly unity. The extent of the rapidly decaying portion usually decreases on storage. *Plectonema* stored in the dark at 0° C for one month still retained 50 per cent of the activity without change in the signal ration. Preliminary examination indicates that the light-minus-dark difference spectrum of the rapidly decaying portion resembles that of an oxidized cytochrome while the difference spectrum of the slowly decaying portion agrees closely with that of the oxidized *P*700[4-6].

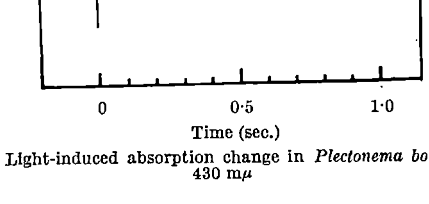

Fig. 1. Light-induced absorption change in *Plectonema boryanum* at 430 mμ

Since blue-green algae are relatively heat-resistant, the transient absorption changes are little affected when the cells are heated for 5 min at temperatures up to 50° C. When heated to higher temperatures, the rapidly decaying portion is affected and begins to disappear, while the slowly decaying portion remains intact. When heated above 60° C, both signals are eliminated.

The actinic-intensity dependence of the composite signal in blue-green algae is similar to that of aged chloroplasts[1,2]. At the threshold intensity of approximately 2×10^{14} quanta cm^{-2} per flash, the rapidly decaying signal starts to appear. At lower actinic intensity, only the slowly decaying portion can be observed.

Cells incubated with 3×10^{-5} M 3(3,4-dichlorophenyl)-1,1-dimethyl urea yielded practically the same composite signal. Sonicated cells, from which phycocyanin was liberated, yielded transients of smaller signal height, but with similar complex decay kinetics. The 430 mμ transient presented here is associated with the long-wave-length reaction centre. This is shown by the fact that 10 mμ-wide flashes at 680 or 690 mμ can also induce the complex transient signal but it has smaller overall height. Preliminary examination with actinic flashes at 620 or 590 mμ, at which phycocyanin absorbs, showed that the accessory pigment has a quite different behaviour of sensitization. Detailed investigations of the action spectrum are in progress and will be reported elsewhere.

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BIOCHEMISTRY

Direct Extraction of Actin in the Fibrous Form from Fish Muscle

Water extraction of an acetone-dried muscle powder preparation of rabbit[1] is one of the standard methods of producing a solution of globular or *G*-actin. Such a solution possesses no birefringence of flow and has a low specific viscosity[2]. On making this solution 0·1 M with respect to potassium chloride, 0·001 M with respect to Mg^{++} and allowing to stand, a marked birefringence of flow develops and the solution has a considerably increased specific viscosity. These changes have been classically considered to be a result of the polymerization of the *G*-actin, as extracted, to the fibrous or *F*-form.

During the course of investigations of the muscle proteins of various animals it was observed that actin can apparently be extracted directly from fish acetone-dried muscle powders in the *F*-form. Acetone-dried muscle powders were prepared from salt- and fresh-water fish of various species by the method of Barany, Barany and Guba[1]. The fish were killed by decapitation or a blow on the head, the dorsal muscle dissected out as quickly as possible and minced twice through an ordinary kitchen mincer. The muscle was then treated twice with 10 vol. 0·4 per cent sodium bicarbonate, washed with 15 vol. of water and dried thoroughly with pre-cooled acetone. The powder was allowed to dry in the air overnight at room temperature and stored at 4° C. The muscle powder must be prepared very carefully, particularly during the stage of acetone drying. For example, in the case of chub, which appears to have an unusually loose texture of the myonemes, unless great care is taken to express water from the muscle granules during the final acetone drying procedure a preparation results which on extraction gives actin in the *G*-form.

Actin was obtained from the acetone-dried muscle powder by treatment with 10 vol. of boiled out de-ionized water for 1 h at room temperature without stirring. Such a water extract of fish muscle powder possesses a marked birefringence of flow and a relatively high specific viscosity, suggesting that a proportion of the extracted actin is in the fibrous form. *F*-actin extracted directly in this manner has been termed *Fn*-actin to distinguish it from *F*-actin prepared by the normal polymerization process. Table 1 shows the results obtained from various species of fish together with the results of a rabbit muscle extraction for comparison.

Table 1. COMPARISON OF ACTIN EXTRACTED FROM RABBIT AND FISH ACETONE-DRIED MUSCLE POWDER

	Protein conc.	Before adding salt		After adding salt	
		Birefringence	η spec.	Birefringence	η spec.
Rabbit	2·7	0	0·03	+	0·31
Fresh-water fish					
Trout	2·95	+	0·45	+	0·65
Chub	8·0	+	0·47	+	0·74
Barbel	7·1	+	0·28	+	0·79
Dace	—	+	—	+	—
Perch	4·4	+	0·29	+	0·54
Salt-water fish					
Cod	15·0	+	—	+	—
Pollack	2·5	+	0·23	+	0·93
Plaice	3·0	+	0·36	+	0·80
Conger eel	4·2	+	0·15	+	0·83

The fact that the fish actin is not extracted completely in the fully polymerized form is suggested by the increase in viscosity on the addition of salt.

It has also been noted that the ability to extract *Fn*-actin from a muscle powder decreases with time of storage at 4° C as may be seen from Table 2. After seven days the proportion of actin extracted directly in the *F*-form is considerably decreased, although the fact that the actin is still polymerizable is demonstrated by the viscosity increase on adding salt. Thus to obtain a good *Fn*-actin extraction it is necessary to use freshly prepared muscle powder.

Table 2. AGEING OF FISH ACETONE-DRIED MUSCLE POWDER AT 4° C

Age (days)	Viscosity (η spec.)	
	Before adding salt	After adding salt
1	0·44	0·61
7	0·23	0·52
30	0·18	0·22

The observation that actin may be extracted directly from fish muscle in the *F*-form appears to reinforce the argument that actin is present in the intact myofibril in the fibrous form, as suggested by Barany, Barany and Guba's[1] experiments on the preparation of actin from muscle without prior extraction of myosin, and Martinosi, Gouvea and Gergely[3], who came to the same conclusion as a result of isotope investigations. Further support to this theory comes from Tsuboi and Hayashi's[4] recent demonstration that acetone-dried muscle powder itself contains no ATP (the bound nucleotide component of *G*-actin) but that it is synthesized from the ADP present during the water extraction procedure, and they further suggest that there must be complicating reactions in the generally accepted preparative procedure for actin.

If it is accepted that actin is present in acetone-dried muscle powder in the *F*-form, as indicated by the absence of ATP, then it would follow that one of the reactions proceeding during water extraction is that of depolymerization. The fact that actin may be extracted in the partially polymerized form from fish muscle would suggest that the depolymerization reaction is possibly under the control of some system which is absent or inhibited in the case of fish. The figures in Table 2 indicate that the ability of a muscle powder to produce *Fn*-actin decreases at a faster rate than the 'activity' (as measured by ability to polymerize) of the *G*-actin. This could be explained by the presence of a labile polymerizing–depolymerizing system in muscle, the balance of which varies in animals from different species or may be altered by the technique of preparation of the muscle powder or its subsequent treatment. In order to substantiate this hypothesis, a close examination must be made of the reactions occurring during the process of extraction of actin from acetone-dried muscle powder together with a comparison of this process in muscle powder extraction systems from various species. This work is in progress and the results will be published elsewhere.

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Conjugated Oxosteroids in Human Urine

IT has long been known that oxosteroids are excreted in human urine in conjugated form chiefly as sulphates or glucuronidates. In order to test the hypothesis that the choice of either sulphate or glucuronidate in different individuals may be wholly or partially determined by genetical factors it was necessary to devise a simple and rapid method for the separation and identification of these two groups of conjugates. In the course of these experiments it was found that a third type of conjugate, oxosteroid phosphate, also occurred in human urine.

Methods for the high-voltage electrophoretic separation of steroid conjugates have been described[1,2]. It was found, however, that the conjugates could be very conveniently separated at lower voltages by the following procedure.

The K or Na salts of dehydroepiandrosterone sulphate, androsterone sulphate[3], and androsterone glucuronidate[4], as well as dehydroepiandrosterone phosphoric acid and its sodium salt[5], were prepared. These substances were assayed without previous hydrolysis by a modified Zimmermann reaction. The steroid moieties were 94 and 91 per cent respectively of the theoretical values for dehydroepiandrosterone sulphate and androsterone sulphate, 99 per cent for the dehydroepiandrosterone phosphoric acid and 95 per cent for the androsterone glucuronidate. The glucuronic acid moiety was 93 per cent of theory. The pyridinium salts of androsterone and dehydroepiandrosterone potassium sulphates were also prepared[6]. The steroid content was 100 and 95 per cent respectively of theory.

Electrophoresis was carried out in a horizontal glass tank using Whatman No. 3 *MM* paper and veronal buffer *p*H 8·6–8·9. The paper was 52 cm in length and separate lanes were made by cutting out 2-mm strips. Androsterone potassium sulphate and androsterone glucuronidate were applied in the first lane, dehydroepiandrosterone potassium sulphate and androsterone glucuronidate in the second lane, and a mixture of the three conjugates in the third lane. Electrophoresis was carried out at 320 V for 4 h at room temperature and the

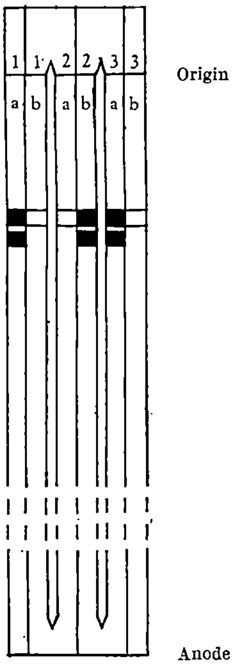

Fig. 1. Copy of electrophoretogram showing separation of: (1) ASO_3K from A gluc.; (2) $DHASO_3K$ from A gluc.; (3) ASO_3K and $DHASO_3K$ from A gluc. Strips *a*, developed with Zimmermann reagent, strips *b*, with naphthoresorcinol. Electrophoresis 4 h, veronal buffer *p*H 8·6, 320 V, Whatman No. 3 *MM* paper

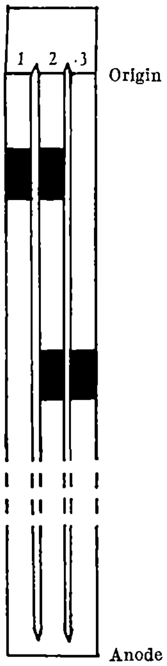

Fig. 2. Copy of chromatogram showing separation of $DHASO_3K$ from $DHAPO_3H_2$ in Schubert's system *E*: (1) $DHASO_3K$; (2) mixture of (1) and (3); (3) $DHAPO_3H_2$

paper then dried at 65°–70° C for 30 min. 1 cm of each of the 2-cm strips was developed for glucuronidate by dipping into a modified naphthoresorcinol reagent and heating to 120° C for 10 min. The remaining strips were developed with the Zimmermann reagents.

Fig. 1 shows that the steroid sulphates separate well from androsterone glucuronidate but not from each other, neither do their pyridinium sulphates separate from each other.

Dehydroepiandrosterone phosphate was also separated effectively from dehydroepiandrosterone sulphate, the latter having the greater mobility. For the location of the phosphate, published methods[7,8] were modified as follows: the paper was dried at 65° C for 30 min, cooled to room temperature, dipped into molybdate reagent and the excess solution allowed to drain off before reheating for 7 min at 85°–90° C. The paper was cooled, drawn through the reducing reagent and dried in a current of air. After a few minutes a light blue colour developed.

Effective separations were also achieved for sodium pregnandiol glucuronidate and glucuronic acid from each other and from dehydroepiandrosterone potassium sulphate, and for glucuronic acid from androsterone-glucuronidate.

Urinary 17-oxosteroid sulphates were extracted as pyridinium salts[6]. Total pyridinium salt in the extracts ranged from 2·2 mg to 5·5 mg/g creatinine in the urine. Electrophoresis of 80 μg of these mixtures showed 3 well-defined Zimmermann reacting zones. The zone with least mobility contained steroid phosphate.

A chromatographic separation of dehydroepiandrosterone phosphate from the potassium salt of dehydroepiandrosterone sulphate was also effected in Schubert's system *E* (ref. 9).

The mobility of dehydroepiandrosterone phosphate was 3 times that of the potassium salt of dehydroepiandrosterone sulphate as shown in Fig. 2.

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Polymorphism of Zinc-α_2-Human Glycoprotein

Genetically determined variants of a large number of proteins, including many blood proteins, have been discovered, especially since the introduction by Smithies[1] of the gel-electrophoresis technique. α_1-Acid glycoprotein, one of the low-molecular-weight normal human plasma proteins, proved to be polymorphic near its isoelectric point. Pooled α_1-acid glycoprotein revealed seven and, after enzymatic removal of its sialic acid, two bands[2]. The human β_2-mucoid the molecular weight of which is similar to that of the mentioned α_1-globulin showed a very similar resolution[3]. We report in this communication the polymorphism of a low-molecular-weight α_2-glycoprotein, zinc-α_2-glycoprotein[4].

Zinc-α_2-glycoprotein was isolated as described earlier[4] from large pools of outdated normal plasma collected in the New England area. This glycoprotein appeared homogeneous on ultracentrifugation, electrophoresis, chromatography, and NH_2-terminal amino-acid analysis. Its extinction coefficient was again found to be 18 (ref. 4). Horizontal starch gel electrophoresis[1] was carried out in the cold over the *p*H range of 2·7–8·6.

At *p*H 8·6 in borate or *tris*-EDTA-borate buffer zinc-α_2-glycoprotein exhibited a single band. Near *p*H 6 in cacodylate, citrate or phosphate, one main band was observed. Between *p*H 5·5 and 4·7 this plasma globulin revealed eight zones, whereas twelve bands were found in the *p*H range 4·0–3·5. The higher resolution noted over the latter *p*H region is probably related to the partial denaturation of this protein, which is known to occur at *p*H values acid to its isoelectric point[4]. At *p*H 3·0 in phosphate buffer, zinc-α_2-glycoprotein showed

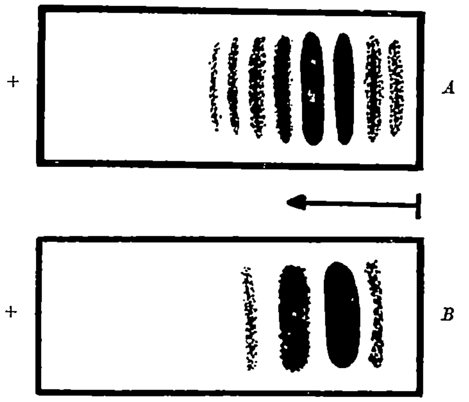

Fig. 1. Starch-gel electrophoretic patterns of zinc-α_2-glycoprotein. Native zinc-α_2-glycoprotein was analysed at *p*H 5·0 (*A*) and its sialic acid-free form at *p*H 5·6 (*B*). The ionic strength of the acetate buffers used for the gel was 0·02 and that of the electrode compartments 0·1

five bands and at *p*H 2·5 one broad zone. The best resolution was achieved on the slightly alkaline side of the isoelectric point of this globulin (Fig. 1*A*). After essential removal of its sialic acid by neuraminidase, zinc-α_2-glycoprotein was again analysed. The most distinct separation resulted between *p*H 5·5 and 6·0, and essentially two bands were observed (Fig. 1*B*). Comparable findings were obtained by investigating the barium-α_2-glycoproteins[5].

The present state of knowledge on the chemical differences between the genetic variants of certain blood proteins appears to indicate that these differences reside in the polypeptide chains of these macromolecules. Several human plasma glycoproteins with molecular weights near 45,000 reveal a resolution on starch-gel electrophoresis which is higher than that of their sialic acid-free native forms. However, transferrin, a plasma glycoprotein with a higher molecular weight and a significantly lower carbohydrate moiety, and its derivative obtained by neuraminidase digestion, show patterns the respective numbers of bands of which are in inverse ratio to those of the mentioned glycoproteins[6,7]. Thus, the present work points to the importance not only of elucidating whether the polymorphism of zinc-α_2-glycoprotein reported here is an expression of genetically determined variants, but also of initiating enquiries into the significance of the sialic acid of the plasma glycoproteins.

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Biological Activity of Natural and Synthetic Insulin *A*-chain Preparations on the Isolated Rat Diaphragm

The possibility that separated insulin chains may be active to a certain extent on some of the insulin activity tests at present available has been repeatedly put to question within recent years either with insulin derivatives[1,2] or with other disulphide hormones[3]. Dixon and Wardlaw[2] demonstrated that, when all disulphide linkages of insulin were cleaved by sulphitolysis, none of the resulting thiosulphate derivatives of the *A* and *B* chains was active on glycogen deposition in the isolated mouse diaphragm or in the mouse convulsion test. If, however, the primary action of insulin on the receptor tissue is mediated by a disulphide–sulphydryl interchange, as suggested by the action of *N*-ethylmaleimide on the cell membrane[4–6], an at least partially intact disulphide structure would be required for this action.

An insulin *A*-chain derivative with an intact intra-chain disulphide bridge, *A* chain-6-11-disulphide-7,20-*bis*-thiosulphate, has been recently isolated from beef insulin by a modified sulphitolysis procedure[7].

We have now found that this compound exhibited insulin-like activity in the following three tests which have previously been proved to be mutually independent[8]: (*a*) glucose uptake by the isolated rat diaphragm (1–2 per cent activity as compared with native insulin); (*b*) incorporation of labelled inorganic phosphate (^{32}P) into 'energy rich' compounds of isolated rat diaphragm[9]; (*c*) incorporation of ^{14}C-labelled methionine in a protein fraction of the diaphragm according to Manchester[10]. However, the biological activity of *A*-chain preparations isolated from natural insulin might originate from traces of intact insulin or contaminating *B* chain which could have escaped detection by chemical means.

We have, therefore, examined a synthetic *A*-chain preparation. It was obtained from *N*-carbobenzoxy-6,7,11,20-tetra-*S*-benzyl-heneicosipeptide[11] by random air oxidation for 18 h in 0·05 per cent concentration in *tris* buffer at *p*H 8·3 after removal of the protecting groups by sodium in liquid ammonia. It may be assumed that during random air oxidation a partial recombination of the 6–11 intra-chain disulphide ring of the native *A* chain would occur. The synthetic *A*-chain preparation exhibited insulin like activity in all three tests: this activity amounted to 0·513 per cent (based on native insulin) according to the glucose uptake of the isolated rat diaphragm ($\lambda =$ 0·12, limits of error for $P = 0{\cdot}95$: 0·22 per cent and 1·49 per cent). The stimulating effect of synthetic *A* chain on the two other metabolic tests is summarized in Tables 1 and 2. Control experiments with performic acid oxidized *A* chain isolated from beef insulin showed no stimulation of uptake of glucose, even at very high levels of the *A* chain-6,7,11,20-tetra-sulphonic acid.

Thus it appears that isolated diaphragm of the rat responds to *A* chain-6-11-disulphide-7,20-*bis*-thiosulphate and to randomly oxidized synthetic *A* chain qualitatively in the same way as it does with intact insulin. The results suggest that the 6–11 intra-chain disulphide bond of insulin *A* chain may play a specific part in the mechanism of insulin action and provides a new argument in favour of a primary disulphide–sulphydryl interchange between

Table 1. INFLUENCE OF SYNTHETIC INSULIN *A* CHAIN ON THE STIMULATION OF THE LABELLING OF PHOSPHATES IN THE ISOLATED RAT DIAPHRAGM
Specific activity (percentage of extracellular or intracellular phosphorus)

Insulin (μg/ml.)		0	10	—	—
Synthetic *A* chain (μg/ml.)		—	—	20	100
Intracellular inorganic phosphorus	(*E*)	4·45	4·89	4·55	3·57
P.C.*	(*I*)	40·0	57·2	60·3	72·5
ATP	(*I*)	45·0	59·5	59·0	67·5
ADP	(*I*)	23·7	27·6	29·8	42·0
U + G†	(*I*)	24·0	41·0	45·5	65·5

(*E*) Percentage of extracellular inorganic phosphorus.
(*I*) Percentage of intracellular inorganic phosphorus.
* Phosphocreatine. † Sum of uridine and guanosine phosphates.

Table 2. INFLUENCE OF SYNTHETIC INSULIN *A* CHAIN ON THE INCORPORATION OF ^{14}C L-METHIONINE INTO A PROTEIN FRACTION OF THE ISOLATED RAT DIAPHRAGM

Insulin (μg/ml.)	0	10	—	—	—
Synthetic *A* chain (μg/ml.)	—	—	5	10	20
Incubation medium (counts/100 mg)	4,350	3,630	4,100	4,390	4,170
Intracellular medium (counts/100 mg tissue)	3,924	4,760	4,480	4,295	4,918
Protein fraction (counts/mg protein)	151	394	236	237	332
	(3·84)	(8·25)	(5·26)	(5·55)	(6·75)

Percentage of intracellular medium in parentheses.

Table 3. Biological Activities of *A* Chain Preparations Alone and on Combination with *B* Chain (percentage based on Native Insulin, 27 i.u./mg).

A chain preparation	Activity *A* chain alone		Activity *A* + *B* chains	
	Glucose uptake	Mouse convulsion test*	Glucose uptake	Mouse convulsion test*
A chain-6-11-disulphide-7, 20-*bis*-thiosulphate	1–2%	0·5–1·0%	—	6–7%
Synthetic *A* chain, randomly oxidized	0·5%	0·4%	2–3%	—

* Carried out by Dr. C. Gloxhuber, Farbenfabriken Bayer AG, Wuppertal-Elberfeld; Zahn, H., Brinkhoff, O., Gloxhuber, C., and Meienhofer, J. (publication in preparation).

this hormone and its receptor in the muscle. In addition, we want to point out that recombination studies with insulin *A* and *B* chains should be considered with some caution in cases where the activities obtained are not larger than those which may be elicited by *A* chain alone. In our work (Table 3) recombinations of natural and synthetic *A* and *B* chains yielded higher activities.

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Mutagenic Action of Hyponitrous Acid and Glucose Oxime

One of us has demonstrated[1-5] with his co-workers that hydroxylamine, nitrous acid, hydrogen peroxide, hyponitrous acid and oxime can induce viral nuclear polyhedrosis in healthy silkworms. Among these, the mutagenic action of the first-mentioned three substances has recently been proved by Freese[6], Gierer[7] and Wyss[8]. We have now found that acidified hyponitrite and glucosoxime produce a mutation in a bacteriophage.

Sodium hyponitrite ($Na_2N_2O_2 \cdot 8H_2O$) was prepared immediately before use, according to the method of Jones[9]. As hyponitrite is unstable, a resin was applied to remove a greater part of sodium ions: 'Amberlite *IR*-120' (Rohm and Haas Co.) was treated with hydrochloric acid, washed with water and dried under 80° C. About 200 mg of sodium hyponitrite were dissolved in 1 ml. of water, passed through a column of 2-g resin and washed with 0·5 ml. of water. The *p*H of the solution obtained was 3·5. The loss of hyponitrous acid by this treatment was negligible.

The phage used was *T*4 (Benzer), the host *E. coli B* and the medium bouillon. In order to estimate the inactivation of *T*4 and the production of its mutants, 1·3 ml. of the hyponitrous acid solution was mixed with 0·1 ml. of *T*4-phage suspension in 0·1 M phosphate buffer of *p*H 7·0 and kept at 35° C. The *p*H of the mixture was 5·2 at the beginning of incubation and 5·7 after 3 h. The concentration of hyponitrous acid in the mixture was 0·4 M and the decomposition of the acid during the incubation was less than 10 per cent. The mutants produced were *T*4*r* (*r*) and mottled *T*4*r* (*m*). Some of the results are given in Table 1.

Table 1. Inactivation and Mutation of *T*4 by Hyponitrous Acid

Reaction time (min)	Inactivation rate (*T*4/ml.)	Mutation rate ((*r* + *m*) × 100/*T*4)
0	$2 \cdot 6 \times 10^8$	0·035
60	$1 \cdot 2 \times 10^8$	0·135
180	$7 \cdot 6 \times 10^6$	0·301

In the control, it was observed that the rate of spontaneous mutation for *r* plus *m* is 0·034 per cent on the average. The experiments further showed that the production of mutants increases to a certain degree with the elevation of hyponitrous acid concentration, and that both the inactivation and mutation do not occur at the neutral reaction.

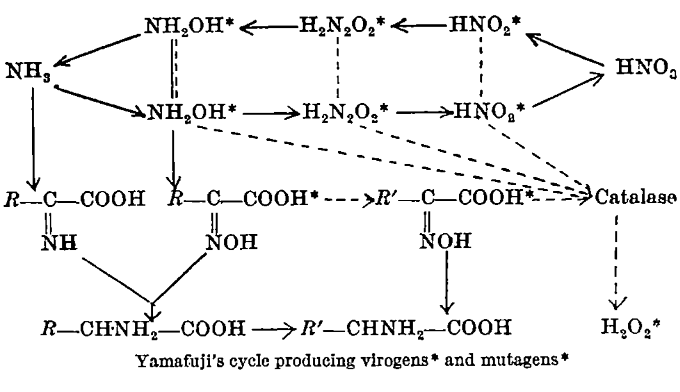
To investigate the mutagenic activity of glucosoxime, 1·8 ml. of 1·1 M oxime solution in 0·1 M phosphate buffer of *p*H 5 was mixed with 0·2 ml. of *T*4-phage suspension in 0·1 M phosphate buffer of *p*H 7 and maintained at 35° C. The *p*H of the mixture was 5·6 and there was almost no change in the acidity during the incubation. Some of the data obtained are given in Table 2.

Table 2. Inactivation and Mutation of *T*4 by Glucose Oxime

Reaction time (min)	Inactivation rate (*T*4/ml.)	Mutation rate ((*r* + *m*) × 100/*T*4)
0	$4 \cdot 7 \times 10^7$	0·040
40	$2 \cdot 8 \times 10^7$	0·077
200	$5 \cdot 0 \times 10^6$	0·533

The reaction mixture contained 0·05 M hydroxylamine at *p*H 5·6, owing to the partial hydrolysis of glucose oxime. It was confirmed, however, that the hydroxylamine of this concentration causes no mutation. The mutagenic function of hydroxylamine itself, as reported by Freese[6], can be detected in a solution of much higher concentration, in the presence of a fairly large amount of sodium chloride. We observed further that both the phage inactivation and mutant production do not take place in the mixture at *p*H 6·6.

On the basis of our discovery[10-12] of six new enzymes, we have established a nitrogen cycle[13]. On the other hand, we found that the intermediates in this cyclic reaction, hydroxylamine, hyponitrite, nitrite and oxime, inhibit cellular catalase without lowering respiration, thus leading to an accumulation of hydrogen peroxide[14-17], and that the enzymes participating in the cycle are widely distributed in animals, plants and microbes[18]. Based on these findings and the foregoing observations, Yamafuji now proposes the following scheme:



Yamafuji's cycle producing virogens* and mutagens*

It is noteworthy that normal nutritional or metabolic reactions produce a series of virogenic and mutagenic substances.

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Biosynthesis of L-Serine Ethanolamine Phosphodiester

From recent work[1] it would seem that L-serine ethanolamine phosphodiester (L-SEP) is found only in the tissues of fish, amphibians, reptiles and birds. D-SEP, on the other hand, appears to be confined to earthworms[2], where it is the biological precursor of lombricine[3]. The status of the work on both isomers has been summarized by Ennor and Rosenberg[4]. These authors had previously shown[1] that dinitrophenol (3×10^{-4} M) completely inhibited the biosynthesis of L-SEP and that cytidine triphosphate (CTP) appeared to be required. Thus it was shown that chicken kidney homogenates, supplemented by CTP, incorporated more inorganic phosphorus-32 into L-SEP than when this nucleotide was omitted.

This communication is concerned with an extension of this observation and with the development of a theory to account for the biosynthesis of L-SEP.

Crude chicken kidney homogenates have been used throughout and were prepared by homogenizing 10 g of fresh kidney in 20 ml. of ice-cold 0·25 M sucrose containing EDTA (10^{-3} M) in a Potter–Elvehjem type homogenizer for 30 sec. The homogenate was centrifuged at 500*g* for 10 min at 0° and the supernatant was used without further treatment. Incubation mixtures containing 1·0 ml. of the supernatant were incubated for 1 h in phosphate buffer *p*H 7·2 at 37° in the presence of various compounds labelled with carbon-14 and in a total volume of 2·0 ml. (Table 1). At the end of the incubation period trichloroacetic acid was added; a crude phospholipid fraction was prepared from the precipitated material and L-SEP was isolated from the supernatant by previously described chromatographic techniques[6]. The radioactivities of the phospholipid fraction and of L-SEP were determined in a Packard 'Tricarb' scintillation counter.

Table 1. Incorporation of ^{14}C-Ethanolamine into L-SEP and Phospholipid in Chicken Kidney Homogenates

Additions	% of added c.p.m. recovered in	
	L-SEP	phospholipid
(1) CDP-^{14}C-ethanolamine (*a*)	8·1	22
(*b*)	8·2	7·4
(*c*)	9·5	30
(2) As (1*a*), enzyme boiled	0·4	2·0
(3) As (1*a*) + unlabelled ethanolamine phosphate	7·7	38
(4) ^{14}C-ethanolamine phosphate	0	0
(5) ^{14}C-ethanolamine	0·1	0·5
	0·4	2·0

The labelled compound added in each experiment had a radioactivity of approx. 5,000 c.p.m.

The results (Table 1) indicate that appreciable incorporation of ^{14}C-ethanolamine into both phospholipid and L-SEP was achieved only when CDP-^{14}C-ethanolamine was the isotopic source and that this incorporation was enzyme dependent. It should also be noted that neither ^{14}C-ethanolamine nor ^{14}C-ethanolamine phosphate was incorporated into the phospholipids or L-SEP and that no isotopic dilution occurred when unlabelled ethanolamine phosphate was added together with CDP-^{14}C-ethanolamine.

These results suggest that the final step in the biosynthesis of L-SEP involves the reaction:

$$\text{cytidine diphosphate-ethanolamine} + \text{L-serine} \rightarrow \text{cytidine monophosphate} + \text{L-SEP}$$

which is presumably catalysed by a specific enzyme. Work now in progress is designed to determine the existence of this reaction and the results, together with full details of the present work, will be submitted for publication elsewhere.

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PHYSIOLOGY

A Biologically Active Metabolite of Vitamin A and Vitamin A Acid

A recent report by Zachman and Olson[1] described the formation of acidic water-soluble metabolites from vitamin A (retinol) and vitamin A acid (retinoic acid) by perfused rat liver. We have isolated an acidic metabolite, with potent vitamin A-like activity, from both retinol and retinoic acid. No compound metabolically related to retinol or retinoic acid with growth-promoting activity has hitherto been described.

Circumstantial evidence has for long suggested[2] that retinol is changed into an 'active form' in the course of its metabolic function. When the vitamin A-like activity of retinoic acid was discovered by Dowling and Wald[3], this acid was at first considered to be the 'active form'. However, it could not be detected in the rat, even after administration of it to deficient animals which were being cured[4]. Apparently it was extremely rapidly metabolized. Therefore, the thought arose that retinoic acid, too, is changed into the actual metabolically active compound. The metabolite reported here may be closer than retinoic acid to the active compound, or may be the active compound itself. Whether it arises naturally from retinol via retinoic acid, or independently from either, has not been determined.

Retinol-deficient rats were given retinol (1 mg in 0·2 ml. cottonseed (Wesson) oil per day) orally by intubation or retinoic acid (1 mg in 0·2 ml. aqueous saline, *p*H 8, per

Table 1. YIELDS OF METABOLIC PRODUCTS FROM RETINOIC ACID AND RETINOL

	6,7-^{14}C-retinoic acid*					6,7-^{14}C-retinol acetate†
Hours after injection	1	2	3	4	10 days‡	2
No. of rats used (wt. 180–200 g)	3	3	4	6	5	2
Total dose injected (μc.) into all rats	4·52	4·52	15·66	22·68	61·80	3·82
Weight of combined intestines (g) from all rats	18	17	25	36	34	12
% of dose in acidic fraction	12·64	12·18	6·60	0·85	0·49	7·35
% of dose in compound 5	1·33	1·35	0·79	0·14	0·08	1·04

* Specific activity 19·9 μc./mg.

† Specific activity 6·45 μc./mg (compound given orally in Wesson oil).

‡ Compound administered orally, 1·24 μc./rat/day, the last feeding 4 h before termination.

day) by intraperitoneal injection for 2 weeks and then received an intraperitoneal injection of ^{14}C-retinol or ^{14}C-retinoic acid, respectively, in amounts indicated in Table 1. The animals were killed after the time indicated, and the large and small intestine were removed and homogenized in 0·25 M sucrose containing 0·025 M potassium chloride and acetone. The supernatant solution obtained after centrifugation was concentrated and then saponified with ethanolic potassium hydroxide for 1 h at room temperature under nitrogen. After acidification, an acidic fraction could be extracted with ether. The ether extract was concentrated and the resulting mixture separated by thin-layer chromatography on Merck 'Silica gel *G*', in benzene (10): $CHCl_3$ (2·5) : CH_3OH (2·5). The mixture separated into 9 spots as shown by the faint yellow fluorescence in ultra-violet light, but 10 spots were revealed by iodine vapour. Spot No. 5, 'compound 5' (R_F 0·32), and spot No. 9, 'compound 9' (R_F 0·85), as detected by iodine vapour and counting the origin as spot No. 1, were the only radioactive spots. They were eluted separately, re-chromatographed and eluted. An ester of compound 5 could be obtained from the intestinal extract prior to saponification. On thin-layer chromatography it remained at the origin. On saponification it yielded compound 5.

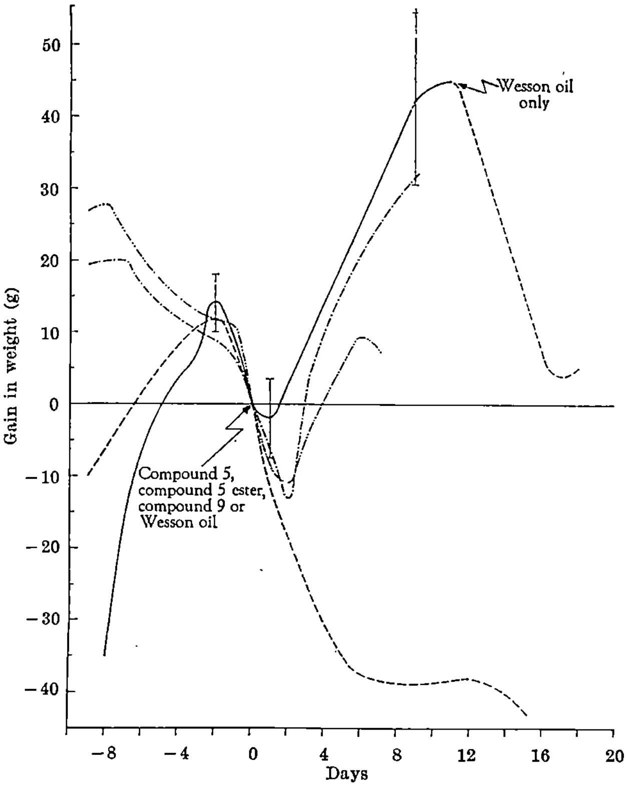

Fig. 1. Gains in weight of rats fed retinol-deficient diets and given compounds indicated, 8 μg dissolved in 0·2 ml. cottonseed (Wesson) oil per day orally by intubation, or Wesson oil alone. Vertical lines show standard deviations of weight gains for 4 rats 2 days prior, 1 day after and 9 days after administration of compound 5. Rats on compound 9 died of the deficiency on the seventh day. —, Compound 5 (4 rats). Average weight on day of administration, 141·5 g; —· —·, compound 5 ester (2 rats). Average weight on day of administration, 173·5 g; —··—··, compound 9 (2 rats). Average weight on day of administration, 135·0 g; — — —, Wesson oil (1 rat). Average weight on day of administration, 148·0 g

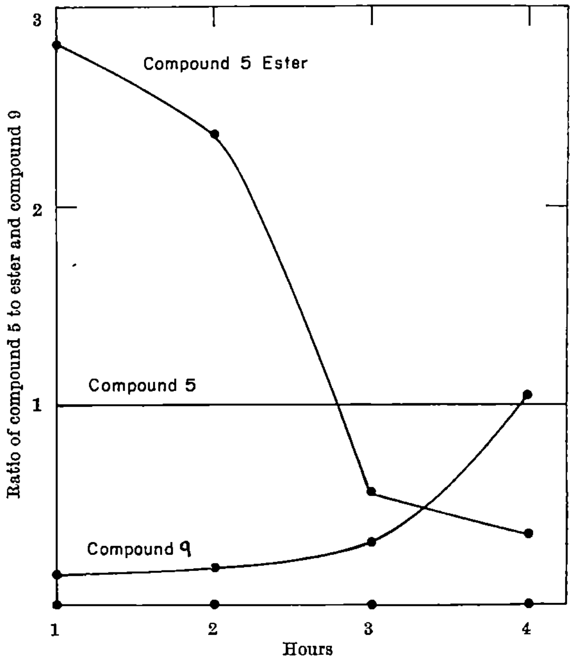

Fig. 2. Ratios of radioactivity of compound 5 ester and compound 9 to compound 5, in relation to time after injection of 6,7-^{14}C-retinoic acid, with compound 5 taken as unity

Growth curves of rats on retinol-deficient diets when given compound 5, its ester, compound 9 or Wesson oil as control are shown in Fig. 1. The curves demonstrate that weights returned to normal after feeding compound 5 or its ester for 3 days, but not compound 9; and dropped to the deficient level after withdrawal of compound 5. The rats cured from the deficiency by compound 5 not only regained their weights, but also lost their eye-lesions and ruffled fur. That compound 5 was not contaminated by retinoic acid was shown by the fact that it was radioactive after administration of labelled retinoic acid, whereas, in the same experiment, retinoic acid, when added as carrier in the isolation procedure, and obtained along with compound 5 by thin-layer chromatography, was not radioactive. Besides, the R_F of compound 5 (0·32) differs greatly from that of retinoic acid (0·82) in the solvent system used above.

The yields of compound 5 from labelled retinoic acid and retinol are shown in Table 1. It can be seen that it appears rapidly, and begins to decline after 2 h. Concomitantly, compound 9 concentration increased. The same relation holds between compound 5 ester and compound 5 (Fig. 2). It would, therefore, be tempting to suggest a sequence such as this: retinol → retinaldehyde → retinoic acid → compound 5 ester → compound 5 → compound 9.

The last column of Table 1 shows that compound 5 can also be formed from retinol. The identity and purity of compound 5 obtained from retinoic acid and retinol was further demonstrated by reversed-phase paper chromatography in 2 different solvent systems (MeOH : BuOH : H_2O, 70 : 25 : 5, and $CHCl_3$: 1 per cent acetic acid in MeOH, 2 : 1; silicone impregnated paper). Only a single spot was found for compound 5 from either precursor, different from the spots given by retinol or retinoic acid. Compound 5 occurs also in liver and kidney.

Rats cured from retinol deficiency by compound 5 are totally nightblind as determined by electroretinogram. Therefore, a conversion of compound 5 back to retinol is excluded. A conversion of compound 5 back to retinoic acid was shown to be unlikely because, when radioactive compound 5 was given to 2 rats for 3 weeks, no label was detectable in added carrier retinoic acid.

Compound 5 is obviously a carboxylic acid derived from retinol and retinoic acid, by loss of at least the carbon atom No. 15, since 15-^{14}C-retinoic acid does not yield radioactive compounds 5 or 9. Compound 5 occurs naturally as an ester with an unknown alcohol, as well as in the free form. It does not produce any colour with the Carr–Price reagent. Its ultra-violet spectrum shows a peak at 252 mμ. The infra-red spectrum shows a broad absorption band between 3,550 and 3,450 cm^{-1} (OH-group), CH_3 and CH_2 peaks (at 2,937 and 2,898 cm^{-1}), a $C{=}O$ peak (at 1,589 cm^{-1}) and a COO^- peak, possibly conjugated (at 1,715 cm^{-1}). Compound 5, therefore, has presumably 2 double bonds in conjugation with the carboxyl group, and a hydroxyl group. Attempts are now being made to determine the structure of compound 5.

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Hæmagglutination of Erythrocytes coated with 6-Aminopenicillanic Acid

SINCE unaltered benzylpenicillin has not been shown to be capable of forming stable covalent bonds with proteins[1], it has been suggested that the hapten complex associated with penicillin hypersensitivity involves the intermediate degradation compound D-benzylpenicillenic acid[2-4]. Levine and Ovary[4] have shown that D-benzylpenicillenic acid will react with serum proteins, especially lysine ε-amino groups, to form the mixture of diastereomers of ε-*N*-(D-α-benzylpenicilloyl)-lysine groups. Such compounds are stable antigens giving wheal and erythema reactions in sensitized individuals. De Weck and Eisen[2], on the other hand, have produced evidence that D-benzylpenicillenic acid enters into mixed disulphide linkages especially with proteins enriched with SH groups and that antibody to such complexes has an affinity for the oxazolone ring structure of D-benzylpenicillenic acid.

Previous investigations have shown that sera from certain patients treated with benzylpenicillin will agglutinate erythrocytes coated with this substance[5,6]. However, the majority of such individuals show no evidence of clinical hypersensitivity and no information is available concerning the possible identity of antigenic determinant groups involved in hæmagglutinin production and in hypersensitivity, although De Weck and Eisen[2] have suggested that the erythrocytes may in fact be coated with D-benzylpenicillenic acid, which forms rapidly in aqueous solutions of benzylpenicillin. If so, the hæmagglutinin would most likely be spatially orientated to fit the determinant oxazolone ring. The reaction of 6-aminopenicillanic acid with carbon dioxide suggested a means of determining whether this view is correct.

If human group O, Rh-negative erythrocytes are added to 6-aminopenicillanic acid, which has been dissolved in 0·85 per cent saline and the *p*H adjusted to 7·0 with sodium hydroxide to increase solubility, no hæmagglutination occurs in the presence of sera capable of agglutinating benzylpenicillin-coated erythrocytes. However, hæmagglutination occurs if 6-aminopenicillanic acid is dissolved in sodium bicarbonate solution[7]. Johnson and Hardcastle[8] have shown that in these circumstances 6-aminopenicillanic acid rearranges to form 8-hydroxypenillic acid according to Fig. 1, which involves the formation of two intermediate compounds. Compound 3 is of great interest since its attachment to erythrocytes could leave exposed a reactive determinant group, either the oxazolone ring or the thiazolidine ring depending on the mode of attachment.

Hæmagglutination tests were performed with positive sera obtained from patients treated with benzylpenicillin as previously described[6], with the modification that tubes were incubated for 0·75 h instead of 1·5 h. Successful agglutination was found to be dependent on the concentration of bicarbonate and also on the time of reaction of 6-aminopenicillanic acid with bicarbonate before addition of erythrocytes (Table 1).

Table 1

A	Conc. of $NaHCO_3$ (g %)	16	8	4	2
	Hæmagglutination	++++	++	–	–
B	Time of reaction 6-APA with $NaHCO_3$ (h)	1	4	8	24
	Hæmagglutination	++++	++++	±	–

Maximum hæmagglutination was obtained with saturated bicarbonate solutions prepared at 37·0° C (approximately 16 g per cent), and also when erythrocytes were added immediately or within 4 h to the mixture. Specifically, no agglutination occurred with bicarbonate concentrations of 4 per cent or less or when erythrocytes were added after 24 h. It appears that 8-hydroxypenillic acid is unable to coat cells and leave an active determinant group exposed to antibody in the same way that penillic and penicilloic acids also give negative results. Of the two intermediate compounds, compound 3 seems most likely to constitute the active hapten. In this event, it seems that 8-hydroxypenillic acid may in fact inhibit optimum coating of the cells since optical density measurements at 322 mμ (which is specific for the oxazolone ring structure of penicillin degradation compounds), after 24 h interaction of 6-aminopenicillanic acid and bicarbonate solution, showed that compound 3 was still present and that the reaction had not proceeded to completion under the conditions of the experiment. However, it is not clear whether this represents inhibition of attachment to the cells or a steric hindrance effect on the cell surface.

Inhibition investigations with various haptens showed that equimolar concentrations of 6-aminopenicillanic acid dissolved in either sodium hydroxide or bicarbonate

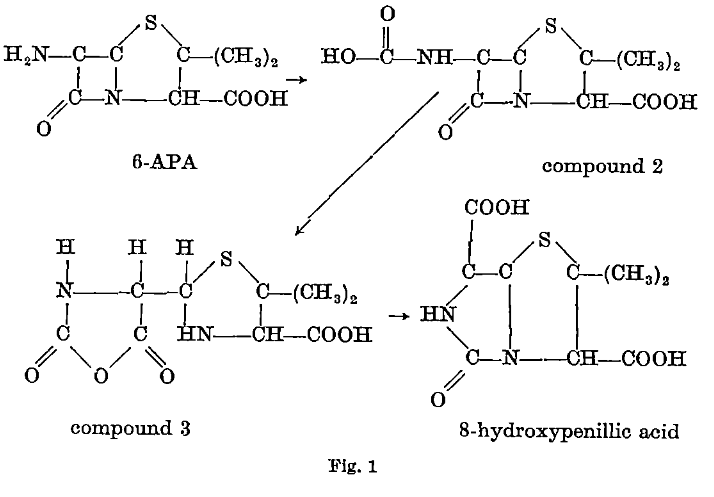

Fig. 1

gave identical degrees of inhibition (effective concentrations of 0·00075 M with a 1/8 dilution of a positive serum which agglutinated benzylpenicillin-coated cells to a titre of 1/256). Since the oxazolone ring structure would not be produced in the presence of sodium hydroxide the results suggest that the antibody is more likely to present a complementary pattern to the thiazolidine ring. This would explain the ability of penicilloic acid to inhibit the reaction and also the observation of De Weck and Eisen[2] that penillic acid and 6-aminopenicillanic acid have only very slight activity in inhibiting precipitation of D-benzylpenicillenic acid-protein conjugates in the presence of D-benzylpenicillenic acid antisera. Furthermore, hæmagglutination was inhibited by cephalosporin *C* at a molar concentration about 250 times greater than with 6-aminopenicillanic acid, suggesting a cross-reaction with the related dihydrothiazine ring.

Since hæmagglutinin is often present in sera in the absence of evidence of hypersensitivity, antibody formation associated with each of these phenomena may not depend on identical determinant groups forming *in vivo* during the degradation of benzylpenicillin.

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HÆMATOLOGY

A Relationship between the *H* Chain Groups of 7*S* γ-Globulin and the Gm System

EVIDENCE has been obtained recently that 7*S* γ-globulin and myeloma proteins can be divided into three types on the basis of antigenic characteristics as well as other differences in the *H* chains[1-3]. A variety of antisera in rabbits to individual myeloma proteins as well as monkey antisera to *H* chains have served to bring out these variations. Splitting of γ-globulin with papain has also brought out differences in these three types[2]. Since the determinants involved in the Gm system have been localized to the *H* chains[4,5], it was thought of interest to test γ-globulins of the three types for the various factors involved in the Gm system. For this purpose, myeloma proteins of the three types were utilized[6,7]. Also included were investigations of the newly described Gm(*f*) factor[8], which has been found in the majority of myeloma proteins which were previously found to be negative for the Gm(*a*) and Gm(*b*) factors[9]. Striking differences in the distribution of the Gm factors were encountered for the proteins of each type.

Myeloma proteins were isolated by zone electrophoresis and were typed for Gm(*a*) (*b*) and (*f*) characters as described previously[6,7,9]. For the Gm(*a*) determinations several normal serum agglutinators were used. The Gm(*b*) reagents included both several normal serum agglutinators and agglutinators from patients with rheumatoid arthritis. Some of the Gm (*b*+) myeloma globulins were typed with three different Gm(*b*) systems with concordant results; one had been typed with five different anti-Gm(*b*), including Harboe's original anti-Gm(*b*) J. K.[10]. Two different anti-Gm(*f*) sera were used, both from donors without rheumatoid arthritis[8].

Table 1. PATTERNS OF REACTIVITY BY AGAR PLATE ANALYSIS OF VARIOUS MYELOMA PROTEINS AND THREE ABSORBED ANTISERA WHICH BRING OUT FOUR DIFFERENT *H* CHAIN GROUPS. ALSO SHOWN ARE THE GM PHENOTYPES OF THESE MYELOMAS

	Anti Vi abs. c̄ Sp	Anti Sp abs. c̄ Vi	Anti Ci abs. c̄ We	L chain group	Myeloma Gm phenotype
Vi	+ + + +	0	0	II	$a-b-f-$
Fe	+ +	0	0	I	$a-b+f-$
Ap	+ +	0	0	I	$a-b+f-$
Hu	+ +	0	0	I	$a-b+f-$
Ma	+ +	0	0	I	$a-b+f-$
We	0	+ +	0	I	$a+b-f-$
Me	0	+ +	0	I	$a+b-f-$
Le	0	+ +	0	I	$a+b-f-$
Cu	0	+ +	0	I	$a-b-f+$
Sp	0	+ + + +	0	II	$a-b-f+$
Ho	0	+ +	0	II	$a-b-f+$
Lv	0	+ +	0	I	$a-b-f+$
Gr	0	+ +	0	II	$a-b-f+$
Ge	0	0	+ +	I	$a-b-f-$
Ci	0	0	+ + +	I	$a-b-f-$
Ro	0	0	+ +	I	$a-b-f-$
Ne	0	0	0	II	$a-b-f-$
Wh	0	0	0	I	$a-b-f-$

Table 1 shows the reactions of three absorbed antisera with different representative myeloma proteins. These have been selected and the numbers in different groups do not indicate the exact relative incidence. It is apparent that these antisera recognize at least four different classes of 7*S* γ-globulin. The first three groups are recognized both in a positive and a negative fashion and appear to be clear-cut classes, and are termed Vi, We and Ge groups, respectively. The fourth type at the bottom of Table 1 was only recognized as deficient by all the antisera. Other antisera in addition to those shown showed similar breakdown of the different types. Particularly striking was a monkey antiserum to *H* chains of the Vi group which gave the exact same distribution of reactivity as that obtained with the Vi rabbit antiserum. Fig. 1 illustrates the reaction of anti-Vi antiserum, unabsorbed and absorbed with Sp, with members of the Vi group of myeloma proteins as compared to those from the second groups of Table 1 (the We group).

Determination of the Gm phenotype of the myeloma proteins of the different groups brought out marked differences. All the Gm(*b*+) myeloma proteins fell into the Vi group. Four of these are illustrated in Table 1. An additional eleven Gm(*b*+) myeloma proteins were also tested and they all fell into the Vi group antigenically. The '*H* chains' from a patient Zu with H chain disease[11,12] also were Gm(*b*+) and fell into the Vi group. Two myeloma proteins which fell into the Vi group were (*a*–*b*–*f*–) including myeloma Vi itself. The incidence in a total of 84 7*S* type, myeloma proteins which were not selected on a basis of Gm type was ten Vi group myeloma proteins with eight Gm(*b*+) and two Gm(*b*–).

The majority of myeloma proteins (approximately 60–70 per cent) were found in the We group. All the Gm(*a*+) or Gm(*f*+) myeloma proteins fell into this group. No single myeloma protein contained both factors but contained one or the other factor.

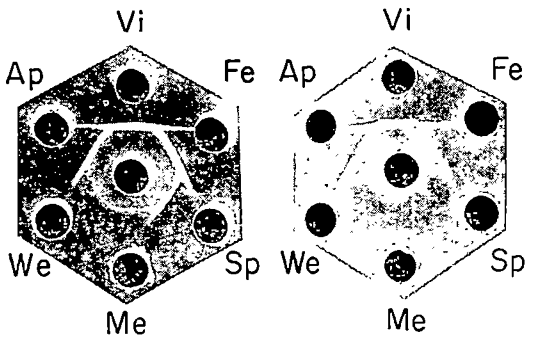

Fig. 1. Reaction of anti-Vi antiserum, unabsorbed and absorbed with Sp, with myeloma proteins of the Vi group (Vi, Ap, Fe) and heterologous myeloma proteins of the We group (We, Me, Sp)

Five myeloma proteins of the Ge group, three of which are shown in Table 1, were negative for all the Gm characters. These myeloma proteins were found to show a unique fast mobility for the F component produced by papain splitting not found with other myeloma proteins[2]. This suggests a considerable degree of individuality for this class of protein although they still cross-reacted in the F fragments with myeloma proteins of the other groups.

In addition to the Ge group, the Gm(*b*–) members of the Vi group, and the rare member of the We group, a number of other myeloma proteins were encountered which were also (*a*–*b*–*f*–). Two of these are shown at the bottom of Table 1. Most antisera showed these myelomas to be deficient but as yet no antiserum has been obtained which recognizes them in a positive fashion. Evidence also was obtained indicating that this was not a homogeneous group but consisted of a mixture of types.

Experiments on the distribution of the various groups among the molecules of normal γ-globulin indicate that they in general parallel the incidence among myeloma proteins. Quantitative precipitin analysis on twenty normal sera indicates a range for the Vi type protein from 0·1 to 1·1 mg/c.c. (ref. 2). A counterpart for the Ge group was also found and this occurred at a lower level. All sera examined, including those from whites of different Gm phenotype, negroes, Chinese and American Indians contained the Vi, We and Ge type proteins.

It has been thought in the past that genes Gm^a and Gm^b are alleles in Caucasians[10]. Certain unusual features, however, have been encountered in other racial groups[13,14]. The results presented here showed that types Gm(*a*+) and Gm(*b*+) did not occur in the same group of 7*S* γ-globulins. All Gm(*a*+) and Gm(*f*+) myeloma proteins fell into the We group which, furthermore, did not include any Gm(*b*+) proteins. It is known that the distribution of Gm(*b*) and of Gm(*f*) is very similar in Caucasians, because white individuals are, with rare exceptions, positive for both or for neither[8,9]. Combining these results, it seems possible that Gm^f (certainly not Gm^b) may be the true allele of Gm^a. It is then a reasonable concept that the 'We locus' (with Gm^a and possibly Gm^f as major alleles) and the 'Vi locus' (with Gm^b and at least one unknown allele) are closely linked. Reasoning along these lines leads to the prediction that Gm(*b*–) γ-globulins of the Vi group contain the product of the true allele(s) of Gm^b and that genes at a third locus, the 'Ge locus', as well as other loci, determine characteristics of the *H* chains of other 7*S* γ-globulin molecules.

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PHARMACOLOGY

Transfer of Oxytocin from the Maternal to the Fœtal Circulation in the Ewe

LITTLE is known of the transfer of peptides between the maternal and fœtal circulation. In their exhaustive review, Hagermann and Villee[1] mention the results of the work of Krzysztoporski[2], who showed that simple peptides cross the placental barrier, and those of Knobil and Josimovitch[3] that insulin crosses the mono-layered placenta of the rat. Other information on the placental penetration of peptide hormones is sparse and contradictory. High concentrations of oxytocin in maternal blood during parturition have been reported[4] and it seemed therefore of interest to investigate the possibility that oxytocin in the maternal circulation might reach the fœtus.

Table 1. OXYTOCIC ACTIVITY IN FŒTAL LAMB PLASMA BEFORE AND AFTER INJECTION OF OXYTOCIN INTO THE MATERNAL UTERINE ARTERY

Ewe No.	Days before full term	Dose of oxytocin (units)	Interval between oxytocin injection and starting collection of blood sample (seconds)	Oxytocic activity of plasma extracts mU/ml. blood	
				Control	After oxytocin injection
1	25	40	10	3·0	3·0
2	17	40	90	2·3	3·4
3	17	40	90	3·3	6·0
4	16	20	90	1·2	2·7

The experiments were carried out in pregnant ewes of the Welsh breed, in which the dates of conception were known. The ovine placenta is cotyledonary and has five layers, the fœtal and maternal circulations coming into juxtaposition in the cotyledons. About 20 days before term, the lamb or lambs were removed from the uterus by Caesarian section under spinal nerve block anæsthesia with the umbilical cord intact. (The operations were kindly carried out by Prof. Messervy and his staff.) Control samples of about 20 ml. of blood were withdrawn from the umbilical artery into heparinized syringes. Oxytocin (20–40 units 'Syntocinon', Sandoz) was injected into the uterine artery of the ewe, and a second sample of arterial blood withdrawn from the cord either before or after the oxytocin-induced contraction of the uterus. After centrifugation at 0° C the plasma was separated and extracted by treatment with acetone and ether according to the method of Ginsburg and Smith[5]. Oxytocic activity in the extracts was assayed on the superfused rat uterus[6] using 'Syntocinon' as standard. The results are shown in Table 1. Oxytocic activities equivalent to 1·2–3·3 mU oxytocin/ml. were found in control plasmas. In one experiment the second sample of umbilical arterial blood was collected 10 sec after the injection of oxytocin and before the uterus contracted; there was no difference in the oxytocic activity of the plasma before and after injection. In the remaining three experiments, in which the second samples were collected 90 sec after oxytocin injection when the uterine contraction was apparently maximal, the oxytocic activity of the second plasmas was 50–125 per cent greater than that of the controls. Treatment of the extract of the second sample of plasma in experiment 3 with thioglycollate[7] reduced the oxytocic activity from 6 mU/ml. to 3 mU/ml. blood, that is, to the level found in the control sample. It seems likely that the oxytocic activity in the control sample was due to the presence of kinins in the extract[8] but that the additional activity after injection was due to oxytocin, suggesting that the placental barrier is not impermeable to oxytocin.

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A Non-cholinergic Synaptic Inhibition in the Central Nervous System of a Mollusc

Tauc and Gerschenfeld[1,2] have shown that in *Aplysia depilans* and *Helix pomatia* acetylcholine (ACh) possibly mediates synaptic excitation on neurones conventionally named *D* cells and also synaptic inhibition in other neurones called *H* cells. *D* neurones are recognized because they have only one type of input which is excitatory in character and cholinergic in nature. The analysis of synaptic activity in intracellular recording in the isolated nervous ganglia of the Argentine land-snail, *Cryptomphallus aspersa*, reveals also the presence of typical *H* and *D* cells. Besides them the perio-œsophagic ring of this snail contains also other types of neurone that in addition to a cholinergic excitatory input as in *D* cells, show a direct inhibitory synaptic input. These cells will be conventionally named here *DInhi* (*D* cells with *Inhi*bition) neurones.

DInhi neurones constitute a group of cells having a typical pattern of synaptic activity and pharmacological response. They are located in the caudal pole of the visceral ganglion, and their number is difficult to ascertain although it is certainly more than 5 or 6. In these cells, the inhibitory input may be mainly evoked by electrical stimulation of the visceral nerve, but sometimes an afference entering through the right pallial nerve could evoke a response. The inhibitory synaptic action is conveyed through an interneurone. The visceral nerve not only brings inhibitory afferences but also some excitatory ones. A single shock stimulation of this nerve provokes after a short latency period a direct inhibitory synaptic potential followed by depolarization and an excitatory burst (Fig. 1*A*). A single shock, or more often a short repetitive electrical stimulation at low frequency, provokes a repetitive firing of the inhibitory interneurone following the excitatory burst (Fig. 1*A*). This repetitive response consists of a long discharge composed by a variable number (generally 20–30) of elementary post-synaptic potentials lasting up to 40–60 sec (Figs. 1*A* and 2*A*). In Fig. 1*A* the repetitive elementary IPSP's completely inhibit the spike firing, but in other cases the IPSP only partially inhibit the spikes, and both spikes and IPSP appear intermingled in different proportions.

Inhibitory post-synaptic potentials appearing in *DInhi* cells have at the resting potential level (40–45 mV) mean amplitudes of 5–6 mV and a duration of 200–300 msec. The time course of this synaptic action is similar to that described by Tauc in *Aplysia* and *Helix*[3]. Impaling a *DInhi* cell with a double-barrelled microelectrode filled with 0·6 M sodium sulphate it was possible to pre-set the membrane potential to the desired levels. In Fig. 2, when the membrane potential is driven to − 70 mV (Fig. 2*E*), the repetitive elementary IPSP's are reversed. At a potential of about − 60 mV the inhibitory synapses do not show any overt action, and this level may correspond to the equilibrium potential of the ionic permeability change provoked by the transmitter (Fig. 2*C*). This inversion potential is similar to the reversal level of cholinergic IPSP's in *Aplysia* and *Helix*[4].

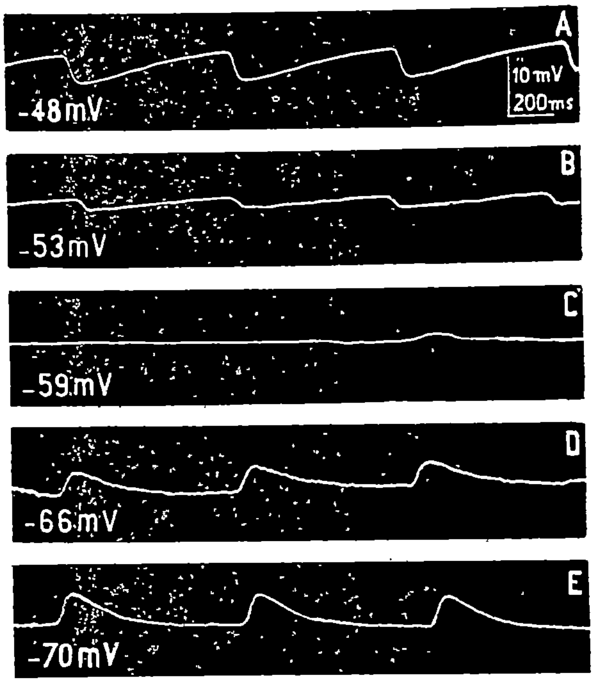

Fig. 2. *DInhi* cell impaled with a double-barrelled microelectrode. *A*, 'Spontaneous' appearing IPSP's resulting from the repetitive firing of an inhibitory interneurone. The membrane potential is driven to different levels injecting inward current through one of the barrels in *B*, *C*, *D* and *E*. Notice the reversal of IPSP's in *D* and *E*, and the inversion potential level in *C*. Potential levels are indicated in the left bottom angle of the recordings

Acetylcholine depolarizes and excites the *DInhi* neurones. Fig. 1*B* shows this effect when the drug is applied during the repetitive discharge of the inhibitory interneurone. From this evidence it becomes clear that acetylcholine cannot be the transmitter responsible for IPSP's. On the contrary, it is possible to demonstrate that D-tubocurarine and atropine sulphate block the excitatory post-synaptic potentials elicited in a *DInhi* cell by electrical stimulation of the left pallial nerve. This excitatory input generally shows up uncomplicated with an inhibitory action, as in the case of the visceral nerve stimulation.

Recent electron-microscopic[5], biochemical[6,7] and microphysiological[4] results have suggested that the gastropodal nervous system may have a complex pattern of possible synaptic transmitters. Of the other chemical substances supposed capable of acting as transmitters and normally existing in molluscan ganglia only dopamine has a constant strong inhibitory action on *DInhi* cells. These are sensitive to the drug down to a concentration of 10^{-9} M. α-Adrenergic blocking agents, such as dibenamine and dihidroergotamine, do not alter the inhibitory input to *DInhi* cells. It is not possible to demonstrate a specific activity of β-adrenergic blocking agents at the synaptic level; that is, dichloroisoproterenol blocks nerve conduction[8] and guanethidine impairs interneurone firing.

Other synaptic blocking agents, active on other vertebrate and invertebrate inhibitory synapses[9], such as picrotoxin, strych-

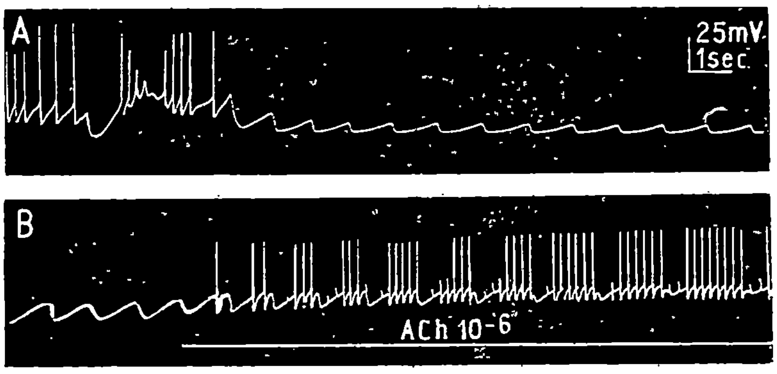

Fig. 1. Recordings from a *DInhi* cell impaled with an intracellular microelectrode. *A*, a single electrical stimulation of the visceral nerve evokes an IPSP immediately followed by an excitatory burst and the repetitive firing of the inhibitory interneurone. The latter results in a series of IPSP's giving an oscillatory appearance to the recording. *B*, during the discharge of IPSP's the application of acetylcholine 10^{-4} by perfusion depolarizes and excites the neurone

nine, tetanus toxin and metrazol, were not effective in modifying the inhibitory input to *DInhi* cells. Further work is being carried out to clarify the possible physiological role of *DInhi* neurones and the nature of the inhibitory transmitter involved in this non-cholinergic synaptic inhibition.

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PATHOLOGY

Failure to induce Atherosclerosis in 'Triton' Hyperlipæmic Guinea Pigs

It has been shown that 'Triton-*WR* 1339', a non-ionic surface-active agent, induces high hyperlipæmia in rabbits[1], rats[2], mice[3], guinea pigs[1,4] and dogs[5]. Typical atheromasic changes of the aorta, coronary and pulmonary arteries have been demonstrated in 'Triton'-treated dogs[5]. These findings are of some interest, since dogs are usually less susceptible to atherosclerosis, and the atheromasic changes have been obtained in animals fed with a normal diet.

On the basis of the foregoing results, 37 guinea pigs of both sexes have been treated with 'Triton-*WR* 1339'. All the animals were divided into three groups and fed with a commercial standard diet to which carrots and green salad were added. Ten (first group) were not treated, 18 (second group) were given subcutaneously a 20 per cent 'Triton-*WR* 1339' in saline at a dose of 400 mg/kg every fourth day, for 19 weeks; 19 (third group) were given 'Triton' as the latter ones, and furthermore received subcutaneously a single dose of 1,000 viscosimetric units of lyophilized testicular hyaluronidase diluted into 3 ml. of saline, 30 days before the end of the treatment with 'Triton'. Treatment with hyaluronidase was given to enhance a possible atherogenic effect of 'Triton', as shown in cholesterol-fed rabbits[6-9]. All the animals were killed by cardiac puncture. The serum level of total cholesterol, phospholipids, total lipids and β-lipoproteins were determined. The entire aortæ, previously fixed in 10 per cent neutral formalin, and stained with Sudan III, were grossly and independently observed by three persons. Furthermore, the aortæ and the other organs were properly processed for histological examination.

Table 1. Average Values and Standard Deviations of Weight and Blood Lipids for the Animals of All the Groups

	No. of animals	Weight (g)		Total cholesterol (mg/100 ml.)	Phospholipids (mg/100 ml.)	Total lipids (mg/100 ml.)	Lipoproteins (%)
		At the beginning of treatment	At the end				
First group. Control untreated guinea pigs	10	435 76	432 114	48·9 13·4	51·4 28·5	192·5 40·6	93·9 3·9
Second group. Guinea pigs treated with 'Triton-*WR*' 1339	18	537 52	522 103	604·0 200·5	1,396·1 489·9	7,634·4 3,560·1	99·2 0·82
Third group. Guinea pigs treated with 'Triton-*WR* 1339' and hyaluronidase	19	517 64	500 113	572·1 187·3	1,176·2 315·2	6,761·0 2,380·5	98·8 0·54

Average values and their standard deviations of blood lipids for the three groups of animals are given in Table 1.

Serum total cholesterol, phospholipids, total lipids and β-lipoproteins are much higher in the second and third group than in the first group.

The organs and aortæ of the animals of all groups were found almost completely devoid of any change.

Chromotrope subintimal ground substance was very scarce in the arterial wall of the control guinea pigs, their endothelial cells lying just directly on the internal elastic membrane.

High blood lipids have previously been demonstrated in 'Tritonized' guinea pigs[4], but at lower levels, presumably because of the shorter period of treatment; moreover, no morphological studies on 'Triton'-treated guinea pigs have been carried out. In our experiments hyperlipæmia has not been followed by any significant change of the aortic wall or even by any lipidic infiltration of the liver, spleen and kidneys. These findings agree with the chemical results obtained by Patnode *et al.* on guinea pigs[4], although they are different from those observed in 'Triton'-treated dogs[5].

It has been shown[10] that lipolithic activity of intimal arterial layer of animals less susceptible to experimental atherosclerosis (for example, guinea pigs) is higher than that observed in the same tissues of more susceptible species (for example, rabbits). This could justify the unexpected lack of any atheromasic findings into the aortæ of the guinea pigs of the present experiments, since their endothelial cells may be particularly efficient in lipidic metabolism.

Moreover, our observations of scarce chromotrope ground substance in the intimal tissues of control untreated guinea pigs could also explain the failure to induce atherosclerosis in these animals, since the ground substance could favour the deposition of lipids into the arterial wall.

Comparative anatomical examination of the vascular structures of species differently susceptible to atherosclerosis might be worth while in this respect.

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Impact of the Laser on Dental Caries

The characteristics of the laser beam which are of great interest and importance from the biomedical aspect are the ability to direct and to focus, intense energy density and some selectivity[1]. Since the laser beam is a light beam, it can be focused most accurately by using lens systems. Energy density is intense. In these experiments, the energy density was approximately 9,000 joules/cm² in the target area. Selectivity indicates that materials which are coloured absorb more of the incident beam of the lasers now available than uncoloured materials. In addition to these properties, of interest to the dentist is the fact that the duration of the impact is in the order of a millisecond.

Following a series of experiments of a study on impact of high-energy lasers, 300-joules exit energy, on bone, preliminary studies were done of the impact of the laser on teeth. These experiments were done with a pulsed ruby laser, 90-joules exit energy, beam width approximately 3/8 in., with an energy density of the focused beam of approximately 9,000 joules/cm². Some experiments were done also with special glass rods which transmitted the laser beam directly into the cavities. The teeth used in these experiments were fastened to holders on the optical bench. Pre- and post-impact pictures were obtained with a standard colour photography set-up. The teeth after impact were observed under a stereobinocular microscope. In these studies, sections have been made of the teeth by John Gray and will be reported later.

The report will be concerned with the effects on areas of caries. Subsequent reports will give details of effects of drilling of the tooth, temperature of the root canal after impacts, effect on calculi, vaporization of amalgams and porcelains and also the development of techniques to permit direct impact on selected areas of teeth in patients. In addition, impacts have been done to attempt to make the surface of the teeth more resistant to infection.

The teeth were not hot on the outside, even after repeated impacts. The effect on the area of caries varied from small 2-mm deep holes to complete disappearance of the caries tissue (Fig. 1), with some whitening of the rim around. With the use of lasers with high repetition rate of firing, the areas of destruction by focused beam will be greater. In several experiments, the unfocused beam of the laser was transmitted through special glass rods. Approximately 38 per cent of the energy was transmitted. These experiments were performed to show how the laser beam may be used in inaccessible areas on the tooth (Fig. 2). The unfocused beam with energy-levels as indicated produced broad but superficial areas of removal of the brownish carious material. Focused impacts produced deeper areas of removal of the infected material. In this series, no cultures were made pre- and post-impact, since the teeth specimens were not fresh. Thermistor readings were taken with large thermistors. Significant temperature rises were recorded. However, the response time of the thermistors was quite long and the experiments will be continued with faster thermistors.

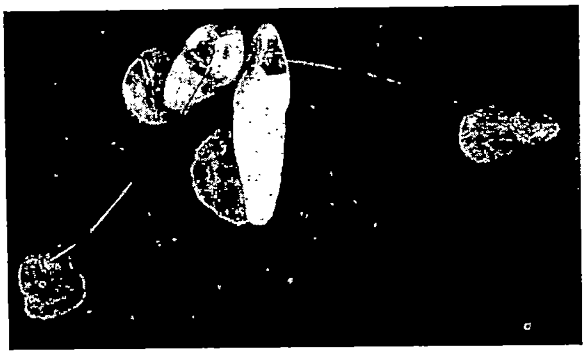

Fig. 1. Depth of impact on the buccal cusp of the lower bicuspid as compared with the impact on the mesial carious portion of the upper right central incisor. Impact from focused beam of pulsed ruby laser with exit energy of 90 joules. The increased penetration of the carious portion is due to the darker colour of this area

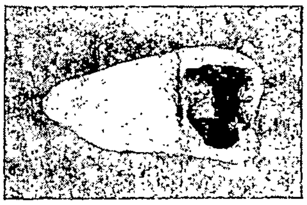

Fig. 2. Broad area of impact in a deep carious area by transmission of the laser beam through a special glass rod. The laser beam is from a pulsed ruby laser of 90-joules exit energy

Preliminary experiments indicate extensive destruction of carious areas of teeth by the unfocused and focused beams of the high-energy pulsed ruby laser. Areas of destruction were smaller but deeper with focused beams. These investigations indicate that the applications of the laser to dentistry should be examined in great detail, not only as regards basic studies, but also for the application for investigative clinical research.

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IMMUNOLOGY

Production of *SV*-40 Complement-fixing Antigen

Mayer *et al.*[1] describe the preparation of complement-fixing antigen of vacuolating agent *SV*-40 in monkey kidney cell cultures. Strain No. 776 of *SV*-40 used for the work described here was obtained from Dr. H. M. Meyer. In our attempts to prepare a satisfactory complement-fixing antigen, two types of cell cultures were used: primary African green monkey kidney (AGMK) and serially propagated *BS–C*–1 (ref. 1). These cultures were obtained from Microbiological Associates, Inc., and maintained at 34° C on Eagle's basal medium containing 2 per cent agamma calf serum, 100 mg of streptomycin per ml., 100 units of penicillin per ml. and 2 mM of glutamine per ml.

Following inoculation they were fed once a week until appearance of definite cytopathic effect. With small doses cytopathic effect appeared within 3–4 weeks. With larger inocula incubation time was shortened to 2–4 days. Tissue cultures were collected at the time of maximum cytopathic effect by freezing at − 70° C. On thawing out, the cells were shaken off the glass and homogenized for 1 min at maximum speed in a Servall high-speed omnimixer. The resulting crude suspension was used as complement-fixing antigen. As antibody source, a 1/40 dilution of pooled 'normal' monkey serum with an original complement-fixing titre of 1/128 was used. At multiplicities greater than 10, relatively little complement-fixing antigen as measured in the 'microtitre' system[2] was obtained. The complement-fixing antigen yield increased significantly, however, at lower virus inputs (Table 1).

This observation was confirmed when 32-oz. flasks of AGMK and *BS–C*–1 cultures were inoculated with approximately 10–1,000 $TCID_{50}$ of *SV*-40 virus (Table 2).

Subsequent preparations of *SV*-40 complement-fixing antigen utilizing minimal amounts of seed virus and *BS–C*–1 tissue cultures have regularly yielded titres of 1 : 16 or greater and in almost every case complement-fixing and infectivity titres were higher than in cultures inoculated with the more concentrated seed (Table 3).

Table 1. RELATION OF COMPLEMENT-FIXING ANTIGEN PRODUCED TO SIZE OF *SV*-40 INOCULUM

AGMK (virus pool No. 1)			*BS-C*-1 (virus pool No. 2)		
Multiplicity* inoculated	Tubes infected/ Tubes inoculated	CF titre†	Multiplicity* inoculated	Tubes infected/ Tubes inoculated	CF titre†
10^{-2}	3/3	< 2	10^{-1}	2/2	< 2
10^{-3}	3/3	< 2	10^{-2}	2/2	< 2
10^{-4}	3/3	2	10^{-3}	2/2	2
10^{-5}	3/3	4–8	10^{-4}	2/2	2–4
10^{-6}	0/3	< 2	10^{-5}	2/2	32–64

* Number of infective doses given per cell, assuming a cell population of $10^{5\cdot5}$ per tube.

† CF 50 = highest dilution giving a 2+ fixation within a range of – to 4+. Where this end-point was between two dilutions the bracketing dilutions are given.

Table 2. PRODUCTION OF COMPLEMENT-FIXING ANTIGEN AND INFECTIVE VIRUS IN CULTURES INOCULATED WITH VARYING AMOUNTS OF *SV*-40 VIRUS

Cells used	Virus inoculum*	Multiplicity†	CF titre‡	Infectivity titre§
AGMK	10	$10^{-6\cdot1}$	2–4	> 7·7
AGMK	1,000	$10^{-4\cdot1}$	< 2	5·2
BS-C-1	10	$10^{-6\cdot1}$	16–32	> 7·7

* $TCID_{50}$.

† Number of infective doses given per cell, assuming a cell population of $10^{7\cdot1}$ per flask.

‡ CF 50.

§ Log 10 $TCID_{50}$/ml.

Table 3. DILUTION OF *SV*-40 INOCULUM AND COMPLEMENT-FIXING ANTIGEN PRODUCTION

Exp. No.	Host system	Size of culture	*n*	Dilution inoculated into test culture: 0	–1	–2	–3	–4	–5	–6
1	AGMK	*RT*	2		< 2*	< 2	2	4–8	< 2	
2	AGMK	32 oz.	8		< 2		2–4			
3	*BS-C*-1	32 oz.	9		< 2				16	
4	*BS-C*-1	*RT*	1			4–8	16	32–64		
5	*BS-C*-1	*RT*	1	< 2	< 2	8–16	2			
6	*BS-C*-1	*RT*	2				8–16	32	32–64	< 2
7	*BS-C*-1	*RT*	2			4–8	8–16	32–64		

n, number of replicates incorporated in mean titre.
32 oz., 32-ounce flask, approximately 120 cm².
RT, roller tube cultures.
*CF 50; titres of test cultures harvested at 4+ cytopathic effect.

Preliminary experimental data suggest that the cause of this phenomenon is not interferon, nor the presence of toxin or a contaminating virus. Neither does age of the *BS–C*–1 or AGMK host cell cultures seem to influence virus yield sufficiently to explain the observed differences. Detailed investigations to elucidate this phenomenon are now being carried out.

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Proliferation of Transferred Spleen Cells after Antigenic Challenge

THE transfer of immunologically competent cells to an immunologically inert recipient is a technique which has been useful in the examination of the antibody-forming capacity of the donor cells. Under these conditions, circulating antibody may be found in the recipient, if the antigenic stimulation is given to the donor prior to transfer, or to the recipient after transfer[1,2]. That the donor cells are responsible for the antibody found in the recipient is evident in that disruption of the donor cells eliminates the antibody response.

In the intact animal it has been demonstrated that proliferation of antibody-producing cells occurs after the injection of antigen[3]. Lymphoid cells have been shown to proliferate after antigenic challenge in a transfer experiment, although there has been no direct evidence that the cells responsible for the antibody response undergo division. This experiment tested whether transferred antibody-forming cells divided after the injection of antigen. The combined fluorescent antibody–autoradiographic method as described by Baney *et al.*[4] was used. With this technique, antibody-forming cells are identified by the fluorescein conjugate method of Coons and the mitotic activity of the fluorescent cell is determined by an autoradiograph. The results indicate that mitosis of donor antibody-producing cells occurs after antigen injection in the recipient as well as in the intact animal.

Isologous donor B_6AF_1 mice (Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine) were 8–10 weeks old at the beginning of the experiment. They were primarily immunized with the intraperitoneal injection of 46 μg bovine γ-globulin (BGG) nitrogen in 0·1 ml. 'Bentonite'–BGG suspension on each of three successive days 3–5 weeks prior to experiment.

A donor spleen cell suspension of 35–45 × 10⁶ nucleated cells was injected intravenously into the tail vein of X-irradiated B_6AF_1 (620 r. given one day prior to injection of cells) recipient mice of the same age.

Immediately after the injection of the primarily immunized donor spleen cells, recall antigenic challenge was provided by the injection of the recipients with 0·1 ml. of a 2 per cent solution of BGG intraperitoneally. Some recipient mice were given in addition 75 μg bacterial lipopolysaccharide (Difco) (one-half LD_{50} for these mice) in 0·15 ml. saline intraperitoneally as a non-specific adjuvant at the time of cell transfer. Each recipient received 3·3 μc. tritium-labelled thymidine (New England Nuclear, Boston, Mass.) (6·7 c./mM) in 0·1 ml. intraperitoneally three times daily. Isotope injections began immediately after cell transfer and continued until the time of killing on days 3 and 4.

After killing, brush smears of recipient spleens were made. Following fixation, the antibody-forming cells were identified by fluorescent staining. Localization of the cells was accurately recorded with the microscope vernier co-ordinates and a photograph. After autoradiography, the previously fluorescent antibody-forming cells were re-identified and an estimation of the previous mitotic activity of the cell made by counting the grains over the nucleus. Cell nuclei labelled with eight grains or more, greater than background, were considered positive.

Two experimental groups were studied. In the first, X-irradiated recipient mice received spleen cells from immunized donors plus antigenic stimulation with 325 μg N BGG. The second group was identical to the first except that 75 μg endotoxin was given to the recipient along with antigen and killed on day 3 and day 4. In both groups tritium-labelled thymidine injections began immediately after antigen injection and continued three times daily until killing. The number of antibody-producing cells which contained tritium label in the two groups is outlined in Table 1. In the first group 43 per cent of the antibody-producing cells also were tritium-labelled. In the second group, with death on day 3 and day 4, 60 and 53 per cent respectively of the antibody-producing cells were labelled with tritium-labelled thymidine. The differences in the percentages of cells labelled at the varying time periods is not significant.

Table 1. ANTIBODY-PRODUCING CELLS TRITIUM-LABELLED AFTER ANTIGEN INJECTION

	Cells + Antigen†			Cells + Antigen† + Endotoxin‡		
Day*	No. cells	No. cells labelled	Percentage	No. cells	No. cells labelled	Percentage
3				20	12	60
4	42	18	43	68	36	53

* After cell transfer and antigen injection. † 325 μg N BGG.
‡ 75 μg (one-half LD_{50}) endotoxin.

The number of non-fluorescent cells found to be randomly labelled was 4·4 per cent based on 1,000 cells counted. Control recipient animals receiving antigen alone or antigen plus endotoxin (without cells) had no clearly identifiable antibody-producing cells by this technique. No attempt was made to identify cells as to their morphological type. Hæmagglutination titration of a pool of donor sera revealed a titre of 1 : 500. Individual titrations of recipient serum at the time of killing failed to reveal significant hæmagglutinating antibody titres.

Tritium-labelled thymidine is known to be incorporated selectively into the DNA of dividing cells[5]. The presence of label in cells producing antibody is direct evidence of DNA synthesis and donor cell division in the recipient X-irradiated mouse.

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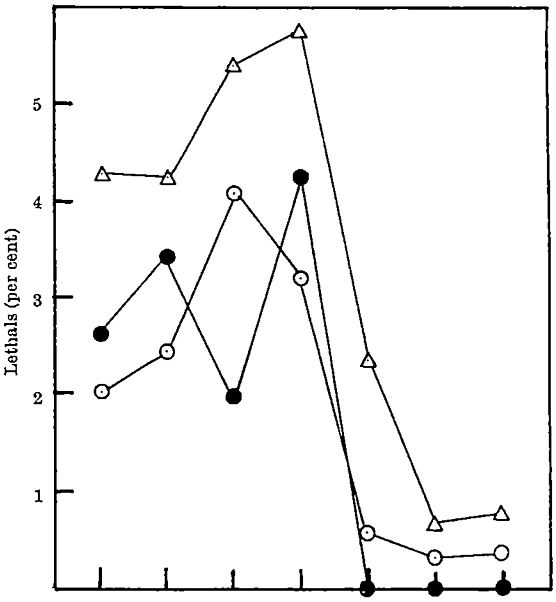
RADIOBIOLOGY

Effect of Sulphydryl Reagents on the Frequency of X-ray-induced Autosomal Recessive Lethal Mutations in *Drosophila melanogaster*

IODOACETIC acid (or its derivative iodoacetamide) is known to enhance the lethal effect of ionizing radiation on mammals[1-3], mammalian cells in tissue culture[4], and bacteria[5,6]; but there are no reports of enhancement of damage which may unambiguously be called genetic. *N*-ethylmaleimide, on the other hand, enhances both the lethal and mutagenic effects of radiation on bacteria[7-9], but has not been reported to affect higher organisms. Indeed, it was shown to be quite ineffective in enhancing radiation damage to red blood corpuscles in contrast to iodoacetic acid[10].

It was decided to test for enhancement of genetic damage in an organism with structural chromosomes. The induction of autosomal recessive lethals in *Drosophila melanogaster* males was investigaged by means of the brood analysis technique[11], which tests germ cells irradiated at all stages of maturation from spermatogonia to mature sperm. Irradiations with X-rays (tube voltage 250 kV, half-value layer 1·2 mm copper) were carried out under anoxia since, with bacteria, *N*-ethylmaleimide is most effective under these conditions. Flies were placed in a container through which a rapid stream of B.O.C. 'white-spot' nitrogen was passed for 20 min before and during irradiation with 800 r. Injections (approx. 0·3 ml.) (ref. 12) of 0·4 per cent saline, or 0·4 per cent saline plus 5×10^{-4} M-*N*-ethylmaleimide (NEM) or iodoacetamide (IAAm), were carried out approximately 35 min before irradiation. Immediate survival from these treatments was 100 per cent.

Mutation frequencies in flies injected with IAAm and NEM but not irradiated did not differ significantly from the expected spontaneous frequency of 0·4 per cent. The mutation frequencies in the irradiated groups are shown in Fig. 1. So few mutants were observed in broods VI and VII that they could not be included in the statistical analysis. Cochran's test, which was applied to broods I–V, showed a significantly higher mutation frequency in the group treated with NEM compared with the saline injected group ($P \leqslant 0{\cdot}01$). The overall frequencies of induced mutants (after subtracting 0·4 per cent spontaneous mutants) were 3·99 and 2·03 per cent respectively. If mutation induction were linear with dose and if NEM acts as a dose-modifying factor[13] the enhancement ratio for NEM would be 1·97, that is, the same as that observed with bacteria[7-9]. It is not possible with the present data to compare the relative effect of NEM on different broods.



	Brood	I	II	III	IV	V	VI	VII
No. tested	Saline	347	328	342	281	351	323	276
	NEM	373	354	334	262	297	304	268
	IAAm	191	205	203	141	152	151	143

Fig. 1. The effect of injected *N*-ethylmaleimide and iodoacetamide on frequency of recessive lethal mutations after 800 rads under anoxic conditions. ○, Saline; △, NEM; ●, IAAm

Mutation frequencies in the IAAm and saline injected groups did not differ significantly from one another ($P \geqslant 0{\cdot}1$).

The induced mutations, the radiation-induced frequency of which is increased by *N*-ethylmaleimide, are probably the expression of chromosomal damage of a much grosser kind than that in *Escherichia coli*[9], where the lesion may well be confined to one of a few specific base pairs[14]. Work on enhancement with *N*-ethylmaleimide and its interrelations with the effect of oxygen is being continued.

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Biological Vectors and Reservoirs of Strontium-90

THE dispersion of radioactive contamination in the environment by mobile organisms has been reported by several authors. Davis[1] noted significant amounts of radioactivity in caddis flies and midges (Orthocladiinae) attracted to lights from the nearby Columbia River. As the result of aquaria investigations on the uptake of radionuclides by aquatic insects, Peredelskii and Bogatyrev[2] concluded that a bioecological purification of contaminated reservoirs would occur as the consequence of emergent insects flying to the surrounding watershed. Auerbach[3] questioned their conclusions because the quantities of radioactivity transported by insects were less than that accumulated from fall-out. However, it was noted that lack of data on emergent insects biomass made it difficult to test the general applicability of the hypothesis. The purpose of this communication is to assess the role of emergent Chironomidae as vectors of strontium-90 and freshwater clams as reservoirs of strontium-90 in the Clinch and Tennessee Rivers.

A comprehensive investigation of the fate of low-level radioactive wastes released by Oak Ridge National Laboratory to the Clinch River has been in progress since 1959 (ref. 4). In connexion with this investigation a large collection of adult chironomids was caught in an insect light trap operated on the Clinch River bank at dusk. These adult chironomids contained $3·55 \pm 1·13 \times 10^{-6}$ μc. strontium-90/g or 8·76 times as much strontium-90 activity as an equal quantity of river-bottom sediment from that location. Investigations elsewhere[5–7] have shown emergent chironomid biomass ranges from several up to about 20 g/m^2/year, the higher productivities arising from eutrophic situations. The Clinch River is not greatly enriched with organic matter, therefore a productivity in the River of 10 g/m^2/year is a reasonable estimate. With this productivity emergent chironomids would remove $3·55 \pm 1·13 \times 10^{-5}$ μc. strontium-90/m^2/year from the river bottom. The average increment of strontium-90 from fall-out in the United States[8] between 1959 and 1960 was $4·2 \times 10^{-3}$ c./mile^2 ($1·62 \times 10^{-3}$ μc./m^2). Thus, fall-out entering the River directly would add 45 times as much strontium-90 as was removed by the emerging chironomids. Movement of insects from a contaminated terrestrial environment was found to play a similar inconsequential part in the dispersal of radioactivity[9]. It is apparent that the general dispersion of strontium-90 by insects is not of great importance.

Previous investigations[10] showed that freshwater clams concentrated strontium in their shells by factors of 2,500–9,000 times that in water and therefore are useful as indicators of strontium-90 in the environment. Since there is a commercial clam fishery on the Tennessee River downstream from the confluence of the Clinch River, calculations were made to estimate the removal of strontium-90 from the River in clam shells. The annual harvest[11] of shells ranges from about $4·5 \times 10^9$ to 9×10^9 g. By using the maximum harvest and the average strontium-90 concentration in clam shells ($7·0 \times 10^{-6}$ μc./g), the calculated removal of strontium-90 was 63 mc./year. If strontium-90 releases were constant, and if growth of the population was in equilibrium with the harvest, a similar amount of strontium-90 would be incorporated into the population each year. These mollusc populations were investigated recently[12] and from this investigation we can estimate a total population of approximately $4·5 \times 10^7$ clams in this area. Assuming a weight of 250 g for each clam (actually a high value) and using the average strontium-90 concentration, the reservoir of strontium-90 in the clams in the river is 79 mc. The annual removal and reservoir of strontium-90 in clams is small when compared with laboratory releases of strontium-90, which in the past have ranged from 22 to 150 c./year[13].

An organism having a significant biogeochemical effect on the distribution of an element in Nature must concentrate an element from its environment. In the instance of strontium-90 in molluscs, even though there is a significant concentration of strontium in their shells, there is no large reservoir of strontium-90. This is due primarily to the low specific activity ratio (^{90}Sr atoms/total Sr atoms) in the river water. In the Tennessee River[10] there are approximately 10^{10} atoms of stable strontium for each atom of strontium-90. This ratio suggests that it would be exceedingly improbable for significant quantities of strontium-90 to occur in biological reservoirs. Parker[14] estimated the total biomass that might be present in the Clinch River at any one time, and by using the maximum observed strontium-90 and cæsium-137 concentrations in fish, calculated that at most 39 mc. of strontium-90 and 8 mc. of cæsium-137 would be present in the fish population.

The transport of radionuclides by organisms within a stream[15] as well as from a stream[1] has been investigated. Detection of contaminated insects is a useful tool for examining their biology, but, when mass movement of radioactive material is considered, quantities are small. Localized problems may exist[1], but a general contamination of the landscape by mobile organisms is unlikely.

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Potentiation of Virus Leukæmogenesis in *C57BL* Mice by X-irradiation or Urethane

LYMPHOID tumours can be induced in high incidence in strain *C57BL* mice by fractionated whole-body X-irradiation[1]. Urethane has also been shown to act as an independent leukæmogenic agent when administered to *C57BL* mice shortly after birth[2]. In adult mice, urethane acts as a co-leukæmogenic agent, potentiating the leukæmogenic effects of radiation or other carcinogenic agents, but exhibits little or no leukæmogenic activity when used alone[3].

The aim of the work recorded here was to examine the effect of X-irradiation and of urethane on lymphoma development induced by a virus (RLV) isolated from radiation-induced leukæmia of strain *C57BL* mice[4] and serially passaged in this and other strains for more than fifteen generations. It has previously been established that a high incidence of lymphoid tumours can be induced in *C57BL* mice by neonatal inoculation of RLV[4,5].

Strain *C57BL/Ka* mice of various ages were used in all experiments. RLV was obtained from virus-induced lymphoid tumours of strain *C57BL* mice. The pooled thymus glands, spleens, and lymph nodes were homogenized in 9 volumes of chilled phosphate-buffered saline.

The homogenate was centrifuged 10 min at 10,000*g*, and the pellet discarded. After 3 cycles of centrifugation, the final supernatant was used directly for inoculation. It was administered in intraperitoneal injections of 0·1 ml. each.

The mice were irradiated in groups of 6–10 in plastic boxes, except in experiments in which the bone marrow was to be shielded; then the mice were anæsthetized with amytal and irradiated under a 3 mm-thick lead shield, in which a window has been cut out in the area over the thymus. The physical factors of irradiation were 250 kV, 1·0 mm aluminium plus 0·25 mm copper filter, 83 r./min.

In the urethane experiments, new-born mice received a single inoculation of a cell-free centrifugate of RLV, followed by ten weekly intraperitoneal injections of urethane, starting at different intervals after RLV inoculation. When urethane administration was started 2 or 10 days after virus inoculation, the dose of urethane (dissolved in distilled water) was limited to 0·5 mg/g body-weight, to minimize leukæmogenesis by urethane itself. When urethane administration was delayed until 20 or 30 days after virus inoculation, the urethane dose was 1 mg/g body-weight.

Animals were caged in groups of 6–10, fed 'Purina Laboratory Chow' and water, and routinely autopsied at death or when moribund. The tissues were examined histologically in all but indubitable lymphomas.

Exp. 1. Mice aged three weeks were given three injections of RLV, 4 days apart. At 1–7 days after the last injection, they received the first of 4 weekly whole-body X-ray exposures of 100 r. each. Control animals received either treatment alone. In another group of mice, the treatment sequence was reversed; X-irradiation was begun at about five weeks of age, and RLV inoculations started 1–7 days after the last X-ray exposure. Control mice received RLV only at the corresponding age.

Table 1. LYMPHOMA DEVELOPMENT IN RLV-INJECTED AND X-IRRADIATED ADULT FEMALE *C57BL* MICE

Treatment RLV pre-X-ray	X-ray	RLV post X-ray	Net No. of mice	Mice with lymphomas No.	Per-cent	Age at death (days) Median	Range
+	+	–	29	28	96·6	164	125–217
+	None	–	30	8	26·7	194	109–359
–	+	–	39	17	43·6	197	155–351
–	+	+	45	35	77·8	201	152–345
–	None	+	32	4	12·5	233	180–357

The results are summarized in Table 1. Combined treatment with RLV and irradiation increases lymphoma incidence well above that resulting from either treatment alone and reduces the average latent period as well. The enhancement by the combined treatments, regardless of sequence, is significantly greater than mere summation of the components ($P < 0{\cdot}001$), suggesting a synergistic interaction between RLV and X-rays. It appears that enhancement is more marked if virus administration precedes rather than follows X-irradiation. The time interval between completion of one treatment and start of the other could be varied from 1 to 7 days without affecting the outcome. Experiments are at present under way to determine the effect of increasing this interval up to 30 days. It is not essential that the X-ray exposures be fractionated; we have obtained similar, though less pronounced, results with single 400 r. exposures.

Exp. 2. The experimental conditions were slightly modified to reduce the effects of the individual treatments. Weanling (30 ± 2 days) mice received only two injections of RLV at four-day intervals. One week later, whole-body X-irradiation was begun in four-weekly doses of 75 r. each. A second group of animals was treated similarly, except that they were shielded during irradiation, so that only the thymus area was exposed. Appropriate controls were included.

Table 2. LYMPHOMA DEVELOPMENT IN RLV-INJECTED ADULT FEMALE *C57BL* MICE AS A FUNCTION OF X-IRRADIATION

Treatment	Net No. of mice	Mice with lymphomas No.	Per-cent	Age at death (days) Median	Range
RLV injections only	41	8	19·5	215	141–304
RLV followed by whole-body X-irradiation	41	40	98	162	105–258
RLV followed by X-irradiation to thymus area	41	32	78	204	114–294
Whole-body X-irradiation only	43	2	4·6	314	294–334
Thymus X-irradiation only	41	0	—	—	—

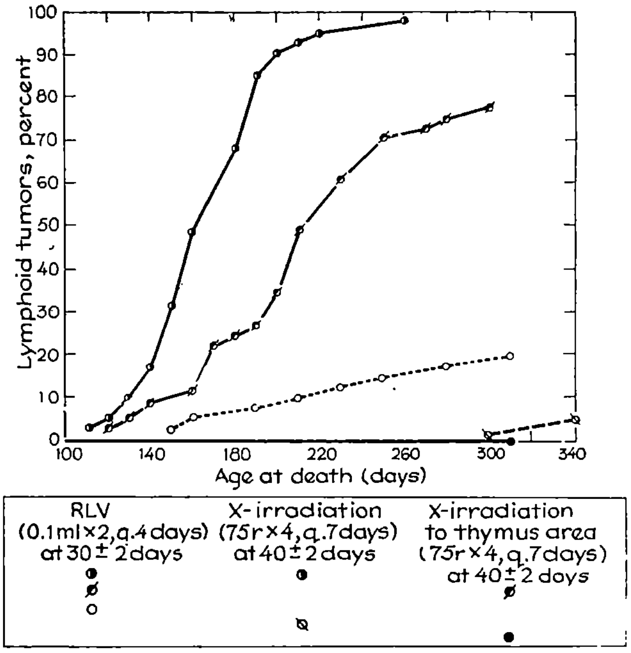

Fig. 1. Cumulative incidence of lymphomas in RLV-injected *C57BL* female mice as function of subsequent X-irradiation

The results are shown in Fig. 1 and Table 2. There is a striking enhancement of lymphoma incidence, coupled with a reduction of latent period. Synergism is even more evident here than in the previous experiment because of the low level of radiogenic lymphomas. It is interesting to note that enhancement also occurred in animals in which radiation was limited to the region of the thymus.

Exp. 3. Lymphoma incidence following combined RLV and urethane treatment was markedly increased, compared with that resulting from either treatment alone (Table 3). The response to combined treatment again exceeds expectation for simple additivity. When urethane administration was begun 2 or 10 days after neonatal virus inoculation, the average latent period for lymphoma development resulting from the combined treatment was markedly decreased, whereas when urethane treatment was delayed until 20 or 30 days after the virus inoculation, there was little decrease in latent period.

The potentiation of virus leukæmogenesis by urethane was also tested in 20-day-old mice, which received three RLV inoculations at 4-day intervals; seven days after the last inoculation, 10-weekly intraperitoneal injections

Table 3. EFFECT ON LYMPHOMA INCIDENCE IN STRAIN *C57BL* MICE OF COMBINED TREATMENT WITH RLV AND URETHANE

Treatment	Age at urethane injection (days)	Urethane dose (mg/g)	No. injections	Lymphoma incidence Nos. pos./total	Per cent	Average latent period (days)
RLV plus urethane	2	0·5	10	18/24	75	160
RLV alone	—	—	—	6/21	28	252
Urethane alone	2	0·5	10	6/29	20	312
RLV plus urethane	10	0·5	10	17/24	70	184
RLV alone	—	—	—	6/18	33	300
Urethane alone	10	0·5	10	8/36	22	330
RLV plus urethane	20	1·0	10	18/19	94	221
RLV alone	—	—	—	10/27	37	275
Urethane alone	20	1·0	10	8/38	21	320
RLV plus urethane	30	1·0	10	25/31	80	210
RLV alone	—	—	—	15/40	37	262
Urethane alone	30	1·0	10	6/31	20	322

Table 4. EFFECT ON LYMPHOMA INCIDENCE IN STRAIN *C57BL* MICE OF COMBINED TREATMENT WITH RLV AND URETHANE

Treatment	Age at RLV injection (days)	No. injections	Age at urethane injection (days)	Dose (mg/g) and No. of injections	Lymphoma incidence		Average latent period (days)
					No. pos./total	Per cent	
Group 1							
RLV plus urethane	20	3	35	1·0 × 10	24/24	100	215
RLV alone	20	3	—	—	6/9	66	212
RLV alone	1	1	—	—	10/10	100	117
Urethane alone	—	—	35	1·0 × 10	8/40	20	320
Group 2							
RLV plus urethane	20	3	35	1·0 × 10	17/30	56·6	215
RLV alone	20	3	—	—	1/16	6·2	366
RLV alone	1	1	—	—	0/15	0	> 388
Urethane alone	—	—	35	1·0 × 10	6/28	21	330

of urethane (1 mg/g body-weight) were administered. Two different serial passages of the leukæmia virus were used in this experiment. The results are presented in Table 4. In Group 1, the RLV preparation proved too active to allow full expression of the potentiating effect of urethane. Significant potentiation was observed in Group 2, using an RLV preparation from another serial passage, which had minimal activity alone in either newborn or 20-day-old mice.

In all experimental groups the tumours were typical lymphocytic lymphosarcomas, identical with those arising after irradiation of adult mice or RLV inoculation of neonatal *C57BL* mice[1,4].

It is concluded that X-irradiation and urethane potentiate the leukæmogenic activity of RLV in mice of strain *C57BL*. Enhancement of susceptibility to other oncogenic viruses by radiation has been reported[6-8]. The mechanism of this potentiation is not established. Depression of the host's immunological defences is probably not an important factor, since X-ray dosage was relatively low. A more likely explanation is perhaps provided by the fact that irradiation and urethane cause thymic injury and regeneration, with a concomitant repopulation by immature lymphocytes. The presence of these cells has been correlated with susceptibility to virus leukæmogenesis[9,10]. When bone marrow is shielded during irradiation, as in Exp. 2, thymic repair proceeds at a more rapid rate, thus shortening the period of prevalence of the immature lymphocytes. This may explain the lesser enhancement observed in these animals.

The present result with urethane is in striking contrast to the inhibition of lymphoma development by urethane in *C3Hf*/Lw* mice carrying the Moloney leukæmogenic virus[11]. Perhaps investigations of the latter strain, comparable to our work on strain *C57BL* with respect to the capacity of urethane to produce concomitant thymic and bone-marrow injury[12], would help to reconcile these apparently antithetical observations.

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BIOLOGY

Effect of Photoperiod on Cyclic Patterns of Body Temperature in the Quail

THE nature of body temperature rhythms in gallinaceous birds can best be described when exogenous factors such as disturbances, movement (muscular activity), and temperature extremes are controlled. Normal fluctuations in body temperature usually are associated with concomitant forms of daily activity. The magnitude of variation differs considerably among species. In chickens kept in an uncontrolled, but moderate, environment the diurnal differential in body temperature is approximately 1·3° C (refs. 1 and 2). The highest body temperature occurs at 1700 h and the lowest at 0400 h. In a controlled environment where temperatures were held constant at 21·1° C, the diurnal body temperature change in chicken was 0·17° C (ref. 3). The body temperature of Gambel quail (*Lophortyn gambelii*) maintained at ambient temperatures between 24° and 26° C fluctuates between 40° and 41·5° C during the day and has a range approximately 1·5° C lower during the night[4]. Most investigators agree that activity *per se* is the principal factor which causes change in body temperature. Since the light period induces or exaggerates activity, it is of interest to determine the influence different photoperiods have on altering the circadian changes of body temperature in coturnix (*Coturnix coturnix japonica*).

The body temperature of six mature coturnix females kept in individual cages (5 in. × 7 in. × 10 in.) in a temperature and light-controlled chamber was measured continuously with a Minneapolis–Honeywell 16-point potentiometer. Average daily room temperature ranged from 19·5° to 25° C. The birds were subjected to artificial incandescent light.

Plastic coated copper-constantan thermocouples (24-gauge) were inserted into the abdominal cavity of each bird through an incision approximately 2·5 cm dorsal and 2·5 cm posterior to the tip of the keel. Each thermocouple lead was shaped snugly to the side of the body and secured to a harness ring on the top side of an adjustable quail harness (Le Captive adjustable quail harness, manufactured by Kennel Crafts, Inc., Orlando, Florida). The leads were laced through a piece of spiral plastic wrapping. One end of the wrapping was fastened to the upper ring of the quail harness; the opposite end was attached to the top of the cage. The coiled wrapping allowed the quail to move freely, except that it restricted rotational movement. Individual body temperatures were recorded every 8 min. The birds were given 7 days to recuperate after insertion of the thermocouple before the experiment was started.

Temperature curves for the five light treatments are shown in Fig. 1. In treatment 1, the daily body temperature fluctuated between 41·8° and 42·4° C. The average body temperatures for the light and dark periods were 42·2° and 41·9° C, respectively. Maximum temperature occurred at 1300 h and the minimum at 2400 h. The daily fluctuation in body temperature was less than that reported for chicken (1·3° C (ref. 2)) and for Gambel quail (1·5° C (ref. 4)).

Shifting the light period to the interval between 2000 and 1000 h without changing the total increment of

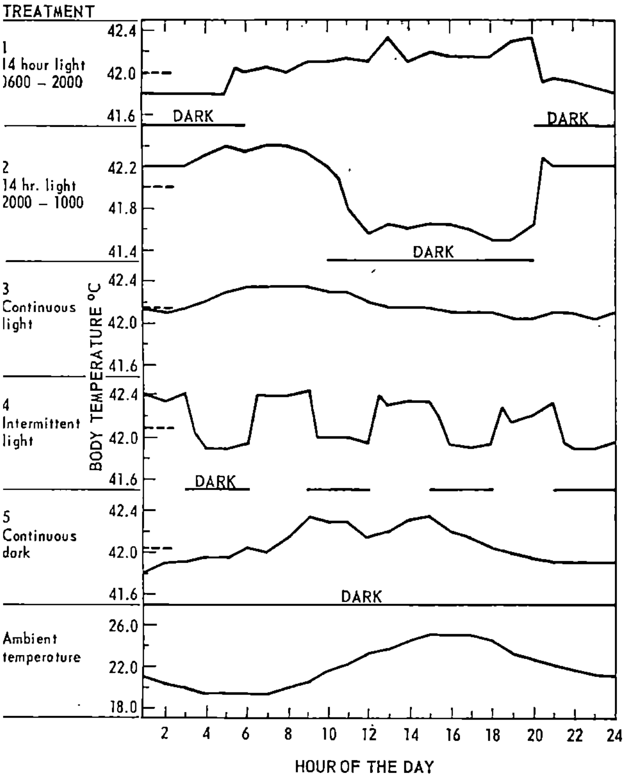

Fig. 1. Mean hourly body temperatures of *Coturnix* exposed to different photoperiods. Data for mean room temperature included. Broken lines represent mean temperatures for the 24-h period

light (treatment 2) resulted in a change of the body temperature pattern. In all birds the rhythm of the body temperature shifted in response to the new light schedule within 24 h. Average body temperatures during the light and dark periods were 42·3° and 41·6° C, respectively. The mean temperature during the dark period (1000–2000 h) was less than the mean of 41·8° C recorded in treatment 1, even though the period of darkness in treatment 2 occurred during hours of natural daylight.

The average daily fluctuation in body temperature of coturnix given continuous light was 0·3° C. The relatively small fluctuation in daily body temperature of coturnix kept in a moderately controlled environment is comparable to that reported for chickens[3]. From general observations it appeared that the birds became relatively inactive several times during the day.

The relationship between photoperiod and the period of activity is demonstrated clearly in treatment 4, where the birds were exposed to an intermittent light schedule of 3 h light and 3 h dark. The mean body temperatures for the light and dark periods were 42·4° and 42·0° C, respectively. The average minimum temperature of 41·9° C recorded during the 1st, 2nd and 4th dark periods approaches the minimum body temperature of 41·8° C recorded for the same birds in treatment 1. These data imply that, on the onset of the dark period, the temperature regulatory mechanism(s) rapidly can adjust the body temperature to a level typical of the resting stage.

The average maximum body temperature of the birds kept in continuous darkness (treatment 5) was recorded at 0900 h. The increase in body temperature started at 0600 h and began to decline at 1500 h. This period roughly coincided with the outside light period of the day. It is doubtful that the minor changes in room temperature would have initiated an activity response, since Bartholomew and Dawson[4] reported that in a sustained environmental temperature of 39° C the daytime temperature of Gambel quail was not elevated above that normally maintained in the absence of heat stress.

Table 1

Treatment	Duration of treatment (days)	No. of females	Light cycle on–off
1	5	6	0600–2000
2	7	6	2000–1000
3	7	6	Continuous light
4	7	5	Intermittent light (3 h L : 3 h D)
5	7	4	Continuous dark

During the experiment the quail displayed excitement when approached for the purpose of checking thermocouples or for servicing feeders and waterers. The degree of excitement depended on whether the birds were handled. When handled, their temperature increased approximately 1·0° C within 8 min; it returned to normal about 16 min later. This fright reaction is not uncommon in fowl. The body temperature of ruffed grouse (*Bonasa umbellus umbellus*) increased to 0·8° C when the birds were removed from the cage and forced to run for 10 min (ref. 5). The body temperature of the eastern house wren (*Troglodytes aëdon aëdon*) was reported to increase 2·2° C following capture[6]. However, the temperature dropped very soon after the birds quieted down.

From results of the tests recorded here, it appears that normal cyclic fluctuations in temperature of birds kept in moderate environments are associated with concomitant forms of physical activity which usually are restricted to the light period. In these tests, it was possible by imposing different experimental photoperiods to alter the rhythm of body temperature in coturnix.

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Mechanism of Capacitation of Rabbit Spermatozoa

Rabbit spermatozoa must be exposed to the environment of the female tract for several hours before acquiring the capacity to fertilize ova[1,2]. This phenomenon was termed capacitation[1], and appears to be a general requirement for fertilization in several species[3,4]. Chang[4] later discovered that spermatozoa capacitated *in utero* could be decapacitated by brief incubation in seminal plasma. Decapacitation appeared to be due to a definite substance in seminal plasma[5].

With the discovery of the decapacitation factor in seminal plasma it appeared possible that capacitation was removal of the spermatozoa from seminal plasma and hence removal of the decapacitation factor as the spermatozoa progressed up the female tract. Two lines of evidence opposed this hypothesis. Austin[1] and Chang[2] showed that epididymal spermatozoa must be capacitated. Bedford and Chang[5] showed that the decapacitation factor existed in seminal plasma from vasectomized males. This evidence suggested that epididymal spermatozoa do not contact the decapacitation factor until the time of ejaculation yet must undergo capacitation. It occurred to us, however, that since the tubular portion of the male tract has a common embryonic origin, the decapacitation factor could exist also in the epididymis and hence contact the spermatozoa before ejaculation. Demonstration of the decapacitation factor in epididymal fluid would support the hypothesis that capacitation is the removal or inactivation of the decapacitation factor. We report in this communication the decapacitation of capacitated spermatozoa by epididymal fluid and their recapacitation on further *in utero* incubation.

Epididymal fluid was collected by distending the excised epididymis (still attached to the testis) with air injected through the stump of the vas deferens. A cut was made in the epididymis, the fluids pressed out, and the epididymis flushed with calcium-free Krebs–Ringer phosphate solution. The final volume was adjusted so that 1 ml. contained the flushings of two epididymes. Spermatozoa were removed by centrifugation at 1,100*g* for 10 min and the supernatant frozen. Uterine spermatozoa were obtained by flushing each excised uterus with 5·0 ml. of Krebs–Ringer phosphate solution 12–15 h after natural, multiple matings and observed for motility. The spermatozoa suspension was divided, centrifuged at 400*g* for 3 min and concentrated to 0·6 ml. The treated spermatozoa were incubated with 0·2 ml. of epididymal fluid for 30 min at room temperature. The control spermatozoa were subjected to an identical treatment except that Krebs–Ringer phosphate solution was substituted for epididymal fluid. Good motility was observed after both treatments. Two preparations of epididymal fluid were used in these experiments each of which produced 100 per cent decapacitation of uterine spermatozoa. 2 h after ovulation surgical insemination was performed on virgin New Zealand does, 6–8 months old, which had been in isolation for at least 21 days and had received 3·3 mg of pituitary luteinizing hormone (Armour-Baldwin Laboratories) by intravenous injection 12 h earlier. The rabbits were anæsthetized with sodium pentobarbital and ether, a midline incision was made, and 0·05 ml. of spermatozoa suspension containing $0·76–12 \times 10^4$ cells was injected into each oviduct through the infundibulum at a depth of approximately 2·0 cm. Treated spermatozoa were placed in one oviduct and the controls in the other. Following surgical closure, each doe received 100,000 units procaine penicillin *G* and 0·125 g streptomycin sulphate intramuscularly. Ova were recovered 26–40 h later by flushing from the oviducts and observed for regular division characteristic of fertilization. Table 1 illustrates the results of these experiments in which 40 out of 50 (80 per cent) of the control eggs were fertile, while none of the eggs inseminated with spermatozoa which had been incubated in epididymal fluid were fertilized. These results indicate that the spermatozoa had become capacitated and that treatment with epididymal fluid prevented fertilization.

Table 1. REVERSAL OF CAPACITATION WITH EPIDIDYMAL FLUID

Additions to capacitated spermatozoa	Ova recovered	Fertile	Non-fertile
None	50	40 (80%)	10
Epididymal fluid	38	0	38

Table 2. RECAPACITATION OF DECAPACITATED SPERM

Spermatozoa treatment	Ova recovered	Fertile	Non-fertile
Freshly ejaculated, washed	13	8	5
Decapacitated with epididymal fluid	16	15	1

To establish that the spermatozoa were not permanently damaged by incubation with epididymal fluid and to demonstrate that epididymal fluid contained a substance with decapacitation factor action, spermatozoa treated with epididymal fluid were recapacitated. The spermatozoa were capacitated as before, incubated in epididymal fluid, and injected into the uterine lumen with a 24-gauge needle 4 h prior to ovulation. Washed, ejaculated spermatozoa served as controls in this experiment.

The results in Table 2 show that capacitated spermatozoa decapacitated with epididymal fluid were recapacitated by *in utero* incubation. (In the control group one oviduct contained 5 ova in which spermatozoa had penetrated the zona pellucida, but the ova failed to cleave.) This demonstration of recapacitation shows that the decapacitation factor in epididymal fluid has the same functional properties as described for decapacitation factor in seminal plasma[5].

The data contained herein suggest the hypothesis that capacitation is the inactivation or removal of the decapacitation factor from sperm. This is not in conflict with Chang's[2] observations that epididymal sperm must be capacitated and that the decapacitation factor is also produced in the male tract below the vas deferens[5]. The hypothesis is also consistent with Austin's[1] suggestion that ejaculated spermatozoa contain an inhibited mucolytic enzyme which permits ovum penetration and capacitation is removal of the inhibitor.

Besides the foregoing experiments on the epididymal decapacitation factor, we have confirmed and slightly extended earlier work on the decapacitation factor of seminal plasma. Bedford and Chang[5] showed that decapacitation factor activity was removed from seminal plasma by ultracentrifugation and resuspending the pellet in the seminal plasma would restore activity. We have found that ultracentrifugation of seminal plasma at 105,000*g* for 4 h gave a pellet which retained activity after washing and resuspending in buffer, thus demonstrating that the supernatant of the seminal plasma was not necessary for decapacitation factor activity.

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Mallophaga on Catarrhine Monkeys: *Colobus guereza*, a Natural Host of *Procavicola colobi**

ACCORDING to Hopkins and Clay[1] and Eichler[2] no Mallophaga exist on Old World monkeys. An adult female *Colobus* monkey (*Colobus guereza guereza* Rüppell, 1835), however, collected in Djam-djam, Shoa Province, Ethiopia, by Prof. D. Starck in 1956 and now in the collection of the Anatomical Institute of Frankfurt, was found to be heavily infested by *Procavicola colobi* (Kellogg, 1910). This species was first described as *Trichodectes colobi* from *Colobus guereza caudatus* Thomas, 1885, at Mount Kilimandjaro. Eichler[3] made *Trichodectes colobi* the type species of a new genus *Meganarionoides* based on the description by Kellogg. Werneck[4] has re-examined Kellogg's specimens and has found that they are members of *Procavicola*, a genus otherwise known from many species of hyraxes (Procaviidae) only. He recognizes *Meganarionoides* as a sub-genus of *Procavicola*. Werneck doubted that the natural host of *Procavicola colobi* was a *Colobus* monkey, especially as he has found a second lot of *Procavicola colobi* at his disposal contaminated with other mallophaga, obviously originating from Procaviidae. Consequently, he suggested that the natural host is *Dendrohyrax validus*. In this he has been followed by Hopkins[5] and Hopkins and Clay[1].

Our *Colobus* monkey from Ethiopia has never been in contact with a *Dendrohyrax* or any other Provaviidae after its death. Yet we have found hundreds of eggs and adult and larval *Procavicola* on it, all clasping the hairs tightly;

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* Results of the Ethiopia Expedition, 1956, No. 10.

most of them on the back and on the throat. The eggs are clearly distinguishable from those of the sucking-louse, *Pedicinus pictus* Ferris, 1934, some specimens of which have also been found on this monkey. There is no doubt in our opinion that *Colobus guereza* is a natural host of *Procavicola* (*Meganarionoides*) *colobi*.

Considering the general host-specificity and the supposedly parallel phylogeny of hosts and Mallophaga, it is interesting to find a member of a group of Mallophaga, which is otherwise confined to Procaviidae, on a monkey. Eichler[3] had united the ischnoceran Mallophaga parasitizing primates in the sub-family Cebidicolinae of Trichodectidae. In 1963, after Werneck's investigation, he removed *Meganarionoides* from this sub-family. But even the relationship of the two remaining genera (*Lorisicola* from the slow lemur (*Nycticebus*), and *Cebidicola* from some New World monkeys) seems highly uncertain. Hopkins and Clay[1] consider *Lorisicola* "doubtfully distinct from *Felicola*", a genus widespread on carnivores. In any event, it seems hazardous to base any theories about the relationship of lemurs and New World monkeys on the relation of their parasites, *Lorisicola* and *Cebidicola*, as, for example, Vanzolini and Guimarães[6] have done.

The Mallophaga probably have never been able to colonize the primates in populations sufficient to enable them to follow the phylogeny of these hosts, due possibly to the intensive care given to the skin by most primates. Suitable niches have opened, for example, in the nocturnal *Aotes* (*Aotiella*, Amblycera) or the thumbless African *Colobus* monkeys, in which sucking-lice of the genus *Pedicinus* also have reached a climax in numbers of species and individuals among Old World monkeys[7]. These niches must have been occupied by different Mallophaga invading from different orders of hosts.

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Nucleic Acid Content of Chloroplasts of Spinach isolated by a Non-aqueous Technique

THERE is strong evidence for cytoplasmic inheritance of chloroplasts in higher plants. This genetic autonomy, together with the demonstration of protein synthesis[1,2] and the presence of ribosomes[3-5] in chloroplasts, has generated considerable interest in the nucleic acids associated with chloroplasts.

Many investigators have reported the occurrence of chloroplast nucleic acids[6], but there is considerable quantitative disagreement in these results. This is undoubtedly in part due to differences in the material analysed and the analytical methods utilized. These methods include cytochemical procedures, which depend on the specific staining of RNA and DNA in the chloroplasts of intact cells, and the chemical analysis of RNA and DNA extracted from chloroplasts isolated in isotonic aqueous media. A more recent technique is the incorporation of tritium thymidine into *Spirogyra*[7] or tobacco leaves[8], and intracellular localization by means of autoradiography. Electron microscopy indicates the presence of DNA in *Chlamydomonas* chloroplasts[9]. Sedimentation investigations in cæsium chloride density gradients indicate that DNA extracted from higher plant chloroplasts[10], and algal chloroplasts[10,11], differs in density from that of the nucleus.

Table 1

	Mg polynucleotide per 100 mg non-aqueous chloroplasts			
Method of analysis / Treatment	Ultra-violet absorption (260 mμ)	Diphenylamine test for deoxyribose	Orcinol test for ribose	Phosphate
(1) Strong acid hydrolysate (5% perchloric acid, 15 min, 90° C)	7·95 (DNA + RNA)	0·40 (DNA)	7·80 (DNA)	8·10 (DNA + RNA)
(2) Mild alkaline hydrolysate (0·3 N KOH, 16 h, 34°C)	7·49 (RNA)	0	7·10 (RNA)	7·62 (RNA)
(3) Residue from (2) after strong acid hydrolysis, as in (1)	0·795 (DNA)	0·37 (DNA)	0	0·41 (DNA)

The amounts of nucleic acid reported in chloroplasts using cytochemical procedures are at the lower limit of the sensitivity of the method[6]. This lack of sensitivity is circumvented by direct chemical analysis of polynucleotide extracted from isolated chloroplasts. However, in this procedure, artefacts are encountered, owing to contamination of the plastids by nucleic acids from other cell components during isolation. This contamination has been elegantly avoided for chloroplasts DNA by Baltus and Brachet[12], whose results were confirmed by Gibor and Izawa[13]. These workers removed the nucleus of *Acetabularia*, prior to chloroplast isolation and analysis. Additional errors in these experiments may also arise by the loss of nucleic acids from chloroplasts either by leakage[14], or enzymatic degradation, particularly when aqueous isolation procedures are used. However, these difficulties are largely avoided if plastids are isolated by the non-aqueous method. This communication reports the chemical estimation of RNA and DNA in spinach chloroplasts isolated by a non-aqueous density technique.

Chloroplasts were isolated by modifications of methods by Heber[14]. Polynucleotides were estimated by the Smillie–Krotkov modifications[15] of the Schmidt–Thannhauser procedures. The purity of the chloroplasts was checked by phase microscopy after staining with methyl green, and an electron micrograph taken by one of us (R. B. P.) of a similar preparation has been published by Heber[16]. This micrograph, together with enzymatic checks by Heber, also demonstrates the purity of the preparations.

100-mg samples of non-aqueous chloroplasts were extracted with methanol, cold trichloroacetic acid, ethanol and ethanol–ether mixtures, and the insoluble residues were either subjected to mild alkaline hydrolysis for RNA nucleotides, or strong acid hydrolysis for total nucleotide estimations. The results are summarized in Table 1.

The confirmation of values obtained by analysis of deoxyribose and phosphate is good. However, the high value obtained from ultra-violet absorption at 2600 Å for DNA suggests interference by some hydrolysis products other than nucleotides. This emphasizes the necessity of working with several complementary analytical methods in such an investigation.

These results show that the chloroplasts prepared by the non-aqueous technique contain 0·5 per cent DNA, and 7·5 per cent RNA by weight. An objection to these data might be that a considerable portion of the plastid lipid was extracted during the isolation procedure, yielding high nucleic acid contents on a weight basis. That this is not the case is shown by other experiments[17], in which non-polar solvents were shown to extract not more than 5 per cent of the chloroplast mass.

The value obtained for the DNA content of non-aqueous chloroplasts is in good agreement with previous reports for aqueous chloroplasts. The value obtained for the RNA content is considerably higher than that reported previously (2–4 per cent), and this difference is probably due to the application of the non-aqueous technique for the isolation of chloroplasts. It is likely that RNA is leached from chloroplasts isolated from aqueous cellular homogenates, whereas this is minimized by using non-aqueous techniques. In addition, the action of the chloroplast RNase[18] is inhibited by operation in a non-polar medium.

In order to localize the polynucleotides within the chloroplasts, stroma and grana fractions were prepared[17] and analysed using the same procedures. The polynucleotides were found to be in the stroma fraction and easily leached out of the chloroplasts by means of aqueous buffers.

In summary, the DNA content of spinach chloroplasts prepared in non-aqueous media is similar to that of chloroplasts prepared in aqueous buffers. However, the RNA content of non-aqueous chloroplasts is higher than that of aqueously prepared chloroplasts by a factor of two. The RNA is localized in the stroma phase of the chloroplast and is not bound to the quantum conversion apparatus. It is suggested that the low RNA content of aqueous chloroplasts is due to leaching[6] of the polynucleotide during chloroplast isolation.

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Thermophylaxis in Bean Rust

Thermophylaxis is here defined as sensitivity to heat resulting from a previous exposure to heat. The phenomenon has been demonstrated clearly only with bean (*Phaseolus vulgaris*)[1] and the uredinial stage of the bean rust fungus (*Uromyces phaseoli*) in bean, but preliminary results with cowpea and with the powdery mildew, *Sphaerotheca fuliginea*, indicate that thermophylaxis may be demonstrable in many species. This report deals only with *Uromyces*, with which the phenomenon seems better expressed than with the other plants indicated.

Pinto beans infected with rust and growing in a greenhouse at about 23° were first heated (predisposed) for 10–20 sec at 45° in a water bath at 2–4 days after inoculation. One primary leaf on each plant was predisposed and the opposite leaf of each plant served as a control. This predisposition dosage was about 1/3 the ED_{50} or 1/6 the ED_{100} for rust of this age, and in itself caused only a barely perceptible delay in rust development if the plants were allowed to remain in the greenhouse. In such plants the rust in the predisposed leaves soon became more tolerant to heat than the control rust[2]. In the work recorded here the inoculated and predisposed plants were placed in a dark incubator at an air temperature of about 38° for 1–7 h (predisposition lag), given a challenge heating of 0–50 sec at 50° in a water bath, returned to the greenhouse, and the number of living pustules counted or rated about 10 days after inoculation. Under these conditions rust in predisposed leaves was killed at a lower challenge heat dosage than rust in the control leaves (Fig. 1), and if the plants were left at 38° for more than 4 h the predisposed rust was usually killed without any additional challenge heat.

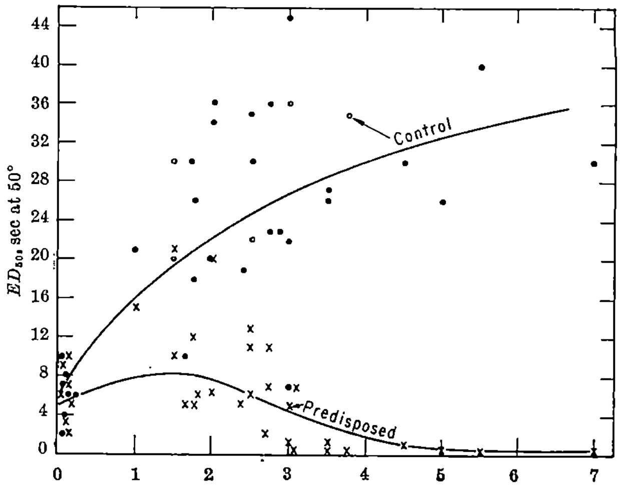

Fig. 1. Simultaneous adaptation and sensitization to heat in bean rust (*Uromyces phaseoli* on *Phaseolus vulgaris*) at 38° C

If predisposed plants were subjected to a range of challenge heats immediately after the predisposition heating of one leaf, the rust in the predisposed leaves was killed at a slightly lower challenge heat dosage than necessary to kill the rust in the control leaves, but this is regarded as an additive effect of the predisposition heat dosage plus the challenge heat dosage. Thermophylaxis can be said to occur only if the predisposed rust is killed at a dosage less than the combined predisposition heat dosage plus challenge heat dosage applied to the control leaf of the same plant. The degree of thermophylaxis can be expressed as an adaptation index[2] which is the ED_{50} of the challenge heat for the predisposed leaf divided by the ED_{50} of the challenge heat of the control. Adaptation indexes greater than 1 indicate adaptation. Adaptation indexes less than 1 indicate an additive effect of heat or thermophylaxis. Logically and experimentally (predisposition heat of 15 sec at 45°, ED_{50} for control of 5 sec at 50°, and a temperature coefficient of 120) an adaptation index less than 0·7 would not and has not resulted from the additive effect alone. Since the adaptation index after 2·5–7 h at 38° was always less than 0·7 in each of 16 trials (Fig. 1), and averaged only 0·13, it is concluded that thermophylaxis occurred.

Almost as interesting is the observation that adaptation in the control (non-predisposed) and sensitization in the predisposed rust mycelium occurred simultaneously in the same environment. That is, the ED_{50} of the control increased with increasing exposure to 38°, while the ED_{50} of the predisposed rust on the same plants decreased. The apparent rise in the ED_{50} of the predisposed rust between 0 and 2 h after the predisposition heat is likely a transient effect or an adaptation artefact because the temperature of the low heat capacity incubator (150 W) dropped temporarily to as low as 32° within a few minutes after the 6–12 pots of plants were placed in the incubator.

The degree of thermophylaxis reported here (0·03 times as much heat necessary to kill the rust in predisposed leaves as in control leaves) is much greater than the degree of heat adaptation previously reported[2], where 4 times as

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β-Amylase
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Lactate dehydrogenase
Malate dehydrogenase
Neuraminidase

INTERMEDIATES
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2-Phospho-glyceric acid barium salt
3-Phospho-glyceric acid barium salt
Sodium pyruvate

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Fructose-1 : 6-diphosphoric acid calcium dihydrogen salt
Fructose-1 : 6-diphosphoric acid dimagnesium salt
Fructose-1 : 6-diphosphoric acid disodium salt
Fructose-1-phosphoric acid barium salt
Fructose-6-phosphoric acid barium salt
Galactose-6-phosphoric acid barium salt
Glucose-1-phosphoric acid dipotassium salt
Glucose-1-phosphoric acid disodium salt
Glucose-6-phosphoric acid barium salt
Glucose-6-phosphoric acid dipotassium salt
Glucose-6-phosphoric acid disodium salt
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much heat was necessary to kill the predisposed rust as the control rust. It emphasizes the high degree of physiological adaptation in organisms, and may be of value in practical heat therapy.

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Formative Response of *Amsinckia intermedia* to the Interrelated Effects of Low Temperature, Thiamin and 2,4-Dichlorophenoxyacetic Acid

THE adverse effect of temperature on plant growth may sometimes be corrected by the external application of certain essential metabolites. As early as 1943, Bonner[1], using *Cosmos sulphureus* Cav. as experimental plant material, found that the growth under sub-optimal temperature conditions was improved by thiamin application. Later work by Bonner[2] and more recently the experiments by Ketellapper[3] showed that both high- and low-temperature effects on several plant species were overcome by chemical treatments. The investigations recorded here were carried out to determine whether or not the response of fiddleneck (*Amsinckia intermedia*, Fisch and Mey) to 2,4-dichlorophenoxyacetic acid (2,4-D) when grown at a low temperature can be changed by treatment with certain metabolites.

Seedlings were grown in plastic pots containing 16-mesh silica sand in the greenhouse for one month and then placed in a growth chamber. Subsequent culture was at 10° C under an 18-h light régime. Illumination was provided by warm white fluorescent tubes supplemented with 60-W incandescent bulbs giving an intensity of 1,250 ft.-candles. Nutrients were supplied to all plants every other day with Hoagland's solution.

In the initial set of experiments, solutions of thiamin were applied with a pipette to individual mature leaves in 0·01 ml. droplets at rates of 0·05–50·0 μg per plant. At the same time, 500 μg of 2,4-D alone or in addition to each of the rates of thiamin was applied to similar plants.

In other experiments, fiddleneck seedlings were established as described here, and weekly treatments of certain metabolites were sprayed on the foliage. Thiamin (20 p.p.m.), vitamin C (1,000 p.p.m.), *d*-biotin (1 p.p.m.), pyridoxin (20 p.p.m.), nicotinic acid (40 p.p.m.), adenine (1 p.p.m.), 2-thiouracil (1 p.p.m.), and a mixture of amino-acids (L-cystine, L-cysteine, L-glutamic acid, DL-aspartic acid and glycine—each at 1 p.p.m.) were sprayed on the leaves. The plants were sprayed with the solutions weekly for four consecutive weeks and then gathered. The root and shoot of each plant were separated and the dry weight measured. The metabolites which caused an improvement in growth at this temperature (10° C) were then applied to similar plants in combination with 2,4-D to determine the interaction. Treatments were 275 μg 2,4-D per plant, plus one of the following additives: amino-acid mixture, *d*-biotin (0·1 and 10 μg), nicotinic acid (10 and 100 μg) or thiamin (10 μg). Observations were recorded one month after treatment.

The untreated and the thiamin-treated plants grew slowly at 10° C with no apparent morphological differences due to the thiamin. 2,4-D applications alone suppressed growth and produced a slight distortion of the apex, 2,4-D plus 0·05 μg thiamin caused 100 per cent mortality (Fig. 1), and 2,4-D plus 0·5 μg thiamin caused mortality to 40 per cent of the plants and severe injury and distortion to 60 per cent. The 2,4-D plus the higher rates of thiamin (2·5, 5·0 and 50 μg) caused severe injury but did not kill the plants.

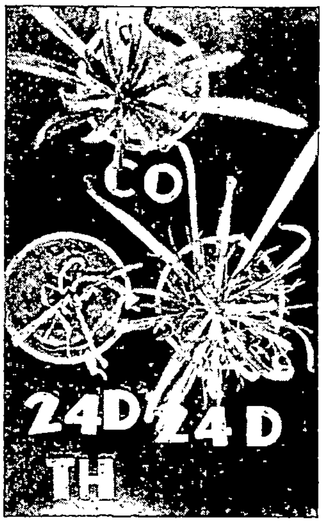

Fig. 1. *Amsinckia intermedia* plants growing at 10° C. Control plant (*CO*), plant treated with 2,4-D (2,4D) at lower right and plant treated with 2,4-D plus thiamin (24DTH) at lower left. Combination of thiamin and 2,4-D causes increased mortality at this temperature

The plants sprayed with the amino-acid mixture, *d*-biotin or nicotinic acid were darker in colour than the control plants and dry weight of both tops and roots was greater. 2,4-D applied to otherwise untreated plants caused a suppression of the apex and some distortion of the youngest leaves. *d*-Biotin (0·1 μg) plus 2,4-D caused severe swelling of the apex, suppression of new growth and discoloration and necrotic areas in the mature leaves. *d*-Biotin at a dosage of 10 μg plus 2,4-D had much less effect. Nicotinic acid at the rate of 100 μg per plant increased the response of the plant to 2,4-D but did not do so at 10 μg. Thiamin-treated plants were the most severely injured by the 2,4-D, although they showed no improvement in growth from the thiamin treatment.

The results show that: (1) low-temperature effects on *Amsinckia intermedia* may be partially overcome by the application of chemical substances; (2) certain metabolites in combination with 2,4-D are more effective in causing formative and toxic effects on *Amsinckia intermedia* growing at a sub-optimal temperature than 2,4-D alone. Of those tested, thiamin was the most effective.

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A Steroid Growth Factor Requirement in a Fungus

THE homothallic oomycete *Phytophthora cactorum* is frequently grown on oatmeal agar[1], and on this medium it forms oospores readily. On a basal medium of sucrose or glucose, asparagine, mineral salts and thiamine its vegetative growth is less and oospores are not formed, or a very few are found after a long time, and then only at the edge of the colony. The basal medium evidently lacks some growth factor or factors present in oats. The addition of aqueous extracts of ground oat grains to the basal medium stimulates vegetative growth but does not lead to the production of oospores. However, the addition of 500 mg/l. of a petroleum ether or ethanol extract of oats both increases the rate and amount of growth and results in the production of oospores. Aqueous and ethanol extracts of peas have similar effects.

Aqueous extracts of peas and oats also stimulate the vegetative growth of *Phytophthora parasitica* and *P. palmivora*, but the alcohol or petroleum ether extract must also be added for sexual reproduction in these species.

In testing fractions of the extracts from ground oats (and later, pure substances) for their activity, *P. cactorum*, strain I.M.I. 21168, obtained from the Commonwealth Mycological Institute, was grown at 25° in 5-cm Petri dishes containing 4 ml. of medium. A known amount of material for testing was dissolved in diethyl ether and added to the basal medium[2] together with agar, and the medium was then autoclaved. (The activity of the crude extract was not lost on autoclaving the extract with the medium at 120° for 15 min.) The inoculum was a plug 4 mm in diameter cut from a culture growing on the basal medium. The resulting colonies were examined for the occurrence of oospores, and their number and time of appearance were noted; the diameter of the colony was also measured.

After removal of the phospholipids, the crude petroleum ether extract was chromatographed on alumina, to give three main components consisting of vegetable oils (60 per cent) and two more polar yellow oils (5 and 10 per cent respectively) eluted with benzene/chloroform (1 : 1) and ethyl acetate respectively. Activity was found to be associated with the second yellow oil; this material was saponified and yielded a neutral red waxy solid (5 per cent) and fatty acid material (75 per cent).

The red waxy solid afforded sterols on crystallization and these were found to be active. Sterols were also obtained from the vegetable oil by saponification and digitonin precipitation of the neutral material, and were likewise found to be active. Previous investigation[3] of the sterols from oat grains had demonstrated the presence of β-sitosterol together with two di-unsaturated sterols tentatively identified as $\Delta^{5,11}$- and $\Delta^{7,11}$-stigmastadienol respectively, and a trace of stigmasterol. The sterol mixture from the red waxy solid was separated into mono- and di-unsaturated compounds by chromatography of the acetates on 10 per cent silver nitrate on silicagel. Abundant oospores were produced when both these fractions were added to the basal medium at 10 mg/l., after 5 days with the former and 8 days with the latter. Examination of these two fractions by gas chromatography indicated that the former, mono-unsaturated fraction consisted largely of β-sitosterol together with small amounts of cholesterol, campesterol and Δ^{7}-stigmastenol, and the di-unsaturated fraction contained two principal components together with small amounts of brassicasterol and stigmasterol and a number of other unidentified trace components. The ultra-violet spectrum of the crude sterol mixture indicated the presence of about 1 per cent of a $\Delta^{5,7}$-diene system similar to that of ergosterol (cf. ref. 3).

Since it appears that sterols are active materials in inducing oospore formation, a number of pure steroids and related compounds were tested, at concentrations (in the final medium) of 10 mg/l., with the results shown in Table 1.

Leonian and Lilly[4] found a growth factor necessary for sexual reproduction of *Phytophthora cactorum* in an alcoholic extract of peas, but they considered the active fraction was not the sterols themselves but rather was merely absorbed on them. By the use of pure samples we have shown that sterols are in fact active growth factors.

It would appear that some step or steps in the synthesis of sterols is blocked; but the absence of exogenous sterols does not prevent vegetative growth. When 51 g (dry weight) of mycelium, produced from growth on 15 l. of liquid basal medium, was extracted with petroleum ether, and the resulting 10 per cent of fatty material saponified, no sterols could be detected in the neutral fraction on precipitation with digitonin followed by gas chromatography. The blockage in sterol synthesis thus appears to be complete. Further, we find that the vegetative growth in the basal medium is not inhibited by nystatin (up to 100 mg/l.), which is believed to act by combining with sterols in the protoplast membrane[5].

Table 1. EFFECTIVENESS OF STEROIDS AND RELATED SUBSTANCES IN OOSPORE PRODUCTION IN *Phytophthora cactorum*

Substance added to basal medium	No. of oospores	Distribution in culture	Time for production
Cholesterol	+ + + +	Throughout	5–6 days
Cholesterol acetate	+ + + +	Throughout	5–6 days
Ergosterol	+ + + +	Throughout	5 days
Stigmasterol acetate	+ + + +	Throughout	8–10 days
Cholest-5-ene-3-one	+	Only at edge	10–11 days
Cholest-4-ene-3-one	+	Throughout	10–11 days
Cholest-4,6-diene-3-one	+	Throughout	10 days
Cholestane	+	Only at edge	14 days
Coprostan-3-one	±	Only at edge	10 days
Calciferol	−	—	—
Diosgenin	−	—	—
Tomatidine	+	Only at edge	14 days
Δ^{5}-Pregnen-3β-ol-20-one	+	Only at edge	14 days
Progesterone	+	Only at edge	14 days
Testosterone	+	Only at edge	14 days
Dehydroepiandrosterone	+	Only at edge	14 days
Œstrone	+	Only at edge	14 days
Squalene	−	—	—
DL-α-Tocopherol	+	Only at edge	14 days
Sodium acetate	+	Only at edge	14 days
None (control)	+	Only at edge	14 days

±, Fewer oospores than in control; −, no oospores.

When sterols are added to the basal medium, growth is increased and sexual reproduction occurs. Of the substances we have tested (Table 1), the sterols themselves are most active. Dehydrogenation at C3 to the ketone (as in the cholestenones) results in lower activity, but it does not always seem to destroy it completely. With the cholestenones, the total number of oospores is not appreciably greater than in the controls, but the oospores appeared several days sooner and were (in two cases) found throughout the culture. Derivatives in which the side-chain is greatly modified (for example, diosgenin) or shortened (progesterone, testosterone, etc.) are apparently ineffective.

Sterols have been recorded as essential growth factors for certain protozoa[6,7], a *Labyrinthula*[8] and pleuropneumonia-like organisms[9,10], but this appears to be the first record of a sterol requirement by a fungus.

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[2] Sucrose, 10·0 gm; L-asparagine, 1·0 gm; KH_2PO_4, 0·5 gm; $MgSO_4.7H_2O$, 0·25 gm; trace element solution, 1 ml.; thiamine hydrochloride, 1 mg; water, 1 litre. The trace element solution contains $Na_2B_4O_7.10H_2O$, 88 mg; $CuSO_4.5H_2O$, 393 mg; $Fe_2(SO_4)_3.5H_2O$, 910 mg; $MnCl_2.4H_2O$, 72 mg; $Na_2MoO_4.2H_2O$, 50 mg; $ZnSO_4.7H_2O$, 4,403 mg; EDTA, 5 gm; water, 1 l. Difco bacto agar or Oxoid agar No. 3, 0·8 per cent.

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MICROBIOLOGY

Relationship between the Products of Aromatic Biosynthesis in *Mycobacterium smegmatis* and *Aerobacter aerogenes*

MYCOBACTERIA, when grown on Proskauer and Beck medium, accumulate salicylic acid[1]. This accumulation is greatly increased by iron-deficiency. The results presented here suggest that this acid originates from an intermediate in the main pathway of aromatic biosynthesis.

Mycobacterium smegmatis was grown either on Proskauer and Beck medium (which contains glycerol and asparagine) or on: quinic acid 5·0 g, L-glutamine 1·25 g, L-asparagine 1·25 g, glycerol 5·0 g, KH_2PO_4 5·0 g, $MgSO_4$ 0·4 g, and water 1 l., made to *p*H 6·8 with sodium hydroxide. In some cases a medium containing shikimic acid 0·1 per cent, $(NH_4)_2SO_4$ 0·15 per cent, with KH_2PO_4 and $MgSO_4$, as above, was used. *Aerobacter aerogenes* was grown on: quinic acid 2·0 g, glutamine 0·5 g, $(NH_4)_2SO_4$ 1·0 g, KH_2PO_4 5·0 g, water 1 l. and made to *p*H 6·8 with sodium hydroxide.

With both organisms, the medium, after growth, was freed from cells, reduced to 1/20th of its original volume, acidified to *p*H 2 and extracted with ethyl acetate. The extract, after drying, was evaporated to about 0·3 ml. and examined by paper chromatography.

Salicylic acid accumulated after growth of *M. smegmatis* on Proskauer and Beck medium. Iron-deficiency greatly increased this accumulation[1]. After growth on either quinic acid or shikimic acid, the amount of salicylic acid found was greater than before; also identified were anthranilic, 3 : 4-dihydroxybenzoic, 4-hydroxybenzoic and 4-hydroxyphenylacetic acids. When *M. smegmatis* was grown on Proskauer and Beck medium and this replaced either by a further quantity of the same medium or by shikimic acid medium, and growth continued for 24 h, more salicylic acid accumulated in the latter medium. Deficiency of iron increased the accumulation of salicylic acid in both cases.

Small amounts of anthranilic and 4-hydroxybenzoic acids were found after growth of *A. aerogenes* (wild-type *NCW*) on quinic acid medium, After iron-deficient growth a considerable amount of 2 : 3-dihydroxybenzoic acid was also produced. With a mutant of *A. aerogenes* (*N*5/36—blocked immediately after the synthesis of anthranilic acid). anthranilic acid, 3 : 4-dihydroxybenzoic acid and an *N*-acylanthranilic acid were found after growth on the quinic acid medium supplemented with 2 μg/ml. of L-tryptophan. Very little change was produced by iron-deficiency. The *N*-acylanthranilic acid derivative which was found had spectra in the ultraviolet region at several *p*H values which were very close to those given by both *N*-formyl- and *N*-acetylanthranilic acids. The material was shown not to be the formyl derivative by chromatographic mobility and by its greater stability to 3 N hydrochloric acid. Hydrolysis with 6 N hydrochloric acid at 100° gave anthranilic acid, but the rest of the molecule has not yet been identified.

Some work has also been carried out with *Escherichia coli* growing on 2 per cent glycerol (it is unable to utilize quinic acid): 2 : 3-dihydroxybenzoic, 4-hydroxybenzoic and 3 : 4-dihydroxybenzoic acids have been located on chromatograms of extracted medium.

Salicylic acid in *M. smegmatis* seems to arise from the main pathway of aromatic biosynthesis, as is shown by the stimulatory effect of shikimic acid, or quinic acid, on its output. Further, it seems to have an origin close to that of 2 : 3-dihydroxybenzoic acid in *A. aerogenes* and probably *E. coli* as well. This is suggested by the fact that iron deficiency affects only these compounds among the number of aromatic compounds produced, as well as by their closely related structures. The origin of 2 : 3-dihydroxybenzoic acid in *A. aerogenes* is after the paths of aromatic biosynthesis diverge and before the synthesis of either tyrosine or anthranilic acid[2]. Thus, this may also be the point of origin of salicylic acid. It is interesting to note that salicylic acid, like 2 : 3-dihydroxybenzoic acid, is not metabolized once it has been excreted into the medium.

It was suggested that salicylic acid might arise through non-introduction of an amino group into a precursor of anthranilic acid, and that its accumulation in iron-deficiency is due to a requirement for iron for the insertion of the amino group[1]. Some support for this idea is given by the finding by Srinivasan and Rivera[3,4] that Fe^{++} is a co-factor in the conversion of 3-enolpyruvylshikimic acid 5-phosphate to anthranilic acid by cell-free extracts of *E. coli.*

The *N*-acylanthranilic acid either is a precursor of anthranilic acid or a metabolite of it: it will be difficult to reach a conclusion on this until the acyl group has been identified. One interesting possibility arising from the first alternative is that in the conversion of chorismic acid[5] to anthranilic acid a bi-cyclic intermediate may be formed as in Fig. 1.

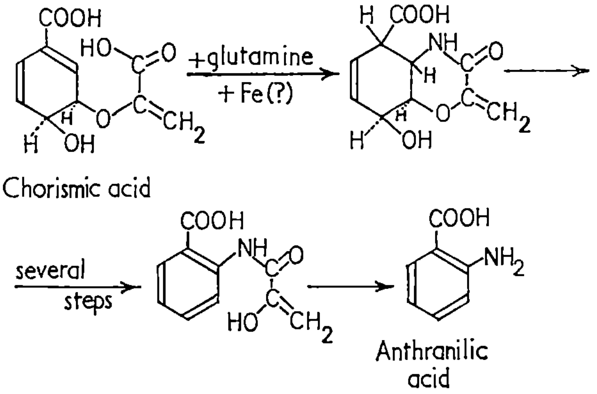

Fig. 1

It is possible that analogous steps, without the introduction of the nitrogen atom, are involved in the synthesis of an alternative bi-cyclic intermediate, which in *Mycobacteria* is hydrolysed to salicylic acid and in *A. aerogenes* yields 2 : 3-dihydroxybenzoic acid.

The wild-type and mutant *A. aerogenes* were part of a gift from Dr. F. Gibson, University of Melbourne. This work was supported by grant *AI* 02842 from the U.S. Public Health Service and by Arthur Guiness, Son and Co. (Dublin), Ltd. I thank Mr. Frank Winder for his criticism and help with this work, and Dr. V. C. Barry for his advice.

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Effect of Deuterium on Starving Bacteria

The presence of high concentrations of deuterium oxide in growth media has been shown to be toxic to bacteria[1]. The effects are characterized by long lag periods before growth occurs and subsequent slow growth with the production of distorted forms. However, experiments designed to study the effect of deuterium oxide on bacteria starving in buffer solutions showed that deuterium oxide was not toxic under these conditions and that the longevity of the bacteria was increased.

Aerobacter aerogenes N.C.T.C. 418 was used as the test organism, and it was grown in continuous culture at 40° C in a synthetic medium with glycerol as limiting substrate[2]. The storage conditions were those used by Postgate and Hunter (*loc cit.*).

Although the increased longevity was easily detected in suspensions containing 15 μg dry weight of bacteria per ml.

and was found to be related to the concentration of deuterium oxide, the effect was more conveniently studied in suspensions containing about 1 mg/ml. dry weight, which represents about $2{\cdot}5 \times 10^9$ organisms/ml., in 75 per cent deuterium oxide. The results of an experiment carried out at this concentration of organisms are shown in Fig. 1. The mean half-life of the suspension stored in aqueous buffer is $12{\cdot}9 \pm 1{\cdot}0$ h and for storage in a buffer containing 75 per cent deuterium oxide, $16{\cdot}6 \pm 2{\cdot}9$ h. The limits represent 95 per cent fiducial limits and were kindly calculated by Mr. S. Peto. The survival curve in 75 per cent deuterium oxide is characterized by an extended initial phase during which the viability falls off more slowly than in the final part of the curve.

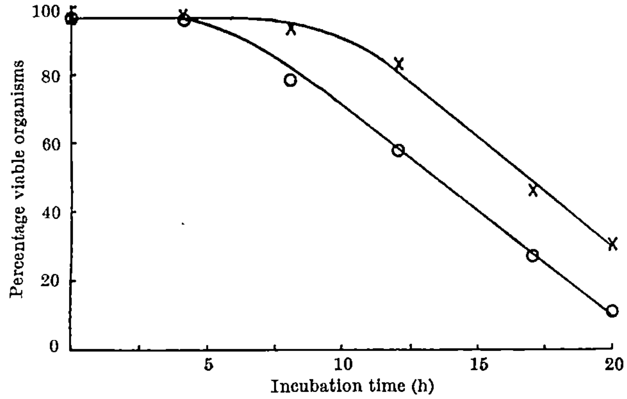

Fig. 1. Change in viability of washed suspensions of *A. aerogenes* incubated at 40° C in saline-*tris* buffer *p*H 7·1. ○, Aqueous buffer solution; ×, buffer solution containing 75 per cent deuterium oxide

Deuterium oxide will permeate the whole of the cell, and rapid exchange will occur with ordinary water present in the cell substance. An isotope effect could then occur if the processes involved in maintaining viability utilized deuterium oxide. Since energy is derived from ribonucleic acid and protein during starvation[2,3] investigations were carried out to see whether an isotope effect could be observed in the breakdown of these substances.

Little difference was found in the protein and ribonucleic acid content of samples of bacteria taken during storage in either aqueous buffer or buffer containing 75 per cent deuterium oxide. The suspending fluids and the materials released from the bacteria by treatment with cetyl trimethyl ammonium bromide were examined for substances absorbing at 260 mμ. It was found that a slightly slower release of these substances occurred from bacteria stored in buffer containing 75 per cent deuterium oxide.

Although a number of enzymes are involved in the breakdown of nucleic acids the effect of 75 per cent deuterium oxide on the endogenous ribonuclease, polynucleotide phosphorylase and phosphodiesterase was conveniently examined *in vitro* using the conditions described by Wade[4], and Wade and Lovett[5]. Deuterium oxide had very little effect on the rate of breakdown of nucleic acid by phosphodiesterase and polynucleotide phosphorylase, but the effect on the action of ribonuclease was considerable (Fig. 2). In view of this result, it was of interest to investigate the effect of deuterium oxide on a bacterium which had no ribonuclease. Wade and Robinson[6] have recently demonstrated that *Pseudomonas fluorescens* (N.C.I.B. 8248) does not contain ribonuclease, and preliminary experiments with organisms grown in continuous culture at 30° C in a medium similar to that used by Postgate and Hunter (*loc cit.*), except that citric acid was substituted for EDTA, showed that 75 per cent deuterium oxide did not increase the longevity of washed organisms suspended in a saline-*tris* buffer at 33° C. EDTA was omitted from the saline-*tris* buffer as it was highly toxic to the organisms.

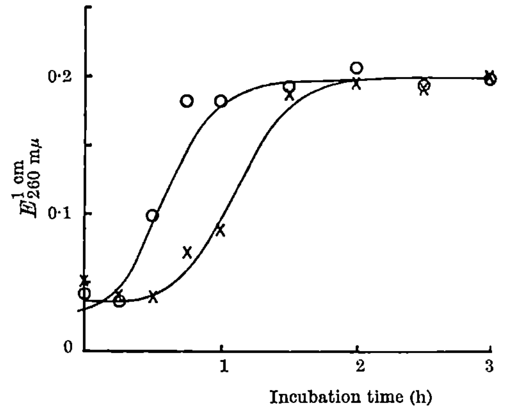

Fig. 2. Curves showing the release of acid-soluble substances adsorbing at 260 mμ from a suspension of 14 mg/ml. dry weight of *A. aerogenes* incubated at 37° C in a buffer containing 0·145 M NaCl, 10 mM magnesium chloride, 10 mM sodium phosphate and 40 mM EDTA. The final *p*H was adjusted to 7·0 with NaOH solution. The bacteria were killed before the experiment by treating with cold 50 per cent aqueous acetone and any remaining acetone removed by evaporation under vacuum at a temperature not exceeding 5° C. ○, Aqueous buffer solution; ×, buffer solution containing 75 per cent deuterium oxide

Although the effect of deuterium oxide on bacterial proteinases has not yet been investigated, the results so far obtained show that under the starvation conditions described for *A. aerogenes* deuterium oxide retards the rate of hydrolysis of ribonucleic acid which is partly used as an energy source in maintaining the viability of the organism. After a certain proportion of the ribonucleic acid is utilized another process ensues which is not retarded by deuterium oxide.

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Antibiotics of *Alcaligenes faecalis*

Alcaligenes faecalis type cultures N.C.T.C. 8764 and A.T.C.C. 9220 were investigated for bacteriocin production against members of the same species[1] as well as a large number of *Escherichia*, *Salmonella*, *Serratia*, *Staphylococci*, Gram-positive aerobic bacilli and *Proteus*[1]. The methods used were those of Fredericq[2]. Organisms were grown on the Pr_3 media of Ben-Gurion and Hertman[3]. Electrophoresis was performed as previously described[4].

The two *A. faecalis* type cultures were found inhibitory for many members of the same species as well as strains of *Proteus hauseri*, *rettgeri*, and *morganii*. Large clear areas of inhibition were produced in the indicator lawns. In addition, A.T.C.C. 9220 was also inhibitory for a number of *Staphylococcus* strains. These antibiotics are bacteriostatic in that sub-cultures from the clear areas of inhibition always yield growth identical to the original strains and, despite repeated attempts, no resistant mutants have yet been obtained. The antibiotics pass through 10 mμ filters and cellulose-dialysis tubing.

Electrophoresis of these inhibitory agents was done with representatives of each of the sensitive *A. faecalis* and *Proteus* strains as indicators of activity (Fig. 1). In each case the same pattern was encountered. This consisted of a clear round area of inhibition towards the cathode. These results indicate that a single inhibitory substance is produced by each of the two strains.

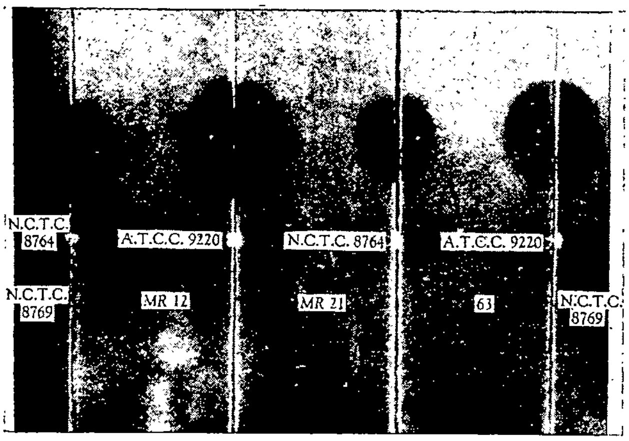

Fig. 1. Type cultures, N.C.T.C. 8764 and A.T.C.C. 9220, were each inoculated on two places in the centre of an electrophoresis plate and grown for 36 h. The growths were then killed with chloroform vapour and a potential of 50 V applied across the plate for 16 h in a refrigerator. The plate was divided into 5 compartments with glass strips through the centre of each inoculum and the plate again sterilized. The compartments were then covered with soft agar containing the indicator strains *P. mirabilis* 63, *P. rettgeri MR* 12, *P. morganii MR* 21 and *A. faecalis* strain N.C.T.C. 8769. The plate was incubated overnight

The antibiotic produced by A.T.C.C. 9220 was further investigated. Sterile cellulose disks were placed on agar plates and the organisms spread on the surfaces. After 3 days' incubation at 37° C, the disks and organisms were stripped from the surfaces and the clear agar stored at −15° C overnight. On thawing, the clear fluid was precipitated in the cold with an equal volume of alcohol–acetone (50 per cent v/v). The precipitate was collected by centrifuging at −10° C and freeze-dried. The freeze-dried material was dissolved in 0·1 M phosphate buffer *p*H 7·4. Dilutions were spotted on a freshly poured indicator lawn and incubated overnight. An inhibitory titre of 1 : 4 was obtained. No activity could be demonstrated with the above method without the use of cellulose disks to separate the organisms from the antibiotic produced in the agar. The activity of the antibiotic solution was destroyed by heating at 85° C for 0·5 h and was not affected by pepsin or trypsin. The spectrum of activity of the antibiotic in solution was identical to that produced by the organism on agar and showed the same electrophoretic mobility.

A large number of other *A. faecalis* strains examined were inhibitory for several members of the same species as well as *Escherichia*, *Salmonella*, *Shigella*, *Serratia*, *Staphylococci*, Gram-positive aerobic bacilli and *Proteus*.

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Origin from Mitochondria of the Double-walled Viral Membrane in *Saccharomyces*

Investigation of the viral infections of *Saccharomyces*[1,2] has shown that this organism is parasitized by both a rod-shaped virus (like tobacco mosaic virus) and a double-walled, spherical, nucloid virus. Electron microscopy, in this laboratory, on the ætiology of yeast infection has been carried out on strains affected by the spherical, double-walled, nucleoid virus[3]. Electron micrographs show that the site of propagation of the virus is the mitochondrion and that the outer double wall of the virus is derived from the double-walled cristæ of the mitochondrion. Fig. 1 shows the mitochondria of a normal yeast cell. Fig. 2 shows a focus of viral infection multi-

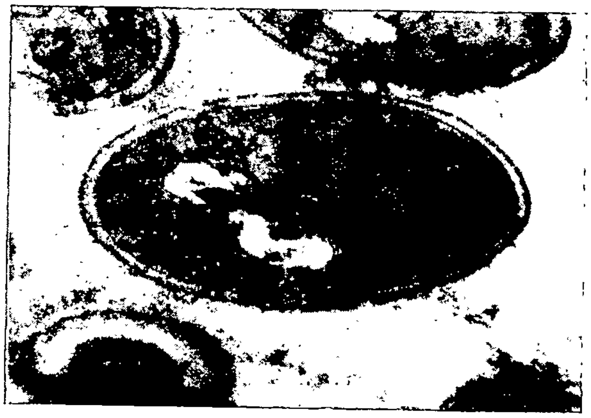

Fig. 1

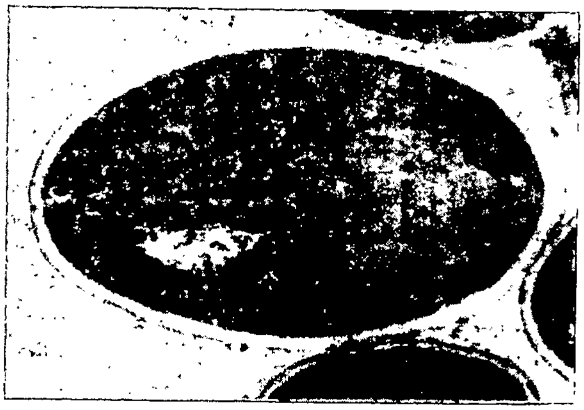

Fig. 2

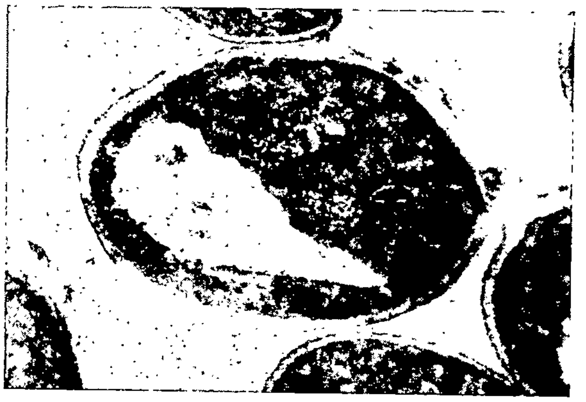

Fig. 3

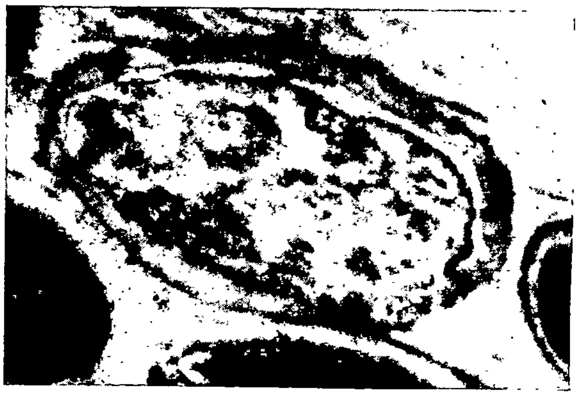

Fig. 4

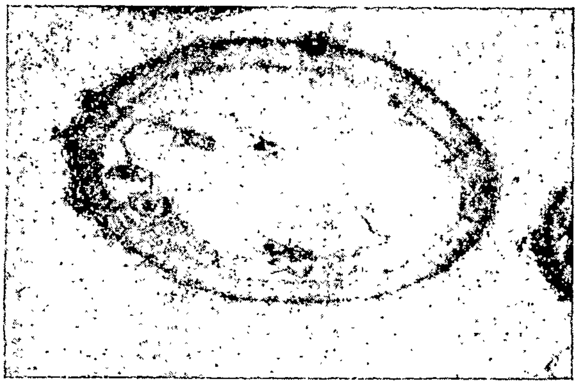

Fig. 5

plying at a centre from which three double-walled cristæ of a disrupted mitochondrion radiate into the cytoplasm. In this cell (and in Fig. 3) there are three mitochondrial foci in which the walls of the mitochondria have become sinuous and are enveloping the viral particles. In Fig. 4 viral particles are found within disintegrated mitochondria. Fig. 5 shows a cell in which the protoplast has been transformed into virus particles and membranes.

We thank Mrs. Masako Osumi and Mr. Wilbert Bowers for the electron micrographs.

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GENETICS

Occurrence of Transferrin D_1 in Korea

So far, sixteen electrophoretically distinguishable transferrin variants have been distinguished by means of starch-gel electrophoresis[1,2]. Observations on the frequency and distribution of the variants have been recently summarized by Giblett[3]. In all populations examined transferrin C is by far the most frequently encountered. Those variants which occur in a measurable gene frequency are usually restricted to certain populations. Transferrin D_1 is widely distributed and has been reported most frequently in American and African Negroes. It has also been reported as occurring in New Guinea, Fiji, New Hebrides, Malaya, Thailand, Philippines, Formosa, Ceylon, India, Central and South America and Sweden.

Recently, however, using a modification of the vertical gel-electrophoresis technique, it has been possible to discriminate a transferrin variant with a mobility very similar to D_1. This variant was originally found in Chinese populations and has been designated $D_{\rm Chi}$ (ref. 4). Kirk, Parker and Bearn[5] re-investigated the transferrin D_1 variants previously reported from different populations to determine whether those transferrin previously classified as D_1 might represent $D_{\rm Chi}$ variants. The $D_{\rm Chi}$ variant was found exclusively in Ceylon, Thailand, north-east India, Malaya, Turk Islands, Formosa, and Japan. Since no transferrin D_1 variants were observed in these areas, it seemed likely that if a transferrin variant occurred in appreciable frequency in Korean populations, it would be of the $D_{\rm Chi}$ type.

Analysis of 120 samples of serum from individuals from Korea disclosed one in heterozygous D variant which proved to be D_1. The occurrence of the D_1 variant in a very low frequency could be ascribed to mutation pressure alone. It will be of interest to examine a larger sample to obtain an accurate estimate of the gene frequency of D_1 in the Korean population and to determine whether the $D_{\rm Chi}$ variant is also present.

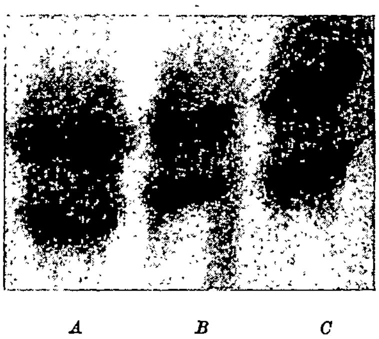

Fig. 1. Comparison of Korean transferrin variant and standard CD_1 and $CD_{\rm Chi}$ by autoradiography after starch-gel electrophoresis. Transferrin C is the faster migrating and transferrin D the slower migrating of the two components. $A = CD_1$, $B = CD_{\rm Chi}$, C = Korean samples (CD_1)

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Does a Connexion exist between Blood Group Modifications observed in Leukæmia and Certain Chromosomal Alterations?

A LARGE number of blood group modifications have been observed during the evolution of leukæmias. They belong most often to the ABO system, and can be divided in two classes: (1) In the first group one can classify the cases where a transformation from groups A or B into A weak is observed, or a transformation into group O by a progressive substitution of the substance H, for the substances A or B. (2) In the second group one can classify the cases where the diminution of the antigen A or B is not accompanied by a corresponding increase in substance H, and the cases where, the patient being group O, one assists progressively in the disappearance from his red-cells of the substance H (ref. 1).

As these modifications occur most often in the course of acute myelogenous leukæmias, and as there are strong reasons for believing that the alterations of the chromosomes observed in the leukæmias of this type also affect the erythroblasts, the blood group modifications are perhaps a result of a genetic alteration of the latter.

It is known that the antigens of the ABO system blood group develop from a fundamental substance formed in the erythroblasts[3,4] (Morgan and Watkins, Salmon). This is transformed into substance H by the action of a pair of genes Xx and then the substance H is transformed into substance A by the gene A or into substance B by the gene B. It follows, therefore, that one can consider very schematically that the sum of the antigens A, B and H is constant in the red cell, all development of the antigen A causing a reduction in the quantity of the antigen H, and all diminution of the formation of the antigen A ending in an accumulation of the substance H.

The transformations from group A into A 'weak' might therefore result from a chromosomal alteration located in the locus containing the genes of the ABO system; one could explain this by a damage of one of the chromosomes, which is frequent in the course of certain leukæmias[2]. There are strong reasons in fact for believing that this locus is found on chromosome 21.

The diminutions of substance H or of antigen A not accompanied by a correlative accumulation of substance H can only be explained by a trouble situated on the locus carrying the genes Xx.

Numerous cases of chromosomal aberrations in leukæmias have been published. Unfortunately, in this case the investigation of the blood groups had not been carried out in detail, or revealed nothing abnormal. In one case, however, we have been able to show, in a subject having acute myelogenous leukæmia, constrictions of a pair of chromosomes of group 6–12, and a transformation from the original blood group A_1 by a diminution of the substance A without the corresponding increase of substance H. This allows us to bring up the hypothesis following which the locus Xx is perhaps carried by a chromosome of the medium group.

Numerous observations will still have to be carried out to try to find the exact connexions between the chromosomal malformations of leukæmias and the blood group modifications. Unfortunately, we are as yet only able to explore morphologically the chromosomes; but probably the genetic deficiency is produced on the molecular level. It would therefore be useless to attempt systematically to find it microscopically in all the leukæmias presenting a blood group modification.

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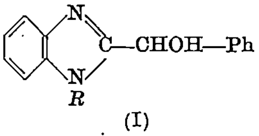
VIROLOGY

Protection of Poliovirus-infected Tissue-culture Cells using the 1-Benzyl, 1-Pentyl and 1-Isopropyl Derivatives of 2-(α-Hydroxybenzyl)-benzimidazole

THE selective protection given by 1-alkyl-2-(α-hydroxybenzyl)-benzimidazoles (I) to *ERK* cells, infected with types 1, 2, and 3 poliovirus, increases from the 1-methyl to the 1-propyl compound, the effect diminishing on passing to the 1-butyl compound[1]. In the case of HBB (I; *R* = H), the D-isomer exerts greater protective action than the DL-isomer[2]. In spite of a disparaging impression of this difference given by the last paragraph of our previous publication[2], the results given therein indicate clearly that protection against types 1, 2 and 3 poliovirus resides largely in the D-isomer. Kadin, Eggers and Tamm[3] have since shown that D-HBB has a greater effect than DL-HBB against the pathogenicity of type 2 poliovirus towards rhesus monkey kidney cells and that L-HBB offers these cells no protection under similar conditions. We have continued our investigation of 1,2-disubstituted benzimidazoles by preparing the 1-pentyl, 1-isopropyl and 1-benzyl derivatives of DL-HBB.



(I)

Table 1. 1-SUBSTITUTED 2-(α-HYDROXYBENZYL)-BENZIMIDAZOLES (I)

R	M.p. (° C)	Yield (%)	Formula	Required (%) C	Required (%) H	Required (%) N	Found (%) C	Found (%) H	Found (%) N
Pentyl	117–8	55	$C_{19}H_{22}N_2O$	77·6	7·5	9·5	77·3	7·8	9·7
Isopropyl	178–9	39	$C_{17}H_{18}N_2O$	76·7	6·8	10·5	76·7	6·9	10·3
Benzyl	166–7	18	$C_{21}H_{18}N_2O$	80·4	5·7	8·9	80·1	5·9	8·8

Table 2. INFLUENCE OF VARIOUS CONCENTRATIONS OF 1-BENZYL-2-(α-HYDROXYBENZYL)-BENZIMIDAZOLE ON THE ONSET OF CYTOPATHIC EFFECTS IN POLIOVIRUS-INFECTED *ERK* CELLS

Virus type	TCD_{50} units per ml.	Average time (to nearest 0·25 day) between inoculation of cultures and 50 per cent cell degeneration at 37°: Infected controls	HBB (100 μM)	1-Benzyl-HBB 25 μM	30 μM	40 μM	50 μM
1	$10^{6\cdot5}$	0·75	1·0		1·25	1·5	3·5
	$10^{5\cdot5}$	1·0	1·25		3·25	4·0	> 5·0
	$10^{4\cdot5}$	1·5	1·75		4·0	> 5·0	> 5·0
2	$10^{6\cdot5}$	0·75	1·25	2·75	3·5		> 5·0
	$10^{5\cdot5}$	1·25	2·5	4·0	> 5·0		> 5·0
	$10^{4\cdot5}$	1·75	3·75	> 5·0	> 5·0		> 5·0
3	$10^{6\cdot5}$	0·75	1·0		1·25	1·25	1·75
	$10^{5\cdot5}$	1·25	1·5		1·75	2·5	3·0
	$10^{4\cdot5}$	1·75	2·0		2·5	3·5	4·25

Examination of culture tubes ceased on the fifth day and uninfected control cultures were always still healthy on this day.

o-Chloronitrobenzene was condensed with pentylamine and with isopropylamine by the method previously described[1]. *N*-Benzyl-*o*-nitroaniline, m.p. 73°–74°, was prepared in 91 per cent yield by heating *o*-chloronitrobenzene (1 mole) with benzylamine (2·5 moles) at 150° for 5 h, cooling, extracting with water, and crystallizing the residue from ethanol. In each case the substituted *o*-nitroaniline (1 mole) was reduced by hydrogenation and the diamine immediately condensed with mandelic acid (1 mole) in M hydrochloric acid (2·5 moles), following previous procedure[1]. 1-Pentyl- and 1-isopropyl-2-(α-hydroxybenzyl)-benzimidazoles were obtained as white needles and the 1-benzyl derivative as white prisms from aqueous methanol. Yields from this synthesis in Table 1 and in earlier publications[1,2] are based on the substituted *o*-nitroaniline.

Preliminary testing was carried out by measuring the delay in onset of cytopathic change in *ERK* cells after infection with one of the type 1 (*L Sc 2 ab*), type 2 (*P*

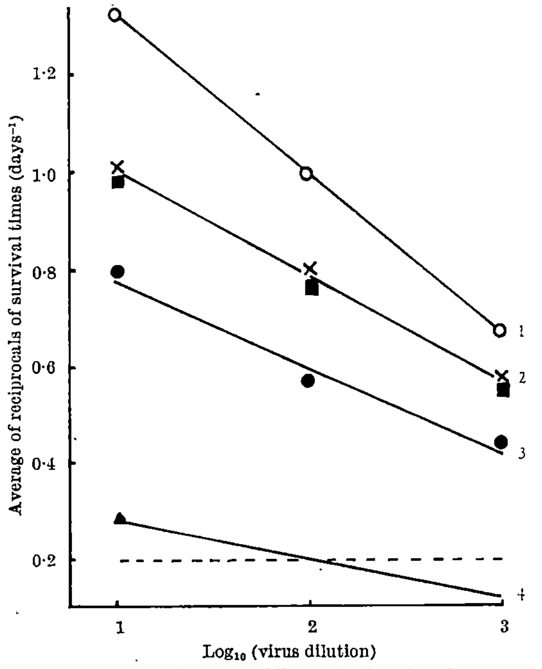


Fig. 1. Lines obtained in assay of the protection given by compounds against cytopathic effects of poliovirus type 1. (1) Control line (test-compounds absent) (○); (2) HBB (100 μM) (×) and also 1-isopropyl-HBB (40 μM) (■); (3) 1-Pentyl-HBB (40 μM) (●); (4) 1-Benzyl-HBB (50 μM) (▲). Examination of cells was terminated on the fifth day after infection. Virus concentration (before dilution) ~$10^{7\cdot5}$ TCD_{50} units per ml.

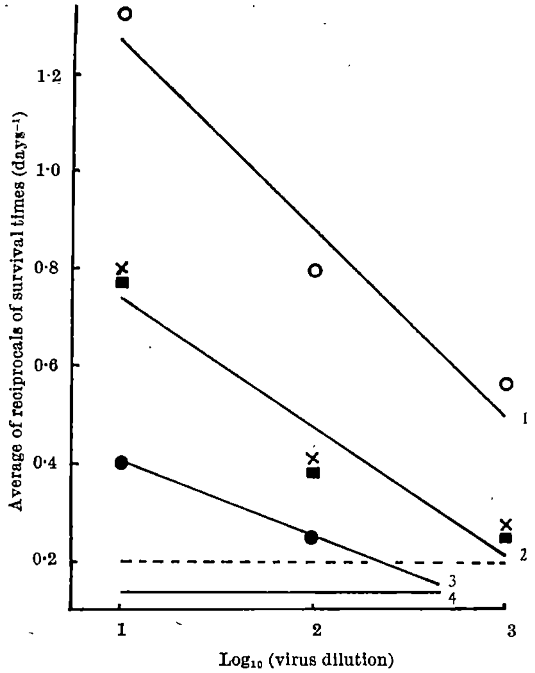


Fig. 2. Protection against cytopathic effects of poliovirus type 2. Legend to Fig. 1 applies, but refers to the type 2 virus

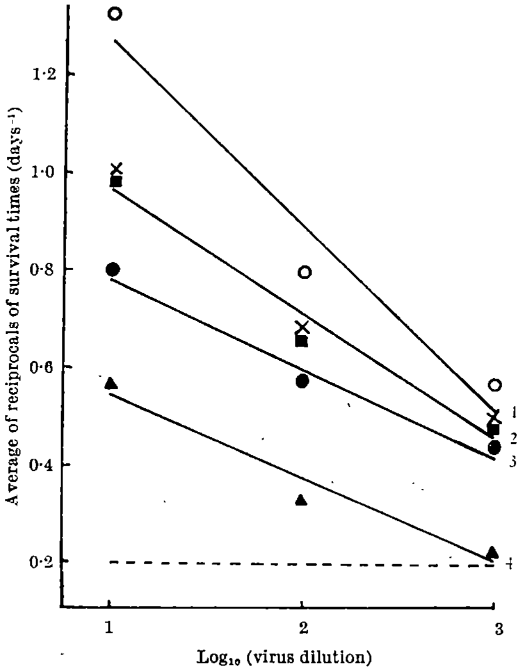


Fig. 3. Protection against cytopathic effects of poliovirus type 3. Legend to Fig. 1 applies, but refers to the type 3 virus

712, *Ch*, 2 *ab*), and type 3 (*Leon* 12 *ab*) viruses using the method previously outlined[1,2]. Results are summarized in Figs. 1, 2 and 3, where protections given by 40 μM 1-isopropyl (line 2), 40 μM 1-pentyl (line 3) and 50 μM 1-benzyl (line 4) derivatives are compared with that given by 100 μM DL-HBB and with infected controls. Plotted points lie on curves, but the functions may be considered to be roughly linear over a restricted range of dilutions. Recent experiments show that 100 μM is the maximum concentration of each of the three 1-substituted compounds that the cells will tolerate and that 210 μM is the maximum tolerated concentration of DL-HBB. Consequently, the relative effects shown in Figs. 1, 2 and 3 need very slight adjustment. The compounds possess no poliovirus-inactivating properties outside the cells. Virus-inhibition and other results will be published elsewhere in due course.

Results presented in Figs. 1, 2 and 3 and the data given in Table 2 indicate that 1-benzyl-2-(α-hydroxybenzyl)-benzimidazole has outstanding performance. The selective protection that it gives to *ERK* cells, against the cytopathic effects of types 1, 2 and 3 poliovirus, approaches that given by the 1-propyl derivative of HBB (PHBB)[1]. The 1-pentyl derivative, while less impressive, still provides noteworthy protection against the types 1, 2 and 3 viruses, while the 1-isopropyl derivative has activity of a similar order to that of DL-HBB itself. We hope to gain more information from study of the 1-isobutyl, 1-phenyl and 1-(α-hydroxybenzyl) derivatives of HBB.

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Effect of 5-Fluorouracil on Antigenic Properties of Tobacco Mosaic Virus

Incorporation of 5-fluorouracil (FU) into TMV particles is claimed to exert a number of biological effects. Although the ability to initiate local lesions by equal quantities of normal and FU substituted virus is not significantly different, less virus is produced in a systemic host when substituted TMV is used as the inoculum[1]. Another aspect of FU incorporation is the increased sensitivity of substituted virus towards ultra-violet irradiation[2]. In the last case at least, the biological response could be traced to the altered nucleic acid component of the virus, in analogy to biological systems of higher organization[3,4].

On the other hand, when the protein coat of the FU substituted TMV was analysed, no alterations in the composition of amino-acids could be detected[5]. However, there are no reports on the possible effect of FU on the sequence of amino-acids in the TMV protein, which would have greater bearing on the biological response of such viruses. That such a possibility should be expected could be deduced from work with bacteria[6], where FU, which is known to enter primarily messenger RNA, radically changes the quality of enzymes produced. In this respect a useful criterion regarding the nature of the protein coat of FU–TMV could be the determination of its antigenic properties.

We have already recorded[7] decreased serological activity of partially purified extracts obtained from TMV-infected, FU-treated plant material. In order to determine whether this was solely due to decreased virus multiplication, or also due to altered antigenicity of virus particles, in this report serological activities of standardized preparations of normal and FU-containing virus were examined.

To propagate TMV (isolate 'Mostar'), leaves of *Nicotiana tabacum* var. 'White Barley' were used. Incorporation of FU into virus particles has been described previously[2]. Virus preparations were purified by differential centrifugation and standardized in their concentrations. Two FU containing virus preparations were used, and a non-substituted preparation served as control. Serological micro-reactions were performed in a drop on a microscope

Table 1. SEROLOGICAL ACTIVITIES OF NORMAL AND FU SUBSTITUTED VIRUSES

Virus preparation	Type of micro-serological reaction	Serial dilutions of virus suspensions									
		1	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512
I TMV—FU	On microscopic slide	+	+	+	+	+	+	+	−	−	−
	Under paraffin oil	+	+	+	+	+	+	+	+	−	−
II TMV—FU	On microscopic slide	+	+	+	+	+	+	+	−	−	−
	Under paraffin oil	+	+	+	+	+	+	+	+	−	−
TMV control	On microscopic slide	+	+	+	+	+	+	+	−	−	−
	Under paraffin oil	+	+	+	+	+	+	+	+	−	−

slide, according to the method of Limasset and Augier de Montgremier[8] and Limasset[9], and in a drop under paraffin oil, according to the method of Slogteren[10]. The latter method is characterized by a specific precipitate formed in the drop at the bottom of a Petri dish.

To follow serological reactivity, virus preparations were serially diluted 1 : 2, up to a total dilution of 1 : 1,024. For serological analyses, TMV antiserum, produced in the Laboratory for Flower Bulb Research (Lisse, Holland), was used. From that antiserum, non-specific antibodies were removed by absorption. For controls, normal serum or physiological saline was used.

In Table 1 it can be seen that positive reactions have been obtained up to a dilution 1 : 64, using the first method. No differences between the two substituted virus preparations were evident, and reactivity is the same as in control preparations. The paraffin-oil drop method proved to be more sensitive, and positive reactions were obtained with the same virus preparations with dilutions up to 1 : 128. Again, as may be deduced from Table 1, viruses containing FU showed the same reactivity as control preparations.

These findings, therefore, argue against an effect which FU incorporation might have on the antigenic properties of the TMV coat protein. However, it is still necessary to determine whether the protein coat in the two types of TMV may still not differ in some subtle property.

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PSYCHOLOGY

Ageing and Noise in the Visual System

THE differential visual thresholds of a group of children aged 8–10 years and a group of adults aged 18–22 years have been compared using a technique similar to that used by Gregory and Kendon[1]. Subjects were asked to vary the intensity of a test spot 3 mm in diameter in a field 100 mm long by 25 mm high at a distance of 2·5 m. A series of eight different intensities of the background field was used and each subject was given one series with the background intensity increasing and one series decreasing. The results of these experiments for the two groups of ten subjects are shown in Fig. 1 by plotting ΔI against I, where ΔI is the difference between the intensity of the background and of the test spot for the spot to be correctly located and I is the intensity of the background field. The results of trials with the background intensity increasing and decreasing are pooled, the justification for this being that the same result is achieved by plotting the results from the two series of trials separately. It will be seen that at every level of illumination used the adults had a lower threshold than the children.

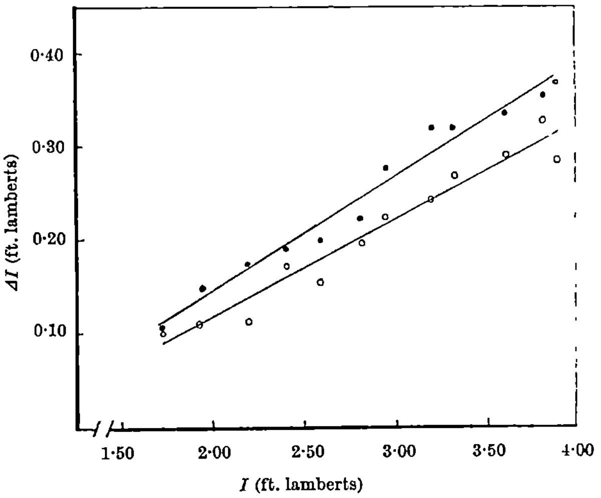

Fig. 1. Data for differential visual thresholds for adults and children. The best fitting line for the two sets of data was found using the method of least squares. ●, Children; ○, adults

It is of interest to compare these results with those of a similar experiment by Gregory and Kendon[1] in which a progressive rise in the slope of ΔI against I was found with age from the 20s to the 70s. Our child subjects seem to have achieved similar results to Gregory and Kendon's older subjects: if we put the two sets of results together it appears that the overall age trend is not progressive but shows a reversal in young adulthood.

It has become customary to attribute such threshold changes with ageing to an increase in neural noise as a person grows older. The treatment of vision as an information source and noisy channel is derived from signal detection theory and when applied to ageing holds that the signal-to-noise ratio diminishes with age. Such an explanation is consistent with two further findings:

(*a*) EEG records of older people tend to show a lowering of general levels of activity and slowing of the dominant rhythms, but some relative rise of what appears to be fairly fast random activity.

(*b*) CFF falls from adulthood onwards. Flicker fusion in these terms can be thought of as occurring at the point at which the difference between peak and trough of illumination is too small to be distinguished. This point will depend on the signal-to-noise ratio in the sense that when this is low the distinction between peak and trough will become blurred. Weale[2] has shown that some, perhaps most, of the fall of CFF during middle and old age can be attributed to alteration of light reaching the retina, that is, to lower signal strength, due to peripheral changes in the eye, but some seem clearly to be due to other causes and may reflect a fall of signal strength or a rise of noise-level in central mechanisms.

These EEG and CFF findings are also true of children who tend to show more fast EEG activity and lower CFF than young adults.

The reasons for decline of signal-to-noise ratio in older people are commonly assumed to be the loss of functional cells which occurs progressively throughout life. The loss would tend to lower signal strength while the lower number of brain cells would mean that there was less averaging of random activity and thus, in effect, a rise of noise-level. It has also been suggested that shortly before destruction nerve cells tend to show an increase of spontaneous activity, which would constitute a form of noise in the nervous system.

Clearly these explanations cannot apply to children. Any explanation of the similarity between older adults and children in this matter must be speculative, but a possible reason might be that, as suggested by Hebb, there is an increased patterning during childhood of the connexions between nerve cells, and that this patterning results in an effective increase in the number of discrete functional units.

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[1] Gregory, R. L., *Proc. Fourth Congr. Intern. Assoc. Gerontol.*, Merano, 1957, 1, 134 (1959). Welfort, A. T., *Ageing and Human Skill*, 158 (Oxf. Univ. Press for Nuffield Found., 1958).

[2] Weale, R. A., in *Behavior, Ageing and the Nervous System*, edit. by Welford, A. T., and Birren, James E. (Charles C. Thomas, Springfield, Illinois; in the press).

Falling Efficiency at Sorting Cards during Acclimatization at 19,000 ft.

DURING the course of the Himalayan Scientific and Mountaineering Expedition 1960–61 (ref. 1) the members sorted cards three times, a practice at a height not much above sea-level, and then two tests at 19,000 ft. Before the first test the average stay at 19,000 ft. had been 2 weeks, with an additional 6 weeks at 15,000 ft. Between the two tests an additional average of 9 weeks had been spent at 19,000 ft., plus 2 weeks at 15,000 ft. Adequate data were available for only three members of the expedition, whose ages ranged from 23 to 32 years. A comparable group of eleven research scientists from the Applied Psychology Research Unit was therefore collected, covering the same range of ages and academic experience. These men sorted cards with the same card-sorter three times in Cambridge, separated by the same average time-intervals. Table 1 shows that the number of delayed responses increased after acclimatization at 19,000 ft., whereas it fell slightly at about sea-level.

Table 1

Height above sea-level (ft.)		Age (years)	Mean percentage of delayed responses		
			Test 1	Test 2	Difference
19,000	J. B. W.	32	2·66	2·87	0·21
	J. S. M.	30	2·86	4·95	2·09
	M. B. G.	23	2·04	3·48	1·44
	Mean of 3 men	28	2·52	3·77	1·25*
< 50	Mean of 11 men	30	3·07	2·83	−0·24*

* Two groups reliably different ($P < 0.05$ on a two-tailed Mann–Whitney U test (ref. 3)).

Each man had to sort packs of cards continuously for 30 min into boxes according to suit. The time at which each card fell into a box was recorded automatically on magnetic tape. A fuller description of the apparatus is given in Poulton *et al.*[2]. The errors of classification, which averaged about 4 per test in the two tests both at 19,000 ft. and at about sea-level, were recorded by the experimenter. To be counted as delayed, the recorded time-interval between a response and the previous response had to exceed 2·0 sec. The increase in the percentage of delayed responses at 19,000 ft. was reliably greater than the change in the control group at sea-level; a difference in mean rank order as large as that between the two groups would have occurred by chance less often than once in 20 experiments[3].

So far as we know this is the first time that a statistically reliable change in performance has been reported in men during acclimatization at such heights. That the change was for the worse is compatible with the observation that "all members of the party lost weight at a rate of 1–3 lb. (0·45–1·36 kg) a week at 19,000 ft. (5,790 m)" (ref. 1). Previous work on acclimatization to height has simply compared the performance of men at different heights up to 17,500 ft. after they had become acclimatized to that height[4,5].

The tape-recordings from the experimental and control groups were made in different places at different times, and any discrepancies which could have been produced in this way inevitably confound the differences reported between the two groups. By comparing the differences for each individual between tests 1 and 2, this criticism has been taken care of as far as it is possible to do so, since any constant added to the results of both tests 1 and 2 will have been eliminated. But the effect of a constant multiplier, if present, has not been eliminated. The possibility of an artefact is difficult to rule out completely when comparing the course of acclimatization in a remote but natural environment with the effect of time alone on a control group.

A second task, number checking, showed no reliable change in performance over that of the control group until a height of 24,400 ft. was reached. This was presumably due to the insensitivity of the task[6], since reliable changes in performance on many tasks are recorded at heights above 10,000–15,000 ft.[7].

This work was carried out under the direction of Dr. L. G. C. E. Pugh and Mr. D. E. Broadbent. We also thank Dr. J. A. Leonard for help in designing the card sorter, the members of the Expedition and of the Applied Psychology Research Unit for acting as experimental subjects, and the Medical Research Council for financial support.

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[1] Pugh, L. G. C. E., *Brit. Med. J.*, 621 (1962).

[2] Poulton, E. C., Catton, M. J., and Carpenter, A., *Brit. J. Indust. Med.*, 21 (in the press).

[3] Siegel, S., *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956).

[4] MacFarland, R. A., *J. Comp. Psychol.*, **24**, 189 (1937).

[5] Tichaeur, E. R., *Ergonomics*, **6**, 51 (1963).

[6] Poulton, E. C., *Ergonomics*, **7** (in the press).

[7] Morgan, C. T., Cook, J. S., Chapanis, A., and Lund, M. W., *Human Engineering Guide to Equipment Design* (McGraw-Hill, New York, 1963).

FORTHCOMING EVENTS

Tuesday, July 28

Society for Analytical Chemistry, Physical Methods Group (joint meeting with the Polarographic Society, at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.30 p.m.—Dr. Dale J. Fisher (Oak Ridge National Laboratory, Tennessee): "Controlled Potential D.C. Polarography".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Officer (male) for work on helminths of economically important marine fish—The Director, Commonwealth Bureau of Helminthology, The White House, 103 St. Peter's Street, St. Albans, Hertfordshire (July 30).

Lecturer (botanist specializing in biochemistry or biochemist) in Botany—The Registrar, The University, Manchester 13 (July 31).

Lecturer (with a good honours degree, and teaching and/or research experience in one or more of the fields of general, social, occupational or experimental psychology) in Psychology in the Faculty of Social Studies, University of Nigeria, Nsukka, Eastern Nigeria—The London Representative, University of Nigeria, 33 Craven Terrace, London, W.2 (July 31).

Mathematician or Scientist (not under 26 years of age, with at least a second-class honours degree or equivalent, and three or more years' postgraduate research experience) in the Radiobiological Protection Division of the United Kingdom Atomic Energy Authority Health and Safety Branch, to apply mathematical techniques to biological problems—The Administration Officer, U.K.A.E.A., Health and Safety Branch, Harwell, Didcot, Berkshire, quoting Ref. H.35/J14 (July 31).

Research Assistant and a Research Student in the Department of Medical Physics, to work on the absorption of ionizing particles in liquids—Prof. H. A. B. Simons, Royal Free Hospital School of Medicine (University of London), 8 Hunter Street, London, W.C.1 (July 31).

Research Assistant (Computer Programming) (with a university degree with mathematics as either a main or subsidiary subject) in Statistical Ecology in the Department of Botany, for work concerned with the preparation and running of computer programmes for the statistical analysis of vegetation by multivariate methods—The Secretary and Registrar, The University, Southampton (July 31).

Assistant Lecturer, Grade "B" (qualified pharmacist, biochemist or biologist) in Applied Biology in the Department of Science—The Principal, Coventry Technical College, Butts, Coventry, marking envelope "application" (August 1).

Research Assistant (with an honours degree in physics or equivalent qualification and some research experience) in Physics to work on nuclear magnetic resonance in liquid metals and alloys—The Registrar, The University, Sheffield (August 1).

Theoretical Physical Chemist (honours graduate, preferably with Ph.D., and previous experience in the mathematical treatment of physicochemical systems), to study the theoretical aspects of the micropore structure/gas absorption of porous carbons—The Assistant Secretary, Ref. A.5, The British Coal Utilization Research Association, Leatherhead, Surrey (August 1).

Departmental Graduate Assistant in the Department of Physics to work in the field of geophysical hydrodynamics and magneto-hydrodynamics—The Registrar, The University, Newcastle on Tyne, 2 (August 7).

Lecturer or Senior Lecturer (with a higher degree in zoology, evidence of competence and interest in research in a field of vertebrate zoology (preferably ecological or behavioural studies), and experience in university teaching) in Zoology at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Brisbane and London, August 7).

Assistant Lecturer or Lecturer in the Department of Genetics to teach the genetics of micro-organisms—The Registrar, The University, Liverpool, quoting Ref. CV/24 (August 8).

Assistant Lecturer (with an honours degree in pharmacy and preferably specialized in pharmacognosy, and an interest in phytochemistry) in Pharmacy—The Registrar, The University, Manchester, 13 (August 8).

Lecturer in Biochemistry—The Secretary, Royal Free Hospital School of Medicine (University of London), 8 Hunter Street, London, W.C.1 (August 8).

Principal Electronics Engineer (physicist/engineer) to take charge of part of the programme of development of fishing vessels and their equipment—The Technical Director, White Fish Authority, Industrial Development Unit, Saint Andrew's Dock, Hull, East Yorkshire (August 8).

Research Fellow (with at least a Ph.D. degree in chemistry or biochemistry) in the Division of Forest Products, Commonwealth Scientific and Industrial Research Organization, South Melbourne, Victoria, Australia, to participate in a programme concerned with the polyphenolic extractives and exudates of wood, their influence on the properties of wood, and also with the various factors controlling the formation of these substances by the tree—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 290/697 (August 8).

Lecturer in Fuel Technology and Chemical Engineering—The Registrar, The University, Sheffield (August 10).

Lecturer in Sociology or Anthropology; and a Lecturer in Social Psychology at Makerere University College, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (August 14).

Physicist (with a good degree and interest in agricultural meteorology and preferably some research experience) for work on water use by plants and the effects of light on plant growth—The Secretary, Rothamsted Experimental Station, Harpenden, Hertfordshire (August 15).

Research Fellow (with a Ph.D. in metallurgy or physics or equivalent qualifications together with some research experience in physical metallurgy or metal physics, and preferably experience with modern electron microscopic or X-ray crystallographic methods) in a newly established D.S.I.R. Research Group on the structure and properties of alloy steels, under the direction of Professor of Physical Metallurgy—The Registrar, The University, Sheffield (August 15).

Tutorial Assistant; and Research Assistants (2) in the Department of Geology—The Registrar, University College of Wales, Aberystwyth (August 15).

Lecturer (Clinical) in the Department of Pathology—The Assistant Registrar, Medical School, The University, Birmingham, 15 (August 17).

M. C. Smith Lecturer in Pathology (Cancer Research)—The Assistant Registrar, Medical School, The University, Birmingham, 15 (August 17).

Senior Lecturer in Civil Engineering—The Registrar, The University, Sheffield (August 17).

Assistant Lecturer or Lecturer in Biochemistry—The Dean, Guy's Hospital Medical School, London Bridge, London, S.E.1 (August 18).

Reader (with a record of scholarship in the field of experimental psychology) in Psychology—The Registrar, The University, Hull (August 22).

D.S.I.R. Research Assistant (physical chemist preferably with research experience of reaction kinetics and/or solid state measurements), to work on a research project concerned with the solid state properties of carbon in relation to catalytic activity—The Secretary, Queen's College (University of St. Andrews), Dundee, Scotland (August 24).

Lecturer in Physics at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 28).

Assistant Librarians (2) (graduates with university library experience and/or professional qualifications)—The Registrar, The University, Sheffield (August 29).

Assistant Keeper (with some training and an interest in taxonomy, preferably in that of cryptogamic plants) in Botany at the Manchester Museum—The Registrar, The University, Manchester, 13 (August 31).

Senior Research Officer (with a special knowledge, preferably with research experience, in some branch of numerical or non-numerical analysis)—The Director, University Computing Laboratory, 9 South Parks Road, Oxford (August 31).

Public Analyst (Fellow of the Royal Institute of Chemistry with the Branch E Certificate or Diploma) for the City of Southampton—The Town Clerk, Civic Centre, Southampton (September 1).

Lecturers/Senior Lecturers (2) (graduates in medicine, science or veterinary science of honours standard or equivalent, with appropriate teaching and research experience in physiology) in the School of Physiology, The University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (September 4).

Senior Lecturer or Lecturer in Microbial or Molecular Genetics; and a Senior Lecturer or Lecturer in Comparative or Cellular Physiology in the Department of Zoology, University of Melbourne—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, University of Melbourne, Parkville, N.2, Victoria, Australia (Australia, September 15).

University Lecturer (with evidence of ability and experience in teaching or research in animal nutrition, and preferably an interest in ruminant nutrition) in Animal Nutrition—The Secretary, University of Cambridge School of Agriculture, Cambridge (September 15).

Chair of Biochemistry—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, 2 (September 26).

Second Chair of Physics at the University of Canterbury, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and New Zealand, September 30).

Chair of Electrical Engineering—The Secretary, The Queen's University, Belfast, Northern Ireland (October 10).

Chair of Organic Chemistry at the University of Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.7 (Australia, October 31).

Assistant Lecturer (suitably qualified metallurgist or mechanical engineer, preferably with research experience) in the Metallurgy Department—Prof. A. V. Bradshaw, Royal School of Mines, Prince Consort Road, London, S.W.7.

Assistant Lecturer (with qualifications in physics, chemistry and chemical engineering together with three years' research experience) in the Department of Chemical Engineering and Chemical Technology, to work in the field of transport properties at high pressures—Head of the Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London, S.W.7.

Gassiot Research Fellows at the Meteorological Office, one for research in seismology and the other for research in geomagnetism—The Secretary, Meteorological Office, London Road, Bracknell, Berkshire.

Head (with high academic qualifications and a substantial combination of teaching, research and industrial experience) of the Department of Chemistry—The Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3.

Immunologist (national of the United Kingdom or the Republic of Ireland, with a good honours degree in zoology or microbiology or a registrable qualification in veterinary science, and some laboratory experience preferably in the immunological field) at the Veterinary Research Laboratories, Kabete, Kenya, to investigate problems of artificial immunity to trypanosomiasis in livestock; duties may also include studies on the bionomics of the vector tsetse flies—The Department of Technical Co-operation, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC217/95/07.

Junior Technical Officer (with at least G.C.E. at "A" level in two science subjects, including one in a biological subject, if below the age of 22, or a pass degree for age 22 and over) in a Group working on mouse genetics—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berkshire, quoting Ref. MFL/3.

Lecturer in the Department of Metallurgy to lecture and to carry out research in physical metallurgy—The Registrar, The University, Liverpool, quoting Ref. CV/242/N (September 19).

Lecturer/Assistant Lecturer in Chemical Engineering, to take a particular interest in process control—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST 64/73.

Lecturer in Physical Chemistry for duties in the B.Sc. Special Chemistry and B.Sc. General courses—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

Lecturer in the Applied Physics Section to teach general physics and research in photoelectronics, vacuum physics, etc.—Prof. J. D. McGee, Physics Department, Imperial College of Science and Technology, London, S.W.7.

Lecturer or Assistant Lecturer in Geography—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

Marine Biologist (with an honours degree in natural science, preferably in biology or zoology, and one year's postgraduate experience in appropriate research work, preferably in fisheries research or work of value in marine biology) at Hong Kong, to investigate and control invertebrates and minute organisms of marine life and their ancillary problems which would normally arise as a result of the changeover of a salt-water lake to fresh-water—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC.289/81/01.

MUSEUM TECHNICIAN (man or woman with experience in the preparation and arrangement of zoological material for museum display)—The Professor of Zoology, The University, Hull.

POST-DOCTORAL FELLOW for work on the chemistry of epimino-sugars—Dr. R. D. Guthrie, The Chemical Laboratory, University of Sussex, Brighton, Sussex.

RESEARCH ASSISTANT (recent graduate or graduating this year Hons.B.Sc, in biochemistry, physiology or veterinary science) to Dr. W. J. O'Connor. Department of Physiology, to study water and sodium balance of dogs—Dr. W. J. O'Connor, Reader in Renal Physiology, Department of Physiology, Medical School, The University, Leeds, 2.

RESEARCH OFFICER (Entomologist) (national of, and permanently resident in, Great Britain or the Republic of Ireland, with an honours degree in biology and appropriate postgraduate experience) with the East African Common Services Organization, to plan and carry out research on the biology of Malaria-carrying mosquitoes in East Africa, and to direct field and Laboratory work of an established entomological research team—The Department of Technical Co-operation, Room 404, Eland House, Stag Place, Victoria, London, S.W.1, quoting Ref. No. RC205/173/02.

RESEARCH STUDENTS (2) (with a good honours degree in geology) IN THE DEPARTMENT OF GEOLOGY—Head of the Department of Geology, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

SENIOR CHEMIST in the Division of Biochemistry, The Laboratories, Belfast City Hospital, for duties which will include the supervision of routine investigations and the development of new analytical procedures—The Group Secretary, South Belfast Hospital Management Committee, Belfast City Hospital, Belfast 9, Northern Ireland.

VERTEBRATE PALAEONTOLOGIST IN THE DEPARTMENTS OF GEOLOGY AND ZOOLOGY with duties in teaching and research—Head of either the Department of Geology or the Department of Zoology, University of Alberta, Edmonton, Alberta, Canada.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 57: Have Eggs of First Quality. Pp. 7. Leaflet No. 80: Hoose (or 'Husk'). Pp. 4. Leaflet No. 138: Separated Milk and Milk Powder for Stock Feeding. Pp. 4. (Belfast: Ministry of Agriculture, 1964.) [225

Biochemistry: The Study of the Molecular Basis of Life. By Prof. Edwin A. Dawes. (Inaugural Lecture delivered in the University of Hull on 7 February 1964.) Pp. 31. (Hull: The University, 1964.) 4*s*. net. [225

Nuclear Power for Ship Propulsion: Report of the Working Group on Marine Reactor Research. Pp. 22. (Cmnd. 2358). (London: H.M. Stationery Office, 1964.) 1*s*. 6*d*. net. [225

University of Cambridge: Department of Engineering. Report of the Head of the Department for the year 1962–63. Pp. 14. (Cambridge: The University, 1964.) [225

Third Annual Report of the Furniture Industry Research Association, 1963. Pp. 16. (Stevenage: The Furniture Industry Research Association, 1964.) [225

General Register Office. Census 1961: England and Wales. County Report—Herefordshire. Pp. xxiii+61. 15*s*. net. County Report—West Suffolk. Pp. xxiii+55. 14*s*. net. (London: H.M. Stationery Office, 1964.) [225

Department of Scientific and Industrial Research. A Select List of Medical Serials held in the N.L.L. Pp. ii+72. (Walton, Boston Spa: The National Lending Library for Science and Technology, 1964.) [225

Meteorological Office. Weather in the Mediterranean, Vol. 2. Second edition. Pp. viii+372. (M.O. 391b.) (London: H.M. Stationery Office, 1964.) [225

Bee Research Association. Save Our Pollinating Insects. Pp. 6. (Chalfont Heights, Gerrards Cross: Bee Research Association, 1964.) [225

Chelsea College of Science and Technology. Prospectus, 1964–65. Pp. 40. (London: Chelsea College of Science and Technology, 1964.) [225

The Institution of Gas Engineers. Publication 653: Natural Gas in the Netherlands. By J. G. de Voogd. Pp. 22. Publication 654: Gasification of Liquid Hydrocarbons. By Prof. Carlo Padovani. Pp. 12. Publication 655: Experiences in Oil Gasification at Bilston. By C. L. Maxey. Pp. 19. Publication 656: Economic and Statistical Research—Some Applications in the Gas Industry. By George Polanyi and H. G. Berrisford. Pp. 20. Publication 657: The Development of Gas Production in the South West. By G. F. Oliver and Dr. M. J. F. Olden. Pp. 29. Publication 658: All Change! By H. Teasdale. Pp. 11. Publication 659: Fire Protection of Liquid Petroleum Gas Storage Tanks. By G. A. Bray. Pp. 9. Publication 660: Official Programme, 101st Annual General Meeting, Blackpool, 1st to 5th June, 1964. Pp. 12. (London: The Institution of Gas Engineers, 1964.) [225

Royal Observatory Bulletins. No. 81: Photographic Zenith Tube: Instrument and Method of Reduction. By D. V. Thomas. Pp. B195–B217. 4*s*. 6*d*. net. No. 83: Time and Latitude Service 1963, July–September. Pp. B221–B240. 3*s*. 6*d*. net. (London: H.M. Stationery Office, 1964.) [225

Filmstrip: Some Experimental Evidence for Atomic Structure. By E. W. Moore and A. Green. 32 frames (in colour). Notes. Pp. 34. (Surbiton: Marian Ray, 36 Villiers Avenue, 1964.) Film and notes 30*s*. [225

Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1043-E: Recognition Criteria of Igneous and Metamorphic Rocks on Aerial Photographs of Chichagof and Kruzof Islands, Southeastern Alaska. By J. S. Pomeroy. Pp. iv+87+110. Bulletin 1142-L: Geology of the Tintic Junction Quadrangle, Tooele, Juab, and Utah Counties, Utah. By Hal T. Morris. Pp. iii+23+plate 1. Bulletin 1154: Geology and Ore Deposits of the Bokan Mountain Uranium–Thorium Area, Southeastern Alaska. By E. M. MacKevett, Jr. Pp. vi+125+plates 1–5. Bulletin 1180-B: The Helderberg Group and the Position of the Silurian–Devonian Boundary in North America. By Jean M. Berdan. Pp. iii+19. Bulletin 1182-D: Relation Between Specific Gravity and Iron Content of Rocks from the Red Mountain Formation, Alabama. By Richard P. Sheldon. Pp. iii+17. (Washington, D.C.: Government Printing Office, 1963 and 1964.) [16

United States Department of the Interior: Geological Survey. Water-Supply Paper 1419: Geological and Hydrologic Features of the San Bernardino Area, California, with special reference to Underflow Across the San Jacinto Fault. By L. C. Dutcher and A. A. Garrett. Pp. vi+114+plates 1–19. Water-Supply Paper 1499-F: Water Resources of the Baltimore Area, Maryland. By E. G. Otton, R. O. R. Martin and W. H. Durum. Pp. vi+105+plates 1–3. Water-Supply Paper 1574: Quality of Surface Waters of the United States, 1958. Parts 9–14: Colorado River Basin to Pacific Slope Basins in Oregon and Lower Columbia River Basin. Prepared under the direction of S. K. Love. Pp. xiii+487. Water-Supply Paper 1597: Geology and Ground-Water Resources of the Upper Grande Ronde River Basin, Union County, Oregon. By E. R. Hampton and S. G. Brown. Pp. v+99+plates 1–6. Water-Supply Paper 1606: Geology and Ground-Water Resources of Montgomery County, Alabama. By D. B. Knowles, H. L. Reade, Jr., and J. C. Scott. Pp. v+76+plates 1–15. Water-Supply Paper 1562-A: Floods of January–February 1957 in Southeastern Kentucky and Adjacent Areas. Pp. iv+195. (Washington, D.C.: Government Printing Office, 1963 and 1964.) [16

United States Department of the Interior: Geological Survey. Water-Supply Paper 1659: An Appraisal of Potential Ground-Water Salvage Along the Pecos River between Acme and Artesia, New Mexico. By R. W. Mower, J. W. Hood, R. L. Cushman, R. L. Borton and S. E. Galloway. Pp. viii+98+plates 1–10. Water-supply Paper 1667-B: Deposition and Solution of Manganese Oxides. By John D. Hem. Pp. iv+42. Water-Supply Paper 1669-V. Ground Water in the Lower Arkansas River Valley, Arkansas. By M. S. Bedinger and H. G. Jeffrey. Pp. iii+17+plates 1–6. Water-Supply Paper 1669-W: The Yield of Sedimentary Aquifera of the Coastal Plain, Southeast River Basins. By Joseph T. Callahan. Pp. iv+56+plate 1. Water-Supply Paper 1688: Magnitude and Frequency of Floods in the United States, Part 13: Snake River Basin. By C. A. Thomas, H. C. Broom and J. E. Cummans. Pp. xi+250+plates 1–5. Water-Supply Paper 1752: Ground-Water Resources of North-Central Connecticut. By Robert V. Cushman. Pp. v+96+plates 1–3. Water-Supply Paper 1757-B: Ground-Water Resources of the Bengasi Area, Cyrenaica, United Kingdom of Libya. By W. W. Doyel and F. J. Maguire. Pp. iii+21. Water Supply Paper 1757-G: Ground Water in the Sirte Area, Tripolitania, United Kingdom of Libya. By William Ogilbee. Pp. iii+14. Water-Supply Paper 1779-R: Snowmelt Hydrology of a Sierra Nevada Stream. By S. E. Rantz. Pp. iv+36. (Washington, D.C.: Government Printing Office, 1963 and 1964). [16

United States Department of the Interior: Geological Survey. Professional Paper 385: Geology and Mineral Deposits of the Mount Morrison Quadrangle. Sierra Nevada, California. By C. Dean Rinehart and Donald C. Ross. Pp. v+106+plates 1–7. Professional Paper 419: Miocene Marine Mollusks from the Astoria Formation in Oregon. By Ellen J. Moore. Pp. iv+109+plates 1–33. Professional Paper 422-A: Morphology and Hydrology of a Glacial Stream—White River, Mount Rainier, Washington. By Robert K. Fahnestock. Pp. iv+70. Professional Paper 427-A: Description of Physical Environment and of Strip-Mining Operations in Parts of Beaver Creek Basin, Kentucky. By John J. Musser. Pp. iii+25+plates 1–7. Professional Paper 432: Upper Cretaceous (Campanian and Maestrichtian) Ammonites from Southern Alaska. By David L. Jones. Pp. iv+53+plates 1–41. Professional Paper 454-G: Uranium and Helium in the Panhandle Gas Field, Texas. By A. P. Pierce, G. B. Gott and J. W. Mytton. Pp. iv+57+plates 1–3. (Washington, D.C.: Government Printing Office, 1963 and 1964.) [16

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TOWARDS A POLICY FOR TECHNICAL CO-OPERATION

ONE reason given in the Plowden Report for the changes recommended in the organization of the Services representing Britain overseas is the increasing importance of the duties which these Services have now acquired in connexion with giving British aid and the administration of technical assistance abroad. Again, while Prof. P. M. S. Blackett did not suggest that technical aid was a reason for expanding our universities in his Granada Lecture in October 1963, he did point out that such expansion would enable Britain to play a much more vigorous part in assisting the progress and welfare of the new and emerging nations, and he instanced three ways in which Britain could help. Two of these directly concerned the universities—the provision of academic and other professional staff on secondment at request, and the provision of wider training facilities in Britain's universities, especially postgraduate. In recent debates in the House of Lords on the problem of leisure and on the Newsom Report, reference was made to the importance of Voluntary Service Overseas, both from this point of view of technical aid and from that of affording opportunity for expression of the altruism of youth.

In view of this widespread interest in technical aid, both as such and from varying points of view, particular interest attaches to the various publications of the Overseas Development Institute. One such recent publication, *Colonial Development*[*], completes the Institute's factual survey of British aid to developing countries, of which four pamphlets have already appeared. A second recent publication, *Not by Government Alone*[†], reviews the work of voluntary organizations participating in the Development Decade. In a third, *French Aid: the Jeanneney Report*[‡], the Institute provides a convenient abridged translation of the report of the Jeanneney Commission set up by the French Government in 1963 to examine independently the whole problem of French aid to the developing countries, its justification, its aims and its administration.

France at present devotes nearly 2 per cent of her national income to aid for the developing countries, and the total of £350 million in 1962 is more than twice Britain's contribution. Because this figure helps to put the subject in a better perspective and also because this Report on the politics of co-operation is a major recommendation on policy for one of the chief donors of aid and is likely to be treated as a basic document by French Governments for some time to come, it seems useful to consider the January Report first. A small section of it, mainly concerned with administration, was regarded as confidential by the French Government and was not published. For the rest, the Report is a logical and cogently argued document, reviewing first, in an introduction, the conditions of economic development, the possible contributions by developed countries, and the principles of action. Next, in successive chapters, the reasons for aid are analysed, the resources available reviewed, and finally the distribution and forms of aid are considered.

There is much more that is of general interest in the Report, as its proposals are not narrowly conceived and mostly bear consideration by other countries, though it is unlikely that all its suggestions and analyses will command general acceptance. They should, at the least, stimulate some hard thinking about Britain's own programme of aid and the structure and work of the Department of Technical Co-operation. It is recommended, for example, that 25 per cent of French aid should go through multilateral channels, and a critical look at individual proposals is urged: some of the present aid may be expensive to the aiding country but contribute little or nothing to the aided country. Again, developing countries should not be expected to model themselves on the aiding country except in so far as the structures copied favour their development. Nor should aid to countries which are temporarily poor be regarded as a short-term relief. It should form part of a new conception of the economic relations between unequally developed countries, firmly based on contractual arrangements. Even the sacrifices involved in trade adjustments would, in the long run, be less heavy than aid in traditional forms, and they would be more effective for the development of countries whom it is desired to help,

There are some points in the Jeanneney Report which should be noted particularly by scientists in Britain. Prof. Blackett's plea for more help in research is fully supported by the observation that, in science and technology, developed countries can not only help by making their knowledge available to developing countries but also by research in fields that are useful, particularly if the developing countries are associated with such research. No estimate is given of the extent to which either manpower or financial resources at present committed to technical assistance is engaged in research. However, of 46,000 people at present provided by France, nearly 32,000 are teachers and only some 1,000 are regarded as real experts on an international level. Provision of teachers is recognized as one of the most valuable forms of aid, but, nevertheless, the Report insists that in developing countries education must be useful for development: the training offered in the advanced countries should be considered only in the light of the contribution its beneficiaries can make to the development of their country when they return.

A similar pragmatic note is struck in respect of specialists from developed countries in the comment that their duty to prepare for their replacement by local people is less often adequately performed than their contribution to the smooth working of enterprises or public services. There are also firm reminders that if experts of the desired quality are to be recruited for this purpose, proper attention must be directed to arrangements for their remuneration, including not only the scale of salary, etc., but also its security, and to their careers on return to France. It is suggested that French policy should encourage the activities of the numerous societies and institutes in France, particularly those concerned with agricultural and industrial techniques, the work of which can be valuable for developing countries. Reference is made to the openings for consultants; it is suggested that it would be useful to establish a set of rules for correct practices and then to keep a list of consultants prepared to adhere to the rules and capable of doing so.

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[*] Overseas Development Institute, Ltd. British Aid—5: *Colonial Development—a Factual Survey of the Origins and History of British Aid to Developing Countries*. By D. J. Morgan. Pp. 96. (London: The Overseas Development Institute, Ltd., 1964.) 7*s*. 6*d*.

[†] Overseas Development Institute, Ltd. *Not by Governments Alone: The Role of British Non-Government Organizations in the Development Decade*. By Peter Williams and Adrian Moyes. Pp. 51. (London: Overseas Development Institute, Ltd., 1964.) 3*s*. 6*d*.

[‡] Overseas Development Institute, Ltd. *French Aid: The Jeanneney Report*. (An abridged translation of "La Politique de Coopération avec les Pays en Voie de Développement".) Pp. 51. (London: Overseas Development Institute, Ltd., 1964.) 5*s*.

In view of the comments of the Plowden Committee on the work of scientific attachés, it is interesting to note that the Jeanneney Commission believes that scientific representation is generally inadequate in French diplomatic missions in spite of the considerable opportunities in many countries open to scientific attachés. Not only competent to organize exchanges in higher education and research, supervise documentation centres and ensure that more space is given in the foreign Press to French science and technique, the scientific attaché should at least be able to talk on equal terms with the scientists of the country to which he is accredited, and should be closely in touch with travelling French scientists. While the Jeanneney Commission proposes some expansion in the number of scientific attachés and the Plowden Committee thought that for Britain the present number was about right, the two reports agree substantially as to the duties of such attachés. Much like the Plowden Report, the Jeanneney Report contemplates the appointment of some leading research workers as French representatives abroad, for some 3–6 months.

Emphasis is placed on the value of allocating aid funds to the supply of French books to university centres and libraries, and besides suggesting that currency losses on books sold commercially in countries where currency is not convertible should be balanced, it is also proposed that books of a suitable kind and price should be specially prepared for developing countries. This obviously follows the general lines of the scheme already adopted by Britain, but one other feature of the Jeanneney Report that should be welcomed as well as noted is the forthright views expressed on principles of action. While the importance of sincere acceptance of the independence of the developing countries could not be more clearly expressed, it is never suggested that respect for such independence means that the aiding country must ignore what it believes to be wrong in the aided country. The Report is certainly as frank as an earlier Political and Economic Planning Broadsheet on the importance of political stability, and of appropriate political régimes and efficient administration in the aided countries.

This statement of principles merits consideration in any discussion of Britain's programme of technical aid or of the activities of such bodies as the Commonwealth Development Corporation. Avoidance of excessive economic integration, for example, certainly makes it easier to resolve disputes with calmness and objectivity, though it does not necessarily ensure success. The search for well-adapted political structures should be supported and it is welcome to find this condemnation of dogmatic support for particular political forms and reminder that neither capitalism nor communism may be suited to developing countries. Above all, procedures are needed to give protection against the effects of domination, and, in urging efforts to discover such procedures, the Report suggests that as a start States with geographical, historical or cultural affinities might make agreements covering such points as: the distribution among the signatories of the burden and benefits of aid: an undertaking by the rich countries to devote a certain percentage of their material product to co-operation and by the recipient countries to devote a percentage of their own material product to investment; the taxation of foreign enterprises in developing countries; the regulation of labour disputes in foreign enterprises; procedures for avoiding the allocation of aid to investments which would duplicate others in the same part of the world; and the conditions in which countries could nationalize enterprises on their territory, the rules and manner of indemnification.

The enumeration of general principles in such matters might facilitate the building up of more durable relation and should promote the confidence which would promote the most efficient supply and utilization of resources. Something on these lines is clearly needed, and the Jeanneney Report demonstrates the existence of a certain amount of thinking on similar lines in different countries. Similarly, the review of British non-government organizations made by P. Williams and A. Moyes for the Overseas Development Institute shows a surprising range of voluntary organizations participating in the supply of unofficial aid to the developing countries. In scale, this probably reaches more than £15 million a year, apart from the £150 million a year which British private firms are thought to be investing in less-developed countries and probably supports about 6,000 British workers in developing countries. This compares with the present £150 million provided by the British Government in loans, grants, investments and technical assistance to developing countries, mostly within the Commonwealth and covering the salaries, in part or in full, of about 16,000 British experts and teachers in developing countries and awards to about 3,000 students and trainees in Britain.

The review is in two parts: a survey of opportunities which includes some suggestions for realizing them, is followed by a factual account of non-government organizations concerned with helping developing countries. Commercial organizations are not discussed, and an introduction explains the challenge of both Governments and private organizations for constructive thinking thrown down by the resolution in the General Assembly of the United Nations last December. Sponsored by the United Kingdom Government and fourteen others, that resolution called for participation by non-government organizations in a world campaign 1965–70 to help developing countries in the fields of food, health and education. The resolution was adopted unanimously and it represents a continuing challenge to Governments to intensify and expand official aid programmes and a new challenge to non-government organizations to become involved in world development to a greater extent.

Government aid depends on the co-operation of non-government organizations for the provision of a large part of the resources, particularly for technical assistance and also for engendering public understanding and support for the aid programme. The relatively favourable climate of public opinion in Britain towards helping developing countries is largely due to non-government organizations; however, the pamphlet suggests that the variety of organizations and aims—more than a hundred are listed in the pamphlet, but the total runs into hundreds—confuse the public, and it is urged that these non-government bodies should present a clearer image o their pattern and activities to the public. They also need greater resources and they should enter into a close working partnership with the Government.

Some thought must be given to the proper role, aims and objectives of British non-government organizations, the means of achieving them and the dovetailing of these activities into national and international effort to assist overseas development. A need is shown for some national association in which the main non-government organizations would all be represented, but covering a wider field than the Freedom from Hunger Campaign or the Standing Conference on Voluntary Organizations Co-operating in Overseas Social Service. Such an association would have as its major task the job of showing how the activities of these bodies were related to each other and to the Government's aid programme. The problem of increasing the financial resources of the voluntary bodies is more difficult and some of them may find the criticism of this booklet, well founded and moderate as it is, hard to accept. None the less, the problem must be faced, and the booklet is equally clear as to the danger of excessive dependence of aid on Government support. Something of the value of Voluntary Service Overseas could well be lost as the Department of Technical Co-operation becomes responsible for financing 75 per cent of British volunteers. It is interesting, if unexpected, to find in this extent of financial support a plea for extended support by the Government of overseas research, though it is not clear whether what is in mind is research conducted for overseas countries in British research institutions or research in overseas institutions.

The possibilities and opportunities as well as the degree of failing to achieve a fitting balance of effort between the private and independent bodies and the Government's own official programme of technical aid are clearly displayed and the booklet should be studied by the Department of Technical Co-operation as well as by the voluntary organizations themselves. The third of the three pamphlets, and that which completes the factual survey of British aid by the Overseas Development Institute, sets the whole subject against the background of colonial development from the Act of 1929 and the Colonial Development and Welfare Acts from 1940 onwards. Indeed, Er. D. J. Morgan provides an admirable and lucid summary of the origins and history of British aid in the light of Britain's relations with the colonies during the past half-century. Successive chapters review assistance to the colonies first up to and then after 1945, while shorter chapters are given to the subjects of sterling balances, colonial currency systems, and aspects of Commonwealth trading.

In a final chapter, "General Conclusions", Mr. Morgan raises some questions about the future aid programme now that the main problem is that of aid in relation to independent countries of the Commonwealth and then outside the Commonwealth. So far as the smaller territories are concerned, Mr. Morgan questions whether past criteria should continue to be applied and past methods used in assisting territories like St. Helena, or whether their organization and treatment might not be better assimilated to that of, for example, the Highlands of Scotland. Again, he suggests that aid should be extended to the independent Commonwealth, but rather under such a system of supervision as the Colonial Development and Welfare system provides, though he points out that the Committee of Public Accounts is scarcely appropriate for the new situation. For those independent countries of the Commonwealth that are unable to balance their budgets without grants, he advances the tentative suggestion that Britain might accept financial responsibility for some such public service, such as elementary education or agricultural development for a term of years, possibly with a system of block grants.

Here again is a challenge to some hard thinking which may well involve a fresh look at the structure and responsibilities of the Department of Technical Co-operation. Furthermore, the note of urgency is again sounded, and this is further attested by the latest report from the Commonwealth Development Corporation, which once again stresses the inadequacy of the financial margin on which the Corporation is called on to work. Apart from this, the continuing demand for the services of the Corporation not only shows that its work is fully appreciated overseas but indicates also that the scope of its activities should fittingly be reconsidered in the light of the comments made in Mr. Morgan's survey of colonial development, especially in the independent countries. There is no reason to doubt the imagination or the human understanding which Mr. Robert Carr brings to his task as Secretary for Technical Co-operation. What is in doubt is whether he is receiving the support from the Government as a whole that the urgency and importance of his work demand. The three pamphlets noted should leave the Government in no doubt as to that urgency and importance; used with wisdom and energy they could also do much to promote the public understanding that would compel adequate support from any Government.

EXPLORERS OF ARABIA

Arabia Felix

The Danish Expedition of 1761–1767. By Thorkild Hansen. Translated by James and Kathleen McFarlane. Pp. 381. (London: Collins, 1964.) 30*s*.

ARABIA FELIX describes the adventures of the first expedition to undertake scientific exploration in Arabia, which was despatched from Denmark in 1761 and included the great Swedish botanist Forsskål among its members. Only one of them, the surveyor Carsten Niebuhr, lived to return to Europe in 1767. His *Beschreibung von Arabien* and *Reisebeschreibung nach Arabien und andern umliegenden Ländern* are among the classics of oriental travel. The author of the present narrative is a Danish literary critic and translator of modern French literature, who has himself travelled in Saudi Arabia and the Sudan. It is decidedly popular in style. In emphatic contrast to Niebuhr's works, it accords prominence to personal relations, jealousies, and the quarrels of the travellers. Space is even wasted in telling the reader what they "must have" thought or felt, sometimes even "half-consciously". Mr. Hansen has, however, consulted unpublished material in the Danish State Archives and has used a number of books that are available only in Scandinavian languages. There is thus some information that will be new to most British readers. The translation is smooth, the plates, nearly all taken from Niebuhr's books, have been well chosen, and the index is adequate, though a more detailed map would have been welcome.

There are some sensible comments, as, for example, on the meagre achievements of the orientalist of the expedition, von Haven. To state, however, that "the concept of colonial expansion was still thought of as implying only a question of which European country was to control these foreign peoples" is to ascribe to these men the assumptions of a later age. In 1762 a colony was, in Dr. Johnson's definition, "a body of people drawn from the mother country to inhabit some distant place". Before the spectacular successes of the Russians in the war of 1768–74 against the Turks, there was no likelihood that large numbers of Ottoman subjects would pass under European rule, least of all in the Asiatic provinces. This is an unfortunate error. Niebuhr's freedom from such presuppositions was one of his great advantages over many subsequent explorers. "In a Dane of the middle of the eighteenth century," wrote D. G. Hogarth sixty years ago in his *Penetration of Arabia*, "the sense of western and racial superiority was not developed enough to debar him from full sympathy with an eastern people. Niebuhr did not see in the Yemen Arabs an interesting lower order of beings, nor in their creed and religious practices curious parodies of those of a Christian".

The author is also wrong in supposing that to travel in Arab dress was then "most unusual for Europeans in the Orient". There were many parts of Asia where very few Europeans penetrated at all, but those who did could often not have travelled in any other way. There are a few mistakes. W. B. Harris should not be called a great English explorer, and his book was not entitled merely *Yemen*, but *A Journey through the Yemen*. Mr. Hansen's "Captain Pasha, the pirate" was the Kapudan Pasha, the imperial Ottoman admiral, as Niebuhr himself says. The "village of Abb" is Niebuhr's town ('Stadt', not 'Dorf') of Äbb; it is the modern Ibb. There is regrettable, though understandable, confusion in the spelling of oriental place names. In his own books Niebuhr used 'sch' and 'dsj' for the sounds usually represented in English by 'sh' and 'j' respectively. His 'Scherdsje' and 'Mauschid' would be confusing enough for British readers, but here they become 'Sjerdsje' and 'Mausjid'. 'Sherje' and 'Maushid' would have been preferable. There are too many misprints in proper names, Palsgrave for Palgrave, Charden for Chardin, and Hodeia consistently for Hodeida.

These, however, are comparatively unimportant blemishes in a readable and pleasantly produced volume which will interest anyone who wishes to know more about the personalities of Niebuhr, Forsskål, and their companions and the background of this astonishing expedition. Naturally, there is much more about Niebuhr than the others, but there is nothing here to make one doubt the justice of Hogarth's memorable judgement, that "if he was not the most brilliant of the party, if any of his fellows surpassed him in energy, courage, and endurance, in intelligence, or in his measure of that scientific temper which is equally free from prejudice and from laxity, then a more remarkable mission was never despatched to any land". C. F. BECKINGHAM

ADVANCES IN ANALYTICAL CHEMISTRY

Advances in Analytical Chemistry and Instrumentation

Vol. 2. Edited by Charles N. Reilley. Pp. vii + 408. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1963.) 115*s*.

THIS volume continues a series in which new ideas and instrumental techniques in analytical chemistry, together with developments in more classical methods, are reviewed by practical experts of international repute. The editor has again secured the services of a distinguished group of contributors and the generally high standard of the individual articles is therefore to be expected, but the volume as a whole might have benefited from a little more uniformity in the treatment of the various subjects.

For example, F. Feigl, R. Belcher and W. I. Stephen, reviewing "Progress in Qualitative Organic Analysis", have compressed this vast subject into 29 pages of text, the result being virtually a catalogue of the significant papers published during the past ten years, in the style of the Chemical Society's *Annual Reports*. On the other hand, G. Mattock has filled 81 pages with a detailed discussion of laboratory pH measurement, including such useful data as a table for the location of faults in glass electrode cells.

The wisdom of devoting two out of six articles to polarography might be questioned, but the articles themselves, on the hanging mercury drop electrode, by W. Kemula and Z. Kublik, and on organic polarography, by Petr Zuman, are excellent reviews by leading authorities on these subjects, both of which are finding increasing application. A more massive review, occupying a third of the volume, is that by Harry B. Mark, jun., Louis J. Papa and Charles N. Reilley on "Reaction Rate Methods". These have applications in the determination of mixtures of closely related compounds such as homologues, isomers and copolymers, which, so far, appear to have been rather neglected in Britain.

One chapter which seems out of place in this volume is that entitled "Mass Spectra of Organic Molecules" by Seymour Meyerson and John D. McCollum. In fact, this deals only with carbonyl compounds and allylic and related ethers, and the section on ethers, far from being a review, consists entirely of the authors' hitherto unpublished work. Moreover, the application of these results to analysis is referred to only in passing, while instrumentation is not even mentioned. The analytical chemist wishing to keep abreast of new developments, for whom this book is supposedly intended, would be sadly disappointed if he expected to find here a comprehensive review of the analytical applications of mass spectrometry. It is to be hoped that this subject will receive in a future volume a treatment more comparable with the high standard maintained by the other articles in this volume and its predecessor. R. J. MESLEY

CHEMICAL ICONOCLASM

Noble Gas Compounds

Edited by Herbert H. Hyman. Pp. xiii + 404. (Chicago and London: The University of Chicago Press, 1963.) 90*s*.

EVERY now and then we are forced into awareness of the areas of unnoticed ignorance in even the best cultivated fields of chemistry and physics. The noble gases of the atmosphere have figured twice in such dramatic happenings: in their original discovery and again, very recently, in the proof that they are not, after all, chemically inert. With the wisdom of hindsight, it now seems surprising that the chemical compounds of the 'inert' gases eluded detection for so long. Pauling and others predicted the existence of xenon fluoride thirty years ago, and all the thermochemical data are on record to extrapolate from the energies of the Te–F and I–F bonds to the conclusion that XeF_4 ought to have a favourable heat of formation. If Yost had worked in nickel or copper apparatus, instead of in quartz, he would certainly have isolated XeF_4 before the Second World War. In retrospect, the reactor chemistry group at Oak Ridge must actually have had xenon fluorides in their hands, but failed to draw the right (but seemingly far-fetched) conclusions from their poor mass balance for fission-product xenon in systems from which free fluorine was being liberated. It still remained for Dr. N. Bartlett, at the University of British Columbia, to do the crucial experiment: having proved that PtF_6 would oxidize molecular oxygen to $[O_2^+]\,[PtF_6^-]$, to infer that xenon, with its similar ionization potential, should behave similarly. The experiment was done and the right inference drawn, even though it now seems he was wrong in identifying the product as $Xe^+\,[PtF_6^-]$.

What happened then exemplifies the difference between the scientific worlds of 1890 and 1960. From

Rayleigh's first observation of the discrepancy in the density of 'atmospheric' nitrogen to the isolation of xenon, about seven years elapsed. During that time, according to the gentlemanly custom, the field was left to Ramsay, who had to work out the techniques, now taken for granted, for manipulating and separating small quantities of volatile substances. If Ramsay enjoyed exclusive prospecting rights in virgin territory, Bartlett started a gold rush. His first published note on so-called '$XePtF_6$' appeared in June 1962. By April 1963 the Argonne National Laboratory could organize a symposium with 58 papers from 105 authors, and the chemistry of xenon had already been pretty fully explored. The contributed papers covered the preparation and properties of the fluorides from XeF_2 to (probably) XeF_8; the oxides and oxy-acids; their thermochemistry; their solution chemistry; their structures as found by X-ray, electron and neutron diffraction; Raman, infra-red and ultra-violet spectra; electron spin and nuclear magnetic resonance; Mössbauer effect; extensive theoretical treatment and toxicology.

Noble Gas Compounds contains the papers presented at that symposium. After some papers on the historical background, nine laboratories contribute to the wide range of preparative experiments that opened up the field, including experiments of microcurie scale on radon fluorides and the isolation of krypton compounds by matrix isolation methods. The stability and reactivity of inert gas compounds are then put on a firm basis of thermochemistry before passing to hydrolytic reactions, the formation of the oxides and the perxenate salts. The way is next paved for theoretical treatment of the interesting valence problems—which are treated by several groups of theoreticians in the final few papers of the book—by establishing the stereochemistry, molecular dimensions and bond properties in great detail, using all the available diffraction and spectroscopic techniques. Rarely can so much have been done to clear up a new field of knowledge at one stroke.

This is not a tidily edited review; it is a set of research papers setting out new discoveries. As usual, one can question whether such symposium papers justify publication as a separate volume and whether the same material will appear repetitiously in the usual journals. In this case, the intense competition for priority certainly led to duplication of effort and identical experiments by different groups of workers. Thus consecutive papers present the crystal structure of XeF_4 as investigated (with identical results) at Brookhaven and at Berkeley. However, the sense of excitement and of a massive attack on an important problem are well conveyed. The papers are mostly terse and businesslike, logically arranged by the editor, and combine to give a view of the whole state of knowledge at that time.

The book prompts thoughts on the power of co-operative research and the advantages enjoyed by laboratories with a great range of skills and interests. More than half the papers come from U.S. Atomic Energy Commission institutions—18 from the Argonne National Laboratory alone. Four papers are from industrial research laboratories, reflecting facilities and a philosophy of research in industry that is almost unthinkable in Britain. Only four papers originated outside North America: from Yugoslavia, from Birmingham and one—sadly demonstrating the impotence of ideas not backed with modern technical resources—from Germany, once the homeland of fluorine chemistry.

All inorganic chemists will be interested in the subject-matter of the symposium; whether all will want to read the original publications rather than critical digests is another matter. On the other hand, the book may stimulate some thinking about the teaching of chemistry. In some subjects the 'case history' method has been found an effective study tool. A student who went intelligently through this collection of papers would emerge knowing a lot about the content and methods of modern inorganic chemistry, by seeing a whole range of ideas—preparative chemistry, theory and experiment in the elucidation of structure, thermochemistry and thermodynamic considerations, the comparative chemistry of the elements—all brought to bear on a single theme. The book should find a wide range of readers, but most will probably consider it rather expensive to put on their own bookshelves.

J. S. ANDERSON

WHAT IS TOPOLOGY ABOUT?

Introduction to Topology

By Prof. Bert Mendelson. Pp. ix+217. (London and Glasgow: Blackie and Son, Ltd., 1963.) 35*s*. net.

THIS book is outstanding. It is a modest introduction to a difficult subject, but the author has cast a spell of magic about the world of topology. He has written so clearly about the basic ideas of the subject, has been so honest in trying to bring it down to the early undergraduate level, that suddenly much of the difficulty and haziness often encountered by students of topology has faded away. At last, university teachers of mathematics have a suitable text for an early course in the subject.

The British edition of *Introduction to Topology* contains a preface by Dr. E. C. Zeeman, which is important because it puts the subject into proper perspective, both relative to school mathematics and in relation to other parts of university mathematics. Dr. Zeeman, who has recently been appointed to the chair of mathematics at the new University of Warwick (*Nature*, 200, 734; 1963), points out that the place of topology has changed a great deal, in that, at least in most British universities, while the subject was little taught a decade ago, it is to-day introduced in most universities in the first year. This is a radical change and a recognition of the fact that topological ideas pervade so much of modern mathematics. Topology is virtually defined as the study of continuity, and it is pointed out that the subject underlies every domain in mathematics and science which uses continuity.

What is contained in this remarkable book? The first chapter deals with sets and functions, including composition of functions and inverse functions. The second chapter deals with metric spaces and covers topics such as continuity, neighbourhoods, open and closed sets, and the equivalence of metric spaces. The third chapter is given to topological spaces. Such a space can be obtained from a metric space by discarding the distance function and retaining the open sets, although there are other ways of introducing a topological space.

Chapter 4 is concerned with connectedness, a term which, although a formal definition is given, may be described as follows: a subspace of a topological space is connected if it is impossible to decompose the subspace into two disjoint non-empty open sets. For example, on the real line single points and intervals are connected subsets, but the set of non-zero numbers and the set of rational numbers are not connected sets. There are various types of connectedness and, apart from the above, arcwise connectedness and simple connectedness are discussed. The former is stronger than connectedness in view of the theorem that an arcwise connected topological space is connected, but the converse is not true. The falseness of the converse is proved by a counter-example constructed with reference to the subspace of the plane consisting of the points (x, y) such that either $x = 0, -1 \leqslant y \leqslant 1$ or $0 < x \leqslant 1, y = \cos(\pi/x)$. Some interesting and important applications of connectedness are given, including the intermediate-value theorem, and various fixed-point theorems (including that of Brouwer).

Chapter 5, the final one, deals with compactness. At first compactness is studied in general topological spaces but later the restriction to a metric space is considered. A metric space is compact if every infinite subset A has at least one point of accumulation, P, the latter being defined as a point such that every neighbourhood of P contains infinitely many distinct points of A. The chapter closes with a section on identification topologies and spaces, including a discussion of the classical surfaces such as the torus, the Möbius strip, the Klein bottle and the projective plane.

All students of topology will find the reading of this book rewarding, and there is little doubt that the text will become a standard introduction to the subject.

L. S. GODDARD

ANALYSIS OF MICROCLIMATE

Physics of Plant Environment

Edited by W. R. Van Wijk. Pp. xvi+383. (Amsterdam: North-Holland Publishing Company, 1963.) 80*s*.

LAND plants are anchored by their roots in a porous medium providing water, nutrients and oxygen, and they grow when leaves use solar energy to reduce carbon dioxide to carbohydrate. Below ground, the physical environment of plant roots governs the conduction of heat, the flow of water, and the diffusion of water vapour, oxygen, carbon dioxide and ions. Above ground, leaves and stems are exposed to radiation and behave as sources and sinks of turbulent fluxes of momentum, heat, water vapour, carbon dioxide, pollutants, spores and small insects. The physics of plant environment is a diverse and youthful science. Although in British universities it is not yet recognized as a child of natural philosophy, it has reached the curriculum of several departments of agriculture. In Holland, Prof. van Wijk has for many years directed the Laboratory of Physics and Meteorology in the Agricultural University of Wageningen, and the book which he has edited and partly written is a summary of research, mainly in his own department, prefaced by three introductory chapters. There is little reference to work later than 1959.

The first chapter of *Physics of Plant Environment* is a statement of philosophy that will be shared by most agricultural physicists, In many farming problems, a simple physical model isolating a few relevant parameters will often give a better working solution than a purely empirical relationship derived statistically. The second and third chapters review the physical properties of the atmosphere and soil; and the theory, measurement, and estimation of solar and terrestrial radiation. In Chapters 4–6, van Wijk, de Vries and Derksen discuss the mathematical physics of soil temperature in the most comprehensive account of this topic that has appeared in book form. This section might more logically have followed Chapter 7, in which de Vries shows how the thermal properties of soil can be predicted from its composition, and summarizes measurements in several useful tables. Chapter 8 is on the turbulent exchange of heat in air with special reference to the theory of sinusoidal temperature variations, and Chapter 9 is a detailed account of the heat balance of glass-houses, in which J. A. Businger shows theoretically that the 'glass-house effect' depends on shielding from wind rather than on preventing radiative losses. Horticultural engineers will find this section a valuable source of reference. The final chapter, by F. H. Schmidt, presents equations for the diffusion of atmospheric pollution and quotes an interesting investigation where damage to vegetation around a steel works was greatest almost 1·5 miles from the source of the pollution.

The book has two weaknesses. First, although the scope of the book was never intended to match its comprehensive title, the index suggests that the editor has been too selective. There are 49 entries under 'temperature' and 'heat', 24 under 'soil', 13 under 'radiation', 4 each under 'evaporation' and 'photosynthesis', and 3 each under 'humidity' and 'carbon dioxide'. Other readers will regret this bias. During the growing season, soil temperature is one of the least important components of plant environment, whereas evaporation and photosynthesis are the main links between weather and plant growth and development. Evaporation is dismissed in a few pages and photosynthesis in a few lines.

A quotation from p. 266 illustrates a second weakness. "A systematic discussion of the application of the principles (of turbulent transfer) set forth in the preceding text to plant environment would largely surpass the scope of this book." (Yet a derivation of the dry adiabatic lapse rate gets space in Chapter 2 and this has little relevance to the central theme.) The writers have tried to describe the environment of plants without reference to plants themselves or to the ways in which they modify their own microclimate. Several chapters open with the development of complex mathematical theory and close with much briefer reference to experimental results. Neglect of plant-environment feed-back is almost inevitable in this attempt to treat environmental physics as an inductive science.

The authors, all Dutch, achieve remarkable fluency in English and there are very few minor flaws in a book that is distinguished for its clean and elegant lay-out. Symbols are qualified by a plethora of suffixes—many unnecessary. A diagram on page 97 shows an inconsistent heat balance. It is debatable whether the surface temperature of a lake can be measured without difficulty (p. 273); or whether the mathematical theory of buoyancy has not reached the stage of application to plant environment (p. 253).

Despite limitations of content and approach, the book will find its way to many shelves alongside "Geiger" and "Sutton", as a standard text on the physics and mathematics of soil temperature. J. L. MONTEITH

JURASSIC FLORA OF YORKSHIRE

The Yorkshire Jurassic Flora

By Prof. T. H. Harris. II: Caytoniales, Cycadales and Pteridosperms. Pp. viii+191+7 plates. (London: British Museum (Natural History), 1964.) 130*s*. net.

IN Volume 1 of *The Yorkshire Jurassic Flora* (1961), Prof. Harris has described the Thallophyta, Bryophyta and Pteridophyta. In Volume 2, he deals with seed-bearing plants, the Caytoniales, Cycadales and Pteridosperms. It has been found necessary to extend the series to four volumes. There are few areas of comparable size to Yorkshire which have yielded such considerable collections of well-preserved fossil plants from one geological formation. It is fortunate, too, that most of the plant fragments, leaves, etc., have well-preserved cuticles which may be isolated and used for purposes of identification. This method of investigation has been very effectively used by Prof. Harris in classification and in establishing the connexion between different organs of one plant species. A great deal of the best material on which the work is based was collected by the late Dr. Hamshaw Thomas and Prof. Harris himself.

The Caytoniales are a particularly interesting group, which has received much attention from morphologists. Thomas considered that the group exhibits features indicating possible relationship to the Angiosperms but perhaps it is more generally regarded as being closely related to pteridosperms. Prof. Harris has been able to distinguish four species in the genus *Caytonia*. The fossil remains consist of detached leaves (*Sagenopteris*), microsporophylls (*Caytonanthus*) and the ovuliferous parts

(*Caytonia*). The greater part of the volume is devoted to the Cycadales and pteridosperms, mainly represented by their leaves, but a few have related fructifications. The principal genera are *Nilssonia* (9 spp.), *Deltolepis* (3), *Paracycas* (1), *Pseudoctenis* (7), *Ctenozamites* (4), *Ctenis* (6), *Pachypteris* (2) and *Stenopteris* (3). Prof. Harris points out that a separation of these into two groups belonging to the Cycadales and pteridosperms respectively is difficult. The principal differences lie in the morphology of the leaf. The reproductive organs of a few are known and some species of *Nilssonia* and *Pseudoctenis* are cyads, while *Lepidopteris*, *Dicroidium*, *Xylopteris*, *Ptilozamites* and *Pachypteris*, which have known reproductive organs, are pteridosperms. He also suggests that *Ctenis* and *Paracycas* may be regarded as cycads, with *Ctenozamites* and *Stenopteris* as pteridosperms. There follow descriptions of cycad-like reproductive organs *Androstrobus* (3 spp.), *Beania* (2) and *Pteroma* (1).

There are three new genera and about twelve new species among those described. The sixty-seven text figures and the plates provide excellent illustration and will be welcomed by those who have not had the opportunity of examining actual specimens of the Yorkshire material. It is intended that Volumes 2 and 3 will be devoted to the Bennettitales, Ginkgoales and conifers.

J. WALTON

LEGISLATION ON DRUG ADDICTION

Narcotic Addiction in Britain and America

The Impact of Public Policy. By Edwin M. Schur. Pp. 281. (London: Tavistock Publications, 1963.) 35*s*.

NARCOTIC Addiction in Britain and America is primarily a reasoned criticism of the approach to narcotic addiction adopted by the Government of the United States since 1914. The first part provides a general introduction to narcotic addiction and summarizes the development of American policy. The second describes the British approach to narcotic addiction and includes a chapter on the characteristics and behaviour of narcotic addicts. The third part is devoted to possible reforms of American policy and the sources of objections to them. A summary of data describing a number of British addicts is contained in appendixes. The first summarizes the replies of thirteen British doctors specializing in the treatment of addiction to questions relating to addicts and addiction; the second summarizes personal data on thirteen narcotic addicts selected as 'representative' by these specialists; the third is a summary of a survey of attitudes to, and knowledge of, addiction in 147 randomly chosen residents in a London borough whose average age was just under 21 years.

The book provides a lucid account of the development of American legislation on narcotics since the Harrison Act (1914), which was the first of a series of repressive measures which have isolated the American addict from the medical profession and classed him as a criminal. As a consequence, in the United States, it is a crime to have unauthorized possession of narcotics or to be an addict. Thus, addiction is seen primarily as a legal problem. In contrast, the British approach is based on the belief that addiction is a medical problem. American doctors may have to stand trial for prescribing narcotics for an addict, a consequence which deters most of them from treating addicts in general practice. In Britain, narcotics may be prescribed under the National Health Service.

Schur examines the effects of the punitive policy practised in the United States and concludes that the lucrative black market in narcotics and the criminal behaviour of some addicts may simply be attributed to repressive legislation. This conclusion is based on a comparison of the 'typical' addict in the United States with a much fuller account of British addicts. Criminal behaviour is rare among British addicts, who can legally obtain cheap supplies of narcotics from their general practitioners. In the United States, however, addicts tend to become attached to criminal sub-cultures, both in order to obtain supplies of drugs and to obtain the money with which to pay exorbitant prices on the black market. Schur effectively explodes the myth of the 'criminal dope fiend' and finds that addiction in Britain is not associated with crime. Furthermore, he emphasizes the fact that the punitive measures adopted to control addiction in the United States do not, in fact, affect the importer and wholesaler as much as the addict himself.

In the last part of the book, legislative changes are discussed which would bring American policy more in line with British practice. A number of objections to liberal reform are disposed of, including the fear that addiction might increase (evidence indicates that, in Britain, narcotic addiction has decreased between 1935 and 1959), while some of the psychological factors which may sustain punitive legislation are critically examined. Relatively detailed recommendations are put forward concerning the short- and long-term care of the addict, which broadly reflect those of the Joint Committee of the American Bar Association and the American Medical Association. One very useful section deals with the way in which the differences between British and American policies have been minimized by authorities in both countries. This has had the effect of perpetuating the most unfortunate legal status of the American addict.

Narcotic addiction is considered a major problem in the United States and estimates of the number of addicts range from 45,000 to 1 million. In contrast, the number of narcotic addicts in Britain is probably between 450 and 700. These two countries also differ with respect to the distribution of known addicts according to age, sex and socio-economic status. The 'typical' American addict is young, male, and a member of an under-privileged minority group. In Britain, a high proportion of known addicts are doctors, dentists and nurses, and there is no preponderance of males. Thus addiction in Britain and the United States differs with respect to both incidence and distribution. Schur describes these differences but does not deal satisfactorily with either of them, though it is fair to add that they could be considered outside the scope of his main theme. However, his main arguments depend on the comparability of British and American addiction, in order that he may predict the consequences of reforming American legislative policy, and it is unlikely that American opponents of such reform will be persuaded until they are given a satisfactory account of the origin of the differences in the incidence of addiction in the two countries.

The main criticism of the book is that while addiction in Britain is described more or less thoroughly, a much less complete picture is provided of the addict in the United States. The description of the official policies adopted in these two countries is excellent, but the comparisons between the consequences of these policies would have been more compelling if comparable information had been supplied about these two addict populations. The British addiction statistics were all obtained indirectly from the Home Office, an all-night London chemist and, by questionnaire, from a number of specialists treating addiction. This information is supplemented by personal information obtained directly from five narcotic addicts. Some readers may consider the investigations of individual British addicts to be rather sketchy and regret that this opportunity to gather information about their problems, attitudes and behaviour was not fully exploited. In spite of this, Schur has provided a valuable summary of information about British narcotic addicts.

This book is written in a fluent documentary style and liberally supplied with quotations which should make it

of interest to both the specialist and the non-specialist reader. The book is generally free from typographical errors, but the practice of collecting footnotes at the end of the book is an irritating one which makes it tedious to locate the sources of the many quotations and references. There is an adequate index and a selected bibliography of 75 items.
D. Legge

UNIVERSITIES OF THE BRITISH COMMONWEALTH

Commonwealth Universities Yearbook 1964

A Directory to the Universities of the Commonwealth and the Handbook of their Association. Pp. xxxi + 1930. (London: The Association of Commonwealth Universities, 1964.) 105*s*.

The forty-first edition of the *Commonwealth Universities Yearbook*, the principal publication of the Association of Commonwealth Universities, follows, so far as its format is concerned, the traditions of its predecessors. This annual publication continues to be the most comprehensive directory as yet available of academic institutions of the British Commonwealth. It not only covers seventeen Commonwealth countries, including the United Kingdom, but also two countries no longer members but which have expressed a wish to remain in the *Yearbook* and to retain close association with the Association of Commonwealth Universities: those of the Republic of Ireland and the Republic of South Africa (Appendixes 1 and 2).

Commonwealth countries have been rapidly expanding their facilities for higher education. In Britain the Robbins Report on higher education recommended in October 1963 that there should be a further 28 institutions with full university status and that there should also be a substantial development of other sectors of higher education; and such plans can be paralleled in other Commonwealth countries. The *Yearbook* has become a yard-stick with which to measure these developments; for example, this edition shows an increase of 105 pages on the fortieth edition. Entries for the first time appear for the Chinese University of Hong Kong; the University of Burdwan, India; the University of Waikato, New Zealand; the University of Lagos Medical School, Nigeria; and the Universities of Essex; Kent at Canterbury, Lancaster and Warwick, in England. Some institutions now appear under a newly constituted form, and Appendix 4 contains a summary of information concerning the ten colleges of advanced technology in England and Wales, and certain Scottish institutions, which are to gain university status now that the Government has accepted the recommendations of the Robbins Report.

In the *Yearbook*, sections are devoted to each Commonwealth country. These in the main are prefaced by a "National Introduction", which is intended to supply a factual guide to the general background of university education in the country concerned. Then follow alphabetically the universities of that country. Members of staff are listed according to departments and details are given of administrative and other staff, associated or affiliated institutions, general information, and "The Year 1962"—the last-mentioned supplying details of developments, retirements, resignations, statistics, and degrees and diplomas awarded during 1962.

Besides the three appendixes already referred to, there are six others which consider: university admission requirements (Appendix 3); students from other countries in United Kingdom universities (Appendix 5); British academic institutions outside the Commonwealth (Appendix 6); a short bibliography (Appendix 7); the Commonwealth Scholarship and Fellowship Plan (Appendix 8); the Association of Commonwealth Universities (Appendix 9).

The *Yearbook* is rounded off by two invaluable indexes. The first, a general index, contains references to institutions, to subjects of study and to such topics as admission, degrees, superannuation, vacation courses, etc. The second is a name index which makes it possible to find at which university any of more than 50,000 men and women, who comprise the teaching and administrative staff of the universities of the British Commonwealth, are serving.

CITATION INDEXING

Science Citation Index

An International Interdisciplinary Index to the Literature of Science. Vol. 1: AABE–CAPL, 1961. Pp. xxx + 496. (Philadelphia: Institute for Scientific Information, 1963.) Five-volume set: 700 dollars.

A number of surveys have shown that the most generally used method of finding relevant papers is by looking at the references quoted in a known paper. This is a technique which is widely favoured by scientists, but it has the disadvantage that it can only go backwards in time. An attempt to overcome this point is the reason for the citation index, which is a directory of cited references each of which is accompanied by a list of citing source documents. Thus, knowing a relevant paper, it is possible to consult the citation index and find references to all the subsequent papers which have cited the known relevant paper. By working backwards and forwards in time it is claimed that a comprehensive collection of references on a given topic can quickly be obtained. Citation indexing has, for many years, been used in law; this series of volumes presents an attempt to apply the technique in the field of science. As such, it may be said to raise two points: first, whether citation indexing can usually be applied to scientific papers, and secondly, whether these volumes represent a good example.

Undoubtedly a citation index must suffer from certain built-in handicaps. Most of the references which an author cites are not directly relevant to the main theme of the paper and the result is that a high proportion of the papers to which one is referred will be of no interest. It is a weakness of this particular citation index that the searcher is given only the citing author and journal reference. It would have been possible to have included a list of the citing papers with their titles; no doubt this was omitted because of the additional costs incurred, but the result is that the user is compelled to refer either to other indexes or to the original papers to obtain any clue as to which reference might be of interest. While it is possible, if somewhat tedious, to compile a citation index clerically, it involves the type of sorting operation for which a computer is well suited. The resulting print-out is, however, in the usual computer print face, and the severe reduction in type size adds to the difficulties of its use.

Volume 1 of *Science Citation Index* covers 102,000 papers which appeared in the issues for 1961 of 613 source journals. It grew out of an earlier citation index in genetics, and there is a consequent emphasis on journals in the life sciences. It is claimed in the introduction that the journals have been selected for their inter-disciplinary interest, and that the number of journals covered will be increased in future years. As it is, for the physicist, this index covers only about 5 per cent of the 800 journals included in *Physics Abstracts* for 1961. While it would be unwise to underestimate the possible value of this method of indexing, I cannot visualize many situations where these volumes could be used more effectively than other existing indexes.
C. W. Cleverdon

The Complete Slimmer

By Prof. John Yudkin. Pp. 191. (London: MacGibbon and Kee, Ltd., 1964.) 30*s*.

IN his opening chapter Prof. Yudkin says that all of us are overweight who weigh more than we did when we were reasonably slim and trim in our twenties. But, having depressed at the outset most of his readers who are more than thirty years of age, Prof. Yudkin proceeds to give sympathetic consideration (and understanding) to our plight and provides a fairly simple way out of the dilemma.

His 'carbohydrate unit diet' is a method of reducing carbohydrate intake without directly altering the intake either of protein or fats. This is achieved by the allocation of so many carbohydrate units daily, the units being calculated from a table which is provided. Each carbohydrate unit is equivalent to 5 g of carbohydrate, and a test period of 15 units a day for two or three weeks is advocated to see what the effect is. If the weight reduction is regarded as being too fast or too slow the unit allocation can be adjusted accordingly. The table of carbohydrate units is illuminating. A raw apple represents 2 units (10 g), a glass of sweet wine 4 units (20 g) and one piece of 'Vitaweat' 1½ units (7½ g). An examination of this table brings an understanding of the increase in weight since the slim and trim twenties. One begins to recall glasses of sherry consumed over the years together with a decrease in the habit of running for buses. Innumerable lunches and dinners are remembered, while recollections of 20-mile country walks fade into the distance. At last the truth begins to dawn; in this affluent society we eat and drink too much and exercise too little.

Prof. Yudkin utters a warning against the numerous fads and miracle diets which appear annually, and he arms us with reason against the blandishments of the copywriters. For those who have a sufficiently strong desire to return to the slim and trim twenties, Prof. Yudkin undoubtedly provides a blueprint. Whether or not it is followed must remain an individual choice; but the efficacy and strength of his argument will be greatly helped by the purchase of a pair of bathroom scales.

P. D. NUTTALL

Le Développement du Vivant par Lui-Même

Par P. Wintrebert. Pp. 456. (Paris: Masson et Cie, 1963.) F. 44.

THIS second volume (the first was *Le Vivant, Créateur de Son Évolution*; see *Nature*, **198**, 117; 1963), which deals with embryogeny, concludes Mr. P. Wintrebert's thesis, the content of which is intended explicitly for the rehabilitation of the living being. Faced with two prevailing currents of contemporary biology the author writes strongly against: (1) genetics, which he considers to be a mechanism illusively rejuvenated by the transfer from the physical to the microphysical level of explanation; (2) causal embryology, a preformist idealism disguised under pseudo-scientific categories. "The two conceptions diminish the intervention of the living . . . in a very different way, either supernatural, by exalting its power, or natural, by degrading it" (p. 282).

The chemical lamarckism of Wintrebert is essentially 'vitalism': the protoplasm alone is alive, alone has the 'intelligence' and the ability to change itself by adaptation. It is also 'epigenetism': everything, at every moment of development, is actually present, and everything leads to a functioning result. There is no entelechy, no 'organizers', only protoplasm, paraplasm (some mitogenous activities of which have misled Speman and his successors) and genes. The absolute precision of the sequences is assured by the successive and orderly combinations, to the primitive protoplasm, of the genes which, during evolution, have accumulated 'adaptive hormones' elaborated by it. Evolution is therefore additive and irreversible: as opposed to Sewertzow and de Beer, Wintrebert considers that there can be no possible deviations other than, at the most, untimely interruptions due to lesions of the genes or to the circumstances of the environment (neoteny) and some lateral coenogenetic adaptations (like aneural motricity of the embryo). From the cellular to the pluricellular state and to the organic, the individual, as Haeckel would have it, moves, through a succession of first physiological and then phylogenetical mutations, the same way as its ancestors; but this recapitulation is essentially chemical, it generally saves the useless transitional forms. Therefore, it is, contrary to the theory of de Beer, phylogeny which constructs ontogeny and, contrary to the theory of Weissman, the soma which builds up the germen.

The book also includes two original chapters of first importance on the development of *Discoglossus* and the dog-fish: after sixty years of observation, Wintrebert is in opposition on quite a number of essential points (fertilization reactions, gastrulation, origin of the gonia) to the classical descriptions. An appendix offers some reflexions on intelligence, cancer and biological ethics.

Though the reader will not always agree with Wintrebert's ideas, he will enjoy the brilliancy of the remarks, the desire for clarity in the descriptions and discussions and, above all, the lesson in independence, which does not exclude fidelity to a tradition of thought: the diffusion of such activating substances within the body of a still embryonic science should not fail to help him to pass successfully through the unforeseeable course of its mutations.

J. LESSERTISSEUR

Treatise on Analytical Chemistry

Part 1: Theory and Practice, Vol. 4. Edited by I. M. Kolthoff and Philip J. Elving. Pp. xxv+1751–2705. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1963.) 189*s*.

THIS volume deals concisely and comprehensively with one of the most neglected fields of analytical chemistry. The book covers magnetic field and electrical methods of analysis. Each subject is divided up into several excellently written, clearly illustrated chapters. It is a complete definitive and authoritative source of information for all analytical chemists working in this rather specialized field.

Magnetic field methods are covered in four chapters, magnetic susceptibility (125 pp., 238 refs.), nuclear magnetic and electron paramagnetic resonance (69 pp., 140 refs), mass spectrometry (106 pp., 305 refs.) and ion-scattering methods (32 pp., 21 refs.). The section on mass spectrometry by Melpolder and Brown is worthy of special note as it critically, and yet lucidly, interprets the principles, practice and uses of this precise instrument, covering both organic and inorganic analytical applications. The comprehensive reference system makes this an excellent review article.

Electrical methods of analysis are sub-divided into eleven short chapters. These cover the scientific and instrumental fundamentals of this field. If any criticism can be levelled at the treatment of this subject, it might be that the large number of sub-sections causes a certain degree of overlap and a smaller number of larger chapters might have been better. Articles dealing clearly and comprehensively with electrochemical techniques (66 pp., 94 refs.), potentiometry (30 pp., 72 refs.), polarography (68 pp., 347 refs.) and coulometric analysis (49 pp., 258 refs.), to mention only a few, provide a wealth of information for the practical and theoretical worker alike.

In short, this edition more than justifies the fine reputation established by its predecessors, its only disadvantage being that it is rather expensive for individual purchase. It is, however, a must for all libraries associated with analytical laboratories.

J. THOMSON

SCIENCE AND HUMAN EVOLUTION

By DR. BERNARD G. CAMPBELL
5 Madingley Road, Cambridge

> "Discovery is an art, demonstration makes the Science."
> Charles Singer[1].

WHILE the scientific status of social anthropology has often been questioned, that of physical anthropology has not, and physical anthropologists would, in all probability, claim that their science, a branch of natural science, conforms to the methodology and traditions of science as a whole. Though the scientific method is perhaps more immediately effective in the experimental sciences it is just as powerful a tool in those sciences which depend not so much on experiment as on observation. Palæontology is a branch of the natural sciences which is not subject to experimental investigation (though it may utilize results of experimental biology), and it seems therefore worth enquiring how scientific method can most effectively be used by students of human evolution. It is scientific method which makes science, not experimentation, not discovery; it is scientific method to which we owe modern knowledge and technology, on which scientific progress depends; and it is scientific method which makes scientific knowledge distinct from religious and historical knowledge—which makes it demonstrable and universal.

How does this method operate in palæontology ? Most discoveries to-day are made as a result of a planned excavation, though this was not always the case. Important human fossils have been discovered by chance (Neandertal, Gibraltar, Cro-Magnon, Mauer); to-day, knowledge of human evolution and geology enable predictions to be made about likely sites for excavation (such a prediction was made many years ago by Dr. L. S. B. Leakey about the Olduvai deposits, and it has since been amply justified). A rather vague hypothesis of this kind—often little more than a hunch—is a necessary pre-requisite to palæontological investigation. After that, results depend not only on the skill of the investigator, but also on luck. (The well-organized and well-financed expedition to Java in 1906 under Mme Selenka returned without any further trace of Dubois's *Pithecanthropus*.) Deduction from a rather general hypothesis will give us a prediction which can act as a guide to palæontological investigation.

The fossils found can then be treated as the given facts or data of human evolution. On them are made observations—which are our attempts to describe them. On a series of these observations more exact hypotheses can be built by induction. These can then be tested by different means: by comparison with other well-founded hypotheses, and by further deduction and prediction. In this way we can move from rather vague general hypotheses to more precise ones. But at all times the power or truth of a hypothesis is its ability to predict, and nothing else. The predictions must be tested, and the result may be that the hypothesis will be corroborated, or it may need to be modified, or abandoned altogether.

In physics or chemistry, not only can experiments be devised to obtain data, but also observations can in some instances be made and interpreted without human interference—the subjective element is thus minimized. In biology this is less often possible, and in palæontology never. The science of palæontology is dependent on personal observations, and so by nature subject to subjective aberration. Even at the level of obtaining data, palæontologists face a number of difficulties: (1) data are limited and finite; (2) they are both difficult and expensive to obtain, and there is an element of luck in their discovery; (3) they are organic and therefore quite complex.

At the same time, in the observation of data, students of human evolution face special problems.

(1) In the first place, observations may be made at a number of different levels. Thus the form of the skull may be recorded in a descriptive manner with words such as dolichocephalic, platycephalic, tapeinocephalic, etc., or better still by measurement. Here the number of measurements and method of taking them are important. Beyond this, drawings, photographs, chemical analyses, spectrographical analyses, etc., may be made on the fossils. Quantitative data obtained in standard and recognized ways will be the least subjective and probably the most valuable. Owing to the limited amount of data at present available, however, it is essential that the maximum amount of observation be made on the fossil; every independent and descriptive measurement and every possible analysis must be carried out which is likely to prove of the smallest value. The quality of the observations will clearly affect the value of the hypothesis to be derived from them.

(2) A second difficulty which is closely related to this is that a number of different skills may be required in the observation of data. Basic anatomical knowledge is essential. Beyond this, genetic, taxonomic, mathematical, chemical, biochemical and physical skills may be required. In practice, the interpretation of fossil data, to be of greatest value to science, must be carried out, not by one man, but by a small team of workers with differing qualifications.

(3) A third difficulty is a special one for anthropologists. The relation of the material to its investigator is complex. Even in other fields of scientific research the investigator is subject to a host of prejudices. Wilfred Trotter[2] has written:

> "The dispassionate intellect, the open mind, the unprejudiced observer, exist in an exact sense only in a sort of intellectualist folk-lore ; states even approaching them cannot be reached without a moral and emotional effort most of us cannot and will not make."

How much more serious this problem is in anthropological research, when man investigates himself. Only a hundred years ago the theory of evolution caused a deep rift in man's mind. The idea of human evolution has still not been absorbed fully into our subconscious and a schism can be detected at many levels of thought and behaviour. The special relationship which we have with the material which we investigate affects our interpretation and observation of data in many ways. One of the most fascinating aspects of this problem is seen in the light of the investigator's world-view. At one extreme man sees himself as an animal which has achieved its humanity with struggle and which can be proud of its achievement. Such a man sees in his ancestry, not only a reminder of his own racial achievement, but also an explanation of his all too common failings in humanity. He may well prove to be tolerant and maintain his optimism in the face of human failure and cruelty which to another can only cause despair.

At the opposite extreme, an individual may see himself as fallen, or at least falling, far short of his potentialities and needs. He finds himself miserable, cruel and perhaps doomed, never to rise above the level of the super-intelligent animal which he is. Those who hold this view, though they may never realize it, may shun the idea of the animal in man and wish to confine his bestial ancestry to

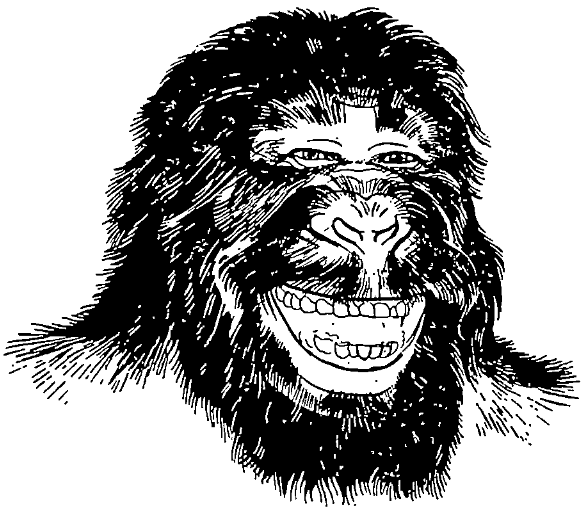

Fig. 1. *Zinjanthropus*, drawn by Maurice Wilson for Dr. K. P. Oakley

the most distant past. Such a man is all too conscious of the gulf between what is and what could be, of the best in man and of the worst. Man, he believes, can become a superman or a god, and his failure to progress is the cause of the deepest pessimism. Clearly these two views of the condition of man cannot fail to affect the frame of reference in which we study our origins (Figs. 1 and 2). Indeed, aberration resulting from the subjectivity of observation may be so serious as to render interpretation of fossil data of no value to science.

(4) A final difficulty which falls into this category is that discoveries in human palæontology receive world-wide publicity—the layman is intensely interested, and the investigator may find himself in the public eye. Publicity of this kind is not only ego-enhancing, but also compelling: the public will not only make a discoverer famous, but will also demand something in exchange. Once a discovery is signalled as great, the discoverer is obliged to maintain this image in his observation and interpretation of the material—the pressure again may be disastrous to that frail vessel which is the human mind. It has even happened that as a reaction to this pressure, a discoverer has completely rejected an early and quite valid interpretation for no good reason, and replaced it by a contrary form. This Dubois did, when towards the end of his life he revised his interpretation of the hominid *Pithecanthropus* find, and stated it to be a giant gibbon. The history of human palæontology presents us with many examples of how not only opinion, but money too, has caused the discoverer to behave in a manner antithetical to science. (A notorious instance was the discovery and disposal of the Le Moustier skeleton, by Otto Hauser. The skeleton was lifted from the Dordogne in 1908 without the recording of any archæological data and sold to the Museum für Völkerkunde of Berlin for 125,000 gold francs. It was destroyed in the Second World War.)

The observation of data is followed by its classification, and the erection of a hypothesis on the basis of such data, together with other data and hypotheses; this new hypothesis brings to discrete observations some coherent meaning. It is at this stage that science begins—scientific investigation itself is an art, and a very skilled one; the erection and demonstration of hypotheses are the beginning of science and it is in this way that scientific method brings its unique kind of universal knowledge. The erection of a hypothesis follows a number of distinct steps.

(1) All observations relative to the matter under investigation must be assembled from different sources, published and unpublished. Those most reliable can be treated as primary or self-sufficient evidence; other data may be set aside and tested at a later stage[3].

(2) One or more hypotheses can then be constructed which account for the maximum of reliable facts or evidence. It is, of course, by no means essential to devote time to the construction of hypotheses. Invaluable work has been done in anthropology of a purely descriptive nature, and in the absence of experiment, observation and description are the essential ground-work of palæontology. Charles Darwin gathered data for five years before he first formulated his hypothesis of evolution, and it was another twenty-two years between the formulation of this 'working hypothesis' in 1837 and its publication in 1859.

(3) The simplest hypothesis should be selected in the first place as a working hypothesis. This is the principle of Occam's Razor. The principle is particularly pertinent to studies of human evolution. As a result of the nature of the data there is no difficulty in the construction of unlimited hypotheses to account for them. Testing of complex hypotheses, which may suggest false predictions, has wasted much scientific endeavour. The most important point is that the hypothesis should not be otiose—it should contain no statement which is unnecessary to account for the observations on which it is based. On the other hand, the simple hypothesis will probably enable one to make more predictions, and will therefore be easier to test.

Occam wrote that "plurality is never to be posited without need". This suggests that in studies of human evolution the simplest coherent scheme which accounts for the reliable facts should be tested before a more complex (here probably polyphyletic) hypothesis. Adherence to this principle would speed the solution of many problems and ensure the most efficient use of scientific effort.

(4) Test the working hypothesis by comparison with other hypotheses and by deduction and prediction against further observations of a reliable nature (new discoveries, etc.)[11]. Meanwhile, the probable or possible status of disputed fossils can be assessed. This testing process is one of the most vital stages in the method of science; but it is here that palæontology restricts the pace. Experiments in physics and chemistry can be specially devised to test hypotheses. All the palæontologist can do is to look for new facts, a lengthy process, or make further observations on known data. (It may be mentioned in passing that there is a very large amount of fossil material

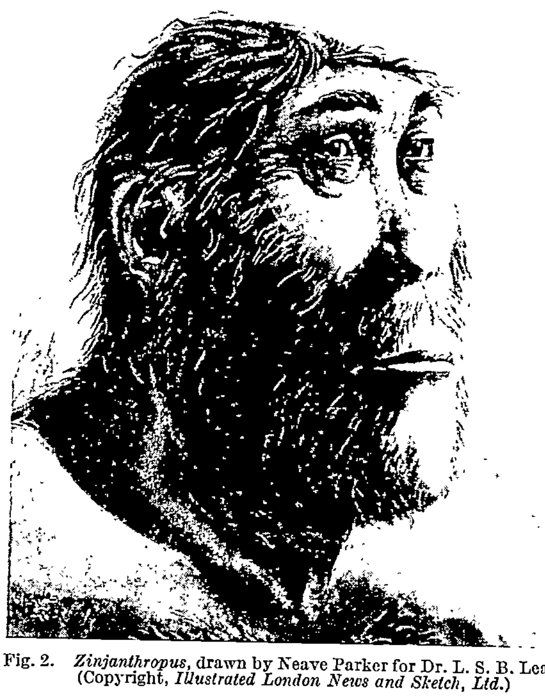

Fig. 2. *Zinjanthropus*, drawn by Neave Parker for Dr. L. S. B. Leakey. (Copyright, *Illustrated London News and Sketch, Ltd.*)

which has received very little attention and may prove of great value in the science of human evolution. Examples include the fossils from Steinheim, Jebel Kafzeh and Montmaurin.)

A logical corollary of the foregoing process involves the examination of any hypotheses or observations which do not support the hypothesis, together with an assessment of their validity. Such examination may result in a modification of the hypothesis, as may the addition of new observations.

(5) Assuming that the hypothesis is able to account for the majority of the most reliable observations, the scientist should then publish it with a demonstration of its validity. This is the second essential stage in the method of science. The purpose of this publication is: (*a*) to communicate what is believed to be an advance in knowledge; (*b*) to make the hypothesis available for further testing by other workers.

If there is one characteristic which distinguishes science, it is that it must be universal—it is the "search for judgements to which universal assent may be obtained"[1]. This characteristic necessitates such publication. If judgements are to be universally accepted, their validity must be effectively demonstrated. As a rule, therefore, scientists refrain from publication of working hypotheses until such demonstration can be given. The publication of a hypothesis with insufficient evidence does, however, carry heuristic value, though if accompanied by expressions of certainty, it may result in unnecessary controversy, because it is, in fact, open to doubt.

Publication is necessary at other stages, however, besides that of the demonstrable hypothesis. First of all facts must be published. In the case of fossils, this means that they must be placed in a public museum for the observation of any suitably qualified person who may wish to examine them. Fossil bones should not be treated as personal property; to withhold scientific knowledge is anti-science. Secondly, personal observations should be made available for the benefit of those who are unable to examine the original fossil data themselves, or who are not qualified to do so. The maximum number of observations should be made by standardized techniques and should be widely published. The withholding of observational data can never be justified (measurements, analyses, etc.).

These remarks may not only help us to analyse the work of others, but they may make our own contribution to the science more valuable. From them emerges one important conclusion: the science of human evolution is fraught with so many difficulties that it is of the greatest importance that a strict and rigorous methodology is followed. Failure to do so leads us from difficulties to confusion and misunderstanding.

Nomenclature and Hypothesis

Harrison and Weiner[3] have pointed out that the economy principle of Occam's Razor "should be extended to the activity of naming new fossil finds". By "naming" they mean, of course, the designation of fossils with Latin names, which Simpson[4] terms *nomina*, to create new species. The reason for this may not at first be clear, but if the meaning of the sexual species concept is fully accepted, it follows that the creation of a *nomen* involves the erection of a hypothesis.

The word 'species' is as old as the Latin tongue, but has changed its meaning with time. Until the eighteenth century, a species was a real and discrete unit—a kind of animal created as such by God and showing only the smallest variation. Although Linnæus accepted this concept he later recognized that considerable variability existed within species, though he did not doubt their original purity and constancy. Darwin observed minutely the geographical variation of species, and when he arrived at his hypothesis of evolution he saw that the term 'species' was to become a convenient label, no longer signifying a discrete population. The concept of the species had lost its meaning.

Our understanding has now changed once more. The sciences of genetics and ecology combined during the early years of this century to nourish a new approach to systematics which developed during the 1930's. To-day we recognize the species as a prime biological unit with objective criteria. The details of this important development are beyond the scope of this article and have been often described by Huxley[5], Mayr[6] and others. Suffice it to say that the use of a *nomen* as a 'label' for one or more fossils is no longer justified—as the concept of the species has developed, so the unit designated by the two Latin terms has come to assume a quite complex meaning.

The history of fossil discoveries contains very many instances in which fossils were named for purposes of convenience without an understanding of the modern species concept. Indeed, no scientist could be blamed greatly for this attitude before about 1935 because the modern species concept was until then in its infancy. The meaning of the term in fact did not worry palæoanthropologists because fossil finds were so few and far between. However, a large number of *nomina* accumulated and have since had to be critically assessed. The resulting confusion has not greatly helped the investigation of human evolution.

But the modern species concept is complex, and has certainly not been understood by all those who have more recently named Hominid fossil species. It would be fair to say that in the field of systematics or taxonomy the concept is now generally accepted and understood, thanks to the brilliant work of Huxley, Mayr, Simpson, Dobzhansky and others. Outside this field the situation is less satisfactory. This is very troublesome for palæoanthropology because fossil discoveries are usually made by either archæologists or geologists, and the fossils are usually described and published either by their finders or by anatomists. None of these skills is necessarily inclusive of taxonomic experience. The understanding of taxonomy usually reaches to a knowledge of the *Code of Zoological Nomenclature*, and of the hierarchical system of classification proposed by Linnæus, but the meanings to be associated with the different taxonomic levels are not understood. (In practice the ineffectual designation of *nomina* does still occur. Recent examples include *Homo sapiens shanidarensis* Senyurek, 1957; *Hemanthropus peii* von Koenigswald, 1957; *Homo homo* var *neanderthalensis* Blanc, 1961; *Sinanthropus lantianensis* Woo, 1964. The validity of all these is in doubt, and some of them are certainly *nomina nuda*.) The *nomina* are in fact still frequently treated as labels. I quote:

> "It always was and still is the custom to give generic and specific names to each new type without much concern for the kind of relationship to other types formerly known."
> Franz Weidenreich[12].

> "I think it will be more convenient to split the different varieties into different genera and species than to lump them."
> Robert Broom[13].

> "Since the names which we apply, *at any and every level in the taxonomic sequence* are inevitably arbitrary and artificial it does not, I believe, matter what we decide to do, provided only that the majority of those who are concerned in the classification, at any given time, are agreed as to how they will use the classification system. . . . All names and terms are after all, only 'arbitrary labels' which are made for the convenience of ourselves and our colleagues." Louis Leakey[14].

There is, of course, no objection to the use of names in any language as labels for either individual fossils or local populations (Simpson's N_1 and N_2 designations[7]) so long as they do not take the binomial Latin form. To avoid confusion such names should be explicitly stated to refer not to species, but to individuals or local populations.

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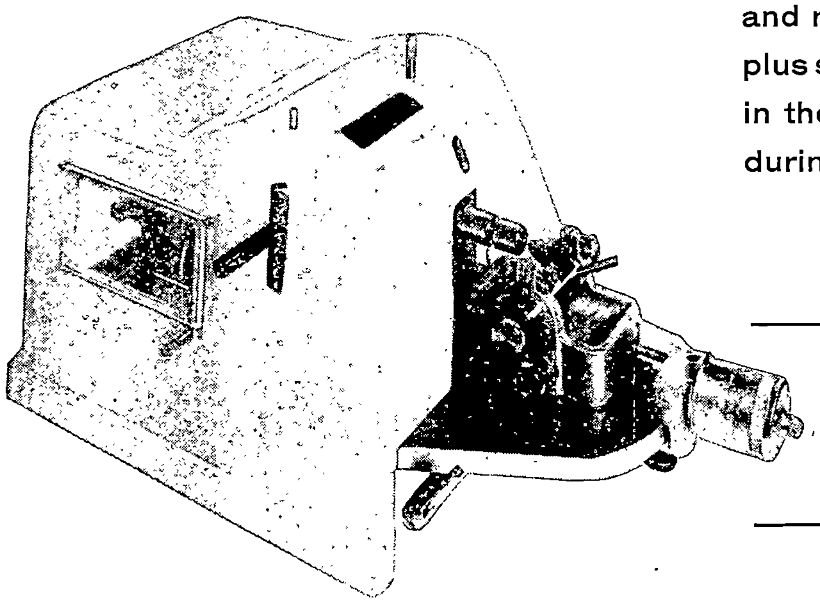

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The understanding of how the species concept can be applied to fossil finds must follow two stages. The first is to be clear about the meaning of a biological species or biospecies; these species can only be recognized at a given moment in time and are defined as "groups of naturally or potentially interbreeding natural populations, which are reproductively isolated from other such groups"[6]. This concept of the biospecies is essential to the theory of evolution, since it is when two closely related groups of animals cease to interbreed that they become two species and henceforward can evolve in different directions to form eventually perhaps two entirely different families. But this concept of the biospecies can only be applied to a moment in time—it does not recognize the dimension of time.

A species in palæontology is an extension of this idea. It is a fundamentally different kind of unit and has been called a chronospecies or palæospecies. Such species can be recognized in two instances:

> "(1) in separate lineages (contemporaneous or not) between which significant interbreeding does not occur ; (2) at successive stages in one lineage but with intervening evolutionary change of such magnitude that populations differ about as much as do contemporaneous species."[4]

That is to say, that we must attempt to divide up each evolving lineage into temporal and morphological stages which show approximately the same amount of morphological variation in time as an average biospecies does in space. I have quoted Simpson[4] as a convenient reference; but this is not a new concept. It was already fully accepted by 1943 [7] and was in use years earlier.

From an understanding of the concept of the palæospecies follows the realization that the creation of a new palæontological species involves the erection of a hypothesis. This unwritten hypothesis could be stated somewhat as follows:

> The species *A-us b-us* (of which specimen Reg. No. 1302 in the Natural History Museum, Timbuctu, is the holotype) is believed to have existed as a group of naturally interbreeding populations over a certain area . . . and with a variability typical of modern living biospecies, and also to have been evolved from, and to have evolved into, other species which are separated by the amount of morphological difference which is usually associated with a species in space at the present time. The species represents a stage in an evolving lineage and will no doubt prove to be divisible (if not already so) into a number of sub-species in both the dimensions of space and time. Furthermore, the species can be distinguished from its neighbouring species (in space and time) by the following characteristics, etc.

The *Code of Zoological Nomenclature* states that for a name to be valid it must be "accompanied by a statement that purports to give characters differentiating the taxon" (Article 13 (*a*) (i)). It is scarcely necessary to state that this implies that the taxon must be differentiated from its nearest neighbours in evolution. Mayr (1942) states: "The diagnosis should include a direct comparison with the nearest relative"[6]. It would scarcely be of value to list the characters common to the family in which the fossil falls to distinguish it from a related family; on the contrary, the diagnosis must be pertinent to its function—that is, to enable other discoveries to be assigned to one or other of two species. The *Code of Zoological Nomenclature* is not in a position to state whether such a diagnosis is effective, just as criminal laws can do little more than punish a breach of the legal code. They cannot instil virtue, and similarly the *Code of Zoological Nomenclature* itself cannot ensure the presentation of an effective diagnosis; but the methodology of science can, for *it is in the diagnosis that we find the demonstration.* It is here that the hypothesis of the new species must stand or fall; it is here that science will train its critical eye to discover the validity of the hypothesis. The diagnosis must support the hypothesis for the species to stand, not in law, but in reality. It is precisely here that publications of new species so often fail. (Many examples of this state of affairs could be quoted. The most topical, and one of the most important, concerns the creation of the taxon *Homo habilis*[8]. In their original publication the authors stated that *Telanthropus capensis* "may well prove, on closer comparative investigation, to belong to *Homo habilis*"; thus the effective demonstration of a novel taxon was negated[9]. The name is valid, but the species has not been effectively shown to have existed, as a distinct taxon.)

I have here attempted to suggest how an understanding and strict use of scientific method may help us to avoid some of the problems which arise in palæoanthropology. Not only will this make our endeavours more fruitful, but also in an anthropological science a proper methodology will obviate much argument and ill-feeling which has, from time to time, been reported in the Press. Scientific communications must be clear, and so far as possible open to no misunderstanding. Since improvement in method, and better presentation of observation and hypothesis are open to palæoanthropologists, it would be the height of folly to neglect them.

I thank Prof. Philip Tobias and Mr. John Wilson for their advice.

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A DARWINIAN APPROACH TO IMMUNITY

By Sir MacFARLANE BURNET, O.M., F.R.S.*

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THE production of antibody by the immunized animal has gradually developed over the past ten years to become one of the key problems of general biology. With the progressive clarification of the process of protein synthesis and particularly of enzyme synthesis, everything points to a progressive transfer and transcription of genetic information stored in DNA, through the now classic machinery of messenger-RNA, ribosome and transfer RNA to the specific sequence of amino-acids in the polypeptide chain. Although there is still controversy on the point, there is good evidence that the secondary and tertiary configuration which will be taken by a single polypeptide chain or a functional complex of more than one chain are determined essentially by the sequences of amino-acid residues, the siting of the cysteine residues capable of forming intra-molecular disulphide bridges being particularly important. The whole specific structure of an enzyme on this view is genetically determined. At first sight, then, the apparent ability of an animal to produce in response to any one of many thousand potential antigens a protein with a precise complementary pattern, allowing firm and specific union to the antigen used, is a major paradox. The so-called instructive theory initiated by Breinl and Haurowitz[1], refined by Pauling[2], and given its most recent expression by Karush[3], claimed that the differences between two antibodies were due, not to differences in amino-acid content or sequence, but resulted from the fact that the final configuration was taken up against the antigen as a template, the initially plastic configuration being fixed by the development of intramolecular S—S and hydrogen bonds. Evidence from several sources seems to render such a view untenable. Koshland and Engleberger[4] have shown differences in amino-acid constitution of two antibodies from the same rabbit; Buckley *et al.*[5] have shown that completely denatured γ-globulin fractions can regain immunological specificity on renaturation.

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* Substance of a University of London Special Lecture in Pathology delivered on June 11.

If, on the other hand, antibody specificity is genetically determined, we are forced to assume either that all antibody-patterns are laid down in the fertilized ovum or that they arise by a process of random emergence which has the general character of somatic mutation and which is afterwards subject to continuing selection for survival within the lymphoid cell population of the body.

The present contribution is intended to support the view that somatic mutation is an adequate, and probably the only, source of immunological pattern and that the nature of the lymphoid system is such that clonal selection, positive and negative, can mould the population of mutants to provide the exquisitely adapted structure of immunological surveillance and defence.

Looked at broadly this gives us what can be called a Darwinian picture of micro-evolution among the lymphoid cell populations of the mammalian body. These cells, being highly expendable and constantly renewed, must necessarily be subject to a process of selection for survival in which clones play the part of species and sub-species in classical evolution.

Somatic Mutation in Mammalian Cells

Mutation is defined as a rare random and irreversible change in the genome of an organism or cell, with the necessary implication that any phenotypic manifestation of the change may potentially or actually be shown by all descendant organisms or cells. If we exclude provisionally changes which might arise in hypothetical ribonucleic acid (RNA) structures, mutation may arise from any form of change in the nucleotide sequence of DNA resulting either from point mutation as a single nucleotide or from some type of 'break and mend' lesion, deletion, inversion or even mitotic crossing-over, compatible with the continued functioning of the cell.

There seems no reason to doubt that mutation in somatic cells is of the same general quality as in germ cells and perhaps of equal or greater frequency. The studies on fleece mosaicism of sheep by Fraser and Short[6] provide a striking demonstration that a certain mutation may occur during the period of primary segmentation of the fertilized ovum and that its frequency throughout is proportional to the number of cells present. Mutation can in general be equated with error resulting from accident and it is reasonable to assume that accidental damage to DNA sequence is likely to occur when the segment concerned is active in transcription of information to RNA as well as in replication. Somatic mutation in relation to functional activities of a cell type may therefore be considerably more frequent than germinal mutation.

Even so the chance of mutation will always be small. If we adopt a frequency of 10^{-4} as the upper limit for any given cistron any modified protein produced as a result of mutation in a single cell will be undetectable in the presence of 10^4 times as many unchanged cells. Somatic mutation is only demonstrable when its effect can in one way or another be magnified.

With only some as yet undemonstrated exceptions in principle, mutation in a mammalian somatic cell is significant only if it gives rise to an observable clone of descendants. The conditions under which this may happen are: (1) The mutation occurs early in embryonic development in a cell from which a significantly large number of descendant cells arise. (2) The mutant cell has, as a result of the mutation, a selective proliferative advantage over its congeners and finds the conditions that may be needed for that proliferative advantage to be manifest.

From our present point of view the most important of these processes is the change which makes a mutant susceptible to the action of a specific mitotic stimulant present in the body. This is one way of interpreting the phenomena of conditioned malignancy, and the clonal selection theory of immunity is based on the assumption that a lymphoid cell by reacting immunologically with a corresponding antigenic determinant may thereby be stimulated to active proliferation. For 'normal' lymphoid cells, the antigenic stimulant is foreign or at least normally inaccessible, but in auto-immune disease the position approaches a conditioned malignancy even more closely, with a normal body constituent acting as the proliferative stimulus.

It should be emphasized that the nature of antibody is such that very small amounts can be demonstrated and titrated in the presence of a great excess of globulin lacking the specific reactivity for which we are testing. If new antibody patterns arise by mutation then it may be possible to detect the activity of a very small clone of descendant mutants or even in specially suitable circumstances a single active cell.

Physiological Phases of the Lymphoid Cell

There is now almost conclusive evidence that three functionally distinct types of cell, all with immunological potentialities, can be derived from stem cells of the type commonly present in the thymic cortex or in the substance of a germinal centre elsewhere in lymphoid tissue, and usually called large lymphocytes or blasts. These cell types are: (1) the small lymphocyte; on appropriate stimulation many, perhaps all, lymphocytes can become proliferating stem cells and the lymphocyte can best be regarded as a 'memory' cell from which the two actively functional forms can be derived; (2) cells of the plasma cell series, the active producers of antibody; (3) mast cells which can be regarded as concerned with pharmacological concomitants of immunological activity.

If it is legitimate to treat the stem cell and its derivatives as phases of the immunologically competent cell, all must be assumed to possess the same (somatic) genetic potentialities of immune reactivity. This can be most simply, if perhaps inadequately, expressed by saying that all carry receptors of the same specificity as that of the antibody produced by plasma cells of the family.

Current Picture of γ-Globulin Structure

Antibodies as present in plasma or serum of an immunized animal are heterogeneous, both physically and functionally, and, for chemical examination, it has been expedient to concentrate on the most 'typical' antibodies such as those found after prolonged immunization with a well-defined antigen. In rabbits, such antibodies are definable as γ-globulin of sedimentation rate 6·6–7S and mol. wt. 160,000, and it is these $7S\gamma 2$- or γG-globulins which have been most examined.

The specific relationship of antibody to the corresponding antigen is implemented by a complementary steric relationship of molecular pattern on relatively small areas of antigen (the antigenic determinant (A.D.)) and antibody (the active patch (A.P.)). Antigenic determinants may be of widely varied chemical nature but the active patch must, in every instance, be composed of portions of one or more peptide chains.

Examination of the proximate components of γ-globulin has resulted in a wide acceptance of Porter's diagram[7] as an appropriate model. In this the $7S\gamma$-globulin molecule is composed of pairs of $A(H)$ and $B(L)$ peptide chains linked by S—S bonds. The A and B chains appear to be governed by distinct genetic determinants and there is some tendency to regard the portion of the A chain which forms Porter's piece III as being distinct (? genetically) from the rest. There is some controversy about the relation of the active patches to the Porter model, but present American opinion (cf. Gally and Edelman[8]) seems to be firmly in favour of the APs being the joint responsibility of parts of both A and B chains. The two active patches appear to be located at each end of a flexible elongate molecule approximately 250 Å × 40 Å, they are of similar specificity[8] and may be thought of as small depressions in the general surface of the protein into which complementary ADs can fit.

No accurate statement can be made about the number of amino-acid residues directly concerned in the active patch. It is probable, in fact, that the number is in a sense indeterminate, but a rough estimate of 10–20 per patch[6] may represent the correct order of magnitude. On such an estimate the 2 active patches would directly involve only about 2 per cent of the 1,500 ± residues in the globulin molecule. Any change in the species or sequence of this 2 per cent would have a correspondingly great effect on specificity of the antibody. In addition it would always be possible that amino-acid changes in other parts of the polypeptide chains would have modifying allosteric effects on the active patches, for example, in increasing or decreasing avidity for a given antigenic determinant without change in specificity as such.

Random Character of Immunological Pattern

In order to account for the facts of immunological tolerance both natural and experimentally induced, both Jerne[9] and myself[10] have held that some process of randomization of specific pattern was necessary. Self-recognition is not susceptible to discussion in terms of instructive theories and, in any type of selection theory, demands the production of a large number of random patterns from which patterns reacting with accessible body constituents can be eliminated by contact with the antigenic determinants at an appropriate stage of physiological maturity.

It is unquestionable that the common features of all $7S\gamma2$-globulin molecules must be genetically determined. These include the general character and relationship of the A and B chains and their main antigenic determinants. The same requirement will hold equally for the other types of immunoglobulin. It is against this background of genetically determined structure that the somatic mutational origin of diversity of pattern must be considered, and it is axiomatic that any type of genetic change which destroys the character of the product as a γ2-globulin is irrelevant to the problem.

Smithies[11] has recently provided a suggestion as to how such a random production of patterns could arise. Small inversions of the portion of the genome coding for significant sequences of the A and B chains could obviously cause major changes in specificity. In more general terms we can accept that any change in the relevant area(s) of the genome whether by point mutation or by any form of deletion, inversion or mitotic cross-over of 'break and mend' type can produce changes in amino-acid sequence and, depending on whether they involve the active patch areas or other regions, will change specificity or modify it. The only limitation which holds similarly for all somatic mutations that can be examined is that the change should not destroy cell viability or so change the protein concerned that it is unrecognizable as γ-globulin.

The essence of this extension of Smithies's hypothesis is that the highly specific pattern of the active patch based on relatively few amino-acid sequences is subject to major change by minor genetic reorganization in the appropriate segment, while change elsewhere may be 50 times as frequent but will produce only such changes as modify the avidity or range of action of the antibody concerned.

Jerne's 'Antibody-plaque' Technique

One of the most striking recent developments bearing on clonal selection theory has resulted from the work of Jerne *et al.*[12] using a technique by which antibody-producing cells can be directly enumerated. This is for the present limited to antibody capable of producing, with complement, hæmolysis of red cells; in Jerne's system mouse spleen provides the antibody-producing cells which are tested against sheep and rabbit cells. The essential features are: (1) in normal 8-weeks-old mice there are small populations, ± 100 cells per spleen, which can produce hæmolysis of sheep or rabbit cells; (2) the two populations concerned are distinct and independent; (3) immunization with sheep red cells provokes a rapid rise in the number of cells effective against sheep erythrocytes but no change in those lysing rabbit cells and vice versa.

These results have already been confirmed in many laboratories and the only simple interpretation is the spontaneous occurrence almost certainly by somatic mutation of small numbers of cells with the pattern needed for each particular type of immune reactivity.

Additional Evidence in Favour of the 'Darwinian' Approach

The concept that all immunological phenomena represent the selection of qualities that have arisen by random process allows a wide range of phenomena, which have tended to be passed over as meaningless, to fall into place. Every laboratory immunologist is aware of the wide variability in the response to a single antigen in any group of animals of the same species. Dixon *et al.*[13], in a classic paper on the immunological production of nephritis, gave a large number of rabbits prolonged daily injections of relatively large amounts of bovine serum albumin. The effect on the kidney depended wholly on the differences in response. Those rabbits producing very small amounts of antibody were the only ones to suffer serious nephritis. Three other substantial groups, comprising high titre antibody producers, medium antibody producers and those which produced no antibody, had transient or no lesions. Whenever we have immunized a group of genetically uniform inbred mice, there has been a wide scatter in antibody titres. As part of the same pattern, we have the difference between poor antigens in which only a small proportion of animals give any antibody at all (yet, with large enough numbers of animals, an occasional 'good' serum can usually be obtained) and the good antigens, like bacterial flagella or $T2$ bacteriophage, where nearly all animals respond well.

Throughout the immunological literature, there are accounts of the extreme heterogeneity of antibodies at both physical and functional levels. Fahey[14] has reviewed the subject extensively and, more recently, Edelman's indirect evidence[15], and the direct findings of Koshland and Engleberger[4], indicate that there is also heterogeneity in amino-acid composition of physically similar antibodies. Evidence of a further type of heterogeneity among antibodies has recently come to light with the recognition both in rabbits[16] and in human beings[17] of antigenic differences between purified antibodies. This, presumably, points also to structural differences in amino-acid sequences. The highly relevant exception is the individuality and homogeneity of myeloma globulins and the retention of that character on transfer of a suitable mouse plasmacytoma to a new host[14]. The fact that, with very refined methods, sub-populations of myeloma globulin molecules can be observed in some is also to be expected from the hypothesis.

Both the irregularity of response and the extreme heterogeneity of antibodies are necessary consequences of a hypothesis of random development and selective proliferation. This is not the place to elaborate the many similarities which can be observed between the micro-evolutionary process by which the body develops its population of immunologically competent cells and antibodies and evolutionary processes in the standard sense, but, on these two points, the analogies with the evolutionary radiations that have occurred with each new step in evolution are obvious.

This theoretical approach is concerned only with: (1) the origin of immunological pattern; (2) its passage to descendant cells and further modification by mutation; (3) the process of selective proliferation under the stimulus of union with corresponding antigenic determinant. Several important and controversial problems are left untouched: (1) The possible transfer of genetic information determining immunological pattern from one cell to another. (2) The form in which an antigenic determinant is capable of stimulating effectively the receptors of a potentially reactive cell. (3) The number of antibodies which can be produced by a single cell. It seems likely that these questions are soluble in principle by experimental work in progress in many laboratories and that the uncertainties they represent do not invalidate the approach which has been adopted.

Summary

An extension of the clonal selection theory of immunity to its logical conclusion relates the origin of specific immunological pattern wholly to random changes in the segment of the lymphoid cell genome which codes for the *A* and *B* polypeptide chains of γ2-globulin and the equivalent constituents of the other immunoglobulins. It is suggested that no greater mutability may be required than in any other type of functioning somatic cell, provided it is accepted that specific union of antigenic determinant with cell bound antibody acting as receptor initiates proliferation of the cells concerned. This would provide the selective forces needed to mould the populations of the various mutants that can arise, to fulfil the body's immunological requirements.

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PROS AND CONS OF INTERNATIONAL RESEARCH CENTRES

AT the meeting of the Society for Visiting Scientists on June 3, great interest was expressed in the potentialities of international research centres. It was admitted from the outset that there is no intrinsic merit in international research as such, since the important thing in any scientific work is the result, not how or where the work is carried out. The justification for any proposed international effort must therefore be carefully examined: the factors making for success will by no means always be the same, and the fact that one such centre may have succeeded is no adequate reason for assuming that others will. In fact, both scientific and political factors enter into the argument and either element would be neglected at one's peril.

The first essential was the clear demonstration of a genuine scientific need. In the case of the European Organization for Nuclear Research (Conseil Européen pour la Recherche Nucléaire) (CERN), the obvious example of a successful international research centre, the starting-point in the demonstration of the scientific need was the poor state in which European nuclear physics found itself after the Second World War. This, of course, was scarcely enough in itself: there was, in addition, the undoubted fact that the equipment requirements of nuclear physics research had by this time reached such a stage that they were quite beyond the financial resources of any Western European country. The CERN synchrotron, which had taken years to build, had, in fact, acted as a cement for the organization. The next stage in the development of the subject, a 300-GeV machine, which was already being discussed, would cost about £100 million, which would strain the resources of the existing Western European countries, and an organization was already being suggested which would include Europe, the United States, and the U.S.S.R. Any stage beyond this would presumably make it necessary to call in some of the other planets, which might raise difficulties.

In any such demonstration of the scientific need it was important to ensure that the scientists themselves were on one's side. Some of them might well feel that expenditure on international research might reduce their own national research budgets. Experience at CERN suggested that this was not the case but rather that the success of an international project might well catalyse increased national expenditure.

The proposal, now under discussion, for an international European institute for molecular biology presented a rather different picture of scientific need, though the sentiment behind the present drive for its formation was essentially similar to that which had led to the setting up of CERN. In this case the proposal might succeed, it was suggested, rather because it was so cheap than because it was so expensive. To begin with, molecular biology is a subject which needs a large group of different techniques and disciplines. Europe at present has nothing to equal the concentration of relevant techniques which the Boston (U.S.A.) area, to take one example, can offer. There is the Cambridge group, the work of Sir John Randall's Department in London, and the work of the Pasteur Institute in Paris. None of these covers more than about one-third of the field. However, unlike the CERN concept, the subject is only moderately expensive in plant and equipment but is very expensive in people. On top of this research situation is imposed the need for a centre where young men in Europe can train in this subject.

To establish such a centre only one or two men from each country would be needed for long-term appointments, so there would seem to be no serious risk of depleting the scarce man-power in each country to an undesirable extent. At present, in any event, no country was getting full value from the men it did have working since these were often too isolated. The cost of an International Institute in this field might be between 2 million and 3 million dollars per annum in contrast to CERN, for which the United Kingdom contribution alone was £2 million per annum. There was as yet no agreement about where such an Institute should be situated and whether, for example, it should be where the subject was already strong or where it was weak. One suggestion was that it might be situated near CERN, since it would be mutually profitable for the molecular biologists and the physicists to be able to talk to each other. A note of caution was sounded in a suggestion that the mere establishment of an institute would itself tend to fossilize the discipline into its present channels and to inhibit new interchanges with yet other disciplines. It was at least generally agreed that there was a strong argument for setting such an institute in an area which could provide a wide-ranging intellectual background.

A third example of an area in which international research was being urgently discussed was provided by cancer research. In this case there was at present thought to be no genuine scientific need for an international institute since there was no work to be done of a kind which could not be carried out in present national institutes. On the other hand, there were specific problems and projects which transcended national boundaries and to which effort could only be devoted by international co-operation. Obvious examples were geographical disparities in the incidence of different types of cancer and the investigation of specific cancer problems in areas in which the national effort was inadequate.

The experience of running CERN presented some interesting lessons. Given general acceptance of the need for an international organization desirable administrative simplicity could be achieved in running it. Each member country of CERN had two representatives on the governing body and voting was on a simple majority basis except for budgetary recommendations. This latter point arose because, on a numerical basis, the smaller members who carry only a very small proportion of the expenditure could out-vote the few large members who paid for most of the budget. Therefore, on budgetary matters, those in favour had to represent more than 50 per cent of the expenditure. Other administrative principles were also simple and desirable: for the ordering of equipment, lowest tenders were accepted and there was no national quota (which meant that the United Kingdom received few of the orders); no patents were taken out, since there was general agreement that this was a cultural activity from which all would benefit; and jobs went to the best men, with no national considerations. The individual workers received contracts for three years, which were renewable for a further three years; thereafter, the individual must either obtain an indefinite contract or leave the organization. Indefinite contracts in practice usually went only to technicians and engineers and not to the research scientists, since the research men must return to their native country to teach the next generation.

An interesting problem was what, for an individual country, would represent a balanced proportion between national and international expenditure. In the case of nuclear physics, the United Kingdom's contribution to CERN represented about one-fifth of total British expenditure in the field. However, in the case of the smaller countries, most of the total national expenditure went to CERN. It followed that many of the smaller countries found it difficult to get their physicists back again from CERN after their term of duty. Moreover, an organization such as CERN is suited only to the more mature research worker, and national facilities will be needed in any event to train individuals to the point at which they can make a useful contribution to an international organization.

This left finally the political factors making for a favourable climate for international co-operation, and these would probably be common whatever the needs of the particular field. CERN had been formed at a time when there was a sense of togetherness among most of the nations of Western Europe, a feeling which sought for some practical expression. It was important that any concrete form which could be given to it should not be controversial, should not be military, should not be a political disaster if it failed and that success if achieved should be clearly recognizable. If these factors were present, the way was open for the scientists, who, after all, led the world in international co-operation, to exert pressure on their political colleagues. In this context great interest was shown in the French proposal for devotion of 0·5 per cent of national defence budgets to cancer research. The enormous sums of money which might become available if this proposal were put into practice led to the suggestion that its scope should be extended beyond the field of cancer research. This was the sort of political climate which one should seek and the individual factors which should operate. If these factors were absent, bureaucracy would find ways, means and doubtless good reasons for stifling the effort.

CHANGING ROLE OF SCIENCE AND TECHNOLOGY*

By Dr. JULIUS A. STRATTON

President of the Massachusetts Institute of Technology

THIS is the first time in forty-two years that the President of the Massachusetts Institute of Technology has been called on to deliver the Commencement Address. I am immensely honoured. Fortunately also I am untroubled by superstitions, for the last occasion of this kind on the campus ended in a terrible downfall. By chance that was also on a twelfth of June. The class of 1922 chose the Great Court for its Commencement exercises, and some two thousand people gathered under an enormous tent. That day was bright and clear, but during the morning the wind began to blow, and just as President Elihu Thomson, over the sound of flapping canvas, launched into his words of exhortation to the seniors, a tremendous gust struck the tent. The outer poles cracked, and slowly the roof began to settle down on the heads of the audience. With flying robes and skirts, the faculty, the students and their parents made their way out safely, and on the steps of the colonnade, under the dome, diplomas were finally delivered in delightful confusion. Only seldom does an orator succeed in bringing down the house—and almost never at Commencement time. Now, in a more serious mood, I speak to you, who are about to receive your degrees, not as a transient visitor on a strange campus, but as one who out of the experience of many years at this Institute has learned at first hand what it means to be a student here. You have worked hard for this moment. I know the pressures that have been upon you; the seemingly infinite demands on your capacity to learn; and the tremendous pace set by a quality of students that constantly challenges the faculty. I believe I know, too, something of the hopes of your families, of their pride in your achievement, and of sacrifices that have made your education possible.

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* Substance of the ninety-eighth Commencement Address delivered at the Massachusetts Institute of Technology on June 12.

You came to us out of a most extraordinary diversity of origins and backgrounds. There is scarcely a State in the Union which is not home to some of you, and you represent countries in every part of the globe. You have grown up in many faiths and traditions. You have known both wealth and poverty. You have attended small schools and large, public and private, in country districts and in great cities.

Yet for all these differences, you brought with you a certain basic community of interests and abilities. You were moved by much the same hopes and ambitions. Some of you were directed toward engineering or science; others toward architecture, toward economics or political science, toward psychology or management. But all of you, I suspect, shared the same concept of what the Massachusetts Institute of Technology stands for in the world to-day and viewed in the same light the role of science and technology in human affairs.

Within the infinitely complex network of intellectual, emotional and spiritual forces which make up this strange body we call mankind, one may discern two primitive impulses that trace back to the beginning of time. The first is the desire to understand—the passionate quest for knowledge for its own sake—about the stars, about matter, about living things. The second is the urge to do, the drive towards action and mastery of our physical environment.

The systematic interpretation of Nature into a framework of law we call science; the effort to convert experience and understanding to useful account is engineering.

For countless centuries the quest for knowledge through science has been moving forward, slowly gathering momentum, while the engineer has provided shelter, assured our supplies of food and water, built our roads and bridges, and created our massive industrial technology. Now, suddenly—almost within your own generation—the whole sweeping line of advance seems to have taken fire. In some strange, unforeseen way, we have come to a critical threshold, beyond which the forces of technical progress appear to be self-sustaining. The processes of discovery, invention and production feed on each other. In every domain of the physical and biological sciences there is a bursting out into new fields and new theories. The translation of ideas into action is taking place at an ever-accelerating pace, so that the functional line of demarcation between scientist and engineer has almost vanished. From the factories and commercial laboratories of the United States pours a mounting stream of new products, new versions of old devices—from jet aeroplanes to transistor radios, from nuclear reactors to household appliances, from a multitude of new drugs to synthetic building materials. We are at the point of being overwhelmed by the very bulk of our accumulated information, bewildered by the diversity of our manufactures; and we are failing to-day to assess clearly the implications of these developments for to-morrow.

Yet through this maelstrom of scientific and technological enterprise runs the almost mystical conviction that somehow every technical advance will contribute ultimately to the good of society. Every responsible physicist believes intuitively or subconsciously that each new insight into the structure of matter will stir someone else—some engineer—to the development of a useful piece of hardware; and every engineer in turn expects that each new product or service will in some way add to our health, comfort and material well-being.

There is nothing new in this idea of technology as the driving force of progress. It is an idea which took form during the enlightenment of the eighteenth century and emerged from the Industrial Revolution of the nineteenth as a well-defined philosophy. The Massachusetts Institute of Technology has its historical roots in that concept of the usefulness of science. Nor is there any need for me to demonstrate how the advance of technology has improved the material condition of mankind.

Yet for all my own faith in, and dedication to, the work and methods of science, I do not believe that we can any longer afford to take such a thesis for granted; and I fear that a blind confidence in the inevitable good of material progress can lead only to disillusionment. The stupendous revolution of the twentieth century is doing more than adding theories, data, and apparatus to the accumulated store of the past. It has provided an entire new dimension to human affairs on a total change of scale.

To use words that you are particularly well qualified to understand, science, technology, and society now form a tightly coupled system. Each new technical advance adds a component to that system. In years gone by we have isolated these components and assessed their usefulness in terms of a specified purpose. We measured the value of a military weapon solely by a military requirement; a new drug by its immediate effectiveness in dealing with a particular pain; a new highway simply by the number of cars it carried; or a chemical waste disposal plant by the interests of local inhabitants. But now, because such components are coupled into an immensely complex system on a huge and massive scale, it is only by an examination of the impact on society as a whole that we can pass judgment on the degree of progress.

To draw on a biological analogy, I am saying that we must advance from the anatomy of components to the physiology of the organic whole—which indeed is now the society itself. One may prescribe an aspirin for a headache or build a turnpike to ease a traffic jam. But the headache may be merely an isolated symptom of a deeper disorder—a disease which may be identified only by a diagnosis which itself is the product of many specialists working together. So, too, our society—the body politic—is subject to old, chronic disorders and to new ailments. These diseases of the system are emerging in increasing number; and we must be courageous in recognizing that they are themselves the by-products of our highly technological environment.

Consider the transformation of our cities—the physical and often social degradation of large areas—the loss of serenity and beauty. We have never before produced so many cars or such fast aeroplanes; yet transportation in the United States is rapidly approaching a point of crisis. The shift to automation in industry is accelerating and will have profound effects on the character of our labour force, on its training, and on its security. We are polluting our air and our water. The pesticides which we are using on a mounting scale are a boon to agriculture but a threat to our natural resources. We find extreme poverty in the midst of affluence. The problem of the economically deprived citizen; be he coloured or white, is one of training and education to cope with a highly technological and rapidly changing society.

These are but a few examples. I could cite many more —all too large, too complicated, too sophisticated to be conquered or even arrested by sporadic investigations and isolated research projects. Of course, science has generated these problems, and we can be equally confident that science can help us to alleviate and resolve them. But our efforts must now move to a higher plateau. We can no longer afford to nibble away piece by piece at the problems of the modern city—of transportation, of underdeveloped economies, of automation, or of disarmament. We indulge excessively in unco-ordinated conferences, surveys, and studies that on the whole are highly unproductive. Our ailments are vast and complex, and they will yield only to planned, collaborative attacks focused on clear objectives and leading to concerted action.

One of the charges which has been most commonly levelled against science is that progress is leading increasingly to the fragmentation of knowledge and the proliferation of a multitude of specialities. But these great new socio-technical problems and the systems they represent are now also generating strongly countervailing forces toward new unities, bringing together many different resources, and giving rise to a new synthesis of knowledge. For in every instance success will depend on the joint contributions of the physical and biological scientists, of economists and political scientists, of engineers and architects, of historians and philosophers. The task of articulating or welding together these components of learning into systems of understanding offers the highest intellectual challenge of our time.

As I have reflected on these matters—as I have pondered about how all this is to be accomplished and where—it has seemed to me that there exists nowhere at the present time any one institution or specific kind of organization which is in a position to undertake alone this monumental task. There is to my knowledge no single agency of government which has the necessary diversity of resources and the freedom of action. By their very character these problems lack the motive of profit which is the essence of private enterprise; and because they lead inescapably from the intellectual domain into the field of action, they present definite risks and perils for the university.

Yet it is only within the framework of the modern university that one finds the wide range of interests, a common ground for the exchange of ideas, a forum of discussion for scholars who draw on the arts and the humanities as well as on science and engineering. This is particularly true of an institution of the character of the Massachusetts Institute of Technology. I do not believe that we can escape the responsibility of taking part in the solution of problems which touch most deeply on the welfare of our society. In the synthesis of knowledge, as well as in the creation of new learning, we must lead the way: and though at times we shall find ourselves drawn more deeply into the main stream of contemporary affairs, we shall continue in the process to educate with relevance to our age. This has indeed been our historic mission.

I have wanted in these few remarks to give a broader and perhaps a new insight into the changing role of science and technology. The essence of this change lies not so much in the expanding scale of discovery and application; it lies rather in the complete penetration of science and technology into every domain of human affairs. But principally it must be understood that these developments have brought a new order of responsibility for the consequences of progress to our society as a whole. Some of this responsibility falls on our institutions—on government, on industry and on the university. But in a free society the burden of obligation rests ultimately on the individual. You are in a superlative position to meet the technical challenges of our day. But you owe something more to the common account. You must be ever mindful of your own deepening responsibility, both to the profession you have chosen to follow and to the society which will look to you for positive action. Your life will be productive in proportion to the goals that you achieve; rewarding in proportion to your commitment to all that is of value; and full in the satisfaction that the world will be better for your efforts.

NEWS and VIEWS

Experimental Physics in the University of Liverpool: Prof. L. L. Green

Dr. L. L. Green, reader in the Department of Physics, has been appointed to the newly established chair of experimental physics in the University of Liverpool. Dr. Green was educated at Alderman Newton's School, Leicester, and at King's College, Cambridge, where he graduated B.A. in the Natural Sciences Tripos in 1944 and gained a Ph.D. in 1948. From 1944 until 1946 he was employed by the Department of Tube Alloys at the Cavendish Laboratory, Cambridge, where from 1946 until 1948 he held a Department of Scientific and Industrial Research research studentship. In 1948 he was appointed assistant lecturer at the University of Liverpool and later held the posts of lecturer, senior lecturer and reader. Dr. Green has lectured on nuclear reactions at conferences organized by the Physical Society and the Argonne National Laboratory and the Universities of Yale, Pennsylvania and Strasbourg. His published work has been mainly concerned with nuclear structure physics. Dr. Green will take up his duties on October 1.

Theoretical Physics in the University of Hull: Prof. G. H. A. Cole

Dr. G. H. A. Cole has been appointed to the newly established chair of theoretical physics in the University of Hull. Dr. Cole is thirty-six years of age and graduated at University College, London, with first-class honours in physics in 1949. He obtained his Ph D. in Queen Mary College, London, in 1952, and the D.Sc. of the University of London in 1963. For a time he was a scientific officer in the Royal Naval Scientific Service and then returned to take up an Imperial Chemical Industries fellowship in University College, London. He left there in 1957 to take up a visiting assistant professorship in the University of California for a year. Since then he has held the appointment of senior theoretical physicist and executive of Clarke, Chapman and Co., Ltd., marine, mechanical and electrical engineers, of Gateshead, Co. Durham. Dr. Cole's major interests have been in the fields of fluid dynamics, statistical mechanics of equilibrium and non-equilibrium fluids and of plasmas.

Physical Metallurgy in the University of Liverpool: Prof. D. Hull

Dr. D. Hull, senior lecturer in the Department of Metallurgy in the University of Liverpool, has been appointed to the newly established chair of physical metallurgy. Dr. Hull, who is thirty-three years of age, was educated at Baines Grammar School, Poulton-le-Fylde, and at University College, Cardiff, where he was awarded the degree of B.Sc. with first-class honours in metallurgy in 1953, and the degree of Ph D. in 1956. In 1954 he was awarded a Students' Essay Prize of the Institute of Metals, and in 1955 the second prize in the British Association for the Advancement of Science Endeavour Competition. During the period 1956–60 he was employed in the Metallurgy Division of the Atomic Energy Research Establishment at Harwell and the Clarendon Laboratory, University of Oxford, being appointed scientific officer in 1956, and senior scientific officer and section leader in 1958. From 1959 until 1960 he was secretary of the Basic Properties Committee of the Inter-Services Metallurgical Research Council. He was appointed lecturer in metallurgy at the University of Liverpool in 1960 and senior lecturer in 1961. He has been a member of the Basic Properties Committee of the Inter-Services Metallurgical Research Council since 1961. He has acted as consultant to Manlabs, Cambridge, Massachusetts, to the Atomic Energy Authority and to the International Research and Development Co., Ltd.

Mathematical Statistics in Monash University: Prof. P. D. Finch

Mr. P. D. Finch, who is thirty-four years of age, has been appointed professor of mathematical statistics in

Monash University, Victoria, Australia. After graduating at the University of Durham he served in the Royal Air Force and was later a senior research officer in the Research Techniques Unit at the London School of Economics, a senior lecturer at the University of Melbourne, and a Fellow in the Department of Statistics at the Australian National University, Canberra. He has written many papers on queueing theory and has lately directed his attention to problems of statistical inference. His appointment at Monash will strengthen an already vigorous school headed by Profs. K. C. Westfold and G. B. Preston.

Second Chair of Geography in the University of Birmingham : Prof. H. Thorpe

DR. H. THORPE, at present reader in historical geography in the University of Birmingham, has been appointed to the newly established second chair of geography from October 1. Dr. Thorpe was born in Derbyshire in 1913, and from Chesterfield Grammar School gained an exhibition to the University of Durham in 1931. There he graduated with first-class honours in geography and was awarded the Maltby Prize for the best thesis presented in ancient history and archæology. After graduation he remained at Durham as demonstrator in the Department of Geography, and was awarded an M.Litt. degree for a thesis on rural settlement in 1936. During the Second World War he served with the Survey Branch of the Royal Engineers, first in the European theatre and then in south-east Asia, where he became senior survey officer, Malaya. After the War he was appointed lecturer in geography in the University of Birmingham, but continued to command 529 Field Survey Squadron, Royal Engineers (Territorial Army), for the Midlands until 1958. He has remained in the University of Birmingham ever since, being promoted senior lecturer in 1951, and given the title of reader in historical geography in 1954. Over many years he has directed his principal research to the elucidation of the evolution of the landscape of the West Midlands and especially of Warwickshire over the past five or six centuries, and the reconstruction of the geography of the area at certain periods in the past. Dr. Thorpe's monograph on the City of Lichfield, published a dozen years ago in the Staffordshire Historical Collections, may be known to some, and his volume on *The Printed Maps of Warwickshire, 1576–1900*, written in collaboration with Mr. Paul Harvey of the British Museum, is a model of its kind. Dr. Thorpe has two comparable volumes in hand, the first on *The Printed Maps and Plans of Warwickshire* and the second on *The Printed Maps of Worcestershire, 1577–1900*. Dr. Thorpe is a well-known figure in geographical planning and archæological circles in Birmingham and the West Midlands. He is chairman of the Birmingham Branch of the Geographical Association, and vice-president of the Birmingham Archæological Society.

New University at Stirling, Scotland

ON the advice of the University Grants Committee, the Government has agreed to establish a new Scottish university at Stirling. The site recommended by the University Grants Committee is the Airthrey Estate, which is at present owned by the Scottish Home and Health Department. The preliminary steps for planning the new university will begin at once and the University Grants Committee will be in close consultation with the sponsoring Committee at Stirling. The target for student numbers recommended by the Robbins Committee for 1967–68 will be met by expansion of the existing universities. The object will therefore be that the new university should be planned so as to enable it to make a growing contribution to the need for university places in the latter half of the ten-year programme envisaged by the Robbins Committee. Stirling will be the eighth new university foundation to be established in Great Britain in the past five years. In recent weeks, also, the Royal College of Science and Technology at Glasgow has been granted a charter and has become the University of Strathclyde. The new university at Stirling will therefore be the sixth in Scotland.

Overseas Students in Britain

A LEAFLET issued by the Association of Commonwealth Universities gives the total number of students from other countries enrolled for full-time study or research in United Kingdom universities in 1963–64 as 14,117, compared with 14,020 in 1962–63, and 13,385 in 1961–62. Of these, 8,280 were from other parts of the Commonwealth and 5,837 from non-Commonwealth countries; 313 were in agriculture and dentistry; 3,310 in arts; 128 in dentistry, 1,965 in medicine, 2,448 in science, 2,574 in social studies, 3,269 in technology, and 110 in veterinary science. Postgraduate students totalled 6,926 and 6,858 held an award of some kind. India sent 1,543, the United States, 1,295, Nigeria, 1,022, Pakistan, 749, Canada, 652, Iraq, 530 and Egypt, 452.

International Survey of Scientific and Technical Personnel

THE June 1964 issue of the *O.E.C.D. Observer*, besides an article on the co-ordination of foreign aid, summarizes the findings of the *Third International Survey of Scientific and Technical Personnel*, recently published by the Organization for Economic Co-operation and Development under the title "Resources of Scientific and Technical Personnel in the O.E.C.D. Area". The Survey provides no answer to the question whether some countries are over-investing in education while others are neglecting it. Moreover, the general correlation between gross national income and educational efforts in member countries of the Organization for Economic Co-operation and Development does not suggest a final answer to the question as to the optional reserve allocation to education in individual countries. Whereas in Britain about 90 per cent of new entrants obtain a first degree, in some other countries, including the United States, only 50–60 per cent of university entrants do so, and while, in Italy, France, Germany and Britain, more than 80 per cent of secondary school leavers qualified for admission to higher education in the United States and some other countries the proportion was 50 per cent or less. The proportion of university graduates qualifying in engineering, science and agriculture varied from 75 per cent in Austria to 20 per cent in the United States and 15 per cent in Greece. The output of scientists in Western Europe is increasing much more rapidly than that of engineers. The number of scientists and engineers, excluding technicians, in member countries of the Organization for Economic Co-operation and Development in 1959 was 2·6 million and is expected to increase 6–7 per cent in the early 1960's.

The Lace Research Association

THE fifteenth annual report of the Lace Research Association, for 1963, refers to the complete change in the basis of membership with the abandonment of the levy, with the effect that the work of the Association is directed much more towards the interests of the Leavers makers, Leavers and Raschel finishers and Leavers and Raschel dyers providing the basic income (Pp. 22. Bilborough, Nottingham: The Lace Research Association, 1964). In the investigation of the packing density of the yarns wound into brass bobbins, a special technique has been developed to fix the yarns in position by 'potting' them in resin before a cross-section of the bobbin is made. Work has been initiated on a device to provide an automatic dropping in of bobbin threads on the brass bobbin winder. Steady progress was made in supplying and fitting beam-thread-breakage detectors on Leavers lace machines and further development work was undertaken with the prototype equipment for detecting holes appearing in the lace due to thread breakage, which is designed to traverse and scan the facing bar. The development of the mechanical pre-cutter has further advanced, and various types of scalloping machines have been investigated for their suitability in handling Leavers' lace. In dyeing Bourdon cord, the affinity of the dye was found to have an important effect on penetration into the core, but the nature of the cord and greasiness of the core also affect the penetration of the dye liquor. No significant advances in the examination of new flame-proofing materials and methods for their suitability for lace were made during the year.

The American Philosophical Society

Year Book 1963 of the American Philosophical Society includes a brief history of the Society, a list of officers since 1769, biographical memoirs, among which are those on N. H. D. Bohr, Lyman J. Briggs, Sir Charles Darwin, Sir Richard W. Livingstone, and reports on meetings during the year (Pp. 738. Philadelphia: The American Philosophical Society, 1964). The reports of standing committees include that on research, recording: 240 grants totalling more than 250,000 dollars approved in 1963 from the Penrose Fund; 65 grants totalling 62,000 dollars from the Johnson Fund; awards of four fellowships totalling 29,000 dollars from the Dallas Fund for research in clinical medicine. Besides these lists of grants, the Committee's report includes notes on the progress of investigations by the recipients of grants. The *Year Book* also includes a list of members, and reports on finance.

Petroleum Consumption in the United Kingdom in 1963

Compared with 1962, the final figures published by the Petroleum Information Bureau on behalf of the United Kingdom Petroleum Advisory Committee show that the demand for petroleum products in 1963 totalled 55,692,382 tons, an increase of 9·4 per cent on the previous year (*U.K. Petroleum Industry Statistics: Consumption and Refinery Production, 1962 and 1963.* Pp. 8. London: Petroleum Information Bureau, 1964). This is exclusive of bunkers for ships engaged in foreign trade. With one exception, white spirit, deliveries into consumption of petroleum products in the United Kingdom record substantial increases. Consumption of motor spirit was up by 5·6 per cent; industrial spirits (including industrial benzole) rose by more than 7,000 tons; white spirit showed a drop of 2,614 tons; demand for kerosine (burning and vaporizing oils) was more than 200,000 tons greater than in 1962; Derv (Diesel engined road vehicle) fuel increased by 8·7 per cent. Total deliveries of black oils (gas/Diesel and fuel oils) rose by 8·5 per cent. Due mainly to substantial increases in consumption by railways, construction industry, domestic appliances, metal industries and more recently the electricity industry, gas/Diesel oil demand rose by 18·1 per cent. Temperature was largely responsible for boosting burning-oil deliveries by 16·9 per cent, having regard to the severe winter conditions in the year under review. For the rest, deliveries of lubricating oils and greases increased by 3·7 per cent, bitumen supplies by 6·2 per cent, liquid gas by 60 per cent, due largely to growing demand by the Gas Boards, the latter authorities also being responsible for a 26 per cent increase in distillate feedstock and a 24 per cent increase in refinery gas offtake. As in recent years, the demand for chemical feedstock, now a major product of the oil industry consequent on the remarkable growth in the field of petrochemicals, rose by 38·9 per cent. Thus the overall picture is one of steadily increasing demand for petroleum products in Britain, a tendency which is world-wide, one likely to persist for many years to come; notwithstanding known petroleum resources, of which the Middle East would at present appear to possess the lion's share, in this context the intensive exploration activities of the oil companies everywhere are clearly justified.

International Bird Preservation

The 1963 annual report of the British Section of the International Council for Bird Preservation again stresses the widespread dangers resulting from the indiscriminate use of pesticides, and the urgent need for Government control (Pp. 29. London: The International Council for Bird Preservation, c/o British Museum (Natural History), 1964. 3*s*.). There has been encouraging progress in the campaign against oil pollution of the sea, several more countries, including the United Kingdom, having accepted the 1962 amendments to the 1954 Convention for the Prevention of Pollution of the Sea by Oil. There have also been additional adherents to the original Convention. The great mortality among birds during the bitter winter of 1962–63 evoked measures for special protection in many European countries, and in Great Britain the previously legal taking of eggs of certain common species was made an offence. The increase in activities of armed hooligans and the acquisition and use of dangerous weapons by irresponsible persons is causing much concern, and the British Section is in consultation with the National Farmers' Union about the possibility of introducing further legislation to deal with this problem. Sweden ratified the International Convention for the Protection of Birds, Paris, 1950, in March 1962, and it is earnestly hoped that the United Kingdom will also take this step.

National Parks in South Australia

An illustrated handbook describing national parks and wild-life reserves of Australia has been published by the Commissioner (*South Australian National Parks and Wild Life Reserves: An Account of the National Parks and Reserves situated near Adelaide, South Australia.* Edited by Bernard C. Cotton. Pp. 160 (16 plates). Adelaide: Government Printer, 1964). All the parks and reserves are within easy reach of Adelaide and the handbook not only describes how they have come into being but also provides considerable detail about the main features of each park and reserve. Plants and animals peculiar to Australia are given special prominence. The handbook also describes the pleasure resorts controlled by the Tourist Bureau. There are many useful black-and-white illustrations and some outstanding colour photographs.

The Western Australian Museum

The annual report of the Western Australian Museum for 1962–63 records that in the research collections work has continued in modernizing the accommodation (Pp. 41. 7 plates. Perth: Government Printer, 1963). In particular, the installation in the basement of fire-resistant rooms with automatically operating carbon-dioxide flooding systems for the spirit-preserved and naphthalene-saturated zoological collections has been completed. In research, members of the staff have continued to make known the results of their work through publication in specialist journals. Co-operation with other museums and organizations at an international level is becoming more frequent. Modernization of the Museum buildings has continued in the completion of the remodelled entrance hall. The Museum has had no addition to permanent buildings since 1899 and the Museum Board has made a strong appeal to the Government to implement a decision made in 1958 to build an extension to the James Street Building.

New Zealand Earthquakes, 1958–59

The network of seismological stations centred on the Seismological Observatory, Wellington, New Zealand, had in 1958 and 1959 seventeen members. These stretched from Apia (13° 48·4′ S., 171° 46·5′ W.) in the north to Scott Base (77° 51·0′ S., 166° 48′ E.) in the south. The organization of the instruments fell into two sections.

First, there were the short-period seismographs distributed widely in New Zealand, and intended to yield records of earthquakes originating in the country. Secondly, there were the teleseismic instruments to provide records of distant earthquakes. These functions, however, interlocked, and every seismograph gave some useful information in both categories. The discontinuance of recording at New Plymouth in 1958 left the network somewhat critically dependent on the operation of the station at Tongariro, but the standard of coverage was successfully maintained. The *New Zealand Seismological Reports* for 1958 and 1959 give details of the installations at all the stations together with timing arrangements and the readings of the seismograms. Further, there is a list of instrumentally determined epicentres, an index of felt earthquakes, and a list of staff together with a note of their publications during the two years.

The principal New Zealand earthquakes of 1958 and 1959 are discussed in the *Reports*, and maps are given showing: (1) epicentres of normal earthquakes; (2) epicentres of deep-focus earthquakes; (3) isoseismals for some earthquakes. In 1958 the level of earthquake activity in New Zealand was low: 55 earthquakes were reported felt only in North Island, 15 only in South Island and 6 in some parts of both Islands. In 1959, 58 earthquakes were felt in North Island only, 19 in South Island only, and 5 in some parts of both Islands. On May 22, 1959, a large shallow shock with magnitude 6 and an epicentre in the Marlborough Sounds region caused as much damage as any shock in New Zealand since the Wairarapa earthquakes of 1942. Picton suffered most severely. In all, some 460 insurance claims were lodged with the Earthquake and War Damage Commission. These mainly concerned old property. Two shallow shocks had magnitude 5·5, but reported intensities from them did not exceed *MM*4. On the other hand, a shock of magnitude 4·8 on July 30, 1959, centred some 20 miles north of Marton, gave rise to 40 insurance claims for damage in the Wanganu district.

Dry-acid Descalers for Oil Wells

CARBONATE scale is frequently a serious factor in inhibiting flow of oil to the pumps in oil wells, especially those past peak production but economically active and where water is produced along with the crude oil. After a time, scale builds up and clogs the perforations at the bottom of the casing, gradually restricting the flow of oil from the surrounding reservoir rock. Until recently the time-honoured practice of pumping hydrochloric acid into the well to remove this scale was customary, but this often involved long-distance delivery in tank-trucks to remote pumping sites, was costly, and frequently hazardous to the oilmen concerned. Much research into the possibility of using a 'dry-cleaner' descaler has been rewarded by the discovery and commercial production of an easy-to-handle compound based on sulphamic acid. This is packaged in 100-lb. drums and is known as 'Visco 900', according to a recent issue of the *Dupont Magazine* (58, No. 2; March–April 1964). The Nalgo Chemical Co.'s Visco Division, Sugar Land, Texas, began marketing 'Visco 900' in early 1961, and the response from oilmen has been very favourable. In 1960, Du Pont approached the Nalgo Chemical Co. with the idea of using inhibited sulphamic acid. After testing sulphamic with Visco Division's additives for nearly a year, a suitable dry-acid cleaner was found. Many practical advantages are claimed for these sulphamic-based descalers, among which are: reduction in costs in equipment and labour; in many cases a 'slug' of the compound, dry or in slurry form, is added at the well-head, displaced by the pump to perforation level, and left to do its work without further attention; shipping and handling dry; convenient storage at well-site; no fume damage to equipment or personnel; non-irritating to unbroken skin; easy removal by washing off with water, etc. Examples of the results of this dry-acid descaling treatment are given in the article, one of which happened at the Standard Oil Co. of Texas oilfield near Sivells Bend, Texas; output of two pumping wells had dropped to 17 and 21 barrels per day, respectively; after cleaning with 'Visco 900', following pretreatment with 'Visco 1111' (a surfactant), at the end of a 17-day period, the first well increased production by 10 barrels, the second by 15 barrels per day. Water-injection wells, used for pumping water back to re-pressure the oil reservoir and flush the oil ahead of it to adjacent wells, and salt-water disposal wells, likewise have their scale-plugging tendencies; here again the use of the sulphamide-based descaler has met with success.

Jacobian Elliptic Functions

VOLUME 7 of the series of *Mathematical Tables* issued by the National Physical Laboratory, Teddington, consists of tables of Jacobian elliptic functions the arguments of which are rational fractions of the quarter period (Department of Scientific and Industrial Research: National Physical Laboratory. *Mathematical Tables*, 7. By A. R. Curtis. Pp. iii+81. London: H.M.S.O., 1964. 15*s*. net). They were prepared in order to facilitate computations occurring in a method of filter design described by S. Darlington (*J. Math. Phys.*, 18, 257; 1939). The 70 pages of tables list the functions $sn\left(\frac{mK}{n},k\right)$, $cn\left(\frac{mK}{n},k\right)$ and $dn\left(\frac{mK}{n},k\right)$ to 20 decimal places for integral values of n from 2 to 15 inclusive, and for m up to $(n - 1)$. In place of uniform intervals of k or $\sin^{-1}k$, Jacobi's nome q, related to k by $q = \exp(-\pi K'/K)$, is used. Equally spaced values of q correspond to a k-distribution which is very dense in the region close to $k = 1$. The range covered by the tables is $q = 0$ to $0 \cdot 35$ at intervals of $0 \cdot 005$, corresponding to $k = 0$ to $0 \cdot 99933$. . . . The calculations were performed using the *Deuce* computer, and the programmes and method used are described in the introduction to the tables. An alternative method of construction of the tables, which is perhaps more convenient when k is used as argument, is also outlined.

Use of Red Phosphorus as a Fertilizer

THE potential use of red phosphorus as a fertilizer has been investigated by H. P. Rothbaum, of the Dominion Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand (*New Zealand Journal of Science*, 7, No. 1; March 1964). The oxidation rate of red phosphorus in four moist soils of different phosphate-retention capacity has been examined and the reaction is shown to be chemical, almost independent of phosphorus/soil ratio, and dependent on temperature, particle size, and the presence of catalysts. The products of oxidation are phosphites and phosphates. Explosion hazards of red phosphorus in superphosphate have been investigated, and the results suggest that mixtures can be made which would be safe and economic long-term fertilizers, with the possibility of reducing phosphate fixation. Small amounts of copper may be useful in regulating the rate at which red phosphorus becomes available. Together with W. Tuft, Rothbaum has also made extended investigations on oxidation rate of red phosphorus. Chemical methods of estimating small amounts of red phosphorus in soils have been developed. The rates of oxidation of red phosphorus in moist and in dry soil, obtained by using chemical methods, were shown to be consistent with results obtained by using radioactively labelled phosphorus. The chemical method also demonstrated that the rates of oxidation determined by radioactive methods apply over longer periods. The rate of oxidation of red phosphorus alone in air was also determined; it was found to be slower than in soil and unaffected by the presence of water.

Handling, Processing and Packing Dates

It has been estimated that at least 1 million people are supported entirely by date cultivation, and in view of the nutritional value of this fruit, particularly in the diet of many people in the Middle East, the recently published Food and Agriculture Organization Development Paper, No. 72, will be of considerable interest, especially as hitherto there has been no single publication dealing with the processing of dates (*Dates : Handling, Processing and Packing*. Pp. xxiv + 392. Prepared by V. H. W. Dowson and A. Aten. Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1962. 20*s*.; 4 dollars). Much of the information concerning the various operations to which the date may be subjected after harvesting has been provided by workers actively engaged in the date industry and should be of particular interest to several countries where attempts are being made to improve the quality of the fruit. Knowledge of the composition and changes which constitute ripening is essential in the processing of dates, as is that of the different varieties, and these subjects have all received full attention from the authors. Gathering and modern packing-house procedures, including the equipment used, are described in detail. In the appendix, information is given of various analytical methods used to examine dates and of the standards used to evaluate their quality. There is also a most comprehensive explanatory list of the Arabic words so widely used by the date industry. The Paper is freely illustrated; the coloured plates showing the development stages in the Beiyuudhi date of Libya are of a particularly high standard. It is perhaps a pity in a work of this standard that such a number of minor errors have occurred in the text, resulting in the inclusion of a rather depressing corrigenda sheet. Nevertheless, as a reference work this Paper will be of great value to all who are interested in the date.

Neuropharmacology

The January issue of *The Practitioner* (19, No. 1147; 1964) deals with neuropharmacology. The reason for the choice is that in no branch of therapeutics have there been more advances than in this. The introduction of the so-called 'tranquillizers' opened up a fascinating new approach to the management of mentally and emotionally disturbed patients. Many of the original claims for this new group of drugs were over-optimistic, but the basic concept was sound. This is clearly exemplified in several of the articles. Almost equally impressive has been the progress achieved in the fields of anti-convulsants and anti-Parkinsonian drugs, but here, as in the case of tranquillizers, the multiplicity of these new preparations has proved a handicap to the general practitioner. To decide which of them is best for his individual patients is proving an ever-recurring problem, and it is hoped that the advice provided in this special issue of *The Practitioner* will prove of real value. Other articles deal with therapeutic problems that the practitioner is liable to encounter.

Fœtal and Infant Liver Function and Structure

All scientists are already greatly indebted to the New York Academy of Sciences for the well-known series of articles published in its *Annals*. The recent publication entitled *Fetal and Infant Liver Function and Structure* contains the forty papers presented to a conference called for the purpose of bringing together numerous papers on the function and structure of the fœtal liver that have been published (Vol. 111, Article 1. Pp. 1–558. New York: New York Academy of Sciences, 1963. 7 dollars). The papers given to the conference are classified under seven major sections: Part 1, "Morphology", contains six papers on the structure, growth and regeneration of the liver; Part 2, "Physiology", contains five papers on the physiology of the liver in the fœtus, new-born infant and chick embryo; Part 3, "Chemistry", contains seven papers on the chemistry of the formation and action of various enzymes; Part 4 contains papers devoted to bilirubin and its related compounds; Part 5, "Pathology", contains six papers on the pathology of various diseases of the liver; Part 6 contains seven papers discussing the clinical problems of jaundice in early infancy and those of biliary atresia; Part 7, "Discussion Workshop", reports discussions on various subjects, such as terminology, regeneration, enzymes, morphology, circulation, neonatal chemistry, bilirubin conjugation, problems of pathology and clinical problems. Edited by Harold E. Whipple with Julian A. Sterling as consulting editor, these contributions by 82 authors together provide a valuable and factual epitome of the present-day knowledge of this subject.

Television Society Premiums

The Television Society has awarded the following Premiums for outstanding papers read before the Society in 1962–63: *The Mullard Premium* to Mr. G. B. Townsend (Rank-Cintel Ltd.), for his paper on "New Developments in SECAM"; *The Wireless World Premium* to Dr. P. Schagen (Mullard Research Laboratories), for his paper on "Electronic Aids to Night Vision"; *The Electronic Engineering Premium* to Mr. A. C. Dawe (E.M.I. Electronics Ltd.), for his paper on "Characteristics of Special Vidicon Camera Tubes and their Applications"; *The Pye Premium* to Mr. K. Fawdry (B.B.C.), for his paper on "Education by Television"; *The T.C.C. Premium* to Mr. C. F. Whitbread (Associated Aerials Ltd.), for his paper "Receiving Aerials for U.H.F. Television".

The Royal Society of New Zealand

The *Proceedings of the Royal Society of New Zealand* for April 1964 (Vol. 91, Part 1) includes the report of the tenth New Zealand Science Congress, with reports of the half-yearly meeting of the Society, 1962, and of the annual and half-yearly meetings, 1963. In his presidential address, Dr. C. A. Fleming, discussing the promotion of science in a Commonwealth democracy, reviews the formation and growth of the Society. He notes particularly the consistent desire of the Council during the past 25 years to become more representative of New Zealand science by some kind of liaison with the increasing number of national scientific bodies serving a particular discipline, the desire that the fellowship should adequately represent the balance of science in New Zealand, and the desire that Fellows should play a more active part in the Society's affairs. He suggests that three changes in the fellowship rules are desirable. Scientific eminence should be the sole criterion for nomination, but all nominees should agree to pay, if elected to the fellowship, a substantial annual fee towards the Society's administrative expenses. The right of nominating Fellows should be extended to groups of the Fellows themselves. The issue also includes Prof. H. Barraclough Fell's Hudson Lecture for 1962: "Saint Cuthbert's Birds and Thunderstones: Sidelights on the Search for Living Fossils".

University News :

Belfast

The following appointments to lectureships have been announced: Dr. M. G. Burnett (physical chemistry (supernumerary)); K. G. Proudfood (plant genetics); Dr. R. M. Pengelly (applied mathematics (digital computing)); Dr. D. L. Smith (botany).

Birmingham

The following appointments have been made: *Lectureships*, Mr. B. Ahamad (econometrics and social statistics); Dr. N. Cottingham (mathematical physics); Mr. B. D. Giles (meteorology and climatology); Dr. L. A. Griffiths (physiological chemistry); Mr. E. Stones (educational psychology). *Research Fellowships*, Mr. A. Ball (physical metallurgy); Mr. P. F. Barker (geology); Mr. J. H. Croft (genetics); Mr. D. J. Dunn (electronic and electrical engineering); Mr. A. G. Fraser (geology); Mr. J. G. Gardiner (Racal Research Fellow in the Department of Electronic and Electrical Engineering); Mr. N. L. Hancox (physics); Mr. W. J. Harvey (pure mathematics); Mr. G. E. Hollox (physical metallurgy); Dr. B. R. Pollard (mathematical physics); Mr. A. Pramnik (Imperial Chemical Industries Research Fellow in the Department of Electronic and Electrical Engineering).

Bristol

Dr. W. Chester, reader in applied mathematics, has been appointed to a chair of applied mathematics. Dr. H. E. Hinton, reader in entomology, has been appointed to the chair of entomology; Dr. F. Coles Phillips, reader in petrology, has been appointed to the chair of mineralogy and petrology. The following appointments to lectureships have been made: Mr. J. B. Bowes (anæsthetics); Dr. J. B. Chappell (biochemistry); Mr. P. B. Garland (biochemistry); Mr. E. M. McKay (electrical engineering); Mr. H. I. H. Saravanamutto (mechanical engineering); Dr. K. A. Upton (civil engineering); Dr. J. Wilks (education).

Edinburgh

The following have been appointed to lectureships: Dr. M. D. Wynne (chemical engineering); Mr. D. I. Campbell (education); Mr. H. Hastie (veterinary practice).

Newcastle upon Tyne

The Rev. Dr. E. E. Aynsley has been appointed to a professorship in chemistry. Other appointments are as follows: *Readerships*, Dr. R. J. T. Pennington (neurochemistry in the Department of Clinical Biochemistry); Dr. M. Fleischmann (physical chemistry); Dr. F. J. McQuillan (organic chemistry). *Lecturers*, N. Laws (applied mathematics); Dr. A. M. Mearns (chemical engineering). Dr. Mary Richardson has been reappointed Junior Luccock Research Fellow in the Department of Physiology for a further year.

Sheffield

The title of reader has been conferred on Mr. I. S. Maxwell (historical geography) and Mr. J. Crangle (physics). The following appointments have been made: *Lectureships*, Mr. J. F. Archer (bacteriology); Mr. D. J. Goldstein (human biology and anatomy); Mr. D. P. Rose (chemical pathology). *Research Fellowships*, Dr. K. H. Swinden (metallurgy).

Sussex

The University of Sussex has received a donation from the Bata Shoe Organization to enable it to establish a unit in the School of Social Studies for the study of race relations with particular reference to the Caribbean, West Africa and Brazil. The first director of the unit, with the title of Professorial Fellow, will be Mr. L. F. Henriques, at present lecturer in social anthropology in the University of Leeds.

Announcements

Prof. G. M. Badger, professor of organic chemistry at the University of Adelaide, has been appointed to the Executive of the Commonwealth Scientific and Industrial Research Organization. His appointment fills the vacancy caused by the death of Dr. S. H. Bastow in January.

An informal open meeting of the Physical Biochemistry Group of the British Biophysical Society on "The Use of Electron Spin Resonance Spectroscopy in Biological Studies" will be held in King's College, London, on September 17. Further information can be obtained from Dr. A. R. Peacocke, St. Peter's College, Oxford.

An informal discussion on "The Electrical Double Layer in Surface and Colloid Chemistry", arranged by the Faraday Society, will be held in the University of Bristol during September 21–22. Further information can be obtained from Dr. R. H. Ottewill, Department of Physical Chemistry, University of Bristol, Woodland Road, Bristol 8.

A course of lectures and practical instruction in "Teratology", intended primarily for medical and scientific graduates working in the pharmaceutical industry but open to others, will be held in the University of Cambridge during September 21–25. Further information can be obtained from Dr. D. H. M. Woollam, Department of Anatomy, The University, Cambridge.

The third European symposium on "Chemical Reaction Engineering" will be held, under the auspices of the European Federation of Chemical Engineering, in Amsterdam during September 15–17. The theme for the symposium will be "The Confrontation of Science and Practice in Chemical Process Development". Further information can be obtained from Dr. J. Hoogschagen, c/o A.K.U.N.V., Welperweg 76, Arnhem.

The Autumn Meeting of the Institute of Metals will be held in Harrogate during September 21–24. The programme will include discussions on: metals versus plastics; the application of modern concepts of metal physics to the development of creep-resistant alloys; hot-rolling of non-ferrous metals; the structure of eutectics. Further information can be obtained from the Institute of Metals, 17 Belgrave Square, London, S.W.1.

A meeting, organized by the European Branch of the Organic Geochemistry Group of the Geochemical Society, will be held in Paris during September 28–30. The first day of the meeting will be devoted to organic matter in rocks, and the following days to other natural organic materials. Further information can be obtained from Dr. M. Louis, Institut Français du Petrole, 1 et 4 Avenue de Bois Preau, Rueil-Malmaison (S. et O.).

A conference on "Luminescence", arranged by the Institute of Physics and the Physical Society, will be held in the University of Hull during September 15–17. The programme will include sessions on: the theoretical aspects of luminescence; electroluminescence; cathodoluminescence; phosphor preparation; single crystal growth; organic phosphor systems; luminescent materials for lasers. Further information can be obtained from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

The seventh international Mineral Processing Congress will be held in New York City during September 20–24. The programme will include sessions on: comminution; gravity and dense-media separation; magnetic and electrical separation; surface chemistry in mineral processing; flotation kinetics; chemistry of flotation; flotation practice; chemical processing; automation and control. A symposium on "Autogenous Grinding" will be held during the Congress. Further information can be obtained from Prof. M. G. Fleming, Royal School of Mines, South Kensington, London, S.W.7.

The first European colloquium on "Electron Probe X-ray Microanalysis", sponsored jointly by the Electron Microscopy and Analysis Group of the Institute of Physics and the Physical Society and the Sous-Commission Microsonde of France, will be held in Delft during September 14–15. The colloquium will be divided into three main sessions: instrumentation; quantitative microanalysis; applications of the electron probe microanalyser. Further information can be obtained from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

SURFACE CONTAMINATION

UNDER the auspices of the American Association for Contamination Control, the Health Physics Society and the Oak Ridge National Laboratory, an international symposium on surface contamination was held at Gatlinburg, Tennessee, during June 8–12, 1964, and attracted about 170 scientists and engineers from nine countries.

Karl Z. Morgan (director of the Health Physics Division, Oak Ridge) opened the proceedings and described the type of accident which his department might have to deal with, according to the practical and fundamental knowledge at their disposal. Five years ago a small chemical explosion resulted in the dispersal of 600 mg of plutonium into the street, with more inside the building. They cleaned down to 30 disintegrations a minute and sealed over with paint. There was much curiosity about the physics and chemistry of the dispersal, attachment and removal of such contamination.

He then introduced A. M. Weinberg (director of the Oak Ridge National Laboratory) as a pioneer in reactor design and a dedicated research worker in the field of applying the vast and potentially cheap resources of nuclear energy to massive projects for the betterment of mankind. Dr. Weinberg spoke of the gas centrifuge, originally constructed for isotope separation but of limited value for this purpose, which was now being looked at by biologists as a potential separator of cell particles and viruses on a continuous scale. The discovery that *SM* 40 virus, a carcinogen in hamsters, had been present in live polio virus suggested the need of a purification process at the end of the production line; it was possible that the gas centrifuge might serve as a model.

The first of the four scientific sessions was opened by C. N. Davies (Medical Research Council and the London School of Hygiene and Tropical Medicine), who reviewed the deposition of aerosols from air in enclosed spaces. Gravitation, thermophoresis, photophoresis, diffusiophoresis and the electrical deposition of adventitious particles over a wide range of sizes were discussed and the respective rates of deposition were estimated. C. N. Segelstrom (Royco Instruments, Boston) gave an account of an electronic counter of airborne particles; light from a 2,850° K lamp, scattered at right angles by individual particles, is measured as a series of pulses which are claimed to be proportional to the square of the particle size, irrespective of its refractive index, complex or otherwise. In the discussion it was accepted that this claim was probably correct for sizes above 10μ, but the instrument was commonly used for smaller particles and a check was needed. The counter is well engineered and will operate, also, on liquid suspensions; it is widely used in hospitals and the electronics industry for monitoring airborne contamination.

A. A. Andersen (Provo, Utah) described a six-stage cascade aerosol sampler. Each stage consisted of a disk perforated with 400 holes which impinged on another disk, immediately below, made of aluminium or stainless steel. The flow rate was 1 ft.^{3}/min and the jet velocities ranged from 3·54 ft./sec for the first to 76·4 ft./sec for the sixth; the corresponding cut-off diameters for unit density spheres were 9·2μ–0·5μ. It was claimed that the use of multiple jets rendered adhesive films on the impaction plates unnecessary for many aerosols.

The investigation of surfaces was introduced by S. Ross (Rensselaer Polytechnic, Troy, New York), who described the effect of contaminating diatomaceous powders used in chromatography with 'Apiezon oil *B*', and tricresyl phosphate. The powders remained free-flowing, apparently dry and non-oily although carrying multi-layers of oil. Adsorption isotherms were obtained which were linear through the origin and the elution peak was symmetrical; the oil film evidently made the surface of the adsorbent more uniform. A single equation was derived for the isotherms produced with varying quantities of oil.

J. F. Pudvin (Bell Telephone Laboratories, Allentown, Pennsylvania) discussed methods of gauging the cleanness of metal surfaces in considerable detail. A new method involving cooling below the dew-point was described. Condensation patterns map the clean areas and the first formation of droplets indicates sites of hydrophylic activity. A neat method of measuring the contact angle had also been used. Gold was unusual among metals in always having a hydrophobic surface; on other metals the presence of oxide rendered the surface hydrophylic. These techniques provided a new standard for detecting contamination in the minutest traces.

An original way of measuring surface contamination had been developed by J. L. Anderson (Orlando, Florida). A film of decyl bromide containing 1–3 atoms of carbon-14 was applied to the surface in question and a thin end-window counting-tube was set close above it. Nitrogen was passed through the gap and, if the surface was clean, all the decyl bromide evaporated in 30–40 sec. Surface contamination slowed down the rate of evaporation and was characterized to some extent by the shape of the decay curve. The solvent used to apply the radiochemical absorbed the soft β-rays and was chosen, therefore, to be of much higher volatility.

The next session was opened by Morton Corn (University of Pittsburgh), whose subject was mechanisms of dust redispersion. The removal of particles from a surface was effected by centrifuging up to 250,000g and by blowing with a tangential air stream; the aerodynamic force on the particle was calculated from the velocity profile of the boundary layer. In the blowing method it was found that time was a factor; this did not apply to centrifuging. Mica particles always lie flat and though their adhesion was less than that of glass, in the centrifuge, it was not possible to blow them off.

L. A. Masironi and B. R. Fish had also constructed a small wind-tunnel with a 0·25-in. working section for the investigation of re-entrainment. Using stroboscopic lighting the behaviour of glass spheres 28μ in diameter had been observed in an air current of 5,000 cm/sec. 32 per cent of the spheres blew right away; 6·5 per cent rolled first and were then detached. 6·5 per cent rolled but stayed on the surface and 55 per cent failed to move. The rolling velocity was only 10^{-5} of the air speed. R. L. Walker and B. R. Fish described an apparatus for oscillating a surface in order to apply acceleration; no 30μ particles had been shaken off using maximal acceleration normal to the surface of 90g.

Three papers followed which dealt with the redispersion of particulate material from surfaces. K. Stewart defined the re-suspension factor, K, as the air concentration in μc. per m^3 divided by the ground concentration per m^2. No good correlation of K with air velocity, particle size or density was obtainable and values ranged from 10^{-4} to 10^{-8}. It was not usually found that deposits from accidental spills out of doors weathered away; on the contrary, the material might weather into the top few millimetres of surface, rain and dew aiding this process and consolidating the surface. The re-suspension factor, in general, was smaller out of doors than indoors because of this effect and the rough nature of outdoor surfaces in comparison with those indoors. The half-life of outdoor ground contamination was 40–100 days for 10μ particles and less for coarser ones.

At Harwell, I. S. Jones and S. Pond found that values of K as large as 5×10^{-5} were associated with vigorous walking and detected a consistent difference between plutonium oxide and plutonium nitrate. Autoradiography indicated that activity was present in particles from 0·4μ to 60μ diameter with the number median size 5μ and the activity median 14μ. The particle size distributions were similar on the floor and in the air. At Windscale, R. T. Brunskill had measured values of K as high as 8×10^{-3}, due to dust from clothing.

Experiments on redispersion of settled dust, by B. R. Fish *et al.* (Oak Ridge), indicated that a person was likely to inhale about one particle per hour, around 3μ in size, for every 4,000 per m² on the floor when working in a room contaminated with dust of copper oxide or zinc sulphide. The mass concentration decreased by a factor of only 2 in going from ankle level to the breathing zone.

The session which followed was devoted to aerodynamic transport and deposition. S. K. Friedlander (California Institute of Technology, Pasadena) had developed an ingenious new sampling device for aerosols of particles between 70 Å and 0·1μ in diameter. It consists of a 1-in. diameter disk rotating at 2,000 r.p.m. A spiral flow towards the surface of the disk is induced which has a component normal to the disk; the normal velocity is the same at all radii and depends only on the distance from the disk. The diffusion of fine particles from this flow, on to the disk, is uniform over the entire surface. An electron microscope slide was set in the disk and runs of 10–70 h enabled size distributions to be obtained of fine atmospheric particles.

The next paper was by C. N. Davies, who reviewed the various mechanisms of deposition from moving air streams and directed attention to complicating factors in gravitational and turbulent deposition.

A method of estimating ground contamination resulting from the destruction of an aerospace vehicle was suggested by E. M. Wilkins (Dallas), who treated it as an instantaneous point source expanding according to turbulence theory, but made no allowance for changing conditions as the ground was approached.

L. P. Davis (University of Tennessee) calculated the deposition of particles in ducts combining inertia deposition, due to turbulence, with Brownian motion; the analogy between heat and mass transfer was used for the latter and coagulation was allowed for. Similar velocities of deposition were obtained for 10μ and 0·01μ diameter particles (about 0·1 cm/sec) with a minimum velocity between 0·1 and 1μ.

The preceding scientific sessions were followed by technical sessions on control, measurement and decontamination, including papers on micro-organisms and chemicals.

There was considerable discussion about working limits for radioactivity. H. J. Dunster (Harwell) claimed that the intake by breathing was negligible, and air sampling was unnecessary, if the surface contamination was below the maximum permissible limit. Surface contamination was easy and cheap to measure. He suggested maximum permissible levels of 10^{-4} μc./cm² for control areas and 10^{-5} for inactive areas and skin; these figures correspond to 20,000 and 2,000 α-disintegrations/min.100 cm² or 6,700 disintegrations per min from the hands.

This view was contested by H. Blatz and M. Eisenbud (New York University), who also outlined some of the legal difficulties associated with standards. H. Glauberman and W. R. Bootman (U.S. Atomic Energy Commission, New York) presented data showing the wide range of air concentrations associated with a given surface contamination in handling uranium and plutonium, a factor of more than 100 being involved, in agreement with the wide range of values of the re-suspension factor, K, which had been quoted in papers presented during the scientific sessions.

The necessity of avoiding undue interference with operations was stressed by G. W. Spangler (University of California School of Medicine). Permissible contamination limits were derived by evaluating the external radiation hazard from surface contamination and the risk of body deposition from airborne material released from the surface. Here limits were much narrower than the International Commission on Radiological Protection air concentration figures and were based on surface contamination causing no more than 10 per cent of the permissible dose. Inhalation came out as equated to the absorption by a 40 cm² wound, which is a considerable area.

The view of the Commissariat à l'Energie Atomique, France, was presented by J. Hamard. Inhalation was regarded as the main hazard from surface contamination which is not bound. It was indicated that, for a 40-h week, 10^6 times the maximum permissible concentration for air in μc./cm³ gave conservative limits in μc./cm² for surface contamination. On this basis external β-radiation from the surface is taken care of.

Four papers followed on measurement techniques. The filter paper 'swipe' had been studied by J. R. Prince and C. H. Wang (Oregon State University). Part of the paper was cut out after rubbing the surface, moistened with phosphor and placed in a standard vial for scintillation counting. Millimicrocuries could be estimated with efficiencies up to 5 per cent or more for tritium and 50 per cent for carbon-14. A dry swipe was better than a wet one. 10^{-4} μc./m² gave some 40 counts/min.

J. E. Dummer (Los Alamos) had made an examination of use of portable radiation survey meters for surface β-emitting contamination. Large areas were simulated by dotting the outline with solution of strontium-90 + yttrium-90 in equilibrium. Sources up to 5-cm diam. were found to act as point emitters; large correction factors were needed for calculating surface dose rates from the meter readings.

R. Wilson and G. A. Vivian (Ontario Hydro-Electric Power Commission) also discussed the relationship between the detector indication and surface activity, considering geometrical factors.

Adhesive paper, smear and 'smair' techniques for assessing surface contamination were compared by G. V. Royster and B. R. Fish (Oak Ridge). The smair sampler contained a filter in a housing which was pressed on the surface. A series of peripheral jets, inclined at 45°, blew air at 90 m/sec or more on the surface inside the hood and released contamination which was trapped on the filter. Although the actual quantity of activity collected by this device was often less than that removed by other methods it gave very much more reproducible and realistic results. For example, equivalent contaminations of dry and on oiled concrete were returned as the same by the filter paper smear method, whereas the 'smair' sampler detected less from the oiled surface.

An instrument developed at the Illinois Institute of Technology was described by D. Werle. This was for estimating traces of non-volatile residue in rocket fuels which created an explosion risk when present as contamination on the internal surfaces of empty fuel tanks. A sample of the fuel was sprayed in drops smaller than 3μ, and after evaporation of the volatile bulk of each drop the residual aerosol was measured by 40° forward light scattering. The apparatus was calibrated with dilute solutions in 'Freon' or trichloroethylene.

W. J. Kerrigan (of Du Pont, Aiken, South Carolina) had prepared a series of standard dusts in sizes of 0·2μ–44μ by sieving and air elutriation. Ground uranium oxide and Alcoa aluminium were available and redispersed well after storage in bulk.

A. K. Baker (Chandler, Arizona) described air-flow problems in outflow hoods designed to protect delicate operations from contamination by room air. The formation of vortices downstream of objects in the working zone led to the entrainment of room air.

A neat new tool for changing the gloves in a glove box without opening up the box was described by J. B. Owen (Dow Chemical Co., Golden, Colorado). The change could be effected in less than 2 min.

The last two papers of this section, from North American Aviation and Grumman Aircraft, were devoted to the routine of controlling dust-free areas in factories. More than 70 per cent of missile failures are due to contamination; the high degree of reliability which is aimed at necessitates the use of 'clean-rooms' and the organization of appropriate training for the staff who build the complex miniaturized electronic navigational devices.

Regular observations of the dose rate from radioactive fall-out had been maintained by Th. Franke of Frankfurt, Germany, using a 4·5-in. sodium iodide crystal for γ-ray spectrometry. An increase from 1·5 microrads/h to 4·0 was observed during 1962–63. Snow cut the rate by 50 per cent.

E. D. Graham (Argonne National Laboratory) described the α-particle monitoring programme which was established when a reactor at Idaho Falls was switched to plutonium core loading. The routine included aerosol sampling, monitoring surface contamination and external radiation, evaluation of internal exposure of personnel and the dosimetry of external exposure.

The control of surface contamination at the Atomic Weapons Research Establishment, Aldermaston, was explained by W. N. Saxby and J. A. Hole. The general strategy was one of containment in limited areas where protective clothing was worn. Monitoring equipment was described and the derived working levels used in contamination control were summarized.

L. K. Burton (Berkeley Nuclear Laboratories) stated that a serious problem at nuclear power stations was the lack of a quick method of assessing the relative proportions of plutonium, uranium and natural α-emitters. In the presence of large masses of concrete the background of radon daughter products was high and confused the picture. Air sampling was to be performed with a small filter attached to the worker which, when combined with a solid-state radiation detector, would indicate large, active individual particles.

Five papers dealt with microbial contamination. T. W. Kethley (Georgia Institute of Technology) had carried out numerous experiments with bacterial aerosols which could be detected down to one particle, or 10^{-13} g/ft.^{3}. The mathematical calculation of concentration in ventilated rooms was explained for steady-state and transient conditions. In practical situations the equivalent settling diameter of bacterial particles was 10–20μ, due to their association with foreign matter.

K. R. Goddard (U.S. Public Health Service, Savannah, Georgia) had used various sampling methods in investigations of bacterial distribution in hospitals. Counts of airborne viable organisms of 40–50/ft.^{3} were regarded as high and at 150 conditions were bad. Investigations of surface coatings had shown epoxy paints and varnishes to be a good substitute for tile; they could be applied directly to concrete block walls, using the correct undercoat.

G. S. Michaelsen and D. Vesley (University of Minnesota) presented two papers. The first was based on work with the Casella slit sampler, supplemented by the Andersen sample for finding size distributions. Low bacterial counts were found to be associated with the pressure ventilation of operating rooms. The counts correlated with the movements of personnel and it was clearly desirable to avoid unnecessary movements. The peaks ranged from 5 to 20 particles/ft.^{3}. Local sources of bacteria included the chutes for soiled linen, which were efficient disseminators and transferred contamination from floor to floor. Work with selective media had been facilitated by a multi-point tool for inoculating plates. The prevalent organisms tended to differ from one hospital to another; the higher Gram-positive cocci were associated with people and the lower ones, rods and moulds with dust.

Their second paper was a statistical evaluation of 'Rodac' agar contact plates which, in addition to having some advantages over Petri dishes, when used in the ordinary way, could be pressed, without sliding, on a surface to record the surface contamination. An analysis of floor contamination in hospitals was made and the efficiency of floor disinfection methods was evaluated.

There is international agreement that space-vehicles landing on other planets must be sterile, and L. B. Hall (special assistant for planetary quarantine, National Aeronautics and Space Administration, Washington, D.C.) gave an account of the difficulty of implementing a guarantee of absolute sterility. He approached the subject historically, from the Mosaic laws pertaining to food. Experiments showed that the die-off of organisms on steel surfaces was complete in 48 h at 25° C and in 8 h at 50° C so long as the relative humidity was in the neighbourhood of 45 per cent. Higher or lower humidities favoured the retention of viability.

The Boeing Co., Seattle, was also concerned with the same problem, and Karl Kereluk, R. Meyer and A. J. Pilgrim went into the problem of constructing a highly complicated and completely sterile planetary lander. 'Rodac' plates were used in this work.

Two papers on beryllium followed. J. J. Cohen and R. N. Kusian (University of California) had found it difficult to correlate surface contamination with health hazard on account of the many factors governing redispersion. R. N. Mitchell and B. C. Eutsler (Los Alamos) examined the air and surface contamination in a beryllium store. Small cyclones, operating at 2 ft.^{3}/min. and May pre-impingers passed, respectively, 6 and 10 per cent of the airborne material, indicating that most of the beryllium was in coarse dust. Floor contamination ranged from 860 to 1,860 μg/ft. and the air concentrations, during resuspension tests, were between 2·9 and 25·8 μg beryllium/ft.^{3}.

A session on insurance and economics was opened by R. G. McAllister (Liberty Mutual Insurance Co., Hopkinton, Mass.). A major release of fission products had been regarded as a risk which involved a potential liability of such magnitude that two pools, involving more than 250 private companies, had been formed to deal with this business. The Price–Anderson amendment of 1951 extended the cover to 500 million dollars for any one nuclear incident. Exclusion clauses in domestic and automobile policies were explained; these were intended to prevent duplication of coverage. The total cost of an incident was linked with the stringency of standards for residual contamination in public places and safe, but realistic, limits were necessary.

R. G. Gallagher (Pittsburgh) gave an interesting account of a number of incidents in which radium, for medical use, had become disseminated by breakage of containers. P. Loysen (U.S. Atomic Energy Commission, New York) described six episodes where decontamination of buildings had been undertaken. The cost ranged from 0·11 to 2·54 dollars per square foot. Here, again, the specification of realistic levels for residual contamination was a vital economic factor.

The symposium was terminated with three papers on decontamination from France, Japan and England. P. Cerre and E. Mestre (Saclay) gave an account of a 500-kg furnace which was used for melting lead. The contamination was held in a slag of the oxides of aluminium, silica and calcium at the surface and was separated when the lead was run off into ingot moulds. An output of 2 tons a day was possible. Acetic acid and hydrogen peroxide were used for cleaning surfaces. An electrolytic polishing process had also been developed.

Y. Nishiwaki spoke of the theory of decontamination, and the use of EDTA and DBS. They had experienced highly active rainfall in 1961, from Russian tests, which contained giant particles of 0·1–1 μc. each. Instruction had been issued for fruit and vegetables to be washed and bread containing activated charcoal had been issued.

G. W. Clare gave a short history of the development of the decontamination service at Harwell. The building and plant, now in use, had paid for itself in only two years when costed on equipment which had been saved and put back into service. They moved everything by overhead tackle, which was much more satisfactory than trucking, and grid floors, with all services below, had been very successful. The centre is adjacent to the Active Waste Disposal Building and carries its own trained staff.

C. N. DAVIES

STRUCTURE AND FUNCTION OF LIPID-CONTAINING SYSTEMS

A JOINT meeting of the British Biophysical Society and the Biochemical Society attracted more than 200 participants to the University of Birmingham during April 20–21. A comprehensive survey of research relating to lipids and cell membranes was undertaken in three sessions devoted to physical chemistry of lipid systems, composition and molecular structure of cell membranes, and the role of lipid in membrane activity.

An introductory talk by Dr. D. G. Dervichian on the general theme of the symposium was followed by 20 papers.

Information derived from X-ray diffraction (V. Luzzati) and infra-red spectroscopy (D. Chapman) served to emphasize the liquid crystalline nature of lipid layers under a variety of conditions and the complexity of phase relationships in mixed lipid systems. D. Attwood and L. Saunders, and D. B. Gammack, discussed the characteristics of dispersions of lipid in water as examined by light-scattering techniques, and A. D. Bangham and D. A. Haydon the interpretation of birefringence measurements. J. A Lucy and M. Glauert presented a molecular interpretation of their electron micrographs of negatively stained lipid preparations and discussed its possible significance in relation to cell membrane structure.

A review by G. M. Gray of data on the compositions of a variety of cell membranes was followed by a report on the lipid components of membranous fractions from brain homogenates by J. Eichberg and R. M. C. Dawson. B. R. Malcolm presented evidence for the α-helical configuration in protein layers at air–water interfaces and A. A. Eddy and P. Johns speculated about the possible arrangements of lipoprotein sub-units in the red cell membrane. J. B. Finean summarized present views on the molecular structure of cell membranes and N. R. Silvester discussed the analysis of the remarkably detailed X-ray diffraction patterns obtained from membrane material derived from cilia. P. F. Millington spoke of the various factors which influenced the widths and densities of lines which characterize cell membranes in electron micrographs, and R. Dourmashkin discussed correlations between the number of sites of interaction of immune complexes with the surface of the red cell as seen in electron micrographs and the rate of lysis of the cells.

In the final session, J. N. Hawthorne undertook an assessment of present ideas on the role of individual phospholipid components in ion transport and A. D. Bangham and J. C. Watkins suggested that their observations on the accumulation of ions inside lipid micelles might have some relevance to this problem. G. Hübscher discussed the problems associated with the assessment of the influence of lipid components on the activities of enzyme systems and A. Martinosi presented biochemical and electron microscope data relating to the inactivation and reactivation of muscle microsomes by removal and restoration of the lecithin components. L. L. M. van Deenen summarized a variety of data relating to the metabolism of phospholipid fatty acids in cell membranes.

J. B. FINEAN

RESEARCH EXPENDITURE AND INDUSTRY

IN his presidential address to the twentieth annual general meeting of the Institution of Metallurgists on May 13, Dr. L. Rotherham dealt with the interrelationship of the growth of numbers of qualified scientists and technologists, the national economy as a whole, productivity and the proper expenditure on research and development. As such, his address was a noteworthy contribution. Although obviously directed to the metallurgist in the first place, it is worthy of close study by all concerned with the more general problems.

The new industries, such as electronics and man-made fibres, have achieved commercial success by exploiting scientific and technological advances and in the process have ploughed back large sums for increasingly expensive researches. Thus during a period of time they have been able to produce and sell a succession of products. In contrast, some of the older industries have been relatively slow to adopt new techniques though the pace will probably quicken in the next few years. The danger is that they will not put aside the money required to develop ever-better processes and products. In 1962 industry employed some 9 qualified men per £1 million of 'gross domestic product' outside the research and development departments. In 1956 it had been 7·9, and on the basis of a 4 per cent economic growth and what appears to be a reasonably steady figure of 10·5 qualified men per £1 million of 'gross domestic product', an additional 100,000 scientists and technologists may expect to find employment in industry between 1962 and 1970. If all employers, even the smaller, were to raise the number of 'qualified men' (that is, those with degrees, diplomas and those who take the examinations of professional institutions) employed to that of the highest percentage, almost half as many more would be needed. This increased number of scientists and technologists is seen by Dr. Rotherham as being increasingly provided by university graduates.

In 1961–62 about one-fifth of Britain's total qualified men were engaged on research and development, and if this ratio remains constant, between 1962 and 1970, about 25,000 extra people will be required. The annual cost, on the average, is of the order of £11,000 per man engaged in such work, and for this increase a rise in productivity of some 3·8 per cent would suffice. The calculation, however, depends on the assumptions that research expenditure remains at the 1961–62 level of 2·7 per cent of the 'gross domestic product' and that research costs per man do not increase by more than 3 per cent per annum. In Dr. Rotherham's opinion, it might be prudent at this time to raise the percentage of the 'gross domestic product' devoted to research in order to counterbalance any shortfall in productivity, say, to 3 per cent (the American figure is already more than this). After making the usual allowances for rising costs, this should provide for an extra 6,000 research workers per annum. The

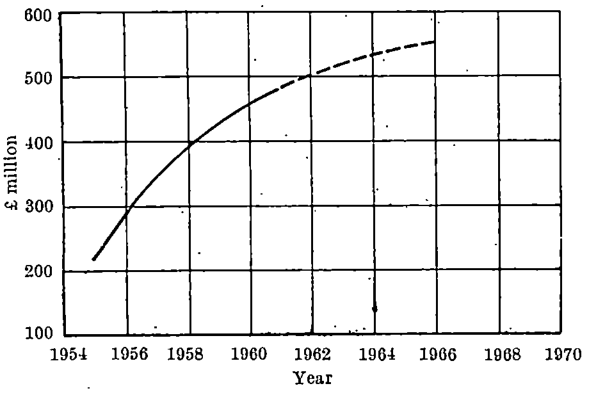

Fig. 1. Total British research expenditure (adjusted to take account of the fall in the value of the £)

wisdom of this is further demonstrated by the graph of total expenditure on research and development, since 1955, which, as can be seen from Fig. 1, seems to be levelling off at £550 million per annum, and also this expenditure expressed as a percentage of the 'gross domestic product' where the graph suggests a levelling around 2·8 per cent (Fig. 2).

In the second half of his address, Dr. Rotherham was concerned with the prospects for metallurgists in the future and the part to be played by the professional institutions. The enhanced status of metallurgy, in which the universities have played a major part, is indicated by the fact that in 1962, despite the recession, only six men who graduated that year in the subject were known to be still looking for jobs six months later. The immediate outlook is for about a 9 per cent increase per annum in the demand, even apart from increased employment in institutions of higher education and the special research expansion called for in the iron and steel industries.

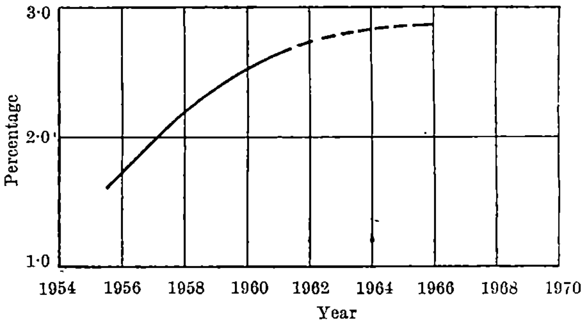

Fig. 2. Research expenditure as a percentage of the 'gross domestic product'

A pressing need is seen by Dr. Rotherham in effecting a better and more realistic image of the applied sciences to school children, their teachers and parents, and the public generally. In this the professional institutions can play a major part, especially since their function, with the increase of university graduates, will become less and less concerned with their own examinations.

It is concluded, therefore, that the numbers of men qualifying in science and engineering are increasing and will continue to increase within the foreseeable future. Membership of professional institutions is showing a corresponding growth. The full employment of these men is governed by industrial expansion and modernization, or putting it another way, commercially successful industries will need more qualified men to design, install and manage new plant. These industries will also provide the money, either directly or from taxes paid to the Exchequer, for increasingly expensive research and technological development. In contrast, any falling off in productivity will lead to a slowing up of plant modernization and research effort. Present indications are that industry will want all the qualified men it can get.

F. C. Thompson

FUNCTION OF AN INDUSTRIAL RESEARCH LABORATORY

THE above title poses questions which will always be controversial between scientists whether 'pure' or applied—since there are no precise answers. The points at issue will always hinge round the query whether an industrial laboratory should rightly embark on problems of academic interest, and vice versa. Can these two extremes be combined without detriment to both ? The principal arguments are familiar. On one hand is the alleged ivory-tower complex of the academic scientist, isolating him from the practical realities of what he is investigating. On the other hand is the severely practical approach of the technologist, who is not allowed time or opportunity to wander into the by-ways of his subject, thereby perhaps missing new vistas which could lead to important discoveries. The giant industrial companies can, of course, afford to set up as independent establishments laboratories for fundamental research akin to those of the universities. At the other end of the scale the small company can afford to cater only for its day-to-day problems, and these inevitably are purely practical.

The problem therefore becomes of real significance with companies somewhere between these two extremes, and there are many in this category. The opening of New Butler's Court, Beaconsfield, Bucks, the Research Centre of the Wiggins Teape Group of papermakers, on March 20, 1963, following a visit of inspection by the Duke of Edinburgh on the previous day, directs attention to a particular case of this kind. Butler's Court, as purchased by the Company in 1956 on the site of Edmund Burke's former home, provided 13,500 ft.^{2} of laboratory space. Since then a further 19,000 ft.^{2} of laboratories and pilot plant buildings have been erected, and the above occasion was the inauguration of these by the chairman, Mr. L. Farrow.

Wiggins Teape are papermakers and paper converters operating, in the United Kingdom alone, 15 actual paper mills and 15 factories where associated manufactures are carried on. Although the general research requirements of the paper industry are handled by the British Paper and Board Industry Research Association which the Wiggins Teape Group supports, the Company has perhaps more specialized problems than any other of its kind, since it manufactures speciality papers and in particular the so-called 'industrial papers'. Examples are: papers for impregnation with plastics, photographic base papers, leather papers, photo-copying and other copying papers, and chart papers resistant to water and oil. These, of course, are in addition to the usual types of high-grade writings and printings, and speciality wrappings such as vegetable parchment and glassine.

With a wide and varied production of this nature the interface between pure and applied science becomes increasingly diffuse. However, Dr. H. F. Rance, technical director, recently summed up the policy of his company quite unambiguously by pointing out that research and development are regarded as a business activity which must show returns commensurate with expenditure. Since they exist to promote change, in the interest of the

Company, they must themselves be flexible and adaptable. For this reason Butler's Court has been built, item by item, to meet specific needs.

Such a policy does not of necessity exclude fundamental research. Thus a basic approach has been made to the investigation of the structure of paper with special reference to the statistical geometry of the arrangement of fibres in a sheet. This has enabled the arrangement of the fibres during the formation of the sheet to be predicted mathematically, and thence it should be possible to predict also many of the physical properties of the finished paper. Such work has a natural practical sequel in the possibility of designing papers with special properties, and in the subsequent mill control necessary to ensure that these properties are achieved continuously in practice. Hence the existence of a printing room and a pilot plant aqueous coater, solvent coater and an extrusion laminator which, together with the papermaking pilot plant area, workshop and electricity substation, cost £235,500 for building and equipment. The coating plant is a special feature, since it can run at 3,000 ft. per min and can be operated with air knife or blade coater methods.

Finally, the implementation of all this development work involves an effective system of mill control to ensure that the papers are manufactured to the correct standard of quality, and, of course, profitably. Quality control in the form of factory instrumentation, the development and application of testing instruments, and a system of routine testing, is a major activity directed from Butler's Court.

This, briefly, is one Company's general conception of the function of an industrial research laboratory. The recent opening of the extension to Butler's Court is at once full justification for the initial step taken in 1956, and a tribute to the direction of the laboratory in the intervening period.

Julius Grant

POTASSIUM–ARGON AGES OF SLATES AND THEIR GEOLOGICAL SIGNIFICANCE

By C. T. HARPER

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A PROGRAMME of potassium–argon dating is being undertaken at Oxford on material from the British Caledonides, and during the course of this work the possibility of using whole-rock samples of slate for age determination was investigated. A few whole-rock slate ages have appeared in the literature[1–4] and recent work[5] has demonstrated the possibilities of using slates on a regional basis to date an orogenic (Hercynian) belt. In the Grampian Highlands of Scotland, a wide range of metamorphic grade allows whole-rock potassium–argon ages of slates to be compared with ages of muscovite and biotite from schists belonging to the same (Caledonian) metamorphic complex.

The metamorphic rocks south of the Great Glen consist of two major units, the Central Highland Granulites, similar to the Moine Series to the north, and the Dalradian Series. The Dalradian Series is divided into two structural and stratigraphic groups, the Ballapel Foundation and the Iltay Nappe Complex, each characterized by a distinct stratigraphic succession. The succession in the Iltay Nappe Complex can be divided into Upper, Middle and Lower Dalradian[6,7]. Slates and schists were collected for age determination from the Upper and Middle Dalradian of the Iltay Nappe, and from the Ballapel Foundation. Schists were also collected from the Central Highland Granulites.

The Dalradian slates are characterized by fine-grained muscovite–chlorite–quartz assemblages, the amount of muscovite relative to chlorite and quartz being directly proportional to the K_2O content of the rock. Although it was not possible to separate micas from any of the slates, X-ray diffraction analysis confirms the presence of muscovite, and shows that in all cases it possesses a high degree of crystallinity. Basal (001) muscovite peaks show no tendency to asymmetry, and the overall diffraction pattern is indistinguishable from that of completely recrystallized muscovite in the schists. Paragonite–muscovite–chlorite–quartz assemblages have been found in three slates[8]. The Middle Dalradian slates collected from the Iltay Nappe Complex have a characteristically strong strain-slip cleavage superimposed on the main slaty (flow) cleavage, while Upper Dalradian slates from the Nappe Complex commonly show little or no strain-slip cleavage. Strain-slip cleavage is particularly strong in slates collected from the Ballapel Foundation, and in some cases becomes the main cleavage in these rocks.

All samples for age determination were crushed and sieved to −40 +80 mesh. Muscovite and/or biotite in the schists were separated by conventional methods. The −40 +80 mesh fraction obtained from the slates was used for analysis without further treatment, the −80 mesh fraction being discarded owing to the possibility of argon loss from this finely ground material. Potassium was determined by flame photometer; argon was measured on a Reynolds-type, bakeable, mass spectrometer by isotope dilution with enriched argon-38; ages were calculated using the following constants:

Isotope abundance: $^{40}K = 0{\cdot}0119$ atomic per cent
Decay constants: $\lambda\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$
$\lambda e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$

The calculated ages are summarized in Fig. 1. Each point represents the potassium–argon age at one locality. Samples are divided into 4 classes, and the isotope ages of the samples in each class are arranged in numerical order and plotted in cumulative frequency. The mean age and standard deviation ($2\bar{\sigma}$) for each class is as follows:

Mica	
(1) Muscovites (from Central Highland Granulite and Dalradian mica schists)	436 ± 9 m.y.
(2) Biotites (from Central Highland Granulite and Dalradian mica schists)	424 ± 6 m.y.
Whole rock	
(3) Slates (from Middle Dalradian and Ballapel Foundation)	431 ± 6 m.y.
(4) Slates (from Upper Dalradian)	461 ± 8 m.y.

The mean ages of muscovites, biotites and Middle Dalradian and Ballapel Foundation slates are equal within the limits of error, and taken together give a weighted mean of 429 ± 4 m.y. (compare averages obtained from Moine schists[9–11]). The mean age for the Upper Dalradian slates is significantly higher.

The observed similarity between Middle Dalradian and Ballapel Foundation slate and mica ages suggests that preferential loss of argon from the slates has not occurred and that whole-rock potassium–argon ages obtained from slates are equivalent to those obtained from metamorphic micas. Ages obtained from the Upper Dalradian slates, however, fall outside the range shown by the other slates and the micas, with two exceptions (Fig. 1). A Macduff slate from the north-east Dalradian, the only dated Upper Dalradian slate which does not come either from the

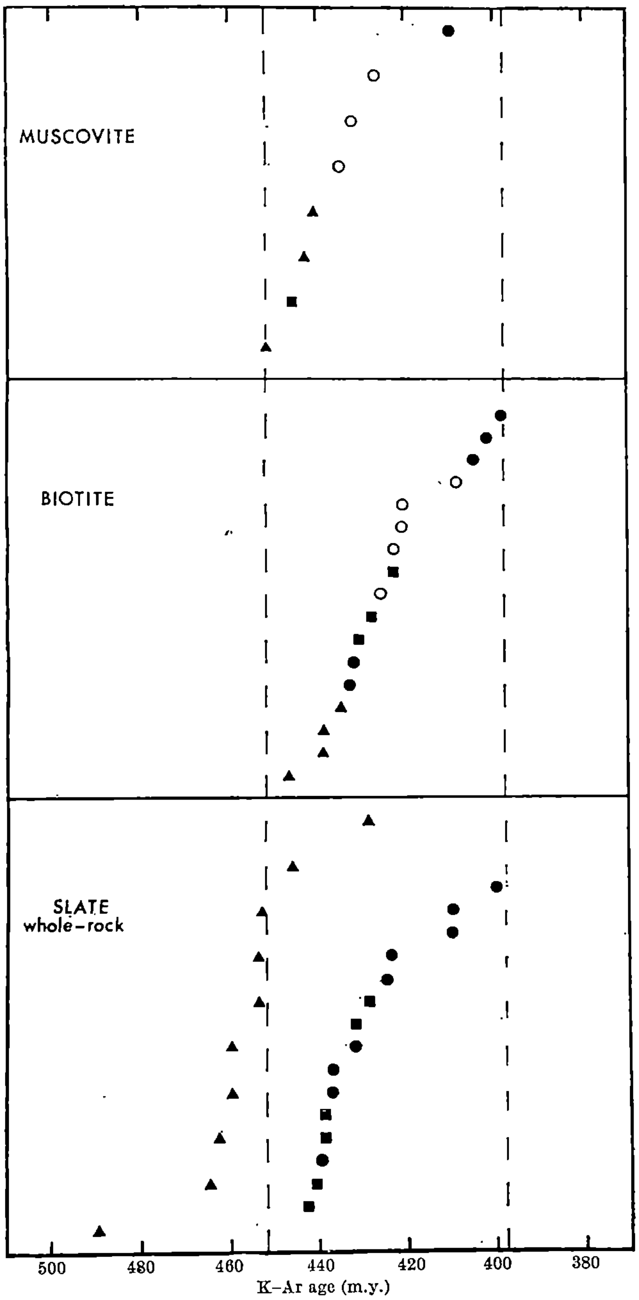

Fig. 1. Potassium–argon ages from the Dalradian Series and Central Highland Granulites
▲, Upper Dalradian; ●, Ballapel Foundation; ■, Middle Dalradian; ○, Central Highland Granulite

Loch Awe Syncline or Aberfoyle Anticline (synform) in the south-west, gives an isotopic age of 429 m.y., 32 m.y. less than the average age for the Upper Dalradian slates, and a Tayvallich slate from the Loch Awe Syncline, which has an exceptionally strong strain-slip cleavage in comparison with other Upper Dalradian slates, gives an age of 446 m.y., 15 m.y. less than the Upper Dalradian average.

Two possible sources of error in the whole-rock slate ages must be considered[12]: (1) the presence of pre-metamorphic argon in detrital minerals which have survived metamorphism; (2) loss of argon during subsequent post-metamorphic history. Although these affect the isotope age in opposite directions, it is most unlikely that both factors are so balanced at each locality in the Dalradian as to give whole-rock slate ages in close agreement with mica ages from schists belonging to the same series. Furthermore, experimental work[13] has shown that loss of argon is facilitated during lattice disturbances of any kind and particularly during loss of water. Such conditions are likely to arise during the formation of slaty cleavage in pelites[14], and result in loss of argon from their detrital constituents.

Although the presence of detrital minerals containing pre-metamorphic argon in the Upper Dalradian slates might account for their higher ages, there is no positive evidence for the presence of such minerals in these slates. Recrystallization and slaty cleavage are as fully developed in these as in slates giving lower ages. Bearing in mind the distinct stratigraphic and tectonic position of the Upper Dalradian slates, their higher potassium–argon ages most probably have a geological, rather than mineralogical, significance.

Experimental and geological evidence[15] suggests that radiogenic argon will not accumulate in the crystal lattices of potassium-bearing minerals above temperatures in the range 100°–200° C. This is below the range where greenschist facies metamorphism is believed to occur[16], and implies that potassium–argon ages of minerals from schists reflect a time of cooling, rather than metamorphism. Isotope ages conforming to such a cooling history have been reported from the Himalayas[17] and European Alps[18], where metamorphic minerals from upper tectonic units give older ages than minerals from lower units which would have cooled more slowly. In the Dalradian Series and the Central Highland Granulites, consistent potassium–argon age differences are found between coexisting muscovite and biotite, irrespective of their relative order of crystallization in the rock, indicating that argon began to accumulate in muscovite some 12 m.y. before it began to accumulate in biotite. In terms of a cooling history, this suggests that argon can accumulate in muscovite at somewhat higher temperatures than it can in biotite.

Cooling of a metamorphic belt is most likely to occur as a result of uplift. Evidence from the New Zealand Mesozoic metamorphic belt[15] shows that argon did not begin to accumulate in the constituent minerals of deep-seated metamorphic rocks until post-metamorphic uplift along the Alpine Fault in Late Tertiary times brought these rocks near the surface. In Scotland, post-metamorphic uplift and cooling of the Dalradian block is likely to have occurred immediately prior to denudation. Severe denudation of the Dalradian undoubtedly occurred at the close of the Silurian with the deposition of Old Red Sandstone conglomerates. However, recent work in the Southern Uplands[19] has revealed the presence of Dalradian schist fragments in Wenlock sediments, suggesting that denudation of the Dalradian was already occurring in Mid-Silurian times. Uplift and cooling would therefore appear to have commenced during the Early Silurian, that is, about 425 m.y. ago using Kulp's time-scale[20]. This stratigraphic estimate for the time of cooling fits well with the average isotopic age of 429 ± 4 m.y. obtained from the Middle Dalradian of the Iltay Nappe Complex, the Ballapel Foundation, and the Central Highland Granulites.

Potassium–argon ages approaching the true age of metamorphism can only be obtained from materials which recrystallized during metamorphism under conditions resulting in complete outgassing of inherent argon, but at temperatures close to that at which subsequent radiogenic argon could accumulate. It is possible that recrystallization resulting in the formation of slaty cleavage occurs at such temperatures, and that high (stratigraphic) level slates which have cooled rapidly after metamorphism may yield whole-rock potassium–argon ages approximating to the age of metamorphism. In the Dalradian, the only high-level slates that have survived denudation are found preserved in the axis of the Loch Awe Syncline and downfolded nose of the Iltay Nappe (Aberfoyle Anticline). The older ages obtained from these Upper Dalradian slates, ranging upwards to 490 m.y., may therefore approach the true age of Dalradian metamorphism, which, from the stratigraphic relationships in Ireland[21], would appear to be pre-Arenig.

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CONCENTRATIONS OF CÆSIUM-137, RHODIUM-102 AND MANGANESE-54 IN SURFACE AIR

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THE concentration of various γ-emitting fission products present in surface air have been determined at this and other laboratories for a number of years[1-3]. Such measurements indicate the amount and composition of atmospheric contamination, and may also provide information regarding the age and even the source of fission debris[4].

At this laboratory airborne particulates were collected on '*H V*-70' filter paper (Hollingsworth–Vose asbestos base filter paper having an efficiency of ~96 per cent for particles of a diameter > 0·25μ) exposed on air pumps operated at flow rates of ~40 m³/h. A number of such pumps have been in continuous operation since late 1952. Known portions of each filter paper were combined to form monthly or semi-monthly composites containing particulates from ~10⁴ m³ of air. Physically the composites formed a compressed disk of finely cut filter paper enclosed in a plastic Petri dish, which was placed on top of a 4 in. diameter by 4 in. thick cylindrical NaI crystal for γ-ray spectrometric analysis. The crystal and source were contained in a 6-in. thick steel shield to provide a low background counting rate, and a 400 channel transistorized multichannel analyser was used for pulse height analysis. The quantities of the individual radionuclides present in the filters were determined by the solution of the appropriate number of simultaneous equations, the coefficients of which were found from the use of standard sources similar in shape to the composites and similarly placed on top of the crystal. During periods when fresh or reasonably fresh fission debris was present, the concentrations of the following radionuclides were determined by the foregoing method: ^{144}Ce–^{144}Pr, ^{141}Ce, ^{125}Sb, ^{103}Ru, ^{106}Ru–^{106}Rh, ^{137}Cs, ^{95}Zr–^{95}Nb, and ^{140}Ba–^{140}La. During the test moratorium as the amounts of the shorter-lived fission products decayed below detectable limits their analyses were discontinued, and analysis of ^{7}Be, produced by cosmic ray interactions in the atmosphere, was added to the computer programme.

Repeated analyses of filters collected during the spring months of 1962, several months after collection, indicated that another radionuclide or radionuclides were also present. Careful examination of a number of spectra in conjunction with estimates of radioactive half-life led us to conclude that ^{54}Mn and ^{124}Sb were present in surface air at that time. Hence, these two activation products formed during the 1961 Soviet tests were also considered in the mathematical analysis.

The concentration of ^{137}Cs observed in surface air a this site during January 1959–December 1963 is plotted in Fig. 1. As noted in the past, the most obvious feature is that of pronounced periods of maximum concentration during the spring months followed by periods of minimal activity during the autumn and winter. These maxima are believed to arise from increased stratospheric mixing during the winter and increased stratosphere–troposphere transfer during the spring months[5,6]. Similar phenomena have been observed in the case of ozone as reported and discussed by Brewer[7] and Dobson[8]. Prior to the resumption of nuclear testing in 1961, the highest concentration of ^{137}Cs in surface air, 0·035 pc./m³, was observed in 1959. Higher levels were present in 1962, ~0·08 pc./m³, and 1963, ~0·16 pc /m³, a fact which is not surprising in view of the large amount of fission debris injected into the atmosphere during 1961 and 1962. Another, less obvious, feature is that the peak concentration occurred later in 1961, 1962 and 1963 than in previous years. With the exception of 1953, when the ^{137}Cs concentration was so low that it might easily have

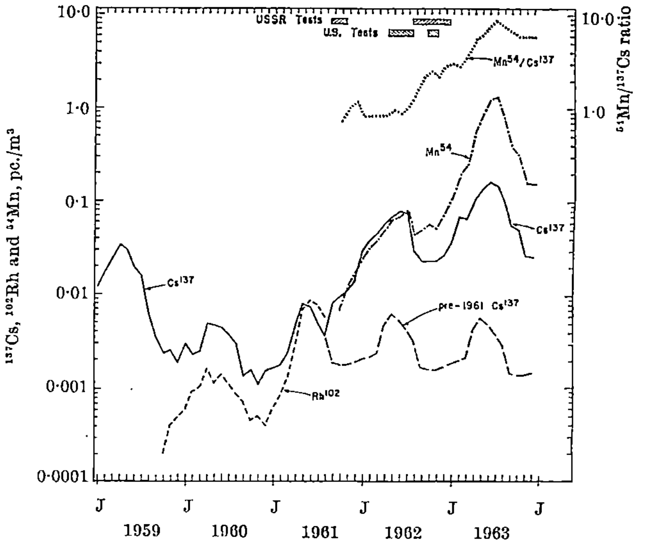

Fig. 1. ^{137}Cs (——), ^{102}Rh (– – –), ^{54}Mn (—·—·—) and ^{54}Mn/^{137}Cs (. . .) ratio in surface air

been perturbed by testing then in progress, the peak has been reached during April and May each year since measurements were begun here in late 1952. In 1961 the peak came in May and June, and in 1962 and 1963 the peak was not attained until June and July.

The delay in the appearance of the peak concentration during the past three years may be entirely fortuitous, or it may reflect some vagaries of the meteorological processes involved. There is evidence, however, that a fairly sizeable fraction of the total ^{137}Cs found in surface air during 1961–63 derived from events in which the nuclear debris was initially present at high altitude.

The presence of debris of high-altitude origin was determined by measuring ^{102}Rh and ^{54}Mn in surface air. The concentration of ^{102}Rh (corrected for radioactive decay to mid-August 1958) and of ^{54}Mn (corrected to mid-October 1961) are also illustrated in Fig. 1. Both radionuclides were activation products associated with high-altitude or high-yield explosions. ^{102}Rh was produced as a tracer in the Orange shot of the U.S. *Hardtack* test series on August 11, 1958, which had a burst height of about 43 km over Johnston Island (16° N., 170° W.)[9]. The highest concentrations of ^{102}Rh were reported by Kalkstein[9] to have been present in middle and polar latitudes in both hemispheres above 19 km from mid-1960 until late 1961. Hence the regions whence ^{102}Rh might enter into the lower atmosphere would similarly be situated in middle and polar latitudes. ^{54}Mn was produced in high-yield shots during the U.S.S.R. 1961 test series, debris from which must have risen well into the polar stratosphere, and its major source may be presumed to have remained there for some time. A second tracer, ^{124}Sb, was produced by activation in the 57-MT shot of this series. Unfortunately, the short half-life (60 days) has precluded to a large degree the investigation of this nuclide in surface air. However, investigation of ^{124}Sb relative to ^{54}Mn in high-altitude samplings have proven useful in ascertaining that no appreciable new input of ^{54}Mn occurred since 1961. ^{124}Sb and ^{54}Mn data obtained at 65,000 ft. from the *Ash Can* balloon flights made from San Angelo, Texas, indicate a fairly constant ^{54}Mn/^{124}Sb ratio from early 1962 until November 1963 (refs. 10, 11). This fact seems to imply that no significant amounts of ^{54}Mn were made subsequent to 1961. Other data from these flights show more ^{124}Sb relative to ^{54}Mn at higher altitude (80,000–100,000 ft.)[10], which is taken to mean that some ^{54}Mn may have been produced in explosions which led to the initial injection of debris to lower altitude than that from the 57-MT shot. In the spring of 1962 the ^{54}Mn/^{137}Cs activity ratio observed at 65,000 ft. had a mean value of 40 : 1 (ref. 10).

As indicated in Fig. 1, the peak concentration of ^{102}Rh in 1961 occurred in June and July. Similarly the concentration of ^{54}Mn reached peak values in June and July in both 1962 and 1963. The amount of ^{137}Cs and ^{102}Rh produced in the Orange shot is not known, although a figure of 3 megacuries of ^{102}Rh has been reported by Kalkstein[9]. One may presume that ^{137}Cs from other events, such as the Teak shot which also occurred at high altitude within days of Orange, have become mixed with that from Orange. In fact, aircraft sampling at 50,000 ft. during May 1961 indicated a ^{137}Cs/^{102}Rh activity ratio of nearly unity[12]. Such a value for this ratio would imply that all, or nearly all, of the ^{137}Cs shown in Fig. 1 during 1961 came from high altitude. If a smaller value of the ratio is used for debris in surface air, then the effect of removing the high altitude portion is that the maximum concentration shifts back to April and May 1961.

Likewise it is difficult to determine directly the amount of ^{137}Cs produced along with the ^{54}Mn, and the value of 40 : 1 for the ^{54}Mn/^{137}Cs ratio has been used. The observed values of the ^{54}Mn/total ^{137}Cs ratio are shown in Fig. 1. They indicate a fairly constant value during early 1962, followed by a steady increase during the latter half of 1962–mid-1963, at which time a new steady value is reached. This increase is somewhat surprising in view of the fact that considerable ^{137}Cs was produced during the summer and autumn of 1962. In order to examine the relationship between ^{54}Mn and ^{137}Cs produced in 1961 during subsequent years, it is necessary to determine the sources of the ^{137}Cs present in surface air.

Further examination of ^{137}Cs and other radionuclides produced during 1961 was attempted during 1962 and 1963 through the use of activity ratios; namely, ^{95}Zr/^{137}Cs, ^{103}Ru/^{95}Zr, ^{103}Ru/^{137}Cs, and ^{140}Ba/^{95}Zr. In addition, certain mean times of production were selected for the various test series: October 15, 1961, for the U.S.S.R. 1961 series; June 15, 1962, for the U.S. *Dominic* series; and October 15, 1962, for the U.S.S.R. 1962 series[13]. At the outset it was necessary to remove the ^{137}Cs which was produced in tests conducted prior to September 1961. For the period September–December 1961 the average ^{137}Cs values found in the same months during 1960 were adjusted by the ratio of the 1961–60 spring maxima. In 1962, the monthly averages of 1960 and 1961 observations were taken to be representative of the pre-1961 ^{137}Cs, and during 1963 these same values were reduced by a factor of 0·9 to account for radioactive decay and loss from the stratospheric reservoir. The pre-1961 ^{137}Cs values obtained in this manner are illustrated in Fig. 1. Removal of the pre-1961 ^{137}Cs from the total during early 1962 had the effect that on extrapolating back to a ^{95}Zr/^{137}Cs ratio of 145, the apparent time of origin was mid-October 1961, in accord with the assumed time of production for the series as a whole, and also in agreement with the findings of other investigators[14].

During June and July 1962 a new input of fission products was apparent from the abrupt rise in the ^{140}Ba/^{95}Zr and ^{140}Ba/^{103}Ru ratios. Estimates of the amount of ^{137}Cs associated with this new injection, which presumably came from the continental and Pacific phases of the U.S. *Dominic* series, were made through the use of the above ratios. The amount of ^{137}Cs ascribed to this series by these measurements was fairly uncertain. Suffice it to say that it was of the same order as that observed during comparable months during 1958 which arose from the U.S. *Hardtack* series as inferred from ^{181}W measurements made at that time[3]. Thus it has been assumed that the ^{137}Cs present during 1963 from *Dominic* was comparable to that observed in 1959 from the *Hardtack* series. From late August 1962 until the end of the year an appreciable amount of new debris became evident, the age of which varied between August to mid-December, the bulk of which was presumed to come from the Soviet tests then in progress[12].

During 1963, ^{137}Cs from pre-1961 sources and from *Dominic* was removed from the total. Similarly, the ^{95}Zr from *Dominic* was subtracted from the totals observed. The best fit to the ^{95}Zr/^{137}Cs ratio for the remaining ^{137}Cs and ^{95}Zr was obtained by using a mean time of production of October 15, 1962, for the interval January-July 1962, and a mean time of production of November 15, 1962, for the remainder of 1963. In the fitting process the excess ^{137}Cs was found, which excess was presumed to have arisen from the U.S.S.R. 1961 tests.

The results of partitioning the total ^{137}Cs, minus the pre-1961 component, into that attributable to *Dominic*, and the U.S.S.R. 1961 and 1962 series, are shown in Fig. 2. During the spring of 1962 less than 10 per cent of the total ^{137}Cs present in surface air collected at this location derived from tests conducted prior to 1961. In 1963, the pre-1961 component fell to less than 5 per cent of the total; approximately 70 per cent of the total came from testing in 1962, and the remaining 25–30 per cent arose from testing in 1961. The situation in 1963 was similar to that in 1959 when some 80 per cent of the total ^{137}Cs came from testing conducted the previous year[3].

The ratio of ^{54}Mn relative to the ^{137}Cs from testing in 1961 is shown in Fig. 3. The variation of the ratio with time is essentially the same as seen in Fig. 1 except that

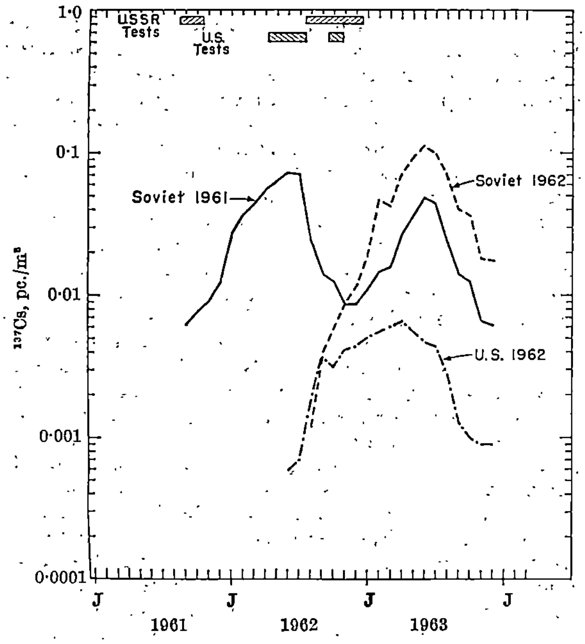

Fig. 2. Partition of ^{137}Cs in surface air. ——, U.S.S.R., 1961; - - - U.S.S.R., 1962; —·—·, U.S., 1962

the final values attained are appreciably higher, as was to be expected according to the previous discussion. The value of the ratio of 25 : 1 to 30 : 1 observed from July 1963 onward is in keeping with the *Ash Can* observations made during the spring of 1962. The total 1961 ^{137}Cs was then further divided into that associated with ^{54}Mn, using a Mn/Cs ratio of 40 : 1, and that fraction which was not, the latter being called the residual ^{137}Cs. Results of this final partition are also illustrated in Fig. 3. It is of interest to note that the peak concentration of the residual ^{137}Cs in 1963 now occurs in May and June rather than in June and July, as shown for the total 1961 ^{137}Cs. The use of a higher ^{54}Mn/^{137}Cs ratio would have the same effect.

It thus appears that in 1961 and again in 1963 there was appreciable ^{137}Cs of high-altitude origin present in surface air. It is difficult to demonstrate by means of tracers that the same was true in 1962. One might consider, however, that by the spring of 1962 the ^{54}Mn may not yet have been well mixed with other debris existing at the same altitude which derived from different sources. The transport of such debris might be the same as for that mixed with ^{54}Mn. This would imply that one might use a smaller value of the Mn/Cs ratio, which would have the effect of causing the spring peak of the residual ^{137}Cs to occur earlier in 1962. One may postulate similar circumstances in 1963 involving debris from the 1962 polar tests for which a unique tracer has not yet been observed.

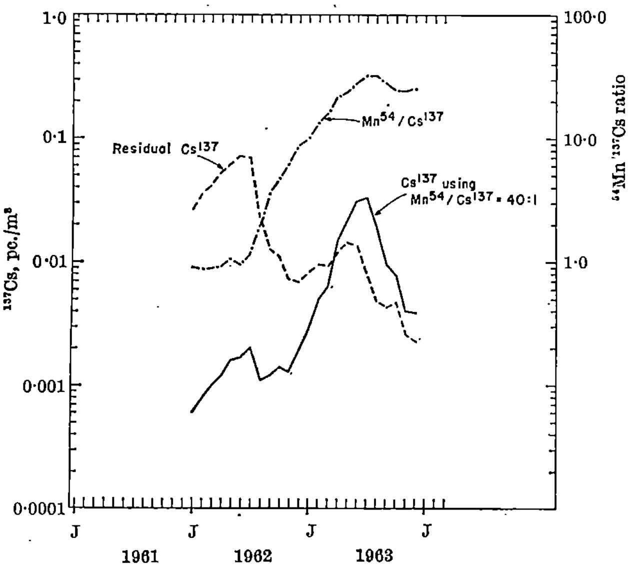

Fig. 3. ^{137}Cs from 1961 tests and ^{54}Mn/^{137}Cs ratio. ——, ^{137}Cs derived from ^{54}Mn/^{137}Cs ratio; - - -, residual ^{137}Cs (^{137}Cs not associated with ^{54}Mn); —·—·, ratio of ^{54}Mn to total 1961 ^{137}Cs

It is not possible to ascertain from these measurements the reason why high-altitude debris is delayed in reaching the surface. It may be related to particle size, or perhaps directly to altitude and the resultant increased path between source and the surface of the Earth. Perhaps measurements of ^{54}Mn and other tracers such as ^{109}Cd during 1964 and subsequent years will help to elucidate the relationship between altitude of origin and the time of appearance of the peak concentrations in surface air. It may be that observations of a natural tracer such as ^{7}Be will be helpful in establishing whether this is actually an altitude effect, or is due to a more general change in meteorological conditions.

A further use may be made of the ^{137}Cs data, namely, that of estimating the levels of ^{137}Cs to be expected during 1964. In some respects conditions during 1963 and 1964 parallel those in 1959 and 1960, barring the perturbation introduced by relatively more debris of high-altitude origin at present. A comparison between the autumn minimum in 1959 and the spring maximum in 1960 applied to the minimum observed in 1963 leads to an estimate of ~ 0·060 pc./m^3 during the peak in 1964. Preliminary values of ^{137}Cs obtained during January, February, March and April 1964 were 0·036, 0·038, 0·060 and 0·065 pc./m^3, respectively. A month by month comparison with findings in 1963 would lead to an expected peak value of ~ 0·13 pc./m^3 in 1964. A maximum concentration of this order is also found by projecting the individual components illustrated in Figs. 1 through 3 into 1964. This was done by making the assumption that the 1962 Soviet debris will behave in 1964 as did the 1961 component in 1963. Furthermore, it was assumed that the 1961 ^{137}Cs associated with ^{54}Mn would reach the same level in 1964 as in 1963. Support for this latter assumption is based on the fact that the ^{102}Rh concentration in precipitation was approximately the same during 1961 and 1962 as observed at Westwood, New Jersey[15]. The result of such a projection is a peak value of ~ 0·11 pc./m^3 in 1964, which is approximately 70 per cent of the magnitude of the 1963 maximum.

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INADEQUACY OF THERMAL INITIATION THEORY IN INTERPRETING THE RESULTS OF FISSION FRAGMENT IRRADIATION OF EXPLOSIVES AT ELEVATED TEMPERATURES

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CONSIDERABLE research has been carried out in recent years[1] to determine the basic requirements necessary to initiate an explosive. Most of the more extensive investigations have been made on the macroscopic behaviour of explosives and have been interpreted satisfactorily in terms of the classical 'hot-spot' model[2–6]. Several explosives have also been subjected to irradiation, but not until recently has a detailed analysis of this process been attempted.

Cerny and Kaufman[7] irradiated several explosives with pions to produce spherical hot spots less than 10^{-5} cm in diameter. In this work it was noted that no initiations occurred. However, it was shown that the high energy–density produced by pion capture (when assumed to quickly degrade to thermal energy)[8,9] should cause a temperature increase sufficient to initiate several of the irradiated explosives.

A comparative analysis of typical pion-capture and nuclear-fission events shows that in the latter process a higher energy–density occurs and hence a greater temperature increase. As discussed in detail elsewhere[7], the energy deposited by the Auger electrons and α-particles which arise from a 'typical' pion capture event produces a spherical hot spot. The intense ionization and excitation along a fission-fragment track, however, produce a high energy–density region of cylindrical geometry.

Irradiations of a few explosives have been performed with fission fragments[10,11] but no initiations have been observed. (The explosions of nitrogen iodide are apparently due to a surface effect rather than a thermal spike[12–14].) However, the calculations of Cerny and Kaufman[7] indicated that some of the more sensitive secondary explosives had not been investigated and that, surprisingly, the frequently investigated lead azide was not expected to initiate on this model.

The purpose of the work recorded here was to determine the behaviour of several of these more sensitive high explosives when irradiated with fission fragments at a controlled elevated temperature. Table 1 shows the temperature required to produce various degrees of decomposition in 10^{-11} sec (the characteristic time for heat dissipation) for all the explosives investigated. It is apparent that the strength of the thermal spike arising from the irradiation need not be great to produce appreciable heat from chemical decomposition. Then, by having the explosives at some elevated temperature, say, 150° C, the likelihood of an irradiation-induced initiation—according to the 'hot-spot' model—should be significantly increased. Table 1 also shows the temperatures used in the experiment. Four high purity (> 99 per cent) secondary explosives were investigated: pentaerythritol tetranitrate (PETN), hexahydro-1,3,5-trinitro-*s*-triazine (RDX), octahydro-1,3,5,7-tetranitro-*s*-tetrazine (HMX) and glyceryl trinitrate (nitroglycerin).

A variable temperature explosive container was used to heat the explosive samples. A small quantity (0·30 ml.) of an acidic solution containing californium-252 (3 per cent spontaneous fission) was added to 300 mg of each explosive. In the case of the three solid explosives the mixture for each pellet was then dried, mixed and pressed on to a 10 ml. thermocouple in right-cylindrical pellets approximately 0·25 in. in diameter and 0·25 in. high.

Table 1. TEMPERATURES REQUIRED FOR VARIOUS FRACTIONAL DECOMPOSITIONS

Explosive	Maximum possible temperature increase (Q/c) °C	Temperature of the explosive (°C) required to produce a decomposition of 1%	50%	99%	Experimental temperature °C
RDX	4,850	750	935	1,035	160
HMX	4,020	805	1,025	1,160	215
PETN	4,930	1,065	1,625	2,050	125
Nitroglycerin	5,380	640	815	920	180

These are the temperatures at which each of the explosives must be kept for 10^{-11} sec in order to produce 1, 50 and 99 per cent chemical decomposition; also listed are the temperature increases that would result from complete decomposition (Q/c), and the temperatures at which the irradiations were performed.

Each pellet was inserted in the container and heated on all surfaces except the top from room temperature to approximately 35° C below the melting-point of the explosive. The pellet was kept at this temperature for 30 min and then heated to ignition. The time to ignition, which ranged from 4 to 10 min, and the temperatures of the pellet and the container were recorded. Five pellets of each explosive were examined in this way. The behaviour of the explosive pellets containing californium-252 was compared with that of pellets of the same explosive prepared in an identical manner but without californium.

The amount of californium-252 in the pellets was determined by measuring the α-activity of small samples of the solution in a liquid scintillation counter. The spontaneous fission to α-decay ratio being known, it was possible to ascertain that at least three fissions per minute occurred in the explosive samples. Table 2 shows the parameters of typical light and heavy fission fragments for californium-252 (ref. 15).

Table 2. TYPICAL PARAMETERS FOR FISSION FRAGMENTS FROM THE SPONTANEOUS FISSION OF CALIFORNIUM-252

Parameter	Units	Light	Heavy
Mass (M)	A.M.U.	111	141
Energy (E)	MeV	100·5	78·0
Velocity (V)	cm/sec	$1{\cdot}32 \times 10^9$	$1{\cdot}03 \times 10^9$
Range (R)	μ of RDX	8·4	7·0
Charge (Z)	—	44	54

The liquid nitroglycerin samples were easier to irradiate since it was only necessary to add the solution containing the californium-252. The mixture was then placed into the container in a small beaker and the thermocouple immersed.

A second method of irradiation was also employed using californium-252 plated on small platinum foils. In this case, a foil was placed in direct contact with the top of the pellet (immersed in the case of nitroglycerin) and the pellet was heated as before. Similar procedures to those of the first method for controlled heating to ignition and measuring the activity were followed. The amount of radiation to which each pellet was exposed was determined to be 33 fragments per min, which was appreciably greater than that of the first method.

No differences between the response of the irradiated explosives and the standards were observed even though the former were exposed to 200–2,000 fission fragments. The 'hot' pellets showed no signs of accelerated decomposition (compared with the standards) either when held at the elevated temperature or when heated to ignition. Both types of pellets of an explosive ignited at the same temperature, in the same general manner, and in the same time—within the normal deviations for such measurements on explosives.

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Although the fission-fragment irradiation did not produce initiation of these explosives, a detailed quantitative analysis was made to determine the predicted behaviour within the framework of the 'hot-spot' model[2–6]. This model has enjoyed some success in explaining the behaviour of explosives on a macroscopic scale, but has not often[7] been used in detail in an analysis of microscale processes.

Initial 'temperature'-radius profiles for the hot spots, which in reality are energy-radius profiles, were obtained by calculating the energy deposited in successive cylindrical shells coaxial with the fission-fragment track. Such calculations are complicated, however, by lack of precise information on the relative amounts of energy that a stopping fission fragment loses to excitations and ionizations. Magee[16] discusses the ionization and excitation resulting from the passage of high-energy charged particles through matter and postulates that two processes—termed 'head-on' and 'glancing' collisions—make equal contributions to the differential energy loss of the particle. Using this model for the initial part of its track, we assume that a fission fragment would lose half its energy in producing δ-rays (knock-on electrons of energy, say, greater than 200 eV) and the other half in low-energy ionizations and excitations. This latter type of energy deposition was assumed to occur within 10 Å of the centre of the track[17]. Although the values chosen for the minimum δ-ray energy and the radial distance for lesser energy drops are somewhat arbitrary, it is evident[17,18] that 200 eV is of the correct order of magnitude for such a figure and that for the explosives investigated 10 Å is approximately the straight-line distance travelled by a 200-eV electron.

The relative number of δ-rays of a given energy that are produced by the fission fragment can be found using the relation of Mott as cited by Bradt and Peters[19]. This expression was normalized (over all possible energies) to one-half the value of a (dE/dx) which was calculated from the range–energy curves of Fulmer[20]. This particular (dE/dx) was chosen at the point along the track where the fragment had lost one-half its initial energy.

The electron range–energy relation used to find the energy deposited away from the track was that of Glocker as cited by Katz and Penfold[21]. To account for the fact that such slow electrons do not move out in a straight line nor in a plane perpendicular to the fragment track, one-half the practical range as given by Glocker was used as an average, or effective, range[22–25].

The time required for the passage of a fission fragment and its subsequent energy deposition is of interest. For a section of the track where *l-d*-100 Å, the passage of the fragment through this region and the stopping of the low-energy δ-rays should occur in appreciably less than 10^{-13} sec. Hence we may assume that the initial energy distribution arises in 10^{-13} sec.

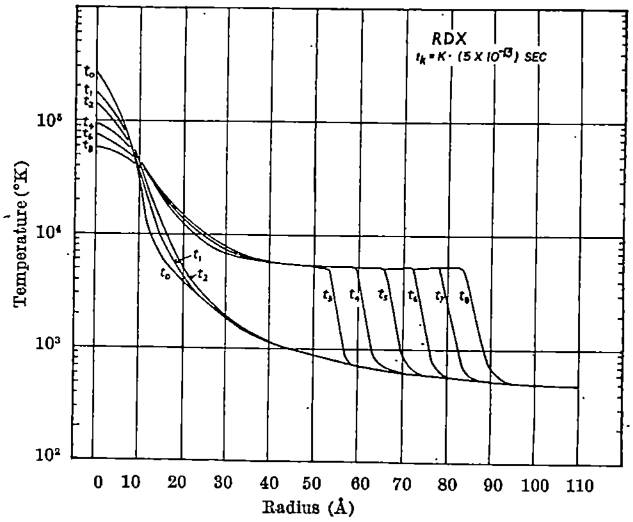

Fig. 1. Temperature versus radius profiles for various times following the passage of the fission fragment through RDX. t_k represents a time period of k times the interval 5×10^{-13} sec

For a first-order reaction the subsequent time-behaviour of the (cylindrical) hot spot is governed by the heat-diffusion equation:

$$\frac{\partial T}{\partial t} = \varkappa \nabla^2 T = \frac{Q}{c} n Z e^{-E/RT} \tag{1}$$

where T is the absolute temperature, t is the time, $\varkappa$ is the thermal diffusivity, Q is the heat of reaction, c is the specific heat, n is the mass fraction of unconsumed reactant, Z is the Arrhenius pre-exponential factor, E is the activation energy, and R is the gas constant. Also, $\varkappa = k/\rho c$, where k is the thermal conductivity and ρ is the density. These constants are listed in Table 3 for the explosives investigated.

Solutions to equation (1) for later times were obtained consistent with the initial temperature profile and subsequent reactant consumption. The method used was an extension of the Schmidt method modified to include the heat liberated by chemical reaction[37]. The material was divided into a series of concentric cylindrical shells, the temperature of each shell being uniform, and the temperatures of the various shells determined by the initial temperature–radius profiles (Figs. 1–3). The temperature for the i^{th} shell, T_i, at time $t + \Delta t$ was then obtained from the temperatures at time t by the approximation:

$$T_i(t + \Delta t) = \left\{\frac{\varkappa}{(\Delta r)^2}\left(1 - \frac{\Delta r}{2r_i}\right)[T_{i-1}(t) - T_i(t)] - \frac{\varkappa}{(\Delta r)^2}\left(1 + \frac{\Delta r}{2r_i}\right) x \; x\,[T_i(t) - T_{i+1}(t)] + \frac{O}{c} n_i(t) Z\right\} \Delta t + T_i(t) \tag{2}$$

Table 3. PHYSICAL CONSTANTS OF THE EXPLOSIVES

Quantity	Units	RDX		Nitroglycerin		PETN		HMX	
		Value	Ref.	Value	Ref.	Value	Ref.	Value	Ref.
ρ	gm/cm³	1·66	26	1·596	28	1·46	1	1·66	26†
c	cal/gm °C	0·264	26	0·300	29	0·272	29	0·315	34
k	cal/sec cm °C	$4{\cdot}9 \times 10^{-4}$	26	$5{\cdot}03 \times 10^{-4}$	29	6×10^{-4}	1	$4{\cdot}9 \times 10^{-4}$	26*
Q	cal/gm	1,280	27	1,615	30	1,341	27	1,266	35‡
Z	sec⁻¹	$10^{21{\cdot}2}$	26	$10^{20{\cdot}5}$	31, 32	$10^{15{\cdot}3}$	33*	$10^{19{\cdot}7}$	36
E	kcal/mole	57·2	26	48·0	31, 32	38·6	33*	52·7	36
Formula	—	$C_3H_6N_6O_6$	—	$C_3H_5N_3O_9$	—	$C_5H_8N_4O_{12}$	—	$C_4H_8N_8O_8$	—

* Cook and Abegg, recognizing the autocatalytic nature of PETN, extrapolated to find the 'unimolecular' (first order) initial rate for the reaction. This, of course, becomes considerably faster in the presence of its decomposition products.

† The values for the density (ρ) and the thermal conductivity (k) of RDX and HMX were set equal because of their similar chemical character and due to lack of consistent data for the pure materials.

‡ Johnson (ref. 35) cites Q_L for HMX as 1,356 cal/gm from *Explosion Effects Data Sheets, NAVORD Rep* 2896 (*NOL*), June 14, 1955 (addendum of 1956). However, Tonegutti (ref. 27) points out that the difference between Q_L and Q_V for RDX is 90 cal/gm. Assuming the same relationship for HMX (since Q_L is nearly the same—as are most of the properties), we obtain 1,266 cal/gm for Q_V for HMX.

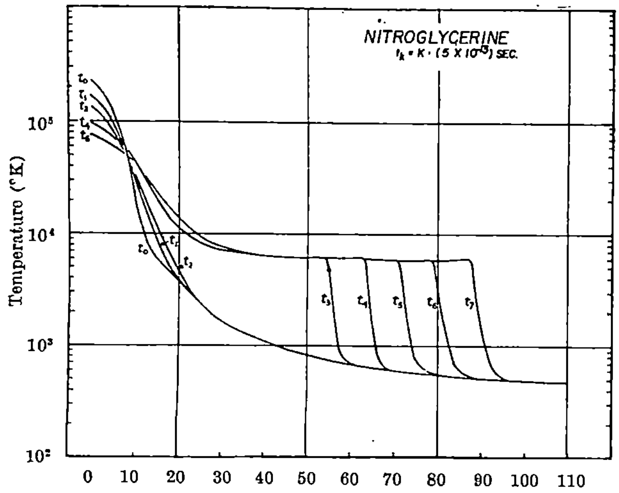

Fig. 2. Temperature versus radius profiles for various times following the passage of the fission fragment through nitroglycerin. t_k represents a time period of k times the interval 5×10^{-13} sec

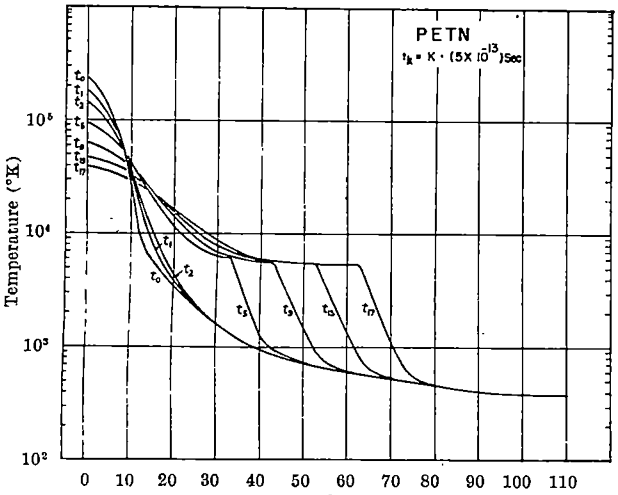

Fig. 3. Temperature versus radius profiles for various times following the passage of the fission fragment through PETN. t_k represents a time period of k times the interval 5×10^{-13} sec

where Δr is the thickness of each cylindrical shell and r_i is the radius to the centre of the i^{th} shell.

We have assumed that since molecular vibration frequencies are $\sim 10^{13}$/sec, it is possible to have the initial energy profile transformed into a temperature profile by 10^{-12} sec. Secondly, we have assumed that this transition from an energy-radius to a temperature-radius distribution can be estimated by using equation (1) neglecting chemical reaction. After 10^{-12} sec the complete solution to equation (2) is obtained. The temperature-radius profiles were calculated using an I.B.M. 709 digital computer. Equation (2) was solved for a series of time-intervals, Δt, chosen such that the temperature change in any shell during the time-interval was less than 5 per cent; the shell thickness, Δr, was taken to be 5 Å. The results for RDX, nitroglycerin and PETN are shown in Figs. 1, 2 and 3, respectively, with initiation predicted in all cases. The behaviour of HMX is expected to follow very closely that for RDX.

An alternative approach was used to check the sensitivity of the results to a variation in the initial energy–radius profile. In this case, energy deposition was assumed to be due to δ-rays alone so that the temperatures for the first three shells (15 Å) were essentially identical ($\sim$ 1·4 × 10 °C in RDX). These calculations also predicted that initiation should occur in each explosive in much the same manner as in the initial case. Although initiation is predicted for all the explosives investigated, such is not the case for explosives in general, as was shown previously[7].

The thermochemical and physical data used in the calculations are listed in Table 3. It was assumed that the thermal conductivity and specific heat were temperature independent, and further that the specific heat of the products of reaction was the same as that of the original explosive. Macroscopic thermal parameters were used throughout, as in previous work, due to the lack of contradictory information[38].

These results show once more the inadequacy of the 'hot-spot' model in describing the effects of microscale events in explosive materials. It should be noted that Bowden and Yoffe[1] have postulated that a critical size of 10^{-5}–10^{-3} cm must be exceeded before the model is applicable; however, it is not apparent from the mathematical model itself just how this limitation comes about. Two theories have been proposed recently which when applied to our experimental conditions appear to yield results consistent with ours. A recent estimate has been made by Dodd[39] using a statistical approach which indicates that the simultaneous decomposition of the order of only ten molecules is sufficient for initiation to occur. Secondly, a statistical model has also been used by Ling[40] to describe initiation phenomena. In applying this model to the case of fission-fragment irradiations, he finds that for temperatures of 200°–400° C in the volume around the track, initiation is unlikely. It appears, however, that if his calculations were extended to include the higher temperatures indicated in this work (say, 1,500° C in a 100-Å radius cylindrical volume), this model would also predict that initiation is likely.

On the other hand, some of the assumptions made in our work are open to question. The extrapolation of kinetic data to these conditions is unavoidable but possibly erroneous. Certainly the processes involved in the conversion of energy to temperature and their interrelation with chemical reaction are not well understood when considered on this time scale[41]. It is conceivable, as noted in shock-wave experiments[42,43], that a delay may exist between the time at which a temperature distribution is established and the beginning of chemical reaction. It should also be noted that calculations on atomic displacements in copper indicate that heat dissipation may occur more rapidly in microscale events than would be expected from classical heat conduction[44]. If there were an analogy, such an effect in explosives would considerably alter our calculations. Lacking more precise information concerning the assumptions made, however, it appears that the 'hot-spot' model may require considerable revision to account for experimental data on explosive irradiations.

We thank Dr. Harold J. Matsuguma for his assistance in the preparation of the experiment.

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THIS attempt to calculate the temperature and the behaviour of a hot spot formed in an explosive crystal by the passage of a fission fragment is a courageous one, but is fraught with some danger.

It is true that the energy which a fission fragment dissipates within a crystalline material is delivered to the lattice, but the rate and the way in which it appears as lattice vibrations (heat) are complex and poorly understood processes. The two major way in which energy is lost are those of fragment/electron interactions (in a Coulomb fashion) and those of fragment/nucleus collisions (in a Rutherford fashion). The division of energy-loss to these two processes is sensitively dependent on the nature of the theoretical model chosen for the atomic interaction potential. Since a considerable amount of the energy delivered directly to the lattice atoms may be carried long distances down close-packed rows of atoms or molecules at shock-wave velocities, the temperatures produced at the centre of the track may well be less than 25 per cent of those suggested by the authors. Moreover, since the diameter of the tube is only 10 Å (approximately 6 atoms) the material should not be considered as a continuum, and it has been shown that the effective thermal conductivity may be greater than the macroscopic conductivity by a factor as large as 10.

The rate at which heat is evolved owing to chemical reaction is proportional to the rate constant, which the authors take as about 10^{20}/sec by extrapolating low-temperature kinetic data over 10^5 °C. This extrapolation is open to some criticism and in fact the rate constant is more likely to be of the order of 10^{14}/sec, that is, the same order of magnitude as the lattice vibration frequency. Hence it is possible that the ratio:

$$\frac{\text{heat evolved due to chemical reaction}}{\text{heat lost due to conduction}}$$

has been overestimated by many orders of magnitude. In addition, the computed temperature profiles would not necessarily lead to a macroscopic explosion. In all three figures the putative flame front is heavily supported from behind (nearer to the particle trajectory) by an intense 'hot spot' (radius approx. 20 Å, temperature greater than 2×10^4 °K). If the calculations were continued until this hot spot became significantly eroded, would the flame front continue to propagate unsupported ?

The critical hot spot size between 10^{-5} and 10^{-3} cm is partly derived on a theoretical basis but mainly from relatively macroscopic experimental observations. Although there is no *a priori* reason why the theory should not be valid outside this range, the theory should not be taken too far. First of all the number of molecules involved in the excitation of a typical track is small and an atomistic approach may be necessary. Secondly, there is the problem of the growth to explosion from the region of initiation. This growth stage can be a delicate one and under many conditions may be quenched.

The scope of classical explosion theory is more modest than the authors imply. It is customary to calculate the evolution of the temperature profile from the basic equation until the end of the induction period, that is, from $\theta = 0$ to $\theta_{.} \sim 2$, where θ is the Frank–Kamenetski reduced temperature increase $E(T - T_0)/RT_0^2$. Neglect of hydrodynamic processes is justified in this case for the conventional explosives because the ratio, temperature increase/adiabatic explosion temperature, cRT_0^2/qE, and thus the fractional decomposition is small ($\sim$ 5 per cent). Thus the classical theory enables us to determine whether a hot spot grows or decays in its early life but cannot be applied to considerations of its subsequent fate.

In conclusion we would agree with the title of this article that there is an inadequacy of thermal explosion theory when applied to fission-fragment bombardment of explosive solids. It would seem, however, that it is not possible to make sensible calculations at this stage since the necessary parameters under these extreme conditions are not known.

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SPECTRUM-LINE-REVERSAL TEMPERATURE MEASUREMENTS THROUGH UNSTEADY RAREFACTION WAVES IN VIBRATIONALLY RELAXING OXYGEN

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THIS article describes some preliminary investigations of the effect of vibrational relaxation on the structure of unsteady expansion waves produced in a shock tube by the bursting of a second diaphragm. Fig. 1 illustrates in distance–time co-ordinates the idealized flow pattern which would result from instantaneous rupture of this diaphragm by the initial (or primary) shock. The expansion being investigated takes place in the gas heated by the initial shock; in other words, behind the secondary contact surface. By a measurement of the time-variation of temperature and of static pressure at some fixed point *B*, the effect of the lag in vibrational de-excitation (and

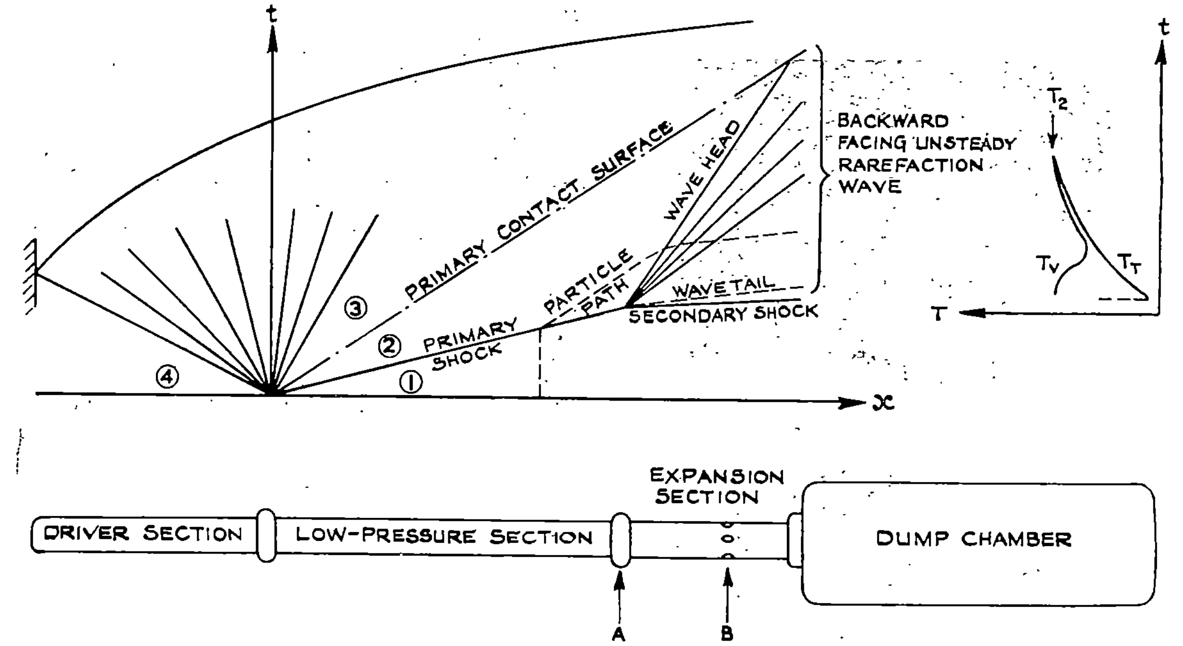

Fig. 1. Schematic diagram of idealized flow geometry in x–t plane. A, Second diaphragm position; B, observation position; T_V, T_T, vibrational and translational temperature at B; T_2, equilibrium temperature of shock-heated gas

of any chemical recombination as well) induced by the rapid cooling in the expansion wave can be followed. In this application the unsteady expansion has the particular advantage, compared with the steady Prandtl–Meyer configuration, for example, that a much higher degree of expansion is possible, thus allowing examination of rate processes over a wide temperature range. As in the case of steady non-equilibrium flows, the static pressure-level is relatively insensitive to internal relaxation effects despite their effect on the flow temperature. Thus a measurement of pressure can be used to check the flow geometry.

The experiments were carried out in a 2-in.-diam. hydrogen-driven shock tube fitted with a second diaphragm of 0·0076-mm thick aluminium foil: this separated the 15-ft. long low-pressure section from a 2-ft. long constant-area evacuated expansion section, also of 2 in. internal diameter. A large dump chamber was used to avoid reflected disturbances.

The oxygen test-gas was at an initial pressure of 10 torr (mm mercury), and the primary shock Mach numbers measured at the second diaphragm position were in the region of 7·0. In the absence of attenuation effects and diaphragm opening delays, this would provide an initial equilibrium state of the oxygen at a temperature of about 2,500° K and a pressure of about 600 torr, and with about 14 per cent (the maximum possible) of the static enthalpy residing in the vibrational mode. Although under these conditions the degree of dissociation was about 1 per cent, it was effectively frozen during the experiment, thus leaving vibrational relaxation as the dominant non-equilibrium process in the expansion wave. Oxygen was chosen as the test gas for this first phase of investigation since its vibrational relaxation time was short enough to allow observation of the expansion wave structure within the shock tube running time.

The temperature variation through the wave was measured by the double beam sodium-line-reversal method developed by Clouston, Gaydon and Hurle[1,2]. There is much evidence from temperature measurements behind shock waves[2,3] and in steady expansive flows in nozzles[4,5] that this technique measures the molecular vibrational temperature directly and so enables the effect of any lag in vibrational excitation or de-excitation to be followed. In the work recorded here, the Pointolite background temperatures were set at about 2,000° K and 2,400° K to bracket the expected variation of vibrational temperature. This resulted in an emission signal on one beam and an absorption signal on the other, and enabled the temperature variation to be followed with better accuracy than by extrapolation from dual emission or dual absorption records. The time resolution was about 5 μsec. The static pressure variation was measured with a miniature piezo-electric transducer having a rise time of about 10 μsec. More complete details of the experimental arrangements are given elsewhere[6].

A typical pair of simultaneously recorded temperature and pressure oscillograms is shown in Fig. 2; about twelve runs in oxygen were recorded, the initial conditions being held as nearly as possible the same for all. Fig. 2*a* shows the time variation of the intensity of the sodium *D*-line radiation against background brightness temperatures of 2,005° K and 2,390° K on the upper and lower beams respectively recorded at a distance of 15·5 in. from the second diaphragm station. Emission causes a downward deflexion and absorption an upward deflexion. The upper trace shows a gradually increasing emission signal commencing from the 'spike' due to the secondary shock marking the start or 'tail' of the expansion wave. The lower trace shows an absorption signal increasing to a maximum from this point and then falling. Both traces show a discontinuity with passage of the primary contact surface.

In the temperature analysis of Fig. 3*a* a comparison is made between the experimental temperature history (open symbols) derived from Fig. 2*a* and theoretical vibrational and translational temperature profiles calculated by Appleton's method[7,8] for comparable initial conditions. For the purpose of this comparison, the time origin of the temperature oscillogram was taken to be the instant when the primary shock reached the second diaphragm determined by extrapolation of the shock speed versus distance data.

There is a striking similarity between the profiles, suggesting that the observed spectrum-line-reversal temperature is essentially the same as the vibrational temperature of the oxygen molecules for the present experimental conditions. The experimental temperature minimum occurs, however, some 20–25 μsec later than expected, and the temperature itself is about 250° K higher than the theoretical value; moreover, the time-scale of the observed temperature variation is shorter than

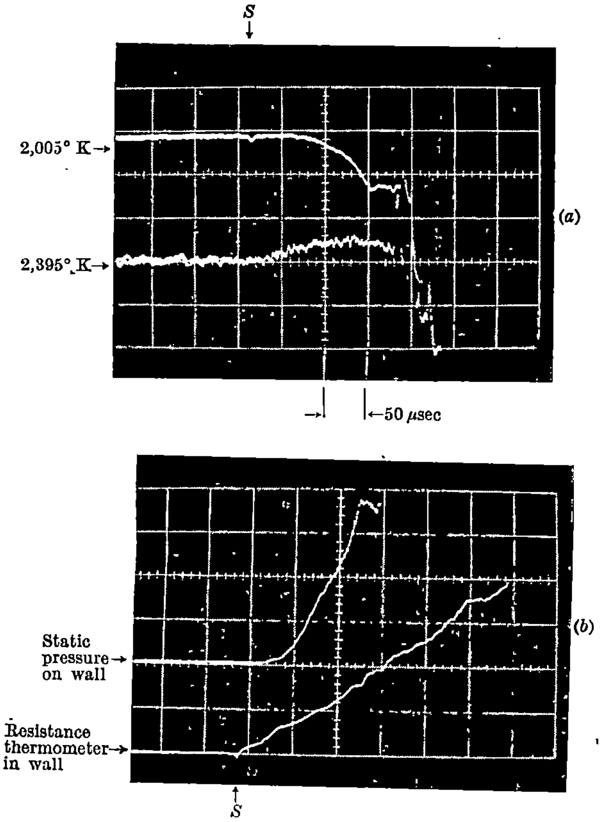

Fig. 2. Typical temperature and pressure oscillograms for unsteady expansion of shock-heated oxygen. Initial conditions, M_s, 6·86; T_s, =2,449° K, p_s, 559·5 torr; (a) X, 15·5 in. (b) X, 12·5 in. The arrow S denotes passage of the secondary shock

that of the theoretical. The significance of these small discrepancies is discussed later, but they may in part arise from the delay in rupture of the weak diaphragm with a consequent modification of the flow geometry; this might be expected to affect the pressure variation as well. Fig. 2b shows the pressure and thin-film gauge signals recorded at a point on the wall 12·5 in. from the second diaphragm. Analysis of the oscillogram showed that the experimental pressure signal was also delayed, and its rate of rise greater than predicted and with a time-scale again less than that calculated (Fig. 3b).

The 'breakaway' and subsequent rise of the spectrum-line-reversal temperature towards a 'frozen' value observed in these experiments shows that while the electronic excitation temperature of the sodium is not coupled to the molecular translational temperature its value is consistent with the supposition that it is the vibrational temperature of the relaxing oxygen that is being measured by this means.

The existence of a minimum value of the vibrational temperature beyond which it again increases (first pointed out on theoretical grounds by Stulov[9] for dissociational relaxation) is a characteristic feature of the wave structure, and therefore the present results lend at least qualitative support to the calculations of Appleton and Stulov.

On account of the low density there was insufficient sodium radiation present in these experiments to permit accurate measurement of temperature as far back in the flow as the fully frozen region, although the observed temperature began to climb towards it. Runs at higher initial pressure which would have made this possible were, however, not attempted with oxygen because of the risk of ignition at the driving interface. Comparable runs in nitrogen at initial pressures of up to 100 torr should overcome this limitation since the D-line radiancy ($\alpha\rho^{1/2}$) would then be greater by a factor of roughly $\sqrt{10}$. Together with the use of interference filters of increased transmission (factor of 3) and more sensitive photomultipliers, now available, the overall signal-to-noise ratio could be improved by a factor of at least 10. This should not only enable detection of the 'frozen' vibrational temperature but will also incidentally extend the measureable temperature range to below 1,800° K.

A further important experimental reason for raising the density is that the sodium radiation may be collision-limited in the 'frozen' region of the expansion, leading in this case to spuriously high observed temperatures. This may arise if the excited vibrational levels of the oxygen molecules are depopulated by transfer of energy to the electronic levels of the sodium atoms which then radiate, rather than by the binary collisions assumed in the theoretical description. Here again, nitrogen is to be preferred to oxygen as a test gas since spectroscopic data for collisions between nitrogen and excited sodium exist[10].

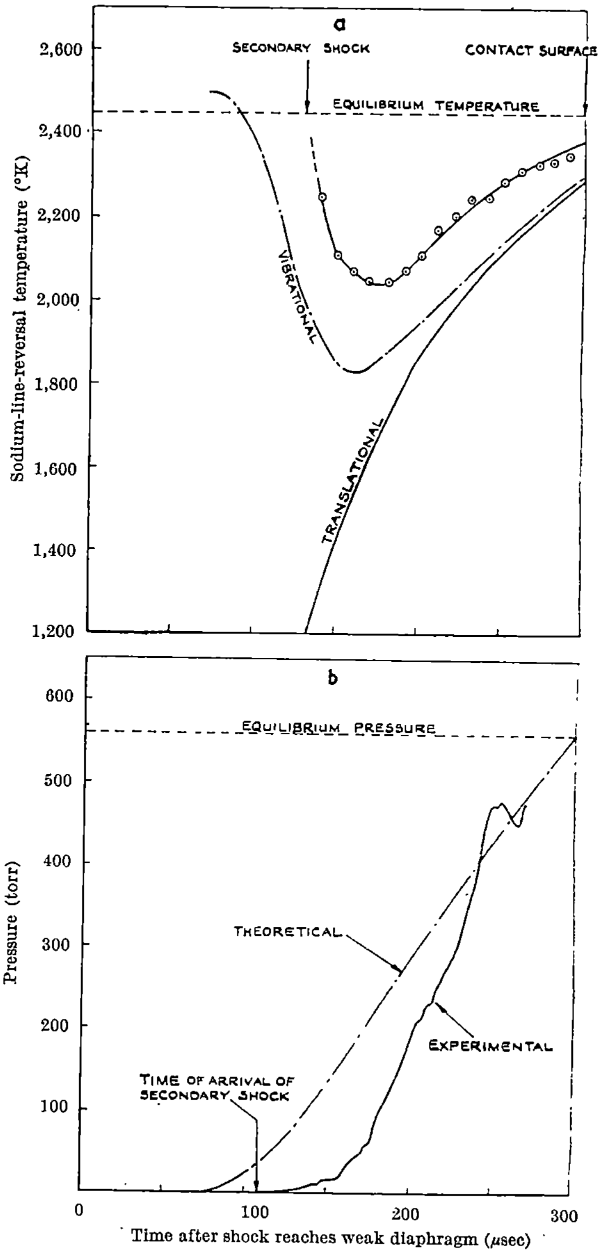

Fig. 3. a, Temperature analysis of Fig. 2a. The theoretical vibrational and translational temperature profiles are for slightly different initial conditions, namely, M_s, 6·96, T_s, 2,492° K, p_s, 585 torr, X, 15·5 in.; b, pressure analysis of Fig. 2b. Theoretical curve for same initial conditions as for Fig. 3a but with X, 12·5 in.

Evidently Kirchhoff's Law will not apply in this region, and an experimental indication of this was that meaningful gas temperatures could not be derived from the ratio of the double-beam signals. This small region round the tail of the expansion wave corresponds to the dashed portion of the experimental temperature profile in Fig. 3*a*.

Allowing for the diaphragm opening delay already referred to, the general agreement between theoretical and experimental profiles obtained shows that the picture of the flow pattern derived from theory is broadly correct, though the degree to which the wave is centred has not been investigated; it is hoped to check this in later work in which measurements will be taken at two downstream positions simultaneously. To avoid spurious distortion of the temperature and pressure profiles due to shock attenuation, a larger diameter shock tube would be desirable.

The observed shock attenuation would have led one to expect temperatures in the region of the contact surface above the equilibrium value, based on the shock speed at the second diaphragm position. In fact, however, in all runs, values less than equilibrium were recorded (Fig. 3*a*).

This may indicate that the running time was not quite sufficient for equilibrium to be reached, but may also be a further illustration of an effect peculiar to spectrum-line-reversal measurements using sodium in hot oxygen observed in some earlier work and not yet explained[11,12]. It was found that while changes of temperature were accurately followed, the temperature values were low by constant systematic amounts ranging up to nearly 400° K in some cases. This effect was not present with sodium in nitrogen.

The present work is of interest in relation to some recent spectrum-line-reversal temperature measurements obtained by Hurle, Russo and Hall[13], in a steady nozzle expansion where it was necessary to postulate a relaxation time for de-excitation of nitrogen fifteen times shorter than that derived from vibrational excitation investigations behind strong normal shock waves. From this they suggest that the Landau–Teller description of vibrational de-excitation may be inapplicable in non-equilibrium situations where the difference between vibrational and translational energy is large, and that the shorter relaxation time indicates that the probability of de-excitation may depend on the vibrational as well as on the translational energy of the colliding molecules.

The present results, however, suggest that for the oxygen relaxation observed in these experiments such assumptions are unnecessary. The general agreement seen with Appleton's calculations implies that his description of the relaxation process was adequate; he used a linear rate equation, Camac's experimental data (from shock wave investigations)[14], and Landau–Teller theory.

The consequences of large changes of the effective relaxation time on the temperature profiles of unsteady expansion waves in nitrogen have been looked into theoretically by Woodley[8]. He has found that a substantial increase in the rate of vibrational de-excitation (of the order of that mentioned by Hurle *et al.*) will shift the temperature minimum to earlier times and reduce its value considerably as might be qualitatively expected. This effect was not observed in the present experiments with oxygen, the results of which are consistent with the accepted value for the effective relaxation time within a factor of two. In fact, the experimental observation of temperatures above theoretical (Fig. 3*a*) might on the contrary, suggest that de-excitation proceeds more slowly than was assumed. According to Appleton's argument, the assumption of frozen dissociation in these experiments is justified, so that the enthalpy of recombination of the oxygen atoms in the wave cannot contribute to the vibrational temperature. A possibility that was considered was that these higher temperatures were due to reflected sodium light entering the photo-multipliers, but this seems unlikely since the aperture of the optical system was small and earlier work[12] involving temperature measurements of the incident shock never at any time revealed a sodium radiation signal preceding the shock.

Extension of this work to reacting gases with the view of measuring the temperature dependence of recombination rates is feasible and might incidentally show whether coupling between the rates of vibrational and chemical relaxation is significant.

The use of unsteady expansion waves for chemical kinetic measurements in shock tubes was suggested by A. R. Fairbairn (1958) in an unpublished Ministry of Aviation note, and the experiment in its present form arose from discussions with D. A. Spence. J. P. Appleton performed the calculations reported in ref. 7 in support of this work, and I am indebted to J. G. Woodley for the further computations in Fig. 3.

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RHYTHMIC ALTERNATIONS IN THE RATE OF SYNTHESIS AND THE COMPOSITION OF RAPIDLY LABELLED RIBONUCLEIC ACID DURING THE SYNCHRONOUS GROWTH OF BACTERIA

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We have observed that a remarkable wave pattern of rate of synthesis and of composition results when the ribonucleic acid newly formed in consecutive segments of the generation period of a synchronously growing bacterial culture is examined. A donor strain of *Escherichia coli K*-12 (*HfrH*) was used in these experiments.

Recent observations on *Hfr* strains of *E. coli*[1] and on certain strains of *Bacillus subtilis*[2], and also some autoradiographic evidence[3], which suggested a sequential, unidirectional replication of bacterial chromosomes, starting from a single growing point, determined the choice of organism for the work recorded here. If the

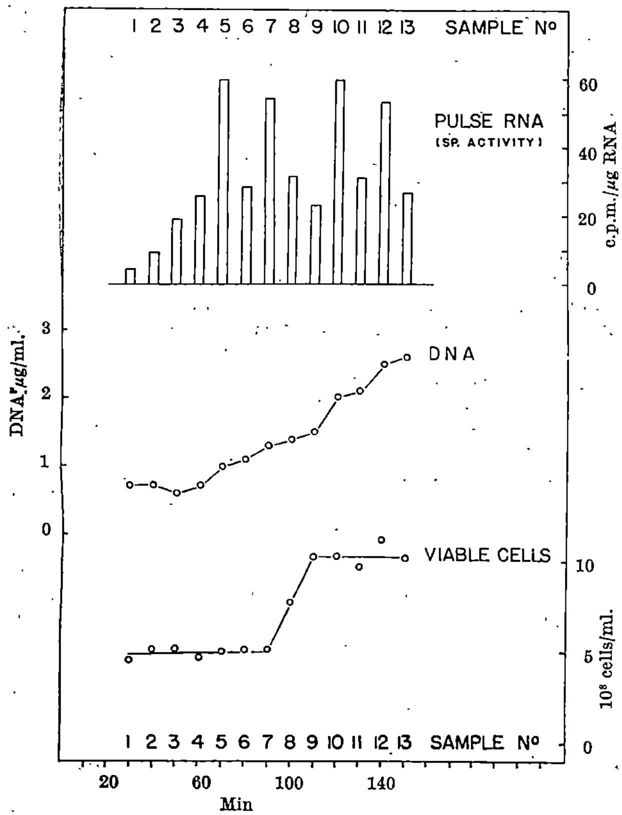

Fig. 1. Synchronous growth of *E. coli HfrH* (m^-, λ, 424), Exp. 1. The samples, taken at intervals of ten minutes, are numbered as in Table 1. The diagrams represent, in upward order: (*a*) viable counts, determined by plating the cells in appropriate dilutions on plates of nutrient agar; (*b*) synthesis of DNA; (*c*) specific activity of pulse-labelled RNA

function of the DNA component of a chromosome is taken to consist in its ability to conserve genetic information (through the duplication of DNA) and to convey it (perhaps through the production of specific RNA molecules), and if the first of these processes advances along the chromosome in an orderly and successive manner, it is not unreasonable to assume that the second does so too. In this event, the rate of synthesis, and the nucleotide composition, of different species of RNA, produced in different intervals within a growth cycle, could well vary. Furthermore, the synthesis of ribosomal and transfer RNA could be expected to occur in a periodic fashion either before or after the replication of those sites on the genome that may determine these RNA species. In order to explore these possibilities, a synchronously dividing population of *E. coli* was subjected to a pulse of ^{32}P-phosphate at regular intervals; the rate of synthesis and the composition of the newly formed RNA were then determined.

The organism examined was an *HfrH* (λ, 424) strain requiring methionine obtained from Dr. T. Nagata, who had shown that the genome of this strain replicates in a sequential, polarized fashion from the *F* locus forward[1]. The bacteria were grown in a *tris*-salts medium[4] to which were added, per litre of medium, 87·1 mg dihydrogen potassium phosphate, 300 mg 'vitamin-free' casamino acids, 50 mg L-methionine, and 2 g glucose. The cells were synchronized by 'fractional filtration'[5], and the filtrate comprising the small cell fraction was incubated in fresh, previously warmed, medium at 37°. Samples were withdrawn from the master culture every 10 min for the following operations: (*a*) 0·1 ml. for viable counts; (*b*) to 1 ml. of chilled 50 per cent trichloroacetic acid solution 9 ml. of the culture were added, and DNA was determined in the precipitate by the diphenylamine reaction[6] (in Exp. 2, RNA was also determined by means of the orcinol reaction); (*c*) a sample of 100 ml. of the culture was exposed to 2-mc. carrier-free ^{32}P-orthophosphate for 80 sec when, in order to terminate the pulse, the cells were poured on crushed ice. The bacteria were then washed to remove extraneous radiophosphate and 1 g (wet weight) of unlabelled *HfrH* cells grown in the same medium was added as carrier. After the extraction of RNA by a modification of several known procedures utilizing 'Duponol' and phenol, determination of radioactivity, and hydrolysis with alkali in the usual manner the (2′ + 3′)-nucleotides were separated by two-dimensional chromatography[7] and estimated spectrophotometrically and by measurement of their radioactivity. The figures for newly formed RNA were expressed as c.p.m./μg of RNA; the estimates of the RNA content of the several preparations were again derived from spectrophotometry. Correction was made for non-nucleotidic ^{32}P-compounds that accompany the RNA specimens in varying amounts (20–30 per cent of the radiophosphate incorporated in a non-dialysable form) through the summation of counts recorded for the purified mononucleotide components.

Figs. 1 and 2 depict, in two independent experiments, the synchronism of cell division in the bacterial culture; the increase in the level of DNA during growth; and the patterns of synthesis of new RNA revealed by the application of a series of short pulses of ^{32}P-phosphate to consecutive segments of the generation period. In addition, the rise of the levels of RNA in the master culture of Exp. 2 and the computed total counts of RNA present in the

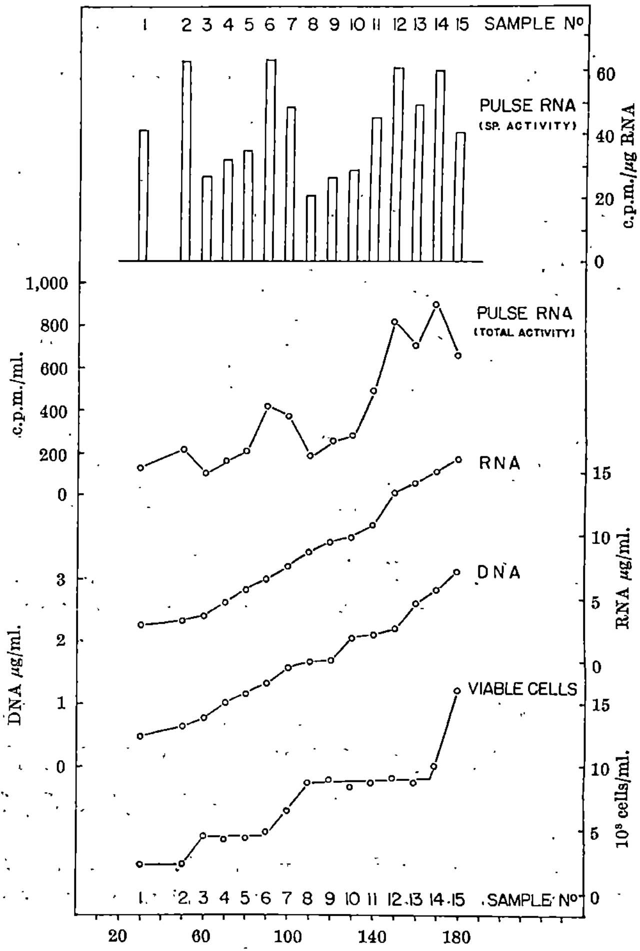

Fig. 2. Synchronous growth of *E. coli HfrH* (m^-, λ, 424), Exp. 2. The samples are numbered as in Table 2. The diagrams represent, in upward order: (*a*) viable counts; (*b*) synthesis of DNA; (*c*) synthesis of total RNA in the master culture; (*d*) total radioactivity of pulse-labelled RNA per ml. culture, computed from the values of specific activity and from the estimation of RNA content (orcinol) of the several samples; (*e*) specific activity of pulse-labelled RNA

Table 1. DISTRIBUTION OF RADIOACTIVITY AMONG NUCLEOTIDE CONSTITUENTS OF RNA, IN RELATION TO DNA REPLICATION, IN SUCCESSIVE STAGES OF SYNCHRONOUS GROWTH OF *E. coli HfrH* (EXP. 1)*

Sample No.	Min after filtration	DNA: increase as % of initial in each generation	RNA: total c.p.m. recovered (× 10³)	Nucleotide constituents of RNA†						
				% of total radioactivity				Molar ratios		
				A	G	C	U	(A + G)/(C + U)	(A + U)/(G + C)	(A + C)/(G + U)
1	30	0	52·5							
2	40	0	84·2	24·0	27·7	25·6	22·7	1·07	0·88	0·98
3	50	0	164·2	24·4	25·3	26·3	24·0	0·99	0·94	1·03
4	60	12	190·8	23·4	26·1	25·8	24·7	0·98	0·93	0·97
5	70	44	423·4	*23·3*	*32·2*	*23·5*	*21·0*	1·25	0·80	0·88
6	80	52	292·5	22·9	29·4	25·1	22·6	1·10	0·83	0·92
7	90	80	423·5	*22·3*	*31·8*	*25·5*	*20·4*	1·18	0·75	0·92
8	100	100	218·0	24·4	28·3	23·3	24·0	1·11	0·94	0·91
Division										
9	110	8	122·4	23·1	26·5	26·0	24·4	0·98	0·90	0·96
10	120	46	506·6	*24·4*	*32·1*	*23·5*	*20·0*	1·30	0·80	0·92
11	130	52	259·0	22·9	29·7	24·7	22·7	1·11	0·84	0·91
12	140	78	443·2	*22·8*	*32·4*	*25·3*	*19·5*	1·23	0·73	0·93
13	150	90	256·3	24·8	25·7	25·0	24·5	1·02	0·97	0·99

* Compare Fig. 1 for other results of this experiment.

† About 1 mg of RNA was hydrolysed with 0·2 ml. of 0·5 N aqueous potassium hydroxide for 20 h at 37°. After separation by two-dimensional paper chromatography, the radioactivity of each (2′ + 3′)-nucleotide was measured in a gas flow counter. The figures express the distribution of phosphorus-32 incorporated into each nucleotide as per cent of total radioactivity, that is, of the summation of all counts given by the sample. The standard deviation ranged from 0·25 to 0·94. In sample No. 1 the counts were too low for calculation. The figures in italics denote the samples for which bursts of RNA synthesis were recorded (see Fig. 1). The three columns of molar ratios list the ratio of purines to pyrimidines, the dissymmetry ratio, and the ratio of 6-amino to 6-keto nucleotides, respectively. Compare Table 3 for the composition of bacterial DNA and RNA. Abbreviations: A, adenylic acid; G, guanylic acid; C, cytidylic acid; U, uridylic acid.

Table 2. DISTRIBUTION OF RADIOACTIVITY AMONG NUCLEOTIDE CONSTITUENTS OF RNA, IN RELATION TO DNA REPLICATION, IN SUCCESSIVE STAGES OF SYNCHRONOUS GROWTH OF *E. coli HfrH* (EXP. 2)*

Sample No.	Min after filtration	DNA: increase as % of initial in each generation	RNA: total c.p.m, recovered (× 10³)	Nucleotide constituents of RNA						
				% of total radioactivity				Molar ratios		
				A	G	C	U	(A + G)/(C + U)	(A + U)/(G + C)	(A + C)/(G + U)
1	30	63	152·4	26·1	27·3	24·5	22·1	1·15	0·93	1·02
2	50	84	276·3	*26·7*	*31·3*	*23·2*	*18·8*	1·38	0·83	1·00
3	60	100	118·8	26·2	26·5	23·7	23·6	1·11	0·99	1·00
Division										
4	70	31	143·0	25·2	26·1	26·3	22·4	1·05	0·91	1·06
5	80	54	139·6	23·1	28·7	25·2	23·0	1·07	0·86	0·93
6	90	75	302·4	*26·2*	*29·4*	*22·7*	*21·7*	1·25	0·92	0·96
7	100	100	160·7	25·6	27·6	25·1	21·7	1·14	0·90	1·03
Division										
8	110	9	149·2	23·0	25·9	26·5	24·6	0·96	0·91	0·98
9	120	10	134·6	25·2	26·3	25·1	23·4	1·05	0·95	1·01
10	130	34	99·4	24·7	26·7	26·2	22·4	1·06	0·89	1·04
11	140	37	132·0	24·4	27·0	25·5	23·1	1·06	0·90	1·00
12	150	42	261·9	*24·6*	*30·1*	*24·0*	*21·3*	1·21	0·85	0·95
13	160	69	242·6	25·2	25·8	25·5	23·5	1·04	0·95	1·03
14	170	82	282·0	*24·4*	*30·7*	*23·4*	*21·4*	1·23	0·85	0·92
15	180	100	195·8	24·1	26·7	25·0	24·2	1·03	0·93	0·96
Division										

* See Fig. 2 for additional results and Table 1 for explanations.

15 pulsed samples are shown in Fig. 2. As concerns the synthesis of DNA, it exhibits a definite retardation at the outset and then proceeds in what would appear to be a stepwise manner (usually two steps in a growth cycle) interrupted by periods of almost no synthesis. It would have been possible to fit a straight line to most of the points indicating the DNA levels; but we believe that our observations represent meaningful variations.

During the initial lag of DNA formation new RNA molecules are apparently produced, in accordance with previous observations[8,9]. RNA is synthesized continually; but when the rates of synthesis are probed into at different points of the growth cycle, rhythmic fluctuations are seen. These are shown graphically in Figs. 1 and 2 in the form of the specific activity of the RNA. At the onset of the growth cycle the increase in the rates of synthesis of RNA, as measured by the uptake of phosphorus-32, was nearly linear; thereafter, the specific activities of pulse-labelled RNA alternated between high and low levels, two bursts being discernible during an average generation time of 70 min (Fig. 1). In another experiment, in which the generation time was somewhat shorter, only one peak was noticed in the first complete generation period under observation (sample No. 6 in Fig. 2). These bursts of RNA synthesis seem to be regularly spaced with respect to DNA replication, occurring generally in the strain under investigation here when DNA had increased by 40–45 per cent and 75–85 per cent. (Compare samples No. 5, 7, 10, 12 in Fig. 1 and No. 2, 6, 12 and 14 in Fig. 2.)

These alternations of the rates of RNA synthesis were found to be associated with remarkable changes in the apparent composition of the newly made RNA molecules. This information will be found in Tables 1 and 2 which list, in addition to figures on the percentage increase in DNA and the total counts recovered in the isolated RNA specimens, the distribution of radioactivity among the (2′ + 3′)-nucleotide components of each RNA sample. These values, it will be remembered, describe the RNA made in the course of a short period of exposure to phosphorus-32, by synchronously multiplying cells at different stages of their cellular history. For purposes of comparison it may be useful to consider these figures in conjunction with those listed in Table 3, in which data on the composition of the DNA and of various RNA fractions of the bacterial strain are presented. It will be noticed that the DNA contains, of course, equal molar quantities of purine and of pyrimidine nucleotides, whereas in the ribosomal and also in the total RNA the molar concentration of purine nucleotides surpasses that of pyrimidine nucleotides by 25 per cent or more.

If, with this in mind, the values compiled in Tables 1 and 2 are scrutinized, two principal groups of composition will emerge. One group, with a ratio of purines to pyrimidines of 1·2 or higher, comprises all those RNA specimens the synthesis of which coincided with the peaks of incor-

Table 3. COMPOSITION OF DNA AND RNA OF *E. coli HfrH**

Nucleic acid	Fraction	Moles per 100 g-atoms of nucleic acid phosphorus					Molar ratios		
		A	G	C	U	T	A + G/C + U(T)	A + U(T)/G + C	A + C/G + U(T)
DNA	Total	24·6	25·5	25·6		24·3	1·00	0·96	1·01
RNA	Total (Exp. 1)	23·5	31·9	24·5	20·1		1·24	0·77	0·92
	Total (Exp. 2)	23·5	31·8	25·6	19·1		1·24	0·74	0·96
	Ribosomal, 16S	25·7	31·4	21·5	21·4		1·33	0·89	0·89
	Ribosomal, 23S	26·0	31·6	21·8	20·6		1·36	0·87	0·92
	Transfer, 4S	19·6	31·8	30·2	18·4		1·06	0·61	0·99

* T denotes thymidylic acid; other abbreviations as in Table 1. The figures for total RNA represent the averages of the spectrophotometric analyses of the nucleotide composition of the 13 RNA samples collected in Exp. 1 (Table 1) and of the 15 RNA samples of Exp. 2 (Table 2). The specimens of ribosomal and transfer RNA were separated by means of a linear gradient of sodium chloride on a column of methylated serum albumin–kieselguhr (ref. 10).

poration of phosphorus-32, that is, samples No. 5, 7, 10, 12 in Table 1 and Fig. 1; samples No. 2, 6, 12, 14 in Table 2 and Fig. 2. These specimens are altogether quite similar in composition—if one were to judge from the results of the distribution of phosphorus-32 in their constituent nucleotides—to that of total or ribosomal RNA. The base-pairing characteristic of DNA is entirely absent. Guanylic acid is the preponderant nucleotide, with the ratio of guanylic to cytidylic acids 1·3 or higher in nearly all instances.

The other group is less homogeneous in appearance, though many specimens share the property of exhibiting molar equivalence with respect to at least one of the two base-pairs: the ratio of adenylic to uridylic acids is near one in samples No. 6, 8, 11 in Table 1 and in samples No. 5 and 15 in Table 2. Similarly, guanylic and cytidylic acids are nearly equimolecular in samples No. 4 and 9 in Table 1, and in samples No. 4, 8, 10 and 13 in Table 2. In a recent paper from this laboratory it was pointed out that the occurrence of only partial base-pairing seems to hold true of most messenger-RNA preparations described in the literature[11].

There are several samples of pulse-labelled RNA in our list that could be regarded as quite similar to *E. coli* DNA in base proportions, particularly samples No. 3 and 13 in Table 1; the examples in Table 2 are less convincing, but samples No. 8, 13, and 15 could be cited. If another characteristic of DNA is adduced, namely, the equimolarity of purines and pyrimidines, samples No. 3, 4, 9, 13 in Table 1, and No. 8, 13, 15 in Table 2 may be said to resemble DNA in this respect. One receives the impression that, on the whole, the pulse-labelled RNA specimens produced at the beginning or at the end of a growth cycle, when DNA synthesis has not yet begun or has tapered off, are more similar to DNA in composition than those made at other periods.

There are other possible correlations, though the experimental material is not large enough for certainty. When DNA has increased by about 10 per cent, the distribution of the label in the RNA made at this stage is very similar in both experiments (compare samples No. 4 and 9 in Table 1, and No. 9 in Table 2). Another RNA composition appears to occur when DNA has increased by about one-third (No. 4 and 10 in Table 2); and yet another, with a high content of guanylic acid, when about one-half of the DNA has been replicated (No. 6 and 11 in Table 1 and No. 5 in Table 2). The ratio of 6-amino to 6-keto nucleotides[13] was near one in many of the specimens, with a mean of 0·97 for all 27 specimens.

The application of a pulse to a bacterial culture or to an organism raises many problems of interpretation with some of which a recent paper from this laboratory[11] has attempted to deal. The use of a narrowly spaced series of pulses administered to a synchronized cell population no doubt offers many advantages; but it remains that this grid, be it ever so fine, is imposed on, or made to slice through, the infinitely complex pulsations of a growing cell. We have probed along the time axis of a dynamic system—a synchronously dividing bacterial culture—and have obtained a set of values. How should they be interpreted and what is their bearing on present-day hypotheses concerning the transfer of biological information?

At the outset, one would wish to ask whether the analytical methods used in this article, as in all others dealing with this subject, in order to reveal the composition of the pulse-labelled RNA specimens, are really adequate; that is, whether regardless of the presumably varying specific activities of the nucleoside 5′-phosphates serving as the precursors of the polymers, the transfer of the phosphate groups to the (2′ + 3′)-positions, as it takes place in the course of the cleavage of RNA by alkali, does give rise to a randomization. This has been questioned repeatedly[11,13]. It may, however, be conceded that the technique is least objectionable when applied, as here, to the comparison of a series of samples produced under conditions having, ostensibly, only one variable, namely, time. Another point that should be emphasized is that, had a longer interval between samples than 10 min been chosen, a much simpler, but doubtlessly erroneous, picture could have emerged: the rapidly labelled RNA might have been in all cases only of the ribosomal type or only of the DNA type or mixed. It is the last-mentioned type that has, in fact, been encountered in investigations with non-synchronous bacterial populations[11,14–17]. If, on the other hand, an even finer net of pulse intervals could have been imposed—but this would be very difficult technically—it is quite conceivable that at one time or another a pulse-labelled RNA species of the composition of transfer RNA (compare Table 3) would have been found. It is quite clear that the examination of the composition or of other chemical and physical properties of an RNA specimen cannot be sufficient for a decision whether any of the many varieties of rapidly formed RNA observed in the course of a growth cycle conform with the concept[18] of messenger-RNA. There is, however, little question that the observation of a characteristic wave pattern in the synthesis and composition of RNA—a pattern apparently related to the extent of replication of DNA—is of some interest.

The alternations observed in this work possibly reflect a progressive transcription of the nucleotide sequence of the bacterial DNA in the form of a series of different pulse-labelled RNA molecules. Whether these, in turn, mirror the composition of individual cistrons in the DNA remains undetermined. The finding that the RNA newly made in the various cultures at identical levels of DNA replication is very similar in composition, but different from RNA produced at other levels of DNA increase, is consistent with the concept of an orderly transcription of the genome. The observation that the pulse-labelled specimens of RNA produced in different phases of synchronism vary in their composition is also in agreement with several previous investigations in which entirely different techniques (elution patterns[19], hybrid formation[20]) were used. Recent attempts to demonstrate the stepwise production of certain enzymes in synchronously dividing cells should also be mentioned in this context[21,22].

The work recorded here was carried out with one donor strain of *E. coli* (*HfrH*) with its given polarity of chromosome replication[1]. The outstanding shifts in the nucleotide distribution in the newly made RNA towards that expected for total or ribosomal RNA appeared referable to the periods when approximately 40 and 80 per cent of the DNA had been replicated. Does this now mean that at these times those nucleotide tracts on the DNA chain become available that are responsible for the formation of ribosomal RNA, and is this spacing characteristic of the particular strain of *E. coli* ? These and related questions, for example about the intervals during which the DNA-like RNA is produced, cannot yet be answered. But if the proposition is correct that different types of RNA are synthesized sequentially in a synchronized population, other donor strains of *E. coli Hfr* may exhibit another characteristic spacing. This problem is being investigated at present with strains having different chromosomal polarities.

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ION-EXCHANGE CHROMATOGRAPHY OF AMINO-ACIDS: IMPROVEMENTS IN THE SINGLE COLUMN SYSTEM

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PIEZ and Morris[1] have described a system for the quantitative analyses of amino-acids using a single column of ion-exchange resin, and instruments based on this principle are now commercially available (Phoenix Precision Instrument Co., Philadelphia, Penn.; Technicon Instrument Co., Ltd., Hanworth Lane, Chertsey, Surrey). With these the complete analysis and regeneration cycle take about 24 h. We wish to describe two sets of modifications in experimental conditions, the first giving a complete chromatogram in 15 h, the second in 5·5 h with no loss of resolution for the protein amino-acids.

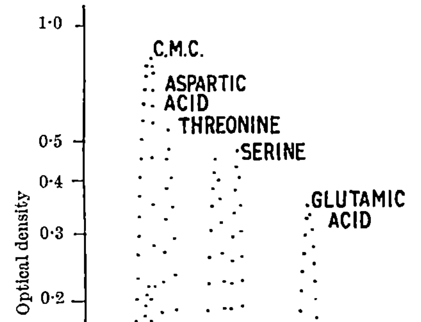

Fig. 1. Part of 15-h chromatogram. 0·2 μmoles each amino-acid, 0·5 μmoles *S*-carboxymethyl-cysteine (CMC)

(1) 15-*h chromatogram*. This may be effected as follows: (i) The flow of buffer to the column is increased to 47 ml./h. (ii) After regeneration with 0·2 N sodium hydroxide the column is equilibrated with 0·05 M sodium citrate (0·2 N with respect to Na^+) *p*H 2·9 in 10 per cent aqueous methanol. Methanol is required to maintain the resolution of threonine and serine and its use gives better separation of these amino-acids than is normally achieved with the 24 h chromatogram. 10 per cent methanol in the corresponding Varigrad buffer further improves threonine - serine resolution, but proline then tends to run into glutamic acid and the separation of cystine and methionine is impaired. (iii) Two additional chambers containing *p*H 5·0 sodium citrate are incorporated in the Varigrad to provide a safety margin for the overnight run. The other conditions are as for the 24-h chromatogram.

Temperature programming can also be used to improve threonine–serine resolution, but we have found this less convenient. Fig. 1 shows part of a typical chromatogram from carboxymethyl-cysteine to proline indicating the resolution obtained. The reproducibility of the system is unaffected by these changes.

(2) 5·5-*h chromatogram*. In investigations of protein structure, the rate of quantitative amino-acid analysis is often a limiting factor. Recently, details of a method

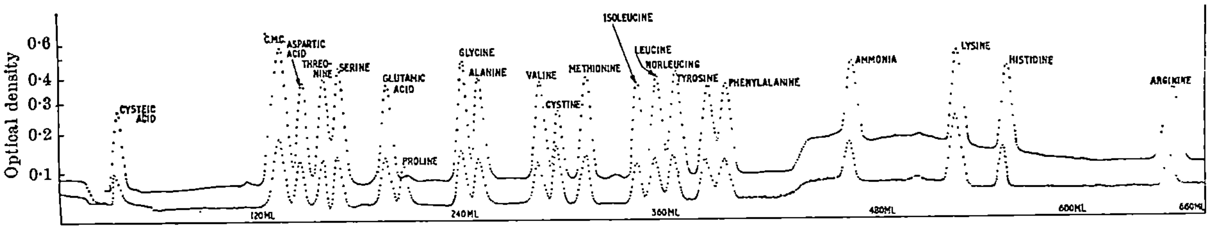

Fig. 2. Typical 5·5-h chromatogram (loading and abbreviation as for Fig. 1)

Table 1. Gradient for 5·5-h Chromatogram
(Volumes for 4·5-h chromatogram in parentheses)

Chamber	0·05 M sodium citrate ($Na^+ = 0·2$ M)			pH 10·0	
	pH 2·75 in 10% methanol (ml.)	pH 2·90 (ml.)	pH 3·80 (ml.)	Na^+ 1·2 N (ml.)	(Na^+ 2·4 N) (ml.)
1	75 (75)	—			
2	– (60)	75 (15)			
3	– (10)	75 (65)			
4	—	5 (5)	70 (70)		
5	—	—	75 (75)		
6	—	—		75	(75)
7	—	—		75	(75)
8	—	—		75	(75)
9	—	—		75	(75)

enabling a complete analysis (application of sample to emergence of arginine), to be carried out in 4·5 h, have become available[2]. This is achieved by major modifications in the gradient, and by an increase in the flow of buffer to the column to 120 ml./h. However, the increased rate of analyses is only achieved at the expense of reproducibility (now ± 5 per cent), and, in our experience, by greatly impaired resolution of glutamic acid and proline, and of tyrosine and phenylalanine. Also, the use of pH 10 sodium citrate (2·4 N with respect to Na^+) leads to much packing of the column with a consequent marked rise in operating pressure (more than 600 lb./in.^{2}). This necessitates frequent repacking of the column and leads to rupture of the buffer transmission lines.

We now use the modified gradient given in Table 1. (The gradient for the 4·5 h chromatogram is shown in parentheses.) This gives improved resolution of glutamic acid and proline, and by using pH 10·0 buffer of lower ionic strength (1·2 N with respect to Na^+), packing of the column is kept to a minimum so that the pressure rise from an initial 400 lb./in.^{2} to 600 lb./in.^{2} is spread over about one month. With this system arginine emerges 5·5 h after loading of the column. Since regeneration can also be carried out at 120 ml./h, the use of a time switch readily enables two analyses to be carried out daily. A third analysis can be carried out with only 1–1·5 h evening work. Fig. 2 illustrates the resolution obtained with this system.

With this modified gradient, gradual packing of the column occurs, necessitating re-pouring, but only about once a month. However, because of the narrow particle size range of the resin (17 ± 2μ), 1–2 h settling followed by pumping for a few hours with starting buffer at 120 ml./h is adequate. Periodic topping-up of the column with resin is generally necessary.

Although the reproducibility of this latter system (± 5 per cent) is rather less than that of the 15-h chromatogram and the resolution is poor for many of the ninhydrin-positive components of interest in biological fluids such as blood plasma and urine, it is nevertheless quite adequate for the analysis of hydrolysates of most peptides.

The advantages of these modifications are:

(1) The 15-h chromatogram frees the auto-analyser during the normal working day for other analyses, without loss of resolution or reproducibility.

(2) With the 5·5-h system, 2–3 analyses/day can be carried out, with good resolution, without change in instrumentation and, since the equipment is operating for considerably less than 24 h/day, there is a distinct saving in costly reagents.

(3) When analysis of both protein and peptide hydrolysates is required the 15- and 5·5-h chromatograms can be run successively in 24 h, thus doubling the number of analyses possible yet retaining the greater reproducibility of the overnight run, for one of the analyses.

We thank S. J. Milsom for his assistance, and Dr. H. Holy, of Technicon Instrument Co., Ltd., for details of the 4·5-h chromatogram.

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CROSSLINKING OF FIBROUS PROTEINS BY FORMALDEHYDE

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THE reactions of formaldehyde with proteins have been investigated for many years and it seems now to be accepted, particularly following the work of Fraenkel Conrat *et al.*[1], that crosslinking between —NH_2 groups on side-chains of soluble proteins is produced by condensation reactions such as:

$$R\text{—}NH_2 + HCHO + H_2N\text{—}CO\text{—}R' \rightarrow R\text{—}NH\text{—}CH_2\text{—}NH\text{—}CO\text{—}R' + H_2O$$

Chemical techniques were used to identify the groups involved in the reaction (usually amino, amide or guanidino) but the conclusive evidence for intermolecular crosslinking was provided, in the case of soluble proteins, by measured increases in molecular weight brought about by treatment with formaldehyde[2]. Increases up to eight times were found in concentrated solutions of protein while no effect occurred in dilute solution. This emphasizes the need for the two appropriate groups of neighbouring chains to be brought into proximity before intermolecular crosslinking can take place.

Fibrous proteins, such as collagen and keratin, present a more complex situation because of their crystallinity and insolubility. A powerful chemical approach was used by Zahn[3], who showed that various bifunctional reagents crosslinked lysine and hydroxylysine residues in collagen; he identified the groups involved by hydrolysing the treated protein and identifying the newly formed pairs of crosslinked amino-acids. Zahn also applied this technique to silk and to wool, although again with reagents other than formaldehyde. For the system collagen–formaldehyde Gustavson[4] has demonstrated that the functional groups involved are the ε-amino groups of lysine.

Direct physical evidence of crosslinking in fibrous proteins has sometimes been sought by examining the effects on the force–extension relationships for fibres immersed in water; this procedure is not generally reliable, however, because the elastic properties are dominated by the crystalline fraction and small changes in crystallinity may easily outweigh the effects of crosslinking, as appears in the experiments reported here. The effects of formaldehyde on other physico-chemical properties of wool, such as 'supercontraction'[5], or swelling[6], have been shown to be consistent with the production of additional crosslinks although not unequivocally demonstrating that such crosslinking has occurred. In the case of collagen, however, the fact that formaldehyde insolubilizes gelatin gels gives an indication that intermolecular crosslinking may be possible in the parent collagen. Furthermore, by heating treated collagen fibres above their shrinkage temperatures they can be converted to the elastomeric state; the crystalline regions are melted, and stress–strain measurements can then be used to determine an average crosslink density for the specimen. This technique has been used successfully by Wiederhorn *et al.*[7,8], by Flory and Spurr[9] (with benzoquinone/collagen systems), and, recently, by Cater[10], who showed that aldehydes of greater molecular

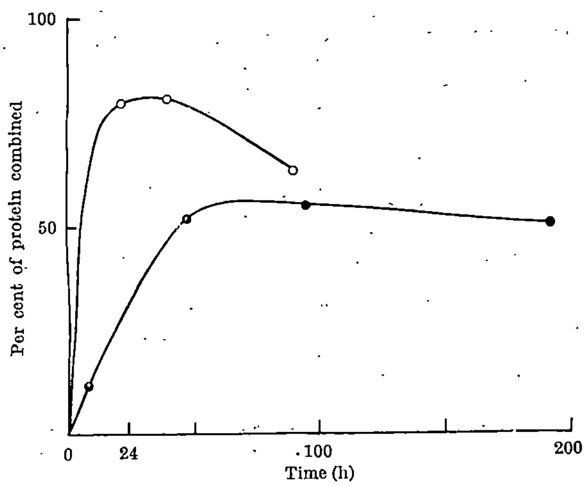

Fig. 1. Reaction of keratin proteins with formaldehyde, temperature 52° C
●, High-sulphur fraction; ○, low-sulphur fraction

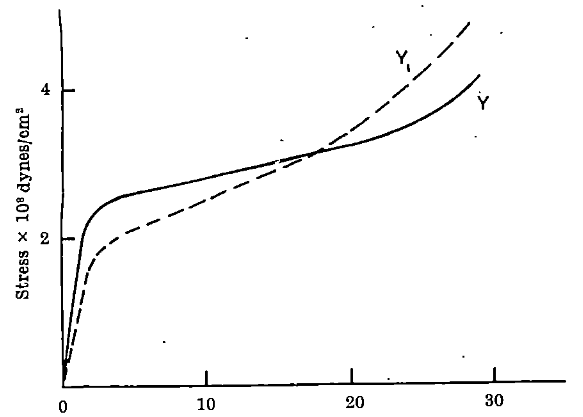

Fig. 2. Stress–strain relationships for wool fibres in water, 20° C; 10 per cent extension/min
——, Control; – – –, formaldehyde treated

size than formaldehyde, for example, acrolein, produced considerably greater crosslinking densities under comparable conditions in native collagen at 20° C. Cater's result emphasizes the importance of steric restrictions to crosslinking at potentially available sites in a fibrous protein.

In keratin the situation is further complicated by the presence of a high density of crosslinks (cystine residues) in the native state. However, the possibility of creating additional crosslinks has considerable theoretical and practical importance, and work now in progress has provided new evidence for the formation of intermolecular crosslinks in keratin by formaldehyde. As with collagen, one indication was given by the insolubilization of soluble derivatives from the protein and the other was obtained from equilibrium stress–strain measurements on fibres.

Two soluble wool proteins were obtained from Merino wool by reducing the disulphide bonds with alkaline potassium thioglycollate solution, alkylating the resulting thiol groups to prevent re-combination, and then fractionating into 'high-sulphur' and 'low-sulphur' components (*S*-carboxymethyl keratines) containing approximately 6 and 2 per cent of sulphur respectively. These fractions were kindly supplied by Dr. J. M. Gillespie of the C.S.I.R.O. Division of Protein Chemistry, Melbourne, who has described the fractionation procedures in detail elsewhere[11]. High-sulphur protein was dissolved in an equal weight of 36 per cent w/v aqueous formaldehyde and portions of the solution were heated at 52° C for periods up to 200 h. The low-sulphur protein was less easily dissolved, and the liquid content had to be increased about ten-fold by the addition of water to produce a homogeneous solution.

Gelation occurred in each specimen, and after the allotted time of heating the gels were washed and allowed to come to swelling equilibrium in water. They were then surface-dried and weighed and finally oven-dried at 105° C to constant weight. Fig. 1 shows how the proportion of the available protein which combined to form gel increased to a maximum and then decreased, presumably owing to degradation; control tests in which the proteins were heated in water produced less than 1 per cent of insoluble material. The gels were very dilute, with maximum weight fractions of protein of 0·08 for the high-sulphur and 0·03 for the low-sulphur component, and they could be dried and re-swollen by water. It may thus be concluded that formaldehyde produces intermolecular crosslinking in these two components of keratin.

To investigate crosslinking in the native, fibrous form, Corriedale wool fibres were heated for successive periods of 24 h in 36 per cent w/v aqueous formaldehyde solution at 52° C. Force-extension measurements were made on the fibres before starting the treatment and after each of the 24-h heating periods. These measurements were carried out at 20° C, at the constant extension rate of 10 per cent/min, and for extensions up to 15 per cent. Each test was made first in water and then in 98 per cent formic acid, which eliminates the elastic contribution from the crystalline material. After washing out the formic acid the length and elasticity of the fibre in water were substantially regained. The necessity of using such a method to eliminate the effects of crystallinity will be apparent from the results presented here.

Fig. 2 shows stress–strain curves obtained from tests in water on a formaldehyde-treated fibre and on a control fibre which had been subjected to the same experimental

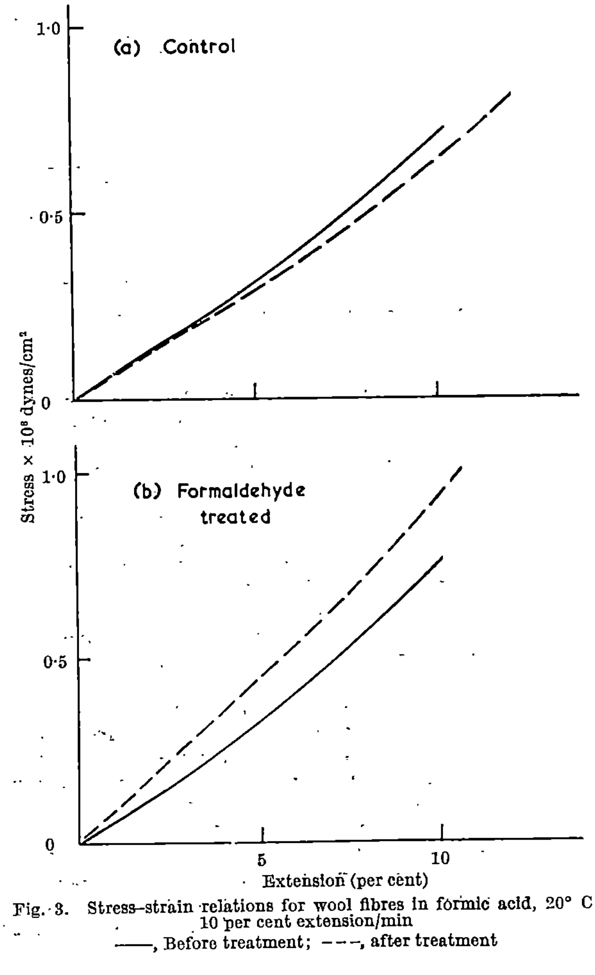

Fig. 3. Stress–strain relations for wool fibres in formic acid, 20° C; 10 per cent extension/min
——, Before treatment; – – –, after treatment

procedure, but with water substituted for the formaldehyde solution. The stresses are based on the cross-sectional area of the wet, unstrained fibre. Three features of these curves indicate structural alterations in the keratin, namely:

(1) The initial Young's modulus, that is, the slope of the linear 'Hookean' region between 0 and 2 per cent extension, is reduced to little more than half the control value by the formaldehyde treatment. The high elastic modulus in this region involves extension of the crystalline components, so that one action of the formaldehyde may be to produce a reduction in crystallinity.

(2) The modulus of the 'yield' region between about 2 and 15 per cent is approximately doubled. Under the present test conditions this modulus is very dependent on the rate of extension; the observed effect could therefore be due either to increased crosslinking or to an increase in the internal viscosity or to both of these factors.

(3) The extent of the yield region is considerably reduced by the formaldehyde treatment. Thus taking as a criterion the extension at the point Y where the stress has increased by 10 per cent above the level indicated by extrapolation of the yield region, the formaldehyde treatment produces a significant reduction from 28·6 to 24·3 per cent. According to the view of the molecular structure of wool developed by Feughelman[12] this effect indicates an increase in crosslinking in the less highly crosslinked parts of the amorphous matrix.

Only the last of these three effects thus gives a direct indication of crosslinking and even this necessitates an appeal to a particular model of fibre structure. A comparison of the curves up to about 15 per cent extension would, in fact, suggest that no crosslinking had taken place but only some degradation of the fibre.

In contrast to the tests in water the tests in formic acid gave positive evidence of crosslinking. Fig. 3 shows typical stress–strain data in which the initial modulus is about 7×10^8 dynes/cm^2, that is, about one-twentieth of the value measured in water. There is evidently no direct contribution from organized crystallites and the material shows a rubber-like elasticity. Furthermore, very little stress-relaxation was found under the test conditions so that the curves may be taken as equilibrium values and their slopes used as measures of an effective degree of crosslinking. It is apparent that the formaldehyde causes a stiffening of the fibre under conditions where the control fibre is slightly softened. There is thus an increase in crosslinking density, produced by the formaldehyde treatment, which amounts in magnitude very approximately to 40 per cent of the crosslink density in the native wool.

Finally, it may be observed that the sharp decrease in Young's modulus produced by formaldehyde occurs during the first 24 h treatment; thereafter the modulus decreases slowly at the same rate as for the control fibres. Reference to Fig. 1 suggests that the sharp decrease is brought about by reaction between the formaldehyde and the low-sulphur protein in the wool, in keeping with the concept that the low-sulphur protein may be identified with the oriented crystalline components of the fibre. This is qualitatively in agreement with the observation by Richards and Speakman[5] that increasing degrees of formaldehyde treatment progressively lowered the supers contraction obtainable in wool fibres under standard conditions.

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DEOXYRIBONUCLEIC ACID AND THE MOLECULAR BASIS OF SELF-DUPLICATION

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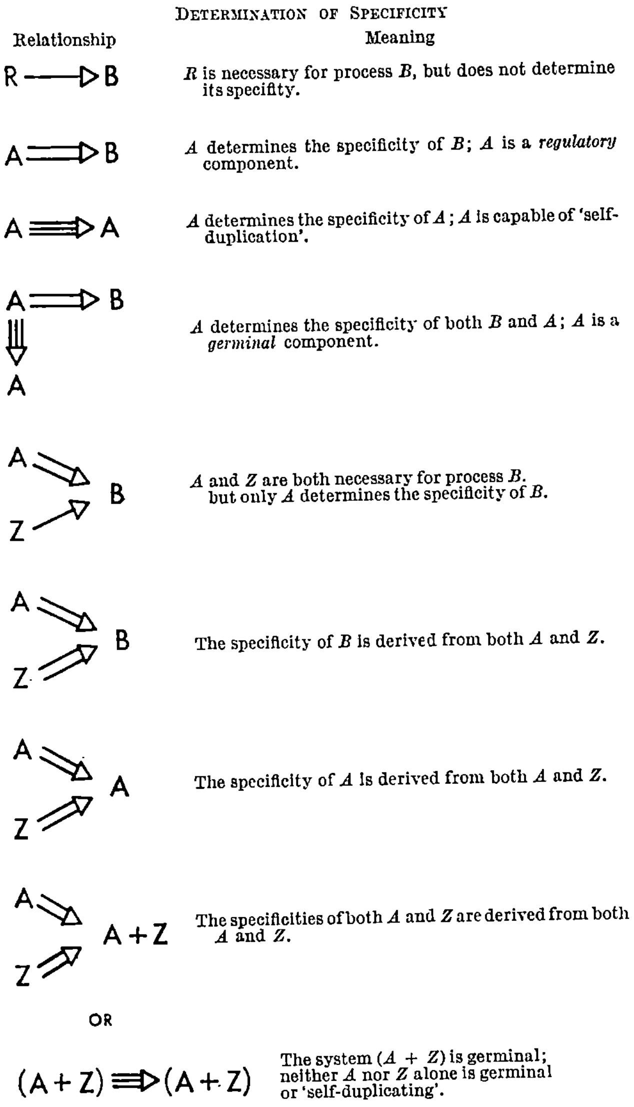
INVESTIGATIONS of the biochemical processes mediated by nucleic acids have led to the generalization that deoxyribonucleic acid (DNA) is capable of self-duplication, and represents a 'code' which specifies the inherited characteristics of the organism[1-4]. The importance of such a conclusion is self-evident, for it would reduce to a molecular level the unique capabilities of living matter for self-perpetuation and inheritance. It is of interest, therefore, to determine what criteria are required to establish that a molecule, such as DNA, is capable of self-duplication and control of inheritance, and to examine the degree to which the available evidence meets such criteria.

Self-duplication is a concept of biological origin regarding the source of inherited specificity in the replication of living organisms. A typical self-duplicating biological system consists of a specific cell and environmental constituents, such as water, oxygen and nutrients, which are essential to the growth and division of the cell. The concept of self-duplication holds that among such a system's several necessary constituents, only one, the cell, is the source of inherited specificity which appears in the product of the replicative event. Thus, in determining whether a system is capable of self-duplication, the chief issue is the source of the biological specificity which is transmitted to the progeny of the replicative event.

In some instances it is possible to analyse inheritable biological specificity in molecular terms, since a number of genetic effects are known to influence particular molecular structures or biochemical processes. Such biochemical specificity is represented by the particular order of the residues which comprise linear polymers such as nucleic acids and proteins, or by a particular path of metabolic reactions in the cell. In biological polymers a very large number of primary structures (that is, sequences of residues) have equal thermodynamic probability, and the specificity of a given molecule represents the choice of a particular sequence among the many possible ones. The numerous molecular components of the cell are thermodynamically capable of a very large number of reactions, and the specification of a given reaction pathway represents the activation of relatively few reactions among the numerous possible ones.

Both types of biochemical specificity are achieved by regulation of the rates of chemical reactions. In keeping with terminology introduced earlier[5], a component, or



R is necessary for process *B*, but does not determine its specifity.

A determines the specificity of *B*; *A* is a *regulatory* component.

A determines the specificity of *A*; *A* is capable of 'self-duplication'.

A determines the specificity of both *B* and *A*; *A* is a *germinal* component.

A and *Z* are both necessary for process *B*, but only *A* determines the specificity of *B*.

The specificity of *B* is derived from both *A* and *Z*.

The specificity of *A* is derived from both *A* and *Z*.

The specificities of both *A* and *Z* are derived from both *A* and *Z*.

The system (*A* + *Z*) is germinal; neither *A* nor *Z* alone is germinal or 'self-duplicating'.

Fig. 1. Diagram to summarize definitions of symbols for non-regulatory participants, regulatory and germinal components of systems involving transfer of biochemical specificity

system, which is capable of determining the specificity of a particular cellular reaction will be termed a regulatory component or system, and its participation in the chemistry of the cell indicated by the symbol (═══▷). A regulatory component or system which also serves as the sole determinant of the specificity of its own synthesis is self-duplicated, and, if transmissible, may serve as an agent of inheritance. An agent with these properties will be termed a germinal component or system, and its participation indicated by the symbol (≡≡≡▷). The participation of a component or system that is essential to a process in which transfer of specificity occurs, but which nevertheless does not contribute any specificity to it, will be indicated by the symbol (———▷).

The foregoing relationships, which are summarized in Fig. 1, permit an analysis of the manner in which a molecular system participates in a replicative or regulatory event. As indicated in Fig. 1, a molecule or system of molecules is to be considered capable of self-duplication only when it can be shown to be germinal, that is, if all the specificity embodied in the new molecule is derived from the parental one. Applied to a typical cellular self-duplicating system, the foregoing designations would show that: parent cell ≡≡≡▷ new cell; nutrients, etc. ———▷ new cell. Since, of the components necessary for the production of a new cell, the parent cell is alone capable of determining the new cell's specificity, the parent cell is a germinal component and: parent cell ≡≡≡▷ new cell.

The theory of the DNA code proposes that DNA governs biological inheritance because the specificity of its molecular structure—the sequence of nucleotides in a particular segment of DNA—is the sole source of both the specificity of further DNA synthesis[1], and of the specificity of a particular inherited metabolic reaction in the cell[2-4]. The theory proposes the stepwise transfer of the specificity embodied in the nucleotide sequence of the DNA to the amino-acid sequence of a particular protein enzyme, which thereby determines the enzyme's biochemical specificity and establishes a metabolic pathway in the cell. The relevant molecular processes are now well known and need not be described here.

For the purposes of this discussion, it will be assumed that: (1) the overall transfer of specificity from DNA to protein and from DNA to DNA that is proposed by the DNA code theory is in fact operative; (2) the governing molecular mechanisms are not necessarily those proposed by the theory; (3) the system is not necessarily the only mechanism which determines inheritance of the biochemical specificity of the cell. These assumptions will simplify further considerations without affecting their result; it will be shown later that these assumptions are in fact the best approximation of what is actually known.

The scheme proposed by the theory of the DNA code is summarized in Fig. 2*A*, using the symbols defined earlier. The scheme leads to the notion that DNA is a 'self-duplicating molecule' because it holds that DNA is the only source of the specificity of newly synthesized DNA. This conclusion is, however, valid only if it can be shown that no other regulatory components participate in DNA synthesis. It becomes important, therefore, that the experimental evidence be scrutinized with this question in mind.

Experiments on the synthesis of DNA *in vitro* show that three classes of components participate: the DNA primer or 'template', the necessary enzyme (that is, DNA polymerase), and the requisite deoxynucleotides. Kornberg's investigations of the DNA polymerase system, which synthesizes DNA from triphosphodeoxynucleotides, usually in the presence of primer DNA, yield evidence that the nucleotide sequence of the DNA product is influenced in a complementary manner by the nucleotide sequence of the primer[1,6]. This shows that some transfer of specificity from primer to product occurs, and that the primer serves as a regulatory component in the determination of the molecular specificity of the DNA product. However, although the theory requires an exact correspondence between the frequencies of a nucleotide in the primer and of the complementary nucleotide in the product, recent experiments show that this condition is sometimes not observed with respect to deoxyadenylic and deoxythymidylic acids[7]. Moreover, if precise replication occurs in the DNA polymerase system it should be possible to demonstrate that DNA newly synthesized in the presence of a biologically active primer is itself active. However, this phenomenon has not yet been conclusively demonstrated[8]. There is, therefore, no direct evidence that the nucleotide sequence of the primer exerts the total control of the nucleotide sequence of the product that the theory requires.

A conclusion that DNA is a self-duplicating germinal component also calls for the negative demonstration that the DNA polymerase enzyme, which is an obligate participant in DNA synthesis, does not contribute to the biochemical specificity of the product (that is, to its nucleotide sequence). However, certain experiments with the DNA polymerase system, reported by Kornberg *et al.*, lead to the empirical conclusion that the enzyme does, in fact, contribute specificity to the DNA product[9].

In the presence of a mixture of deoxyadenylic and deoxythymidilic acids—and in the absence of a DNA primer—the polymerase causes the formation (after a lag

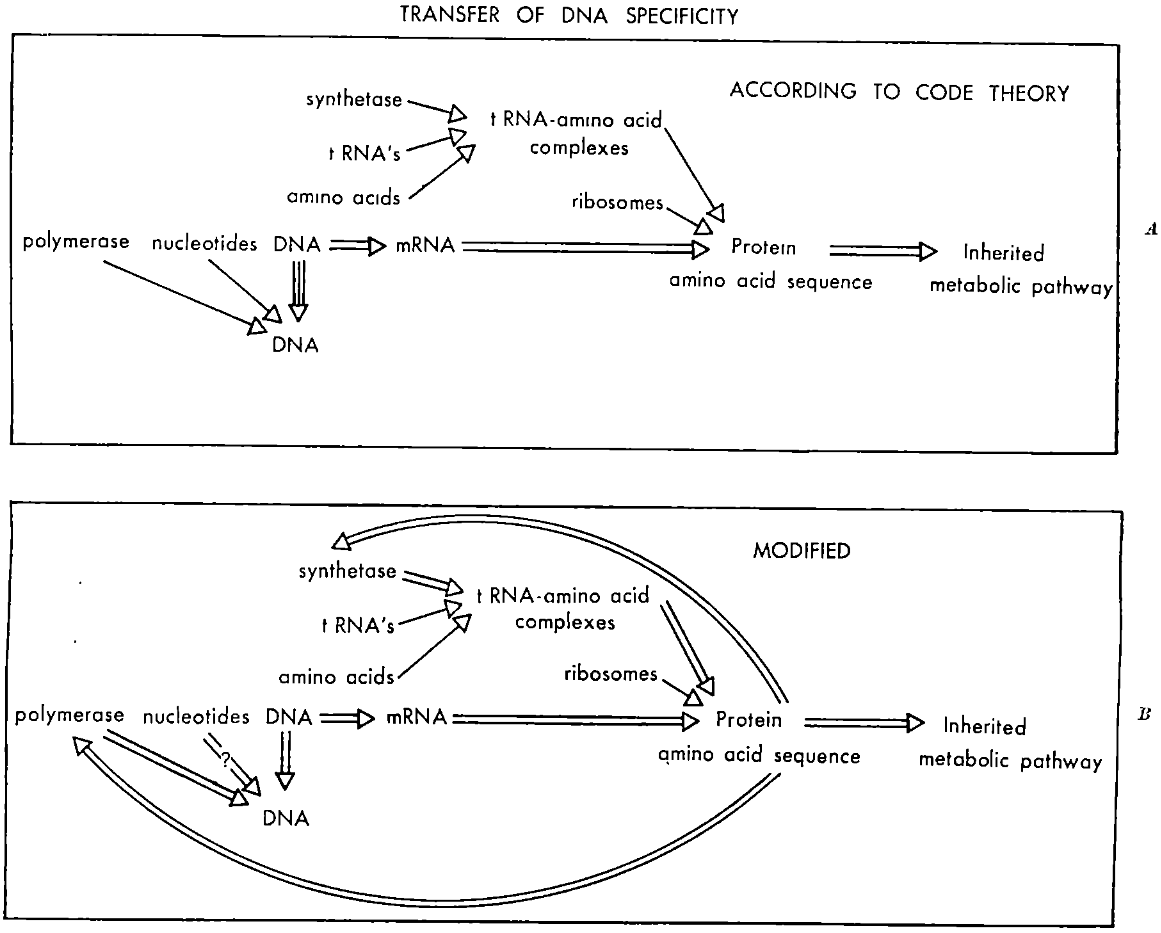

TRANSFER OF DNA SPECIFICITY
ACCORDING TO CODE THEORY
synthetase
t RNA's
amino acids
t RNA-amino acid complexes
ribosomes
polymerase
nucleotides
DNA
mRNA
Protein amino acid sequence
Inherited metabolic pathway
DNA
A
MODIFIED
synthetase
t RNA's
amino acids
t RNA-amino acid complexes
ribosomes
polymerase
nucleotides
DNA
mRNA
Protein amino acid sequence
Inherited metabolic pathway
?
DNA
B

Fig. 2. (*A*) Illustrates the transfer of biochemical specificity in the system proposed by the theory of the DNA code, which holds that the biochemical specificity of DNA (its nucleotide sequence) is the sole source of specificity in the replication of DNA and in the synthesis of protein, the specificity of the latter determining a particular inherited metabolic pathway. In this scheme DNA is itself a germinal self-duplicating component and the sole source of the cell's inherited biochemical pattern. (*B*) Illustrates modification of the system suggested by the evidence discussed in the text. This scheme illustrates the consequences of evidence that part of the biochemical specificity of DNA originates in the polymerase enzyme which catalyses DNA synthesis, and possibly in the relative availability of the several requisite nucleotides. Also shown is the effect of contribution of specificity originating in amino-acyl RNA synthetase, an enzyme essential to protein synthesis. In this scheme, DNA is neither a germinal, self-duplicating component, nor the exclusive determinant of the cell's biochemical specificity. Instead, these effects have a multi-molecular origin and the entire system requires pre-existing specificity in both DNA and at least 2 protein enzymes. Symbols according to definitions given in Fig. 1

period) of an *A–T* copolymer, with a regular alternating order of nucleotides, ... *ATATAT* .... In the absence of primer DNA the polymers .... *GGG* .... and .... *CCC* .... are formed when *G* or *C* are present alone; only these polymers are formed in the presence of a *G* + *C* mixture. Each of the polynucleotide products synthesized in the absence of DNA primer has a simple but definitely non-random nucleotide sequence and therefore possesses biochemical specificity (or in the parlance of DNA code theory, 'genetic information'). This specificity does not arise in the nucleotide sequence of a primer DNA molecule, for none is present in the reaction. The source of the product's specificity appears to be the enzyme, which is therefore—along with the DNA primer —a regulatory component in the system which synthesizes DNA. In the absence of a primer the DNA polymerase enzyme system appears preferentially to catalyse the formation of certain specific internucleotide bonds, ... *A–T*, ... *T–A*, ... *G–G*, ... *C–C*; it fails to catalyse the formation of certain other bonds, ... *A–A*, ... *T–T*, ... *G–C*, ... *C–G*.

These results indicate that in a system which synthesizes DNA in the presence of both primer and enzyme, both these constituents may be expected to contribute to the specificity of the DNA product. Direct evidence of the dual origin of DNA specificity in such a system requires observation of the effects of experimental variation of the specificity of the two components on the nucleotide sequence of the DNA product. While the absence of the necessary techniques precludes complete data on DNA nucleotide sequence, evidence regarding overall nucleotide composition, the variation of which must reflect gross changes in nucleotide sequence, is available. Lehman *et al.*[10] have reported the nucleotide composition of DNA produced when primers derived from several different biological sources are added to a system based on polymerase from *E. coli*. When both the DNA primer and the polymerase are derived from *E. coli*, the primer and the product differ only very slightly in base composition. Thus, if either total *E. coli* DNA, or DNA from the *E. coli*-specific *T*2 bacteriophage, is used as primer in the *E. coli* polymerase system, the *A* + *T*/*G* + *C* ratio (which is the most sensitive overall index of nucleotide composition) of the product differs from that of the primer only by 5 and 1 per cent, respectively. When DNA primers from other organisms are used, the *A* + *T*/*G* + *C* ratios of the products resemble those of the primers, although the latter are different from that of the *E. coli* DNA by a factor of 2. However, the agreement is by no means exact. When the primer is derived from *M. phlei*, the *A* + *T*/*C* + *G* ratios of primer and product differ by 20 per cent of the primer value; with *A. aerogenes* primer, the difference is 25 per cent; with calf thymus primer the difference is 17 per cent. Thus, when both primer and enzyme are derived from the same biological source (*E. coli*), the *A* + *T*/*G* + *C* ratios of product and primer agree to within 5 per cent; but when the two components are derived from separate species, considerably larger differences are observed. This is evidence that the relatively precise replication of the primer which occurs in the homologous system depends on both the specificity of the DNA primer, and on the specificity of the DNA polymerase enzyme, at least in so far as such specificity is reflected in the $A + T/G + C$ ratio. The influence of the polymerase specificity on DNA nucleotide composition appears to be smaller than that due to the primer, but it is, nevertheless, appreciable.

The evidence of biochemical genetics suggests that DNA nucleotide sequence is probably replicated within a given organism with a high degree of precision. The foregoing *in vitro* evidence indicates that the precision of replication in the living system is dependent, at the least, on specificity contributed by both the DNA primer and the species-specific polymerase which catalyses DNA synthesis.

Evidence regarding the effect of relative concentrations of nucleotides that are essential for DNA synthesis on DNA nucleotide sequence (and therefore on its biochemical specificity) is rather scanty. Base ratios of extracted DNA do not appear to change significantly when normal micro-organisms are grown in different types of nutrient media. On the other hand, Kanazir[11] observed that when bacterial mutants incapable of thymine synthesis are transferred to a thymine-deficient medium, the rate of mutation (that is, of reversion to thymine dependence) increases sharply among the cells produced just before thymineless death occurs. This observation, which has since been confirmed[12], suggests that thymine deficiency that is so severe as to be nearly lethal may alter the nucleotide sequence of DNA, which according to substantial evidence is the basis of such mutations.

The relevant *in vitro* evidence appears to be a single experiment in which enzymatic DNA synthesis with the four requisite nucleotides present in equimolar concentrations was compared with systems in which either deoxythymidilic acid or this nucleotide and deoxyadenylic acid were reduced five-fold in concentration[10]. No significant changes in the ratios of $A + T/G + C$ or $A + G/C + T$ in the synthesized DNA were observed. In view of the relatively limited variation in nucleotide concentration this observation is not conclusive evidence that nucleotide sequence is wholly independent of nucleotide availability during DNA synthesis.

The heterocatalytic regulatory effects of DNA, that is, the transfer of specificity from DNA to protein, have been the subject of a considerable number of recent investigations. Most of these results have been interpreted as lending support to the scheme shown in Fig. 2*A*. However, the available data fail to support the conclusion that the transfer of specificity between DNA and protein is rigorously determined by the specificity of the 'messenger' RNA (which, according to the theory, is itself derived from DNA nucleotide sequence).

Of particular importance is the origin of specificity in the system which provides the 'code' relating nucleotide sequence to protein amino-acid sequence. According to the conventional theory, the nucleotide sequence of a DNA segment (which represents a genetic agent) determines the nucleotide sequence of a 'messenger' RNA (*m*RNA). The latter, in turn, mediates the order of assembly of short segments of 'transfer' RNA (*t*RNA), previously charged with the appropriate amino-acids, on the ribosome where the amino-acids are joined to form the protein's primary structure. The addition of a specific amino-acid to the terminus of a particular *t*RNA is catalysed by an enzyme, amino-acyl RNA synthetase. According to the conventional theory, the ordering of the amino-acid sequence in the protein is determined by the nucleotide sequence of the *m*RNA, a given nucleotide pattern in the latter having an affinity for a particular one of the 20-odd *t*RNA's, thereby achieving the appropriate positioning of the amino-acid uniquely associated with a given *t*RNA unit. The theory of the DNA code requires that, of the several components of the system which participate in the assembly of the protein's amino-acid sequence, only the messenger serves as a source of protein specificity (that is, its amino-acid sequence). If any other source of specificity were to intervene, then the basic conclusion of the 'code' theory, that protein specificity (and therefore metabolic control of the cell) is wholly determined by DNA nucleotide sequence, fails. It is appropriate, therefore, to consider the available evidence regarding the origins of the specificity which regulates the ordering of amino-acid sequence in protein synthesis.

A crucial test of this question has recently been reported by Barnett and Jacobson[13], who examined the influence of synthetases derived from two different species on the specificity of amino-acid attachment to particular *t*RNA's isolated from one of them. They find that soluble RNA from *E. coli* contains only a single *t*RNA component which is charged specifically with phenylalanine by the action of *E. coli* synthetase. However, when the synthetase is derived from *N. crassa*, three separable *t*RNA units found in soluble *E. coli* RNA become charged with phenylalanine: (*a*) One *E. coli* fraction, apparently identical with the component that is specifically charged with phenylalanine by the *E. coli* enzyme, exhibits the same property when *N. crassa* synthetase is present. (*b*) A second *E. coli* RNA fraction is charged with either phenylalanine or alanine by the *N. crassa* synthetase, but is not affected by the *E. coli* enzyme. (*c*) A third *E. coli* RNA fraction is charged with phenylalanine by the *N. crassa* enzyme, but it not acted on by the comparable *E. coli* enzyme. The authors regard these observations as evidence for "interspecies degeneracy, ambiguity, and lack of universality in this coding system". In effect, these results show that the precision with which an intact *E. coli* cell is capable of regulating the specificity of the proteins which it synthesizes depends not only on the specificity provided by the DNA genetic agent (presumably transmitted by *m*RNA), but also on the species-specificity of its amino-acyl RNA synthetase. There is considerable evidence in the literature which shows that *in vitro* protein synthesis involving components derived from different species fails to produce protein with the specificity expected from the source of the *m*RNA[14].

Similar questions arise regarding the participation of transfer RNA's and of ribosomes in the determination of amino-acid sequence in protein synthesis. While the available results are not definitive, there is no strong evidence against the conclusion that these agents, like the synthetase, may contribute to the specificity of protein synthesis[15].

Observations that a single gene mutation may give rise to a single change in a protein's amino-acid sequence are sometimes cited in support of the conclusion that protein amino-acid sequence is rigorously specified by DNA nucleotide sequence. However, these effects are exhibited only by entire living systems. The only rigorous conclusion that may be derived from them is that DNA specificity does exert a precise regulatory effect on amino-acid sequence, provided that protein synthesis takes place in a cell of appropriate specificity. However, it is evident from the above that the species-specificity of non-DNA constituents of a cell is essential for precise determination of protein structure. Hence, data regarding genetic effects on amino-acid sequence do not militate against the conclusion that non-DNA constituents of the cell participate in the determination of protein specificity.

To sum up, the present evidence concerning the reactions involved in the development of the specificity finally embodied in DNA and in proteins indicates that such specificity can arise, in part, from sources other than DNA nucleotide sequence. While complete support for this conclusion must await detailed analysis of the actual reaction mechanisms which govern the formation of the covalent inter-residue bonds in DNA and of the several successive reactions involved in protein synthesis, the evidence now available shows that at least two of the relevant enzymes possess species-specificity which can influence the ordering of residues in both DNA and proteins.

These considerations suggest certain amendments to the system of regulatory effects proposed by the theory of the DNA code[16]. Fig. 2*B* shows how the additional sources of biochemical specificity indicated by the evidence brought forward here affect the conventional system. DNA specificity is no longer self-determined, but depends on DNA nucleotide sequence and on the specificity of the polymerase (and perhaps on the relative concentrations of free nucleotides as well). Similarly, the specificity of a protein is determined not only by DNA nucleotide sequence (transmitted via 'messenger' RNA), but also by the species-specificity of the synthetase (and possibly by specificities of the 'transfer' RNA's and of ribosomes).

Accordingly, the systems which govern both the specificity of protein synthesis and of DNA synthesis are fundamentally circular in nature. Although protein specificity is derived, in part, from DNA specificity, the latter is, in turn, partially dependent on the protein specificity of the DNA polymerase, which is itself dependent on the specificity of at least one other protein, the amino-acyl RNA synthetase. The system is operative as a self-sufficient source of specificity for DNA and for proteins only if DNA with a specific nucleotide sequence, and at least two proteins which possess species-specificity (polymerase and synthetase), are already present. This situation prevails in the intact cell. It can apparently be imitated with varying degrees of success in *in vitro* systems which derive all the necessary components from the same biological source, and thereby retain the complex sources of specificity which are present in the living cells of a given species.

As indicated by a comparison of Figs. 2*A* and *B*, the net effect of the introduction of these additional sources of biochemical specificity is to negate the simple conclusion that DNA is by itself a germinal, self-duplicating component or that DNA is the sole source of the biochemical specificity, embodied in proteins, that regulates the specificity of cellular metabolic processes. Such a conclusion is valid only if the specificity-transferring system is monophyletic, proceeding linearly from DNA to protein without branches or feedback. But the available evidence negates this conclusion; specificity ultimately embodied in DNA nucleotide sequence and in protein amino-acid sequence has a multi-molecular origin in a system which is characterized by circular feed-back relations.

A number of experiments show that the introduction of certain DNA components, such as bacterial transforming principle, into an appropriate host cell, can induce in the latter the appearance of a new operational genetic agent. This result does not conflict with the foregoing conclusion that DNA replication and its regulatory effect on the cell require sources of specificity other than DNA. A given DNA transforming agent is effective only when introduced into a living cell of an appropriate type. Hence, replication of the agent may depend on specificity contributed by the host cell. Moreover, quite massive changes in the nucleotide composition of bacterial DNA (for example, the replacement of 50 per cent of *E. coli* DNA thymine with 5 bromouracil) lead to an increased incidence of normally observed mutations, but do not cause gross changes in the range of mutations or in the species characteristic of the cell[17]. If the total genetic specificity were under the exclusive control of the DNA nucleotide sequence, such a massive change in DNA composition would be expected to disorganize severely the main features of the organism. On the other hand, if 5 bromouracil is regarded as a biologically effective substitute for thymine in the DNA nucleotide sequence, then no massive effects would be expected. However, in this case there is no explanation for the bromouracil-induced increase in mutation rate.

If the view summarized in Fig. 2*B* is correct, and agents such as DNA polymerase or amino-acyl RNA synthetase are, like DNA itself, regulatory components and therefore sources of specificity in DNA replication or in protein synthesis, why is there no evidence that such proteins are capable of acting as transferable genetic agents ? The following considerations suggest that this empirical observation may be due to a secondary difference between the status of DNA and the related enzymes in the cell, rather than to any basic difference in their capabilities as regulators of biochemical specificity.

The evidence of genetics shows that DNA agents which are the basis of typical Mendelian effects are present in the cell in only one or two replicas; that is, they are non-redundant. Hence, an invading DNA agent, such as transforming DNA, can readily compete with the DNA-borne genetic agents pre-existing in the host cell, or failing that, appear in an appropriate proportion of subsequent daughter cells unaccompanied by the indigenous agents. Thus, the striking genetic effect of an invading DNA agent is due not only to its regulatory capability, but also to the low level of multiplicity of the cell's normal DNA-borne genetic agents with which it must interact.

However, in the case of enzymes such as DNA polymerase, or amino-acyl RNA synthetase, matters are very different, for a single cell may contain hundreds or more of separate enzyme molecules. For this reason an invading enzyme molecule, even if capable of contributing to the specificity of DNA and protein synthesis, is not likely to have a noticeable effect on the biosynthetic activity of the host cell and little chance of becoming segregated out as a dominant form in the enzyme population of any daughter cells. Hence, no genetic effects are ordinarily discernible. Nevertheless, such an observation does not prove that such proteins are incapable of contributing to the specificity of DNA or protein synthesis.

Conclusions. The foregoing considerations lead to the conclusion that the role of DNA in biological inheritance does not reflect a unique capability for self-duplication and for regulation of the specificity of protein synthesis. While DNA does contribute to the specificity of DNA and of protein, this property is shared with certain protein agents, for example, DNA polymerase and amino-acyl RNA synthetase. The special role of DNA as a vehicle for the Mendelian gene is indeed based on a unique attribute of 'template' DNA. However, DNA is not unique because it is capable of regulating biochemical specificity, but because, among the cell's regulatory agents, those composed of DNA occur at a very low level of redundancy.

These conclusions suggest certain theoretical and operational consequences. With regard to the general theory of molecular control of inheritance, they propose that the source of transmissible biochemical specificity in inheritance is multi-molecular, rather than being derived uniquely from the specificity of DNA. In particular, the assumption described in the so-called "Central Dogma" (which states that biochemical specificity can be transferred from nucleic acid to nucleic acid, and from nucleic acid to protein, but not from protein to nucleic acid[3]) is denied by the available evidence. Hence, the general consequence of this assumption—that the capability of the living cell for self-duplication and inheritance is uniquely determined by DNA—is not valid. The precise replication of DNA and proteins in living cells requires the pre-existence of both specific DNA and specific cytoplasmic proteins. Hence the capability of living matter for precise self-duplication is derived not only from nuclear material, but from the cytoplasm as well. This is a restatement of a conclusion long familiar to biologists—that life, in its present specifically replicative form, is derived from pre-existing life. Considerations which have been published elsewhere also show, on other grounds, that the 'template' function of DNA is only a special case of a more general and primitive DNA function, nucleotide sequestration, and suggest that early forms of life lacked the precise type of replication observed in present living things[5].

The position developed in this article also leads to two chief operational conclusions which may be subject to experimental test. If the code theory were correct, then it should be possible, by chemical modification of DNA, to develop specific nucleotide sequences hitherto absent from living things; and, in transformation experiments, to achieve wholly new inheritable biological characteristics and even new forms of organisms. In contrast, the view that inheritance is based on multi-molecular sources predicts severe limits on the biological effectiveness of artificial modifications of DNA nucleotide sequence. Such gross changes are likely to be incompatible with the specificity requirements imposed by enzymes, such as DNA polymerase and amino-acyl RNA synthetase, which would remain unchanged in a DNA-mediated transformation experiment. Hence, an artificially modified DNA might, when introduced into an appropriate cell, induce the synthesis of new DNA; but, because of the specificity contributed by the cell's enzymes, the artificial DNA would not be exactly replicated, the product being a polynucleotide more closely resembling normal DNA. For the same reason, this view predicts that in such experiments the resultant biological mutations will not depart appreciably from the range and type of mutations observed in the normal biology of the organism. These contrasting views of the consequences of extensive chemical modification of DNA should be subject to experimental test.

The theory of the DNA code holds that only DNA, or possibly an RNA component on which DNA specificity is impressed, can serve as a molecular vehicle for the transmission of inheritance. In contrast, if the view developed in this article is correct, it should be possible to demonstrate genetic transformation of a cell by a protein agent, analogous to the known instances of bacterial transformation by DNA. Such an experiment would require the introduction of an enzyme which is capable of imparting specificity in DNA or protein synthesis into an appropriate host cell under conditions in which the natural redundancy of these agents in the cell is sufficiently reduced to permit the expression of the invading protein. Experimental methods for accomplishing the low levels of protein redundancy essential for such an investigation might include suppression of protein synthesis in the host by means of inhibitors or by substrate starvation, or the use of micro-organisms of extremely small cell size, in which cytoplasmic redundancy might be correspondingly reduced[18]. I thank Prof. M. Swann, of the Zoology Department, University of Edinburgh, for the last suggestion.

As I have pointed out elsewhere[16], the assumption (derived from biochemical evidence) that the unique capability of living matter for self-duplication is due to the presence within it of a molecule, DNA, which itself has this attribute, conflicts with certain principles of biology, especially the cell theory and the epigenetic basis of development. The considerations developed in this article may help to elucidate this discrepancy, for they suggest, from an analysis of the biochemical data themselves, that the operative unit of self-duplication is not DNA, but a multi-molecular system which is so complex as to require the participation of the entire living cell.

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ACTIVITY OF THE HEXOSE MONOPHOSPHATE SHUNT IN THE NORMAL AND DYSTROPHIC RETINA

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THE patterns of glucose catabolism via the Embden–Meyerhof and Krebs cycle pathways have been compared in developing normal and dystrophic rat retinæ[1]. The dystrophic animals have a recessively inherited degenerative condition of the retina resembling human retinitis pigmentosa. No qualitative differences between normal and dystrophic retinæ were found and the quantitative differences which could be detected were subsequent to histological degeneration.

The hexose monophosphate (HMP) shunt pathway of glucose catabolism has been implicated in the normal functioning of the visual cycle in the mammalian retina[2]. It appears that, after bleaching, reduction of retinene to vitamin A alcohol is coupled to the oxidation of glucose via the HMP shunt mechanism through the utilization of $NADPH_2$. In addition, Cohen and Noell[3] reported relatively high activities for the HMP shunt in the young, undeveloped rabbit retina.

In the investigation described here, the activity of the HMP shunt was examined in developing normal and dystrophic rat retinæ over the age range 6–28 days. This period covers the development of the lesion in the abnormal retina. The experiments were carried out *in vitro* on retinal tissue obtained from normal, black-hooded (*PVG*) rats and pink-eyed piebald agouti rats affected with the retinal dystrophy[4].

All experiments were carried out on litter-mates irrespective of sex. Preparation of retinæ for biochemical investigation has been previously described[1]. 25 mg quantities of tissue were incubated for 1 h at 37° in 1 ml. Krebs–Ringer bicarbonate medium[5] in an atmosphere of 95 per cent oxygen–5 per cent carbon dioxide, in special

Table 1. RESULTS EXPRESSED AS THE RATIOS OF ACTUAL YIELDS OF $^{14}CO_2$ FROM 1-^{14}C GLUCOSE/6-^{14}C GLUCOSE

Age	Normal	Dystrophic	Increase in dystrophic (%)	
6–7 days	3·5 (0·4)	3·3 (0·6)	−6	$P = 0·8 - 0·7$
14 days	1·6 (0·4)	2·4 (0·5)	50	$P = 0·3 - 0·2$
27–28 days	1·5 (0·2)	2·4 (0·2)	56	$P = 0·01$

(*S.E.M.*, in brackets)

Table 2. RESULTS EXPRESSED AS RATIOS OF SPECIFIC YIELDS OF $^{14}CO_2$ FROM 1-^{14}C GLUCOSE/6-^{14}C GLUCOSE

Age	Normal	Dystrophic	Increase in dysotrophic (%)	
6–7 days	2·9 (0·6)	4·1 (0·8)	41	$P = 0·3 - 0·2$
14 days	1·7 (0·3)	2·5 (0·5)	49	$P = 0·2 - 0·1$
27–28 days	1·2 (0·1)	1·8 (0·2)	50	$P = 0·05 - 0·02$

(*S.E.M.*, in brackets)

micro-Warburg flasks fitted with two side-arms, one of which was closed with a rubber cap. 2 μc. of either 1-^{14}C glucose or 6-^{14}C glucose diluted with inert carrier glucose to give a final concentration of 0·1 per cent in the medium was added from the side-arm to initiate the reaction. Carbon dioxide was collected at the end of the incubation period by injecting alkali on to the paper in the centre well, followed immediately by injection of acid into the medium, with 10 min additional incubation to ensure complete collection. Medium and tissues were separated by centrifuging and the tissues washed twice in isotonic saline, the washings and medium being pooled and finally made up to constant volume. Radioactive carbon dioxide was precipitated as barium carbonate, plated out on aluminium planchets and counted under a thin end-window Geiger–Müller counter, suitable corrections being made for self-absorption. Aliquots of medium before and after incubation were chromatographed on Whatman No. 1 paper in a solvent system of *tert*-butanol, 80 ml.–picric acid, 4·0 g–water, 20 ml. The radioactivity of the glucose 'spots' was evaluated by running the chromatograms through an automatic scanning device[6].

Evaluation of the relative activity of the HMP shunt pathway in the catabolism of glucose was carried out in two ways; (*a*) by determination of the actual yield of carbon-14 recovered as respiratory carbon dioxide and expressed as the ratio of $^{14}CO_2$ yield from 1-^{14}C glucose to that from 6-^{14}C glucose (Table 1); (*b*) by expressing the $^{14}CO_2$ yields (called specific yields) in terms of glucose utilized (Table 2). The latter method is based on those of Wang *et al.*[7], modified by determining residual glucose after incubation as suggested by Katz and Wood[8]. This overcomes possible criticism of the assumption that the same amounts of differently labelled glucose are utilized in parallel experiments. In all cases, the activities of the carbon dioxide collected were calculated to conform to a constant specific activity of glucose substrate, the latter being determined from aliquots of medium before incubation.

The results clearly show an elevation in the activity of the HMP shunt pathway in the dystrophic retina compared with the normal at corresponding ages. The differences, however, are greater during the later stages of development of the lesion, that is, at 14 and 28 days, and both methods of assessing the results show higher statistical significance at these times. It is, however, noteworthy that results taking glucose utilization into account (based on specific yields) show higher significance throughout. The results also demonstrate conclusively the decline in relative importance of the shunt pathway for glucose breakdown, with increase in age and development, in the normal retina.

The existence of a primary biochemical lesion associated with the genetical anomaly in this degenerative retinal condition has been sought for some time. Recently, Reading and Sorsby[9] reported an early depression in rate of protein synthesis confined to the retina in affected rats, occurring some 6–8 days before histological changes. This depression becomes less marked as development proceeds, until, at 24 days of age, rates of protein biosynthesis in normal and dystrophic retinæ are indistinguishable. Depression in synthesis of a specific protein moiety is a phenomenon associated with a number of other genetic abnormalities, especially those concerned with hæmoglobin synthesis[10].

Chronologically, maximum depression in rate of protein synthesis in the dystrophic retina occurs before maximum elevation of the shunt. The significance of this observation remains to be assessed. It could possibly represent some form of 'feed-back' mechanism within the tissue. In this respect, it has recently been suggested[11] that a metabolic coupling exists between the shunt mechanism and protein biosynthesis since there is a striking parallel between the activities of these two metabolic pathways in a number of mammalian tissues. Experiments are in hand to determine whether the depression in protein synthesis or the elevation in activity of the shunt mechanism is directly concerned with the structural breakdown in the dystrophic retina.

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ISOTOPIC EXCHANGE AT EQUILIBRIUM AS A CRITERION OF ENZYMATIC MECHANISMS

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KINETIC criteria proposed for the diagnosis of enzymatic mechanisms have been based mainly on initial reaction velocities, but some use has been made of correlations between equilibrium constants and initial rate behaviour, that is, the Haldane relations. The possibility of developing criteria of these categories to differentiate between the variety of branching and linear mechanisms which may operate in the important class of group-transferring enzymes was examined in a recent generalized investigation by us[1]. Now an additional kind of kinetic criterion for the differentiation of mechanisms has been recognized by Boyer[2] and Boyer and Silverstein[3,4], who have pointed to the potential diagnostic value of studying the rates of isotopic exchanges in enzymatic systems at equilibrium. It is proposed here to consider this new criterion in the light of the generalized theory already referred to which, built on the determinant method of King and Altman[5] for solving the steady-state equations, permits a direct visualization of the rate consequences of enzymatic mechanisms.

A group-transfer reaction may be represented:

$$GX + Y \rightleftharpoons X + GY \qquad (1)$$

In the system at equilibrium there will be no net change in the concentrations of these reactants, but, if a minute quantity of GX or X labelled isotopically in the X-moiety be added, the exchange of the labelled X-moiety between GX and X may be measured. For a number of specific mechanisms Boyer[2], Morales, Horovitz and Botts[6], and Boyer and Silverstein[3] derived rate expressions for such exchanges and these can be used to evaluate rate parameters. This led to the proposal of the exchangeability criterion to differentiate between reaction mechanisms. Consider, for example, the exchange of X-moiety between GX and X; if the concentrations of the other pair of reactants, GY and Y, are made very large, the exchange of X-moiety may or may not become completely blocked, depending on the mechanism. Thus the blocking or lack of blocking of exchanges may serve as a criterion of mechanisms.

In what follows, a simple and direct method is described for ascertaining for any given mechanism whether or not an exchange between one pair of reactants will be subject to blocking when the concentrations of the other pair of reactants are raised to saturating levels, the system remaining at equilibrium. This method, in common with the generalized featurewise method of kinetic analysis developed earlier, depends on defining the precise features, possibly common to many mechanisms, which will be responsible directly for the particular rate effect in question; in this case, the blocking or lack of blocking of a specified exchange reaction. As will become clear, this featurewise approach avoids some special assumptions made in deriving rate equations for exchange reactions (cf. Morales, Horovitz and Botts[6]) and it is applicable easily to an unrestricted range of mechanisms, including complex examples for which the derivation of full-rate expressions has not been attempted and would present difficulty.

The principles of the method can be illustrated by considering the effects of high concentrations of GY and Y on the exchange of X-moiety between GX and X:

(1) Let $[GY, Y]$ represent the concentrations of GY and Y (present always in the proper proportions to maintain the equilibrium state). As $[GY, Y]$ becomes very large, some of the enzymatic species in the reaction mechanism will tend to vanish, while other species will persist in finite amounts.

(2) In the general case of a mechanism involving n enzyme-containing intermediates, $ER_1, ER_2, \ldots ER_n$ (including free enzyme as one of these species), the probability of the conversion of one species to another is called a kappa, which consists of the kinetic constant of the conversion step multiplied by the concentration of any non-enzymatic reactant taking part in the conversion, if there be one. Solution of the steady-state equations for the mechanism permits the fractional concentration of any one of the ER's to be expressed in terms of the kappas. Consider, for example, the mth species, ER_m. Its fractional concentration is represented as the ratio of two groups of terms, as follows:

$$\frac{[ER_m]}{[\text{total enzyme}]} = \frac{\Delta_m}{\Delta_1 + \Delta_2 + \ldots + \Delta_n} \qquad (2)$$

The denominator in equation (2) is common to the expressions for the fractional concentrations of all n species and equals simply the sum of the n respective numerators. Using a determinant method, King and Altman[5] derived a rule, rigorously established, which specifies clearly and comprehensively the composition of the terms present in Δ_m. Each term in Δ_m is a product of $(n - 1)$ different kappas. Schematically, these $(n - 1)$ kappas correspond to $(n - 1)$ reaction steps which lead either individually or in confluent and continuous sequences to ER_m; no kappa originating from ER_m is present in these terms which must therefore contain one kappa originating from each of the other $(n - 1)$ ER's present in the mechanism. The full expression for Δ_m is given by the sum of all possible kappa-products which conform to this description.

(3) Although, for any given mechanism, the full expression for Δ_m (and hence also the denominator terms $\Delta_1 + \Delta_2 + \ldots \Delta_n$, in full) can be written out by inspection, supplemented by sketching out schematic representations of all the possible pathways leading to ER_m*, it is rarely necessary or even helpful to develop such expressions in full detail. In the present connexion, as will become evident, the only relevant property of the terms comprising Δ_m (and the terms comprising $\Delta_1 + \Delta_2 + \ldots + \Delta_n$) is the highest degree of any of the terms with respect to GY and Y. The concept of the degree of a mechanism with respect to the concentration of a reactant was proposed[1] as a fundamental basis for the classification of enzymatic mechanisms, and its usefulness is illustrated again in the present connexion. Here we are concerned with the degree of terms in Δ_m with respect to two reactants, GY and Y, and this refers merely to the maximum number of times the concentration factors $[GY]$ and $[Y]$ appear in any terms in Δ_m, that is, the maximum number of times $[GY]$ and $[Y]$ can appear in any kappa-product present in Δ_m. If there are z different ER's in the mechanism which react with GY or Y or both GY and Y, the degree of Δ_m with respect to $[GY, Y]$ cannot exceed z. The procedure then is to ascertain by inspection and sketching of schematic terms whether there is a kappa-product leading to ER_m in which $[GY]$ and $[Y]$ can appear z different times, or failing this, whether there is a kappa-product leading to ER_m and in which $[GY]$ and $[Y]$ can appear $(z - 1)$ times, etc. Thus, starting from z, the degree of Δ_m with respect to $[GY, Y]$ can soon be reached. The degree of each of $\Delta_1, \Delta_2, \ldots \Delta_n$ with respect to $[GY, Y]$ is similarly reached. With practice, this is easily carried out and it usually requires no more than a few minutes to examine a mechanism and to sketch out schematically the kappa-products which are relevant.

(4) Once the degrees of the various Δ's with respect to $[GY, Y]$ have been established, it will become evident which ER's in a given mechanism will vanish and which will persist, when $[GY, Y]$ is made saturating. If the degree of the numerator is equal to that of the denominator in the expression for a particular intermediate ER_m, then ER_m will persist in finite amount when $[GY, Y]$ becomes very large. If, on the other hand, the degree of the numerator is lower than that of the denominator, ER_m will vanish when $[GY, Y]$ becomes very large. (It may be noted from equation 2 that the degree of the numerator cannot exceed that of the denominator, since the denominator, common to all the expressions, contains all the individual terms which are present in the respective numerator Δ's.)

(5) All reaction steps originating from a persisting enzymatic species in the mechanism will proceed at significant rates. Furthermore, since we are considering the system at equilibrium, the principle of microscopic reversibility dictates that all reaction steps leading to a persisting species must also proceed at significant rates. In terms of interconversions between different species, this means that the direct interconversion of two given species will continue at a significant rate if one or both species persists when $[GY, Y]$ becomes saturating. On the other hand, interconversions between pairs of species which both vanish will be insignificant.

(6) It is evident that the exchange of X-moiety between GX and X will not be blocked if an uninterrupted chain of significant interconversions constituting a pathway for the exchange persists when $[GY, Y]$ is increased to a saturating level, whereas the exchange will be blocked if the chain of interconversions is interrupted. Such interruptions will result if any two adjacent ER's present in such a chain of interconversions are caused to vanish as $[GY, Y]$ is made very large.

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* In practice, for more complex mechanisms, it is more convenient to make use of a simple mathematical procedure to ensure comprehensive inclusion of all admissible terms; this is described by Wong and Hanes (ref. 1).

The considerations outlined in the foregoing paragraphs provide a general method for determining: (*a*) which ER's in a given mechanism will persist and which will vanish; (*b*) which interconversions will continue at a significant rate and which will fall to zero; and accordingly, (*c*) which potential pathways for the interconversion of GX and X will remain and which will be blocked when $[GY, Y]$ is made very large. The application of these principles may be illustrated by considering the series of mechanisms A–E. Here the enzymatic species which persist in the presence of very large $[GY, Y]$ are underlined, and significant interconversions are represented by solid arrows, whereas insignificant ones are shown by broken arrows. For each ER in these mechanisms there is shown in brackets the degrees with respect to $[GY, Y]$ of the numerator and denominator terms of the expression for its fractional concentration (cf. equation 2) derived by inspection as described above.

(In mechanism E, GY acts not only as a substrate, but also as a modifier by adding to a site represented as being on the left side of E to form GYE, which is able to function by combining with GX to form $GYEGX$.)

It will be seen by inspection of these diagrams that the exchange of the X-moiety between GX and X will be blocked in mechanism A, on saturation with $[GY, T]$, since no continuous chain of significant interconversions for bringing about the exchange remains, but in each of the other cases an uninterrupted chain for the exchange reaction will remain, as follows:

Mechanism

B $$\underline{EY} \overset{GX}{\rightleftharpoons} \underline{EGXY} \rightleftharpoons \underline{EGYX} \underset{X}{\rightleftharpoons} \underline{EGY}$$

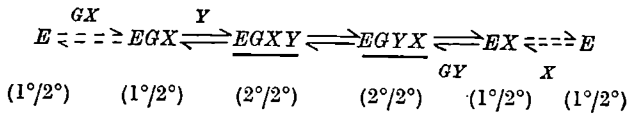
C $$\underline{EY} \overset{GX}{\rightleftharpoons} \underline{EGXY} \rightleftharpoons \underline{EGYX} \underset{X}{\rightleftharpoons} \underline{EGY}$$

D $$\underline{E} \overset{GX}{\rightleftharpoons} \underline{EGX} \underset{GY}{\overset{Y}{\rightleftharpoons}} \underline{EX} \underset{X}{\rightleftharpoons} \underline{E}$$

E $$\underline{GYE} \overset{GX}{\rightleftharpoons} \underline{GYEGX} \underset{GY}{\rightleftharpoons} EGX \overset{Y}{\rightleftharpoons} \underline{EGXY} \rightleftharpoons \underline{EGYX} \underset{X}{\rightleftharpoons} \underline{EGY}$$

Mechanism

A



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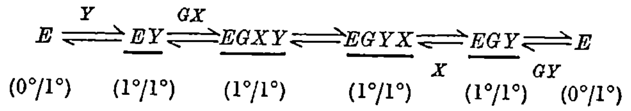

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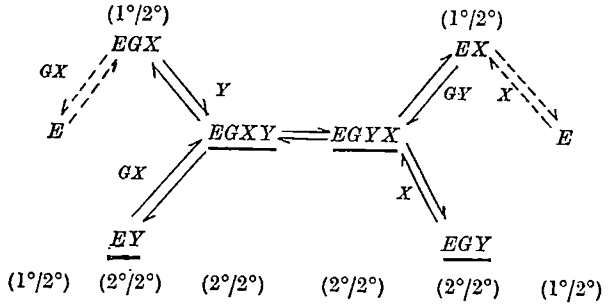
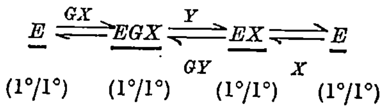
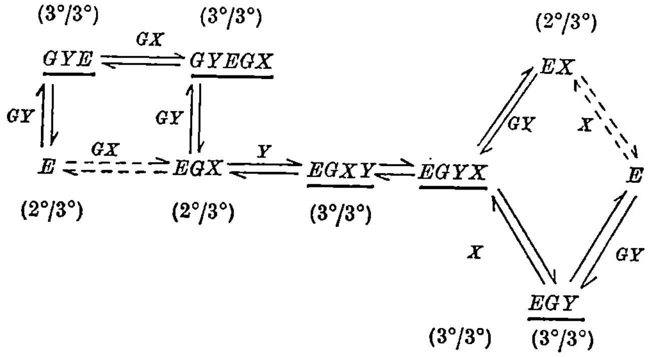


D



E



It was suggested by Boyer[2], and Boyer and Silverstein[3,4] that the blocking of an exchange between one pair of reactants by high concentrations of the other pair might prove to be characteristic of a linear reaction pathway (compulsory order) and that lack of blocking might be characteristic of branching (alternative) pathways. The above examples show that this is not strictly the case. Mechanism C (with dead-end branches) has a compulsory order of reaction, but the various exchanges will not be blocked by saturation with any pair of reactants. In mechanism D, the exchange reaction between GX and X cannot be blocked by high concentrations of GY and Y, but can be blocked by high concentrations of GX and GY, or of X and Y, that is, one of GX and X has to be saturating in order to block its own exchange reaction.

Mechanism E is of particular interest in showing that the action of GY as a modifier has changed a mechanism for which blocking of the exchange of X-moiety by high $[GY, Y]$ is predicted, into one which will not be blocked. It illustrates the further interesting feature referred to earlier that virtual deletion of a single intermediate by high $[GY, Y]$ does not interrupt the continuity of significant interconversions through this intermediate.

We have been interested especially in mechanism E as representing one of the very few mechanisms which appears capable of providing an explanation for a wide range of features of initial rate behaviour exhibited by the alcohol dehydrogenase of horse liver, to be reported shortly elsewhere. It seemed at first that the observations of Boyer and Silverstein[4], showing that isotopic exchange between NAD+ and NADH is not blocked by high concentrations of alcohol–acetaldehyde (in equilibrium proportions) in this same system, might constitute evidence against this mechanism but, as will be evident from the foregoing analysis, this is not the case. It is pertinent to note that mechanism D, which corresponds to the mechanism proposed originally by Theorell and Chance[7] for the liver alcohol dehydrogenase reaction, is now shown also not to be subject to blocking by high [alcohol–acetaldehyde]. However, on grounds to be presented elsewhere, this mechanism appears inadequate to explain a number of diagnostic features of rate behaviour which have been observed.

We thank Prof. P. D. Boyer and Dr. E. Silverstein for permitting us to read in advance of publication their observations on equilibrium reaction rates occurring in different enzymatic systems, and also for helpful discussions on the interpretation of isotopic exchanges at equilibrium.

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EXPRESSION OF POLYOMA-INDUCED CELLULAR ANTIGEN(S) IN HYBRID CELLS

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THE occurrence of somatic hybridization in mixed *in vitro* cultures of two mouse cell lines was first observed by Barski *et al.* in 1960 (ref. 1), and soon thereafter confirmed by Sorieul and Ephrussi[2]. In 1962, Ephrussi and Sorieul[3] obtained a second somatic hybrid, designated *M*-109. This new hybrid was obtained in mixed cultures of the two following cell lines: (*a*) clone N.C.T.C. 2555, the 'low cancer' inducing line isolated by Sanford *et al.*[4] from a culture of subcutaneous connective tissue of a *C3H* mouse; (*b*) clone *Py*-198-1 isolated by Dulbecco and Vogt[5] from a culture of mouse (non-inbred Swiss) embryonic tissue 'converted' *in vitro* by polyoma virus. *Py*-198-1 is a clone of non-virus releasing cells characterized by multi-layer growth.

The conclusion that *M*-109 represents a line of hybrid cells was based on karyological evidence, namely, on the observation that these cells present a modal chromosome number approximating the sum of the modal chromosome numbers of the two parental lines[3,6] and contain marker chromosomes contributed by both parents. N.C.T.C. 2555 carried 11–16 bi-armed chromosomes and clone *Py*-198-1 several 'rabbit ear' chromosomes absent in the cells of the former line. Recently, this inference from the karyological investigation found support in the finding that *M*-109 cells carry four iso-antigens, three of which are carried by one of the parental lines, and one by the other[7].

The object of the present investigation was to establish whether cells of the parental line *Py*-198-1, 'converted' by polyoma virus, and those of line *M*-109 contain the cellular antigen(s) induced by the virus[8]. The presence of this antigen would provide additional proof of the hybrid nature of line *M*-109. Furthermore, such information is important for the elucidation of the mechanism whereby cells are 'converted' and become malignant as a result of virus infection. It will be recalled in this connexion that a similar investigation on a hybrid between a cell line derived from a polyoma-induced tumour and the well-known *L* line, obtained by Gershon and Sachs, indicated that "the polyoma-induced cell antigen may exist in the hybrid cells"[9]. This conclusion was based on a slight inhibitory effect on the size of tumours induced by the hybrid cells in the polyoma-immunized mice.

Since there was no available information concerning the ability of the *Py*-198-1 or of the hybrid cells to produce tumours, it was necessary to determine their ability to do so in order to conduct the standard experiment for the demonstration of the polyoma-induced transplantation antigen(s)[8]. Suspensions of viable *Py*-198-1 and *M*-109 cells were inoculated into 3–4-month-old *C3H/He* and *A/J* mice (obtained from Bar Harbor, Maine) either intracerebrally at a dosage as high as 10^6 cells per mouse or subcutaneously at a dose as high as 5×10^6 per mouse. In addition, an equal number of animals that had received 450 r. on the day of inoculation were treated in a similar manner. The mice used in these experiments differed at several histocompatibility loci from the cells[7]; however, no strains co-isogenic to these cell lines were available. No tumours were seen during a period of six months in the 40 *C3H/He* mice or 31 *A/J* mice (either irradiated or non-irradiated) which had been inoculated with various doses of *M*-109 cells or in the 49 *A/J* mice inoculated with *Py*-198-1 cells; indeed, not even temporary growth occurred. It is not possible to decide, at this stage, whether the failure to produce tumours with the *Py*-198-1 and *M*-109 cells should be attributed to an intrinsic inability to form tumours or to an antigenic constitution which differed from the host's.

It was therefore necessary to utilize a different method to test for the possible polyoma-induced transplantation antigen(s), that is, immunization of mice with tissue culture preparations and challenge with isologous polyoma-induced tumours.

Cells from the *Py*-198-1 and *M*-109 were collected by scraping from the glass surface, washed several times in phosphate buffered saline solution, and inoculated into panels of *C3H/Bi* and *C57/BL* mice at a dose of $7 \cdot 5 \times 10^6$ cells per mouse. Two subcutaneous injections and one intraperitoneal injection were given one week apart. One week after the final immunization, the mice were subcutaneously inoculated with the isologous polyoma-tumour cell suspension free of virus. The origin and characteristics of these tumours, as well as the demonstration of polyoma-induced transplantation antigens in these tumours, have been reported elsewhere[10]. The animals were examined bi-weekly for three months and tumour growth recorded. At the end of the experiments, mice of the various groups were tested for the presence of polyoma virus hæmagglutination inhibiting activity in

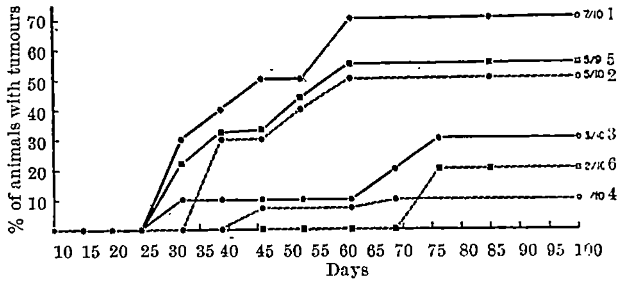

Fig. 1. Panels of *C57/BL* mice were immunized with *Py*-198-1 or *M*-109 cells twice subcutaneously and once intraperitoneally one week apart ($7 \cdot 5 \times 10^6$ cells per time/mouse) and were then challenged with $1 \cdot 5 \times 10^5$ or $0 \cdot 5 \times 10^5$ cells from an isologous polyoma-induced virus-free tumour. Curves 1 and 2, control; 3 and 4, immunized with *M*-109 cells; 5 and 6, immunized with *Py*-198-1 cells; 1, 3 and 5, $1 \cdot 5 \times 10^5$ cells; 2, 4 and 6, $0 \cdot 5 \times 10^5$ cells

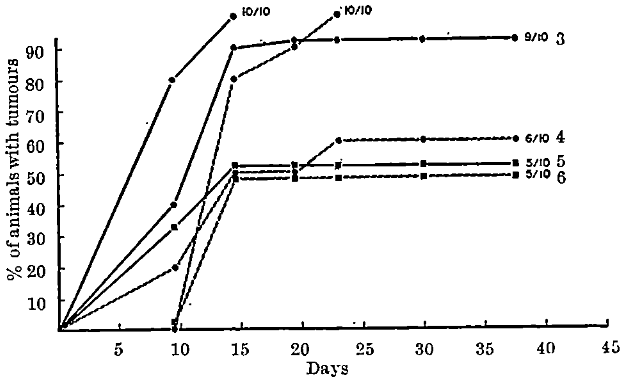

Fig. 2. Panels of *C3H/Bi* mice were immunized with *Py*-198-1 or *M*-109 cells twice subcutaneously and once intraperitoneally one week apart ($7 \cdot 5 \times 10^6$ cells per time/mouse) and were then challenged with 3×10^5 or 10^5 cells from an isologous polyoma-induced virus-free tumour. Curves 1 and 2, control; 3 and 4, immunized with *M*-109 cells; 5 and 6, immunized with *Py*-198-1 cells. 1, 3 and 5, 3×10^5 cells; 2, 4 and 6, 10^5 cells

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* Leukemia Society Scholar.

Table 1. EFFECT OF PARENTAL AND HYBRID CELLS IMMUNIZATION ON TRANSPLANTABILITY OF ISOLOGOUS POLYOMA TUMOURS

Mouse strain	Tumour cell dose	Control	*M*-109 immunized	*Py*-198-1 immunized*
C3H/Bi	3×10^5	10/10†	9/10	5/10
	10^5	10/10	6/10	5/10
C57/BL	$1{\cdot}5 \times 10^5$	7/10	3/10	5/9
	$0{\cdot}5 \times 10^5$	5/10	1/10	2/10

* Mice given subcutaneous and intraperitoneal injections of $7{\cdot}5 \times 10^6$ cell one week apart and then challenged with isologous polyoma tumours.

† Animal with tumours/animals inoculated.

Table 2. RESULTS OF COMPLEMENT-FIXATION TEST WITH PARENTAL AND HYBRID CELLS AGAINST SERUM FROM MICE BEARING POLYOMA TUMOUR*

	Antigen dilution						Anticomplementary activity of the antigen		
	0	2	4	8	16	32	0	2	4
N.C.T.C. 2555	0	0					0	0	0
L	0	0					0	0	0
Py-198-1	4	4	4	4	3	0	Tr.	0	0
M-109	4	4	4	0			Tr.	0	0
M-109 clone 3	4	4	4	0			Tr.	0	0

* These results were obtained with a 10 per cent cell homogenate in buffered saline solution tested in the presence of 8 C.F.U. of serum.

the sera. All the sera were negative; this confirms that polyoma virus was not present in the immunizing cells or in the challenging tumours. The results are given in Table 1.

In comparison with the non-treated mice, the *C3H/Bi* and *C57/BL* mice that had been pre-immunized with the *M*-109 and *Py*-198-1 cells showed resistance to transplants of the respective isologous tumour cells at least at the two doses of challenge cells used. Furthermore, the appearance of tumours in the immunized animals was considerably delayed in comparison to the controls, as illustrated in Figs. 1 and 2. In general, immunization with *Py*-198-1 cells seemed to be more effective than with an equal number of *M*-109 cells. The resistance phenomenon was also more clearly shown in the *C3H/Bi* mice than in the *C57/BL* mice. The degree of resistance demonstrated in these tests is comparable to that reported by Sjögren[11] when different mouse strains were immunized with homologous polyoma-induced tumours and then challenged with isologous polyoma tumours. It may then be concluded that the polyoma-induced transplantation antigen(s) was present in the hybrid cells as well as in the parental polyoma-transformed cells, possibly, though, only to a minor extent in the former.

Further characterization of these cells was done by utilizing the fact that sera obtained from hamsters and mice bearing large polyoma-induced virus-free tumours fix complement when tested with saline extracts of these tumours. The specificity of this reaction is assured by the fact that these sera do not react with the original inducing virus, nor with extracts from tumours induced with different viruses in the same species, although they will cross-react with the tumours induced by the same virus in different species. Furthermore, the activity of these sera is not removed after absorption with sheep red cells[12].

In these tests, sera from hamsters carrying a transplantable polyoma tumour and sera from *C57/BL* mice with transplantable polyoma tumours were used. Tissue-culture cells to be tested for the presence of antigen were suspended in 10 per cent volume in phosphate buffered solution, dispersed and broken by repeated vigorous pipetting, and the suspension tested against the sera of the animal carrying the tumour. A typical test conducted with mouse sera is illustrated in Table 2. Even when undiluted, extracts of the N.C.T.C. 2555 cells and of a line of *L* cells carried in our laboratory did not fix complement, while extracts of *Py*-198-1 cells fixed complement at a dilution of 1 : 16 and the hybrid *M*-109 and a clone, *M*-3, derived from it were positive at a dilution of 1 : 4.

Thus, the complement-fixation test for polyoma-induced antigen also indicates that the hybrid line presents some of the characteristics of one of the parental lines. Although no definite conclusions have as yet been reached, the polyoma tumour complement fixing antigen and the transplantation antigen are probably different since one can be demonstrated in the absence of the other[13].

The presence of the polyoma-induced antigen(s) in the hybrid cells does not provide an answer to the still unresolved question of whether the viral genome, or a part thereof, is continuously present in the converted cells[14]. However, the persistence of this (or these) antigen(s), established by the foregoing experiments, will have to be taken into account in any interpretation of polyoma oncogenesis.

This work was supported in part by U.S. Public Health research grant *CA*–04534 (National Cancer Institute), the American Cancer Society (*E89F*), and the U.S. National Science Foundation (*G*–23129).

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AN EFFECT OF INTERFERON ON UNINFECTED CHICK EMBRYO FIBROBLASTS

By DR. JOSEPH A. SONNABEND

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INTERFERON inhibits the synthesis of virus specific RNA in chick embryo fibroblasts (CEF) infected with Semliki Forest virus[1]. The following is a report on the inhibition of RNA synthesis in uninfected CEF by a partially purified interferon. Crude preparations were used in previous investigations[2,3] on the action of interferon on the uninfected cell, and this probably accounts for the variable results reported.

The interferon used in these experiments was kindly supplied by Dr. J. Beale of Glaxo Laboratories. It had been purified from allantoic fluid obtained from influenza virus infected eggs and contained 1 mg protein and 2,000–4,000* units interferon per ml. It contained only very small amounts of ribonuclease (400 mμg/ml. in the undiluted material)

The conditions of culture of the CEF were as described by Taylor[1]. RNA was extracted from cultures of CEF which had been exposed to tritiated uridine or tritiated adenosine following a period of pretreatment with interferon. Where incorporation into total acid precipitable RNA was examined, the extraction was by a modified Schmidt–Thannhauser procedure[4]. RNA was estimated

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* One unit of interferon is the amount which reduces the number of plaques by 50 per cent in a standard assay. The method used for purifying the interferon has recently been described (Fantes, K. H., O'Neill, C., and Mason, P. J., *Biochem. J.*, **91**, 20; 1964). However, the material used in these experiments had not passed through a DEAE-cellulose column.

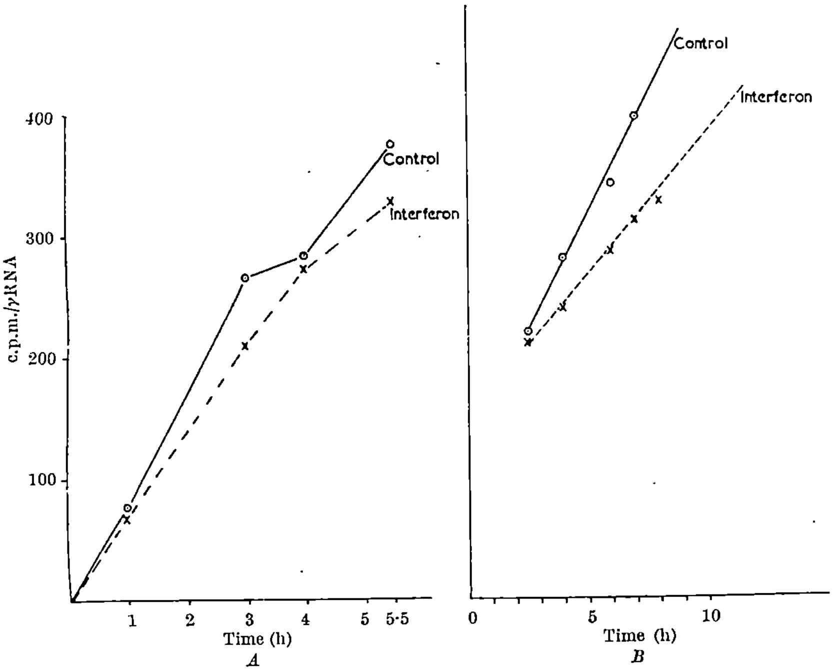

Fig. 1. Specific activity of RNA extracted from CEF treated with 10 units/ml. interferon for 24 h before addition of tritiated adenosine (*A*), and 50 units/ml. interferon for 5 h before addition of tritiated uridine (*B*)

on the alkali hydrolysate by the orcinol method[5]. In some experiments RNA was extracted with water-saturated phenol at room temperature. The interphase was re-extracted with phenol and sodium lauryl sulphate at 60° C when sucrose gradient analyses were undertaken; alternatively it was hydrolysed with alkali for determination of the specific activity of the RNA. Linear sucrose gradients (5–20 per cent sucrose) were run for 4½ h in the *SW*39 rotor of the Spinco model *L* ultracentrifuge and 12 drop fractions collected. Tritium was counted in a Tri-Carb scintillation counter.

Figs. 1*A* and *B* show an inhibition of incorporation of precursor into RNA following a period of pretreatment with interferon. In the experiment shown in Fig. 1*A*, 24-h-old CEF were treated with 10 units/ml. interferon for 18 h, 10 μc. tritiated adenosine was added to each plate and at the indicated times plates were removed and the specific activity of the total acid precipitable RNA determined. The effect of pretreatment for 5 h with 50 units/ml. interferon is shown in Fig. 1*B*. Following exposure to interferon, tritiated uridine (10 μc. per plate) was added to the cells, which were kept at 4° C overnight. After starting incubation at 37° C plates were removed at the indicated times. A further control showed that normal allantoic fluid had no effect on RNA synthesis.

There was little difference in the specific activities of the RNA extracted from interferon-treated and control cells following a 1-h labelling period. The largest differences in specific activity were observed after exposure of the cells to labelled precursor for 6 h. In a representative experiment, where cells were treated with 50 units/ml. interferon for 5 h, the specific activity of the RNA extracted from interferon-treated cells was 95 per cent of that in the controls after a 2·5-h labelling period, whereas after 8 h it had fallen to 70 per cent. An attempt was made to ascertain whether the synthesis of a specific molecular species of RNA was preferentially inhibited in the interferon-treated cells. Cells were treated with 100 units/ml. interferon for 18 h. Tritiated adenosine was added for 6 h and the RNA extracted with water-saturated phenol at room temperature. The interphase was re-extracted with phenol and sodium lauryl sulphate at 60° C and both RNA fractions analysed by sedimentation in a sucrose gradient. The sedimentation profile of both RNA fractions from the interferon-treated cells showed no marked difference from the controls. Fig. 2 shows the sedimentation pattern of the RNA extracted with phenol at room temperature from interferon-treated cells. It did not differ from the controls.

Levy, Snellbaker and Baron[3] suggested that a possible explanation for the delay they observed in the appearance of label in cold phenol-extractable RNA following interferon treatment was that there was an inhibition of transfer of RNA from nucleus to cytoplasm. The results of an experiment shown in Fig. 3 do not support such a mechanism. In this experiment CEF were treated with 50 units/ml. of interferon for 18 h before addition of tritiated adenosine. At the indicated times, samples were removed and extracted with water-saturated phenol at room temperature. Specific activities were estimated on the phenol-insoluble RNA at the interphase and on the RNA released into the aqueous phase. It can be seen that there is a reduction of incorporation of precursor into both the rapidly labelled and phenol-extractable fractions. Treatment with interferon resulted in a reduction of total counts as well as of specific activity.

The results showed an inhibition of RNA accumulation in interferon-treated cells; an effect on a particular species of RNA was not demonstrated. However, since RNA synthesis was examined only after some hours of interferon treatment, it is possible that the general inhibition observed was a consequence of an earlier specific

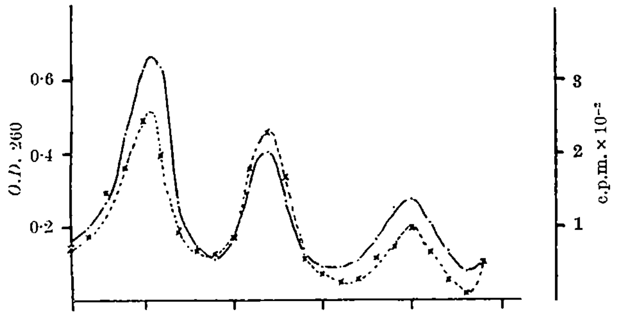

Fig. 2. Sucrose-gradient analysis of RNA extracted with phenol from CEF treated with 100 units/ml. interferon for 18 h. Tritiated adenosine was added for 6 h before extraction of the RNA. —·—, Optical density; ×, c.p.m.

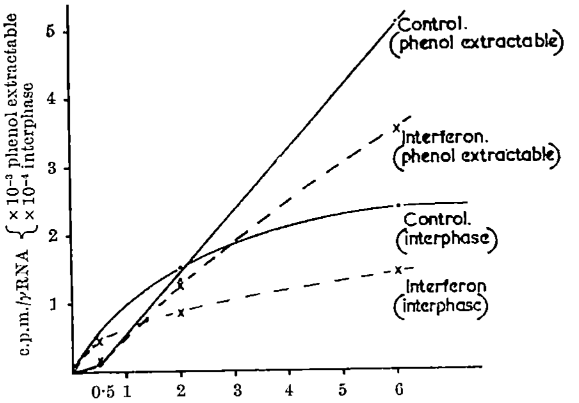

Fig. 3. Specific activities of phenol-insoluble and phenol-extractable RNA from CEF treated with 50 units/ml. interferon for 18 h

effect on a particular species of RNA. It is also possible that the effects observed in these experiments were due to impurities in the preparations; however, normal allantoic fluid did not inhibit RNA synthesis.

Taylor has shown that the antiviral action of interferon depends on continued host cell RNA synthesis and suggested that there may be a similar dependence on host cell protein synthesis[1]. That protein synthesis is required for interferon action has been demonstrated[6], and it was suggested that the antiviral action of interferon results from the induced synthesis by the host cell of a protein which inhibits viral RNA synthesis. The results presented here suggest that the activity of this protein may not specifically inhibit the synthesis of viral RNA; the accumulation of host cell RNA is also inhibited. A possible mechanism is that interferon induces the synthesis of an unstable enzyme which degrades newly synthesized RNA, host cell as well as viral. The induced synthesis by interferon of an unstable activity limiting the accumulation of RNA would account for the inhibition of both RNA and DNA viruses, as well as providing a basis for a self-limiting process allowing for a recovery of cell RNA synthesis. An explanation for the species specificity[7] of interferon action might be that it is only interferon of appropriate specificity that can induce the synthesis of the antiviral protein. Interferon production as well as interferon action is sensitive to actinomycin *D* (ref. 8). One implication of this observation is that those viruses which inhibit host cell RNA and protein synthesis will be poor interferon producers.

It should be possible to demonstrate whether the observed inhibition of RNA accumulation in interferon-treated cells results from an increased breakdown of newly synthesized RNA or from an inhibition of its synthesis.

A further point is that interferon action as well as interferon production provide possible examples of induced protein synthesis in mammalian cells and suggest a system for the investigation of these processes.

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EFFECTS OF HYPOXIA ON THE RABBIT DURING EXPOSURE TO COLD

By Dr. BRYAN BROOM*

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HYPOXIA is known to decrease the augmentation of oxygen consumption seen in warm-blooded animals on exposure to cold[1-3]. It has been suggested that hypoxia is an essential ingredient in the duet of hypercapnia and hypoxia that is found in the autonarcosis technique of profound and reversible hypothermia with prolonged cardiac arrest[4,5]. The role of hypoxia in the cooling process is of clinical importance, as the depression of oxygen uptake by anæsthesia is the basis of all cooling procedures, whether internal or external in type.

Despite the invariable increase in oxygen consumption seen in warm-blooded animals on exposure to cold[6], there is as great an increase seen on exposure to heat[7,8]. Although the initial uptake of oxygen on cold exposure is near maximal[2], there is no inhibition of cooling[9], and in exposure to acute cold there is a linear decrement of oxygen consumption which parallels decrease in body temperature[10], a relationship which is not altered in the hypothermia seen in the cold-adapted animal subjected to severe chilling[2]. In the adaptation to cold seen in prolonged exposure, there is a decline of oxygen consumption towards basal levels[11]. The phylogenetic recapitulation of thermo-regulation seen in the developing house wren[12] and suckling mouse[13] shows that the stabilization of adult temperature correlates more precisely with adult size and insulation than with uptake of oxygen.

If the chemical regulation of heat production is an inefficient process[14], and it is in the physical regulation of heat loss that one must assess the characteristics of the well-adjusted homeotherm[15], then an attempt quantitatively to differentiate these aspects is needed. Hypoxia provides a basis for the assessment of the relative importance of the two processes.

The following experiments were performed to determine the effects of hypoxia, with and without concomitant hypercapnia and/or anæsthesia, on the body temperature and survival time of the rabbit in a known and limited supply of oxygen, both at neutral (14°–18° C) and reduced (–18° to –24° C) ambient temperatures. With a finite oxygen store, the survival time of the rabbit will depend on the rate of oxygen utilization, as influenced by the ambient temperature, the inspiratory carbon dioxide concentration and the presence or absence of anæsthesia. This situation offers a simple and reliable method of assessing the effects of body temperature changes on oxygen consumption that is free of the errors otherwise avoidable only by elaborate instrumentation[3].

Ninety-six rabbits of various strains were used, 48 for each of the two ambient temperatures. The rabbits were clipped of their fur under pentobarbitone anæsthesia more than 48 h before the experiment, prior to which they were allowed free access to food, 'Diet *S.G.*1', and water. The rabbit was placed in a cylindrical 7-l. tin, 25 cm × 15 cm, having at one end a detachable lid, and at the other a 'Perspex' attachment, 12·5 cm × 12·5 cm × 12·5 cm. 100 g fresh silica-gel was placed in the 'Perspex' attachment before the experiment, and also, in those experiments in which carbon dioxide enrichment was not practised, 100 g fresh soda lime. After an initial period of 10 min in the tin, air, or 30–32 per cent oxygen (both with or without 10 per cent carbon dioxide), was passed through the tin at about 3 l./min for 2 min, after which the rubber tubes passing to and from the tin were rapidly occluded with spring- and gate-clips, the lid having previously been sealed with adhesive plastic tape. The choice of pentobarbitone and/or oxygen or carbon dioxide enrichment was made by prior random selection. The initial and final ambient and colonic temperatures were noted, as was the time to respiratory arrest. The absence of leaks was checked by subsequent immersion of the tin in water.

From the results shown in Tables 1 and 2, it can be seen that hypoxia markedly reduces the body temperature in the cold, but only moderately at neutral ambient temperature. However, the cooling rates in hypoxia are half those found with normoxia; the carbon dioxide-induced acceleration of hypothermia is abolished and the pentobarbitone-induced acceleration of hypothermia is nearly halved[16]. In contrast to normoxia, prolongation of

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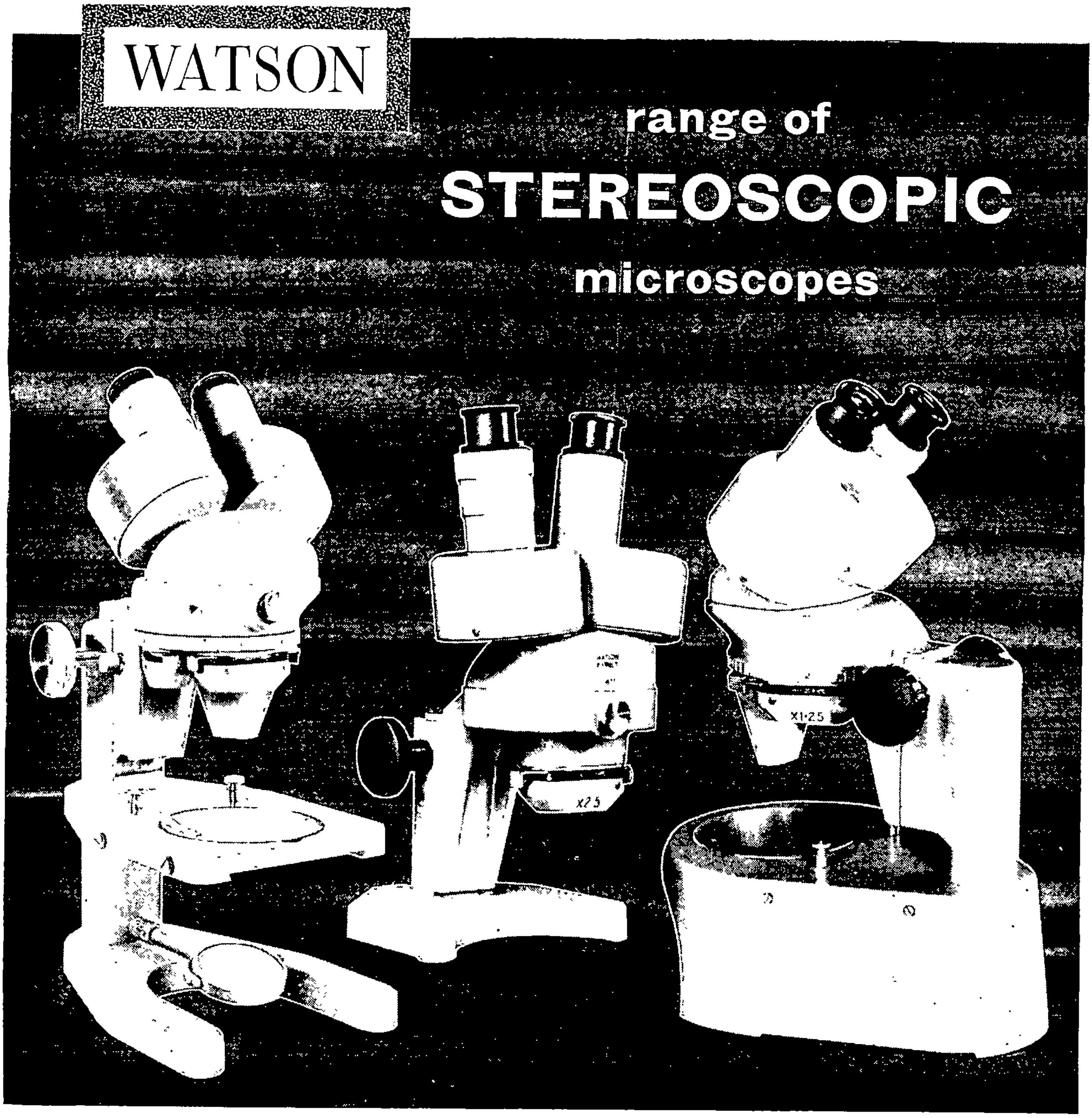

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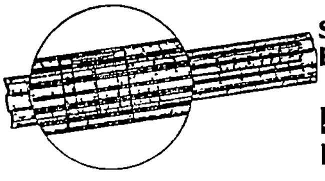

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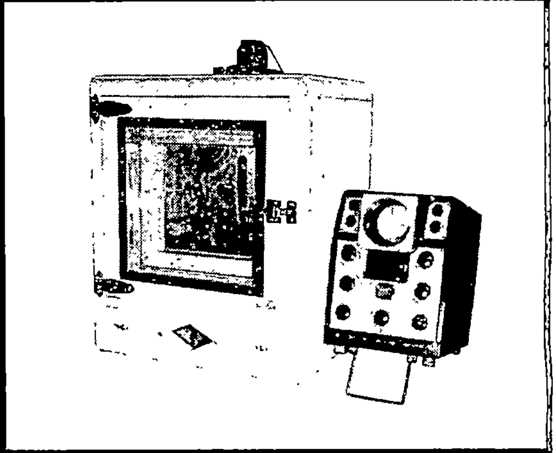

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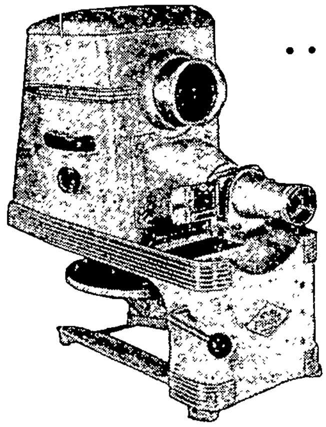

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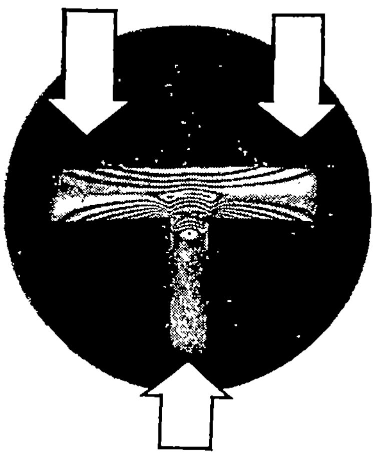

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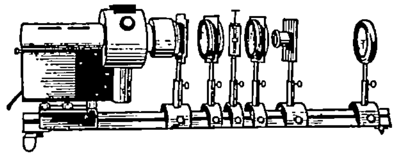

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Tables 1 and 2. SURVIVAL TIMES AND COOLING RATES IN HYPOXIA
(Corrected for body-weight)

Table 1. MEAN SURVIVAL TIMES (MIN)

Gas mixture/ anæsthesia	14°–18° C	Significance (analysis of variance, %)	−18° to −24° C	Significance (analysis of variance, %)
Pentobarbitone	94·8	0·1	148·8	0·1
No pentobarbitone	66·7		89·4	
Oxygen	90·6	0·1	164·6	0·1
Air	70·8		100·8	
Oxygen/CO$_2$	No difference		114·2	5·0
Air/CO$_2$			96·7	

Table 2. COOLING RATES (° C/MIN)

Pentobarbitone	0·041	0·1	0·108	0·1
No pentobarbitone	0·018		0·078	

Note. No correlation of cooling rate with carbon dioxide or oxygen.

survival time is replaced by a diminution of survival time by carbon dioxide inhalation as compared with pentobarbitone anæsthesia. Thus, despite the increase of oxygen uptake by carbon dioxide that is seen in hypoxia, this is insufficient to maximize cooling rates, and under the hypoxic stimulus only the diminution of oxygen consumption by pentobarbitone operates to increase cooling, a function that is curtailed in these circumstances. These facts substantiate the view that carbon dioxide operates by enhancing optimal oxygen uptake, with which both survival time and cooling rate may be maximal, in normoxia[16].

The results also demonstrate the protection conferred by prior anæsthesia[17], and hypothermia[18], against hypoxia. While carbon dioxide protects against hypoxia during the initial phases of exposure[19,20], it cannot be expected to continue this protection if persistent hypoxia is ultimately unrelieved[18–20].

That the combination of an increased oxygen uptake and increased cooling rate can occur is shown by earlier work in which thyroxin administration[1,9], and thyrotoxicosis[21], were found to increase cooling rate and heat loss, respectively, despite an increase of oxygen consumption to levels found in cold exposure. Similarly, the hyperthermic response by primary metabolic stimulation that is found on ergotoxine administration in an ambient temperature above 28° C is converted into a hypothermic response at an ambient temperature of 7° C, by adrenaline reversal, from peripheral vasodilation with consequent diminution of body temperature and hence of uptake of oxygen[22]. These phenomena show that circulatory factors may outweigh metabolic ones in the context of temperature regulation in the cold[23,24], and is compatible with the recent demonstration of simultaneous adaptation both to heat and cold in man[25].

During most of hypoxia, the vasomotor tone is high[26]. The fact that oxygen administration alone may initially inhibit temperature depression in the cold[27] and briefly, during hypoxia[26], can only be explained by an increase in peripheral vasomotor tone[26] and a resultant decrease in heat loss, as the inhalation of oxygen by unanæsthetized animals diminishes oxygen consumption[28]. Carbon dioxide is perhaps the only agent, apart from a condition of acidosis[29], that will simultaneously increase both oxygen uptake and blood flow[30].

Previous work on the mechanism of the hypothermic action of carbon dioxide during cold exposure[31 33] suggested it to be due to a diminution of oxygen consumption. Chapin and Edgar[33] used 32·5 per cent carbon dioxide, which is an anæsthetic concentration[34] and would thus be expected to decrease oxygen uptake. Repin[31] gave clear evidence of increased heat loss being responsible, yet equated this as being due to decreased heat production. Stupfel[32] found that 10 per cent carbon dioxide induced hypoxia and mimicked the oxygen uptake and the temperature depression seen with the inspiration of 11 per cent oxygen. Yet he found that the hypoxia associated, in his hands, with 20 per cent carbon dioxide could be relieved without abolishing the lowering of body temperature, and also that concentrations greater than 20 per cent did not further augment cooling but led to respiratory depression which killed the animal. Hence his maximal cooling rates were obtained with a concentration of carbon dioxide that was not associated with hypoxia. The results presented and discussed in this article resolve these discrepancies.

I thank Dr. Ian Sutherland for all statistical calculations, and Prof. E. Neil for his advice.

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EFFECT OF TOBACCO MOSAIC VIRUS PROTEIN ON TOBACCO MOSAIC VIRUS INFECTIVITY

By DR. V. HOLOUBEK*

Department of Virology, University of California, Berkeley

THE infectivity of isolated TMV–RNA is only about 0·1 per cent of that of the same amount of RNA contained in TMV protein. After the addition to the TMV–RNA of the TMV protein under certain conditions the TMV reconstitutes, and full infectivity of virus particles can be restored[1]. If the TMV protein is added to an inoculum of TMV, depending on its concentration it causes either enhancement or decrease of virus infectivity.

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Table 1. EFFECT OF ADDED PROTEINS ON THE INFECTIVITY OF TMV (*A*) AND TMV–RNA (*B*)

Protein tested	Concentration (μg/ml.)	No. of lesions (% of control)	No. of assays
	(*A*) Inoculation with TMV*		
TMV protein	10	133 ± 10·1	5
	20	141 ± 13·7	3
	50	70 ± 7·8	5
	100	45 ± 7·7	5
	500	25 ± 5·3	5
	1,000	0·7 ± 0·3	3
Ovalbumin	10	108 ± 12·3	4
	50	63 ± 7·7	4
	100	44 ± 6·7	4
	500	22 ± 6·0	4
TMV protein aggregated to rods	10	104 ± 9·8	3
	50	84 ± 11·2	3
	100	76 ± 9·8	3
	500	50 ± 5·4	3
TMV inactivation by ultra-violet radiation	10	99 ± 8·1	4
	50	89 ± 7·7	4
	100	80 ± 5·2	4
	500	45 ± 8·0	4
TMV reconstituted from ultra-violet inactivated RNA and native protein	10	75 ± 6·4	4
	50	36 ± 3·6	4
	100	20 ± 4·3	4
	500	9 ± 3·4	4
	(*B*) Inoculation with TMV–RNA		
TMV protein	10	217 ± 34·0	5
	50	239 ± 32·4	5
	100	245 ± 32·6	5

* The numbers represent means of results from different assays, each assay based on 12 half-leaves compared with 12 half-leaves of the control.

The results, in numbers of lesions per half-leaf obtained with various mixtures, are expressed in terms of per cent of the lesion number obtained in the control half-leaves.

The present experiments were designed to evaluate some possibilities for the mechanism of this effect of TMV protein on TMV infectivity.

In all experiments, the TMV was prepared by the standard method of differential centrifugation from leaves of *Nicotiana tabacum* var. 'Turkish' infected with the common strain of TMV. The TMV labelled with carbon-14 was supplied by courtesy of Dr. P. R. Whitfeld. The viral RNA and protein were prepared as described[2,3]. The concentrations of the virus, the virus protein and the RNA were determined spectrophotometrically[4]. Ultra-violet inactivation was performed with a Westinghouse germicidal 'Sterilamp' placed 50 cm from the sample to be irradiated. The lamp had an ultra-violet output of 5·2 W and 95 per cent of its radiation was at 2537 Å. The RNA was irradiated at a concentration of 1·2 mg/ml. in distilled water in a 1–2 mm magnetically stirred layer. Reconstitution was carried out according to the method of Fraenkel-Conrat and Singer[1]. For some experiments the TMV protein was re-aggregated into rods in 0·1 M phosphate buffer at *p*H 5·2. Ovalbumin was a preparation obtained from Armour Laboratories, Chicago, Illinois. The ultracentrifuge analyses were made in a Spinco model *E* ultracentrifuge, operating at room temperature. Sedimentation coefficients were corrected to a standard state corresponding to water at 20°. Electron microscopic pictures were obtained by J. Toby through the courtesy of Dr. R. C. Williams. For infectivity tests the mixtures of TMV or TMV–RNA with different concentrations of proteins or inactivated TMV in water solutions were mixed with 0·1 volume of 1 M phosphate buffer at *p*H 7·0 at 0° and applied to 12 half-leaves of *N. tabacum* var. 'Xanthi *nc*'. Control TMV or TMV–RNA solutions in the same concentration was applied to the opposite half-leaves. Inoculation was carried out as described[4].

Suppression of TMV infectivity by TMV protein and other proteins. If an inoculum of TMV at a concentration of 0·01 μg/ml. was mixed with TMV protein in a concentration higher than 50 μg/ml., the increasing concentration of the TMV protein inhibited the infectivity of TMV, and at a concentration of 1,000 μg/ml. the protein suppressed the infectivity to a level lower than 1 per cent of the original infectivity (Table 1). The suppression by ovalbumin, protein having isoelectric point similar to that of TMV protein, was very similar.

Santilli *et al.*[5] suggested that the inhibition of TMV infectivity with TMV protein and other proteins was due to a reduction in number of particles resulting from aggregation. However, ultracentrifugation analysis of a mixture of TMV with TMV protein showed no aggregation of TMV particles, but two distinctive peaks, one of TMV protein and the other with a sedimentation coefficient of S_{20}–210 or 205 in two experiments, were observed. The decline of TMV infectivity to 1 per cent would require the aggregation of one hundred particles to an infectious unit, an aggregation which surely would manifest itself in the sedimentation coefficient. Also when samples of TMV which had been incubated with 10, 50, 250 and 1,000 μg TMV protein/ml. for 1 h at 4° were examined under the electron microscope, no indication of aggregation of TMV particles was found. Likewise, it was not possible to observe visible aggregation between ovalbumin and TMV.

From the results of ultracentrifugal analysis of the mixture of TMV protein with TMV it is possible to assume that, at higher concentrations of proteins in mixed inocula with TMV, they became adsorbed on the surface of TMV to such an extent that they prevented attachment of the virus at the susceptible sites in the plant cell. If instead of TMV *A*-protein solution the TMV protein aggregated in rods was used, the suppression of TMV infectivity was similar but lower than with TMV *A*-protein (Table 1). Probably the protein rods were not able to adsorb to the surface of TMV so easily as protein dissociated to basic sub-units (the TMV *A*-protein).

Suppression of TMV infectivity by inactivated TMV. TMV inactivated by ultra-violet radiation caused as much suppression of the TMV infectivity as TMV protein aggregated in rods. In contrast the suppression of TMV infectivity by TMV reconstituted from ultra-violet inactivated TMV–RNA and non-irradiated native TMV protein is notably bigger (Table 1). This may be explained by the assumption of different mechanisms of these two inhibitions. It is probable that the virus with inactivated RNA but with native protein coat can compete with TMV virus for the same potentially infectible sites of virus attachment in a similar way as the systemic strain U_2 of TMV suppresses the formation of lesion by the strain U_1 (ref. 6). The inactivation of the virus as a whole by ultra-violet radiation might lead to such changes in its protein coat that the irradiated virus cannot compete with the infective virus for the sites of virus attachment. The TMV infectivity suppressed by higher concentrations of this virus was probably suppressed by the same mechanism which caused the suppression of TMV infectivity by TMV protein. In this relation, the observation of Wu and Rappaport[6] is of interest, that the inhibitory effect of U_2 was decreased by its irradiation before mixing with the non-irradiated U_1.

Enhancement of TMV and TMV–RNA infectivity by TMV protein. In contrast to the inhibition of the TMV infectivity by high concentrations of TMV protein, the same protein in a concentration around 10 μg/ml. stimulated the TMV infectivity (Table 1). Similar results have been obtained by Santilli *et al.*[5]. It was not known whether or not partial reconstitution could be accomplished when the protein is mixed with TMV at low temperature and the mixture was inoculated 30 min after mixing, conditions which do not permit TMV complete reconstitution from TMV–RNA and protein. Samples ranging from 1 to 6 mg of TMV were mixed with 600 μg of TMV protein labelled with carbon-14 (activity of 8,140 counts per mg per min) and incubated for different time-intervals of 1–24 h in water solution at the temperature of 0°. After this incubation the TMV was washed by repeated ultracentrifugation (Spinco 40,000 r.p.m. for 1 h) and resuspended in water. The results of carbon-14 activity measurements showed that after 4 cycles the protein which

Table 2. EFFECT OF RUBBING TOBACCO LEAVES WITH PROTEINS AT DIFFERENT TIME-INTERVALS BEFORE AND AFTER TMV AND TMV–RNA INOCULATION

Tested protein	Time-interval between rubbing and inoculation	Protein concentration used for rubbing (μg/ml.)	No. of lesions as percentage of those of the control	No. of assays
	(*A*) Inoculation with TMV			
TMV protein	40 min before inoculation	15	101 ± 11·3[*]	3
		50	154 ± 12·8	3
		150	140 ± 13·0	3
	20 min before inoculation	15	95 ± 7·2	3
		50	163 ± 16·5	3
		150	143 ± 15·5	3
	20 min after inoculation	15	96 ± 6·0	3
		50	107 ± 8·1	3
		150	94 ± 7·4	3
	(*B*) Inoculation with TMV–RNA			
	40 min before inoculation	15	117 ± 12·8	3
		50	228 ± 26·6	3
		150	256 ± 20·6	3
	20 min before inoculation	15	103 ± 9·4	3
		50	278 ± 29·8	3
		150	254 ± 34·8	3
	20 min after inoculation	15	101 ± 9·9	3
		50	96 ± 7·3	3
		150	105 ± 11·6	3

[*] The numbers represent means of results from different assays, each assay based on 12 half-leaves compared with 12 half-leaves of the control.

The results are expressed in the number of lesions on half-leaves pre-treated at various time-intervals by rubbing with different protein concentrations, as percentage of number of lesions on half-leaves pretreated only by rubbing with water.

was at the beginning adsorbed to the viral particles was washed out to less than 0·1 per cent of the originally added amount. This low residual amount of carbon-14 activity was not dependent on the time of incubation. Such washed TMV had the same infectivity as the original TMV before addition of protein. For the rise as well as for the suppression of TMV infectivity the protein must be present in the inoculum at the time of infection. From these results it is possible to exclude reconstitution as an explanation of the enhanced effect of low concentrations of TMV protein on the TMV infectivity.

If the TMV–RNA at a concentration of 0·5 μg/ml. was mixed with TMV protein, the rise in the infectivity of this TMV–RNA was more than 100 per cent. In contrast to TMV, the TMV–RNA infectivity was stimulated also with concentrations of TMV protein which suppressed the infectivity of the virus (Table 1). The addition of ovalbumin was without effect on the TMV–RNA infectivity. The stimulation of the TMV–RNA infectivity was not due to reconstitution of TMV from the RNA and the protein added just prior to inoculation. Under such conditions (0° temperature and 0·1 M phosphate solution) no sedimentable particles were formed.

Changes in TMV and TMV–RNA infectivity after rubbing the tobacco leaves with TMV protein. If the TMV protein in water solution was rubbed on the tobacco leaves at different time-intervals up to 40 min before the inoculation with TMV or TMV–RNA, the number of lesions on these pre-treated half-leaves was, in the case of TMV–RNA, about 250 per cent, and in the case of TMV, about 150 per cent higher than on control half-leaves which were rubbed at the same time-intervals before inoculation only with water (Table 2). Rubbing the leaves with TMV protein earlier than 40 min before inoculation or 20 min after inoculation was without any influence on the number of lesions caused by TMV or TMV–RNA. The number of lesions was also not significantly different from the number of lesions on control half-leaves if a pre-inoculation with ovalbumin was used at any time-interval.

The increase of TMV and TMV–RNA infectivity caused by TMV protein rubbed on the leaves before the TMV or TMV–RNA inoculations makes it probable that the infectivity enhancement by TMV protein is at least in part result of TMV protein-induced changes in the tobacco leaves. In this connexion, the findings of Cochran *et al.*[7] are of interest in that they found TMV protein induces in tobacco leaf cells some specific changes which reflect themselves in the shift of the infra-red transmission spectra of the cell.

The enhancement of TMV infectivity by low, and the suppression of this infectivity by high, TMV protein concentration was caused by two different mechanisms. The suppression is probably the result of non-specific reversible adsorption of a part of the added protein to the surface of virus particles which inhibits the ability of these particles to attach at the infectible sites in the leaf cells. Different proteins from TMV protein also suppressed the infectivity of TMV. In this connexion it appears significant that TMV protein at the same concentration suppresses virus infectivity, but enhances TMV–RNA infectivity. The enhancement of the infectivity of both TMV and TMV–RNA by TMV protein is probably the result of an influence of this protein on the tobacco leaf cells, which resulted in creation of more favourable conditions for the initiation of infectivity. This enhancement is characteristic for TMV protein only and is not caused by other proteins.

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I thank Dr. H. Fraenkel-Conrat and Dr. P. Whitfeld for their advice.

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A NEW CLASSIFICATION OF SEED PROTEINS: APPLICATION TO THE ALEURINS OF *Arachis hypogaea*

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THE early biochemists used the techniques of maceration and extraction to isolate the proteins of an organ. These proteins were catalogued according to their origin, and from these first investigations evolved more meaningful classifications based on the biological functions of the proteins or the structure with which they were associated. There is little to be gained, for example, in discussing liver or kidney or brain proteins. as compared to enzymes or specialized proteins of a tissue or organelle. There is, however, at least one outstanding example of a classical catalogue of proteins of an entire organ which has persisted virtually unchanged from the

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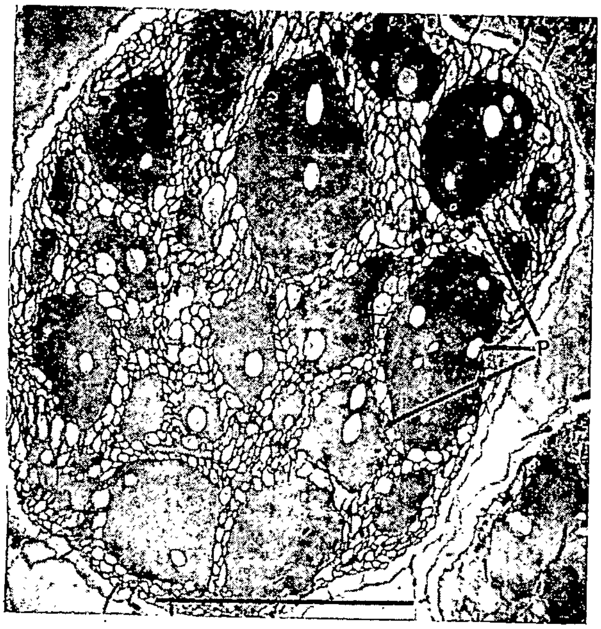

Fig. 1. Section of parenchyma cell of cotyledon of *Gossypium hirsutum* showing protein bodies (*P*). Bar, 10 mμ. Tissue fixed in $LiMnO_4$ and post-stained with lead citrate. (Courtesy of Dr. L. Y. Yatsu)

very beginning: the classification of seed proteins. A major portion of the mature seed is comprised of storage materials located either in the cotyledon or the endosperm. Most of the proteins of the seeds have been considered as storage proteins and have been classified on the basis of their solubility properties. Moreover, the major proteins are separated rather easily either by precipitation with acid or salts, by changing temperatures, or by crystallization[1,2].

It is now known that many of these proteins originally considered single entities are actually mixtures. Numerous examples are available of separation of these so-called pure species into several protein fractions by various techniques, such as chromatography or electrophoresis[3-8].

Moreover, the concept of solubility need not necessarily be fundamental to the protein, since associated materials or methods of obtaining the protein, or even the condition of the seed prior to extraction, have a profound influence on the solubility, as was shown, for example, by Stockwell *et al.*[9] for the solubility of edestin from hemp seed.

Obviously, as the reproductive structure of the plant, the seed has many proteins. It contains the biological machinery necessary for initiation of germination: mitochondria, plastids, and ribosomes, and their associated enzymes. A classification by an artificial criterion such as solubility does not provide a basis for selecting from the total proteins of the seed those which have their analogues in other tissues, plant or animal, and those which are unique to seeds.

A better approach to classification of seed proteins would be to use an element of uniqueness in seeds as the basis for categorization. It is now well established that much of the protein of seeds exists in so-called aleurone grains or protein bodies[3,10]. This is by no means a new observation. Protein bodies were described in the nineteenth century by Hartig[11] and Pfeffer[12], and were called aleurone grains by Hartig. These bodies vary from 2 to 20μ in diameter and, in general, vary in population density in the storage tissue in relation to its protein content. They were identified as containing protein by cytological staining. Their morphology is described better by the application of electron microscopy, and such techniques have been used to describe the following seeds: *Arachis hypogaea*[13], *Gossypium hirsutum*[3], *Glycine max*[14], *Pisum sativum*[15] and *Triticum aestivum*[16]. Fig. 1 is a photograph of typical protein bodies in a section of a cotyledon of a mature cotton seed (*Gossypium hirsutum*).

That these bodies do indeed contain protein was shown by Dieckert *et al.*[17], who isolated two types of protein-rich bodies from *Arachis hypogaea* seeds by a non-aqueous fractionation technique and showed that they contain 11 and 13 per cent nitrogen, respectively. They also showed that phytic acid is associated with the fraction containing 11 per cent nitrogen. By such a technique, however, they were unable to fractionate the proteins themselves; the DEAE–cellulose chromatograms of the protein of the particles as well as those of the total soluble proteins of the seed are similar. Varner and Schidlovsky[15] prepared a pellet from the cotyledons of *Pisum sativum* and showed by DEAE–cellulose chromatography that it contained the major fraction of the proteins of that seed. Morton and his colleagues showed that protein bodies isolated from the endosperm of immature *Triticum aestivum* contain the acid-soluble proteins of the wheat kernel[18].

We propose that those proteins contained within protein bodies or aleurone grains of seeds be named 'aleurins'. We chose this word because aleurone grains have been the classical name for the protein bodies in seeds. At this point, we are considering 'protein bodies' and 'aleurone grains' as synonymous terms. We conceive that each seed will have its aleurins; these will be identifiable and separable from the rest of the proteins. There is the possibility and likelihood that some aleurins of various seeds will have similar structure and function.

It may be that introduction of this classification will at the beginning change little of the present classification since many of the present techniques may have succeeded by chemical means in isolating mostly those proteins existing in protein bodies. But the isolation of proteins from an entire tissue suffers from the possibility that artefacts may have been produced by the admixture of the proteins with each other and with other tissue components; this possibility is minimized when the proteins are isolated from sub-cellular particles instead. There is also the possibility that several types of sub-cellular particles will occlude and be isolated together. As the separation procedures are refined, we may expect that some proteins originally classified as aleurins will fit better into other categories.

This approach to classification can be illustrated by considering the soluble proteins of the cotyledon of *Arachis hypogaea*.

The total soluble proteins of the peanut cotyledon have been categorized by Dechary *et al.*[19] into four groups based on their behaviour on chromatography on DEAE cellulose. An elution diagram of the total proteins is given in Fig. 2; in the same figure are shown the elution diagrams

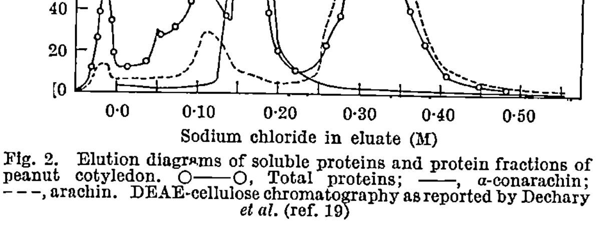

Fig. 2. Elution diagrams of soluble proteins and protein fractions of peanut cotyledon. ○——○, Total proteins; ——, α-conarachin; - - -, arachin. DEAE-cellulose chromatography as reported by Dechary *et al.* (ref. 19)

of classical arachin[1,2], and of α-conarachin isolated by Dechary *et al.*[19], by preparative DEAE chromatography. Altschul *et al.*[20] showed that approximately 75 per cent of the total proteins of this seed were not solubilized in neutral buffer containing 'Carbowax'* and that some of them were not solubilized in the presence of 0·25 M sucrose. This procedure provided the basis for the isolation of proteins from particles and their comparison to the total proteins and to the fractions shown in Fig. 2.

The protein bodies of the peanut cotyledon are fragile and particularly sensitive to the ionic strength of the buffer. The first attempt to isolate these particles involved maceration in 0·05 M phosphate buffer, *p*H 7, 0·25 M in sucrose. After removal of the debris and starch grains at 1,000*g* centrifugation, the supernatant was refrigerated overnight; the protein bodies which sedimented at 100*g* contained only 3·5 per cent nitrogen. In a second attempt, an unbuffered 0·25 M sucrose solution was used. After removal of starch grains and debris at 100*g*, the protein body fraction was isolated by centrifuging at 10,000*g* for 10 min; after further purification a sample containing 10·7 per cent nitrogen was obtained. The proteins of the particles were dissolved in buffer at *p*H 8. The elution pattern of DEAE chromatography typical of the soluble particle proteins is shown in Fig. 3; note the resemblance between this pattern and the arachin fraction of Fig. 2.

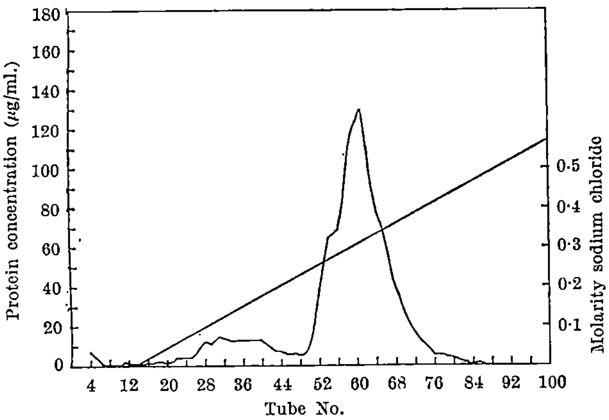

Fig. 3. Elution pattern of particulate fraction stable in 0·25 M sucrose. Chromatography on DEAE-cellulose as described for Fig. 2. The straight line is the sodium chloride gradient as measured in the eluate

In the major peak of the chromatogram of the particulate fraction (Fig. 3) there is evidence of a shoulder eluted at about 0·26–0·29 M sodium chloride. Therefore, it was of interest to determine whether density gradient centrifugation would separate the pellet into several fractions. Density gradient centrifugation did indeed enrich the material eluted at 0·26–0·29 M sodium chloride: the heavier material gave a pattern similar to that shown in Fig. 3; the lighter material showed almost an equal amount of the material eluted at 0·26–0·29 M sodium chloride and at 0·32–0·34 M sodium chloride, respectively. It was found, however, that better separation was obtained by taking advantage of the relative stability of portions of these particulates to slightly higher *p*H. In this manner two fractions were obtained: a heavy fraction and a fraction stable at *p*H 6 which contained primarily the proteins with the elution peak at 0·26–0·29 M sodium chloride. The elution patterns for these two fractions are shown in Fig. 4; the *p*H 6 fraction is predominantly the shoulder seen in the other fraction.

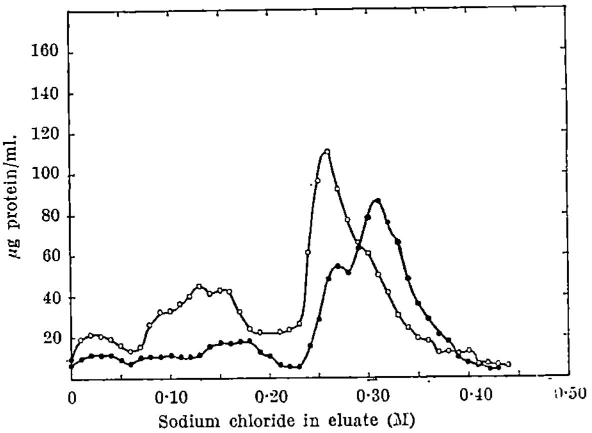

Fig. 4. Fractionation of particulate proteins of peanut cotyledon. Chromatography on DEAE-cellulose as described for Fig. 2. ●, Denotes the heavy fraction which was obtained as follows: cotyledons were macerated in 0·05 M citrate buffer, *p*H 5, 0·25 M in sucrose and 0·005 M in sodium ethylenediamine tetraacetic acid (EDTA). (The inclusion of EDTA increased the yield and reduced the clumping of particles.) The debris, nuclei, and starch grains were removed by centrifugation at 500*g* for 30 sec. The pellet obtained by centrifuging the supernatant at 1,000*g* for 10 min was resuspended in the same solvent, re-homogenized, and brought down at 500*g* for 10 min. This pellet was layered over 1·8 M sucrose in the same buffer and centrifuged at 125*g* for 20 min. the material at the interface was removed and collected by centrifugation at 11,500*g*. ○, Denotes the *p*H 6-stable fraction prepared as follows: The cotyledons were macerated as described above. After removal of debris and heavy particles, the supernatant was collected at 5,000*g* and resuspended in 0·25 M sucrose containing 0·005 M EDTA adjusted to *p*H 6 with NaOH. This was stored for 18 h at 4° C, collected at 5,000*g* for 20 min and washed several times

After dialysis against distilled water, the fractions were dried and analysed for protein, phosphorus, and lipid content; fresh samples were also tested for phosphatase activity with ATP as a substrate. The results are given in Table 1. Both fractions have a high protein content and some phosphorus; the *p*H 6 fraction contains more lipid and has more phosphatase activity. It will be recalled that Yatsu and Altschul[21] recovered particles from the cottonseed cotyledon which contained 25–29 per cent lipids.

The sedimentation patterns of both fractions in phosphate buffer (*p*H 7·9, μ = 0·03) are given in Fig. 5. The major peak has a sedimentation coefficient, S_0, of 9,

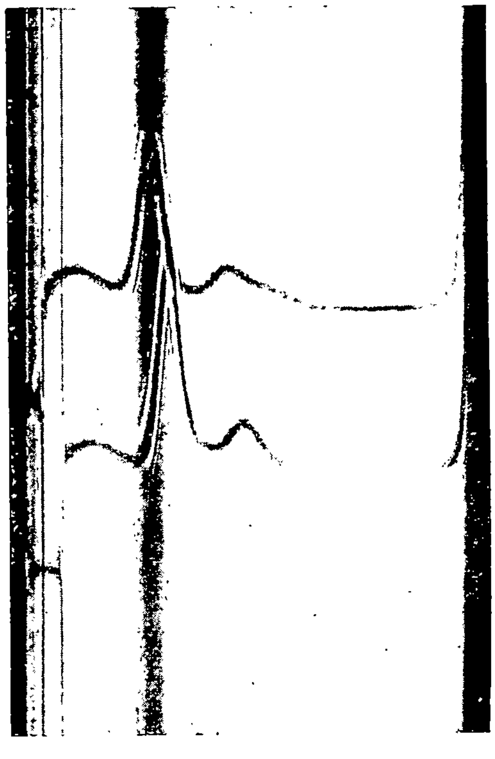

Fig. 5. Sedimentation patterns of *p*H 6 stable particle (upper pattern) and heavy particle in 0·03 M phosphate buffer, *p*H 7·9, 24 min after top speed at 59,780 r.p.m. (Analyses performed by W. B. Carney)

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* Mention of trade names does not imply recommendation of the particular product over others which may be equally suitable.

Table 1. COMPOSITION OF TWO PARTICULATE FRACTIONS FROM COTYLEDONS OF *Arachis hypogaea*

Fraction	Protein %	Total phosphorous %	Lipid %	ATPase
Heavy particle	56	0·35	8	0·17
*p*H 6 particle	55	0·40	45	0·45

Protein was determined by the method of Lowry *et al*, (ref. 24), total phosphorus by acid digestion followed by assay for inorganic phosphate by the Technicon modification of the Fiske–Subbarow method (*Technicon Manual*, 1958). The ethyl ether–extractable material is reported as lipid; ATPase analyses were by Dr. H. D. Brown; enzyme activity is reported as mg inorganic phosphate produced in 2 h per mg protein (ref. 25).

which is identical with that given by Johnson and Shooter[22] for the monomer of arachin. It is interesting to note that Johnson and Shooter, who used high salt concentrations to isolate arachin, obtained predominantly the dimer; this particular method of isolation produces predominantly the monomer. Both samples were also examined by gel-electrophoresis and gave essentially the same patterns which showed three major components. Here is an instance where two fractions differ in their behaviour by DEAE column chromatography but are similar both by ultracentrifugation and gel electrophoresis. Moreover, the particles differ considerably both in enzyme activity and in lipid content. We may be dealing here with allomorphic forms of the major components, or perhaps proteins which differ slightly in amide content. This matter is being investigated further; the morphological significance of the fractionation is also being investigated.

The aleurins of *Arachis hypogaea* comprise primarily those proteins of group IV according to Dechary *et al.*[19]. Apparently α-conarachin is not contained in these particles and, therefore, on the basis of this evidence is not an aleurin. It is interesting to note that with the same system of DEAE chromatographic analysis, the aleurins of the pea, *Pisum sativum*, have the same chromatographic properties as the aleurins of the peanut[15]. The only other instance known of particles from protein bodies having been isolated and characterized are those in the immature wheat endosperm[18]: the aleurins of wheat would be the acid-soluble proteins of the endosperm.

There is phosphatase activity in the particles and no doubt other enzyme activities are either a part of the particles or are occluded to them during the process of isolation. We known that the particles undergo well-defined alterations on germination[13]. What proportion of these are induced by enzymes existing within the particles, by some produced *de novo* within the particles, or by exogenous enzymes, is not known. We also know from the work of Morton and Raison[23] that the particles contain systems for deriving energy from phytates and for synthesizing the particle proteins themselves. Therefore, there is every reason to believe that the particles as they exist in the resting seed are not entirely inert. By investigating further the properties of the particles it may turn out that biological activities will be found for many of the proteins contained therein.

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EFFECTS OF GROWTH-REGULATING SUBSTANCES ON INULIN-STORING PLANT TISSUES

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REINDERS[1,2] was the first to establish that the uptake of water by plant storage tissues was promoted by the application of growth substances such as indolyl-3-acetic acid. This and related effects have since been investigated by various workers, attention having been directed both to changes in cell walls[3–6] and cell constituents[7,8].

The effects of growth-regulating substances on water uptake[9–11], rate of respiration[12,13] and protein synthesis[14] have been found to be greater for disks of Jerusalem artichoke than for other storage tissues. Jerusalem artichoke was one of the few plants examined which stores inulin. We have confirmed the marked effect of growth substances in promoting water uptake[2] and increasing the rate of respiration[12,13] of this tissue and have found even greater responses to occur with tissues from other inulin-storing plants such as chicory. The marked effect of 2 : 4-dichlorophenoxyacetic acid (2 : 4-D) in promoting water uptake in disks of Jerusalem artichoke and chicory is shown in Table 1 and chicory in Fig. 1. We have examined a wide range of compounds in this way, including aryl- and aryloxyalkane-carboxylic acids, and the chemical structure in relation to this type of biological activity has been investigated. With phenoxyacetic acids the effects of nuclear and side-chain substitution on activity were similar to those found previously using other

Table 1. EFFECT OF 10^{-5} M 2 : 4-D ON THE WATER UPTAKE AT 25° C OF TISSUE SLICES OF ARTICHOKE AND CHICORY WITH RESPECT TO TIME

Water uptake as a percentage of initial weight

Time (days)	Artichoke 2 : 4-D	Artichoke H_2O	Chicory 2 : 4-D	Chicory H_2O
0·7	—	—	13	11
1·0	26	—	—	—
1·9	—	—	82	15
2·0	38	6	—	—
2·8	—	—	188	21
3·0	72	9	—	—
3·8	—	—	172	18
4·0	81	6	—	—
6·0	74	7	—	—

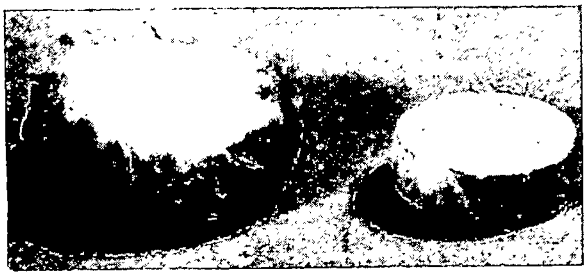

Fig. 1. Chicory root disks of the same initial weight after incubation for three days. Left, treated with 10^{-5} molar 2 : 4-D; right, treated with water

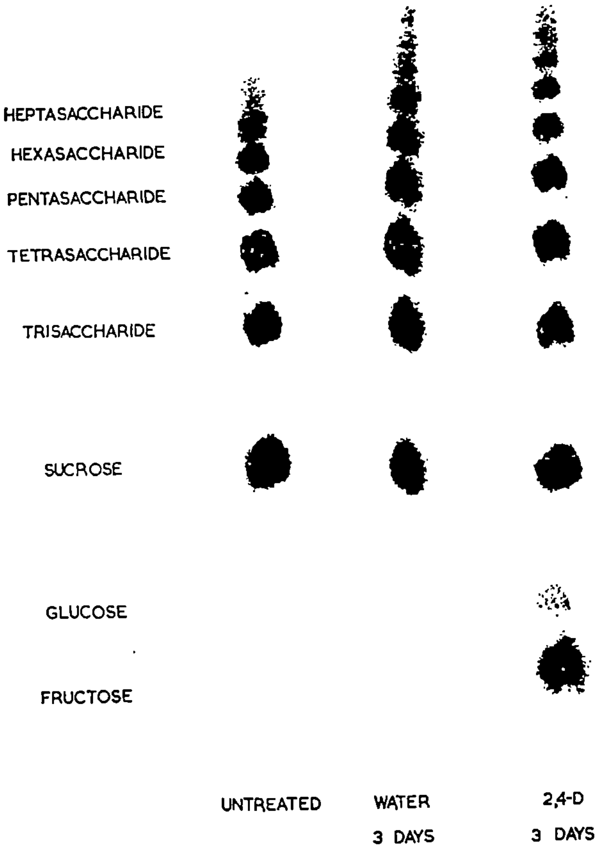

Fig. 2. The effect of 10^{-5} molar 2 : 4-D on the breakdown of fructosans in Jerusalem artichoke

tests for plant growth-regulating activity[15]; thus, for example, 2 : 4-D is highly active, whereas 3 : 5-dichlorophenoxyacetic acid (3 : 5-D) is inactive. These aspects of our work will be published elsewhere.

As early as 1935 Czaja[16] suggested that auxins promoted extension growth by inducing increased osmotic pressure of the cell sap. Later osmotic pressure determinations[8] on the liquid expressed from disks of potato tubers indicated that auxin treatment does not appreciably change the number of osmotically active molecules present, but the increased rate of respiration induced by treatment indicated that changes in carbohydrate had occurred. Although there is evidence[17] that auxins do not appreciably affect amylase activity, it is possible that they may influence the enzymes present in inulin-storing tissues[18]. We have examined this possibility by following the changes in carbohydrate occurring within the storage tissues of chicory and artichoke.

Continuous extraction of the tissue slices under reflux with 80 per cent ethanol was used to separate the carbohydrates into soluble (less than about 10 hexose units) and insoluble fractions. The soluble carbohydrates were further fractionated into reducing and non-reducing sugars, the former having been shown to consist solely of glucose and fructose[19]. These reducing sugars were determined directly by the method of Somogyi[20] and the non-reducing and insoluble fractions were estimated by the same method after acid hydrolysis. The individual soluble sugars were separated by paper partition chromatography, eluted and determined in a similar manner. Detailed results will be published elsewhere.

The proportion of carbohydrate in the dry matter was found to decrease during incubation and this loss was much greater in the 2 : 4-D-treated tissue. For artichoke, treatment with water or the inactive growth substance 3 : 5-D (10^{-5} M) for six days lowered the total carbohydrate content by some 25 per cent: 2 : 4-D at 10^{-5} M, on the other hand, caused a corresponding decrease of about 70 per cent.

Such differences are explicable in terms of the increase of the rate of respiration which we observed to be about three times greater than the control in artichoke disks treated with 2 : 4-D.

The amount of insoluble carbohydrate present in the artichoke tissue, comprising about 4 per cent of the dry matter, was not significantly affected by treatment with growth substance or water, and the overall decrease in total carbohydrate arising from the increased rate of respiration is accounted for by a decrease in the amount of certain soluble sugars present. Chromatographic examination of changes in individual sugars showed that the 2 : 4-D treatment caused a progressive decrease of the amount of

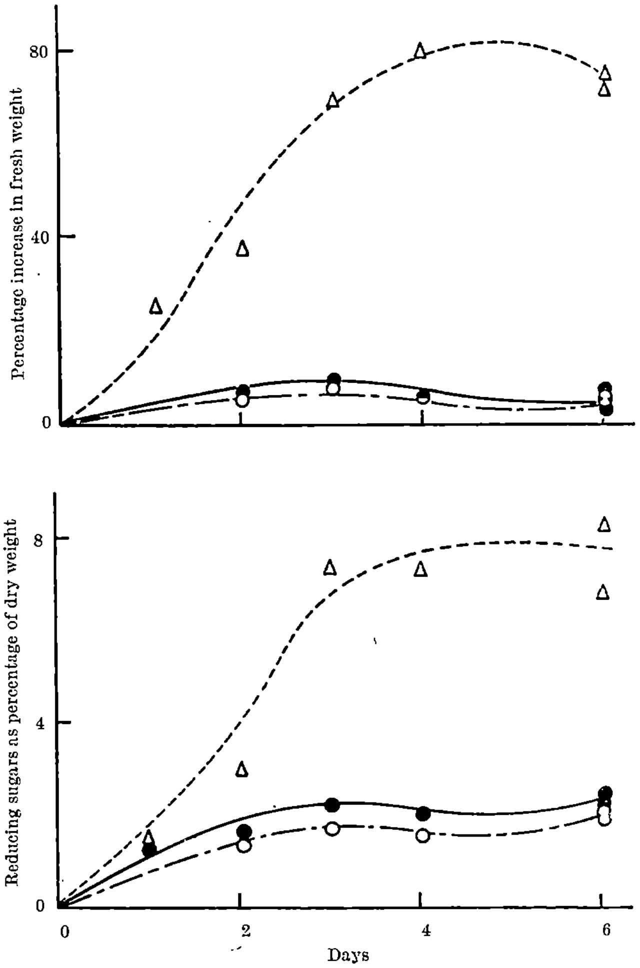

Fig. 3. A comparison of the water uptake and production of reducing sugars induced in Jerusalem artichoke tuber slices by water and 10^{-5} M solutions of 2 : 4-D and 3 : 5-D. ●, Water; ○, 3 : 5-D; Δ, 2 : 4-D

Table 2. TOTAL MOLAR CONCENTRATION OF SUGARS IN ARTICHOKE DISKS TREATED WITH WATER AND 10^{-5} M SOLUTIONS OF 2 : 4-D AND 3 : 5-D AT 25° C

Untreated: 0·252

Time (days)	Treatment Water	2 : 4-D	3 : 5-D
1		0·153	
2	0·186	0·079	0·177
3		0·098	0·183
4	0·160	0·074	0·157
6	0·188	0·086	0·179

tri- and higher soluble polysaccharides; sucrose after an initial fall remained constant, while there was a considerable increase in glucose and fructose. In artichoke these reducing sugars increased from about 1 to 8 per cent. In untreated tissue and that treated by the inactive growth substance, 3 : 5-D, there was a slight fall in the levels of all sugars. The effect of 2 : 4-D on the breakdown of fructosans present in Jerusalem artichoke is shown in Fig. 2.

Within the first 48 h of cutting, the molar concentration of soluble sugars in tissue slices cut from Jerusalem artichoke decreased regardless of whether they had been treated with 2 : 4-D, water or 3 : 5-D. However, this decrease was much greater for 2 : 4-D-treated tissue (Table 2). Thereafter the total molar concentration of soluble sugars remained constant in spite of considerable changes in individual sugars, respiration and water uptake. For 2 : 4-D-treated tissue this was achieved by the breakdown of oligosaccharides, resulting in an increase of glucose and fructose (Fig. 3).

The results here reported clearly indicate an important effect of 2 : 4-D in promoting the hydrolysis of fructose oligosaccharides present in certain storage tissues and are consistent with the view that during most of the period in which water is taken into the tissue sugars are involved in some kind of osmotic regulation. Attempts are now being made to isolate the system which controls the hydrolysis of fructose oligosaccharides in this tissue so that the influence of growth substances on its activity can be fully investigated.

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PHYSIOLOGICAL AND GENETIC EFFECTS OF 8-AZAGUANINE ON *Aspergillus nidulans*

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PURINE and pyrimidine base analogues have been reported as having a variety of physiological effects in angiosperms[1,7], algae[2], ferns[3], fungi[4,8–10,13], bacteria[5,14] and viruses[6]. The majority of investigations have been concerned with the effect of base analogues on growth and the biosynthesis of nucleic acids and proteins, and comparatively little information is available on heritable changes induced by these antimetabolites. Action of the analogue may involve the synthesis of analogue-containing nucleic acids or fraudulent nucleotide derivatives. Consideration of the following areas of metabolism would appear profitable in this context: (1) inhibition of nucleotide synthesis creating a base deficiency; (2) incorporation of analogue nucleotides producing fraudulent nucleic acids; (3) imbalanced nucleic acid synthesis due to stimulation, or inhibition of certain enzymes, for example, xanthine oxidase inhibition causing a decrease in normal purine catabolism; (4) impaired protein synthesis due to, say, availability of guanosine di- or tri-phosphates. The present programme was initiated with the aim of determining the nature of 8-azaguanine activity and correlating this with any induced genetic effects.

Two stable, haploid, homocaryotic strains (13 and 13*y*) of an ascosporic *Aspergillus nidulans* stock, producing green and yellow conidia respectively, were treated with 8-azaguanine (8-AG) over the concentration range 10^{-8}–10^{-2} molar in liquid Czapek minimal medium. The *p*H of the medium was not adjusted. The analogue was dissolved in a minimal volume of sodium carbonate (0·5 per cent w/v) before addition to the medium. Using standard spore inocula, cultures were set up in 30 ml. aliquots of media contained in 100 ml. Erlenmeyer flasks and incubated at 25° ± 0·5° C on a reciprocal shaking machine. Growth was measured by collecting the mycelia on tarred sintered glass filters, oven drying at 100° C for 24 h, then re-weighing. Measurements were made in triplicate, and growth expressed as mg dry weight of mycelium per flask. Unsupplemented control cultures were run with each experiment.

Both strains had similar growth characteristics in minimal medium: 13*y* showed a slightly higher logarithmic growth rate (2·15 mg dry wt./h) and shorter lag phase (60 h) than 13 (1·95 mg dry wt./h and 61·5 h respectively). After 22 days' incubation each strain had produced approximately 350 mg dry wt. of mycelium per flask; afterwards autolysis commenced. Similarly both strains showed a common response to 8-AG and results from a typical experiment are given in Table 1.

High concentrations of 8-AG (10^{-2} and 10^{-4} M) inhibited growth, whereas lower molarities had a stimulatory effect. The initial enhanced growth rate was due to increased germ tube production—conidia in minimal medium germinated via a single (occasionally two) germ tube, while two or three germ tubes developed in the presence of the

Table 1. EFFECT OF 8-AZAGUANINE ON THE GROWTH* OF *Aspergillus nidulans*

Molarity of 8-AG.	*A. nidulans* 13				*A. nidulans* 13*y*			
Sampling time (h after inoculation)	10^{-8}	10^{-6}	10^{-4}	10^{-2}	10^{-8}	10^{-6}	10^{-4}	10^{-2}
24	75	100	37	0	200	300	35	0
48	56	73	54	1	150	100	42	12
72	102	46	20	12	68	63	23	17
144	134	98	84	16	82	80	59	26
192	114	104	88	27	115	136	55	35
312	102	99	94	30	101	93	95	37

* Expressed as percentage of unsupplemented control (control = 100).

analogue. Subsequent decrease in growth rate (cf. data for 72 h) in the majority of treatments was the result of retarded sporulation; thereafter growth in supplemented media was stimulated until the onset of senescence. In the presence of 10^{-2} M 8-AG growth never exceeded 30–40 per cent of the controls and hyphal morphology was abnormal compared with development in all other cases. Normal hyphal cells were rectangular, had homogeneous contents and measured 2–3 × 20μ. The 10^{-2} M 8-AG induced irregular cell division and growth, cell shape varying from spherical, oval to isodiametrical and size from 6 × 5μ to 7·5 × 8μ with frequent swelling of the hyphæ to 12μ in diameter. The contents of such cells appeared granular and/or vacuolated in stained and viable preparations. The analogue frequently induced a growth form reminiscent of the pseudomycelia of certain yeasts. Similar abnormal development has been observed in wild-type *Aspergillus glaucus* when the fungus was grown on solid media containing 8-AG (Bull and Johns, M., unpublished data); certain hyphæ grew in advance of the colony front and, while cell elongation was strikingly inhibited, cell division and branching were stimulated, thereby producing 'star-shaped' mycelia and whorls of short, swollen hyphæ.

The inhibitory effect of 8-AG was reversed by normal purine bases; results are shown in Table 2.

Table 2. EFFECT OF PURINE BASES ON 8-AG INHIBITION DURING THE LOGARITHMIC GROWTH PHASE*

Purine	8-Azaguanine (10^{-4} M) : purine					
	100 : 1		1 : 1		1 : 100	
	13	13*y*	13	13*y*	13	13*y*
Adenine	25	24	113	127	52	49
Guanine	28	26	118	107	0	0
Uric acid	73	65	48	57	16	28
Xanthine	92	111	99	96	46	73
8-Azaguanine			84	59		

* Reversal expressed as percentage of unsupplemented control (control = 100).

The effect on growth of the purine bases themselves is illustrated in Table 3.

Table 3. EFFECT OF PURINE BASES ON *A. nidulans* DURING THE LOGARITHMIC GROWTH PHASE*

Purine	Strain 13		Strain 13*y*	
Molarity	10^{-2}	10^{-4}	10^{-2}	10^{-4}
Adenine	43	96	52	157
Guanine	42	95	77	154
Uric acid	26	80	24	108
Xanthine	15	88	27	99

* Expressed as percentage of unsupplemented control (control = 100).

All the purines inhibited growth at high concentrations except in strain 13*y* where the 10^{-4} M treatments were stimulatory. The fact that 8-AG relieved the inhibition induced by supra-optimal concentrations of adenine, guanine and xanthine in strain 13 suggests that the analogue can act by reducing the physiological level of these purines. This response is similar to that found in the imperfect fungus *Diplodia natalensis*[8], where it has been suggested that adenine and guanine are interconvertible since the growth inhibition of antipurines is reversed in the presence of either normal base. A contrary situation has been demonstrated in biochemical mutants of *Neurospora*[9] and *Ophiostoma*[10]. The effects of the di- and trihydroxypurines are difficult to interpret at this stage. These bases are normally encountered as products of adenine and guanine degradation. The absence of high xanthine oxidase activity in these strains of *Aspergillus* would make available xanthine for adenine and/or guanine synthesis as in rabbit bone marrow[11] and rat[12], whereas a high enzyme activity would suggest non-specific competition of the hydroxypurines with 8-AG. 8-Azaguanine inhibition in other genetically characterized strains of *A. nidulans* is completely removed by adenine and guanine but not by xanthine[13]; in contrast, hypoxanthine releases 8-AG growth inhibition in *Bacillus cereus*[14]. The potentiating effect of uric acid on 8-AG inhibition may be due to the inhibition of analogue deamination.

After growth for 24–432 h in liquid Czapek minimal medium containing 8-AG both strains were screened for morphological variants. Samples of mycelia were transferred to solid Czapek minimal medium and their subsequent growth observed for the occurrence of morphological variation. The variants isolated constituted either the whole of the growth, or arose as sectors from apparently wild-type mycelium. No variants were obtained from control cultures. Table 4 shows the variants isolated from liquid cultures of different ages in two experiments. Each sample of mycelium was taken from a separate culture.

Table 4. VARIANTS* OF *A. nidulans* ISOLATED ON SOLID CZAPEK MINIMAL MEDIUM FOLLOWING TREATMENT WITH 8-AG IN LIQUID CULTURE

		Strain 13				Strain 13*y*			
Molarity of 8-AG.		10^{-8}	10^{-6}	10^{-4}	10^{-2}	10^{-8}	10^{-6}	10^{-4}	10^{-2}
Sampling time (h after inoculation)									
Exp. 1	24								
	48								
	72								3
	144								
	192				1				
	312	1	1						
Exp. 2	96					3	3	4	
	168						3		
	312	1						4	
	432	1	1	1	2		1		1*a*

* Variant 1: pink, fluffy mycelium; cleistothecia ('perithecia' of common genetical usage) bearing Hülle cells which autolyse; pink pigment secreted into medium. Variant 1*a*: as variant 1 but Hülle cells normal.

Variant 2: cleistothecia absent; little extracellular pigment compared with wild type.

Variant 3: mycelium covered with web of aerial hyphæ; reduced production of cleistothecia; red-pink pigment produced extracellularly.

Variant 4: increased production of cleistothecia; few conidial heads; little extracellular pigmentation compared with wild type.

The asexual spore progeny of all isolates of each variant was examined. All the isolated 1 and 2 variants bred true except one obtained after 432 h in 10^{-6} M 8-AG (strain 13, Exp. 1). The latter gave both sexual and asexual colonies among its asexual spore progeny. The asexual spore progenies from variants 3 and 4 contained a number of phenotypic classes. The two isolates of variant 4 from strain 13*y* gave rise to different phenotypic classes among their asexual offspring. In subsequent asexual spore progenies all the isolates of variants 3 and 4 have produced yet further phenotypic classes, some of which bred true, but the majority of which again segregated among their asexual offspring. So far only the sexual variant 1 isolates have been screened by Jinks's[15,16] heterocaryon test. All these isolates proved to be nuclear in origin. The continuously segregating isolates of variants 3 and 4 obtained from strain 13*y*, which was known to have uninucleate, haploid asexual spores[17], are prospectively cytoplasmic in origin[18]. The results in Table 4 suggest that 8-AG may act specifically in the production of genetic variation, and the behaviour of the variants suggests that the sites of activity of the analogue may be both nuclear and extranuclear.

Work is continuing to determine the metabolic fate of 8-azaguanine in this micro-organism and to establish more precisely the genetic nature of the variants.

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LETTERS TO THE EDITOR

GEOPHYSICS

Laser Radar Echoes from the Clear Atmosphere

Using experimental pulsed ruby laser radars, numerous observations have been made here of echoes from the 'clear' atmosphere. There is no doubt that these returns are caused by backscattering from particulate matter in the aerosol. Even in the clearest conditions echoes are invariably obtained from the immediate vicinity, and these are much enhanced in hazy conditions. Strong signals are commonly noted at heights corresponding to low-level inversions, revealing haze layers which are not discernible by eye.

A particularly interesting series of observations was made on August 19, 1963. These observations were made with an early version of our laser radar having the characteristics given in Table 1. They were made by pointing the radar at an elevation of 15° (on a bearing of 036° T) in the direction of San Francisco Bay, which is some 2·5 nautical miles distant from the laboratory. Although very slight haze had been present in the morning, the afternoon of August 19, 1963, was bright and clear, with blue skies and unrestricted visibility (more than 8 nautical miles). A northerly sea breeze, which was estimated at 5–10 knots in the late forenoon, had freshened to 10–15 knots by the time of observation (1430 P.S.T.). Fig. 1 shows a plot of relative signal intensity versus range for three successive observations made over a period of some 12 min. Log/log co-ordinates have been used to facilitate interpretation. On these co-ordinates a plot of signals from a non-attenuating medium would be a straight line having a slope determined by range.

Fig. 1*a* thus shows an instantaneous, direct observation of discontinuities in clear air which was in motion. We believe that the variations in signal intensity were due to variations in the particulate matter content, and hence in the backscattering characteristics of two or more intermixing aerosols. Figs. 1*b* and *c* show the changes which occurred with time as mixing proceeded or as more homogeneous air passed into the area. A number of other observations made on August 19, 1963, before the series illustrated, showed similar discontinuities fluctuating with time. For comparison, Fig. 1*d* shows returns made in very similar apparent conditions on the following afternoon, August 20, when the aerosol was consistently homogeneous.

Table 1. *SRI* Lidar *Mark I* (1963)

Laser	3 in. × 1/4 in., 90° *C*-axis ruby crystal, transparent and semi-reflecting coatings on flat ends. *Q*-switched	Receiver	10 stage RCA photo-multiplier type 7326
Pulse length	24 n sec	Aperture	4 in. diam.
Peak power	5 MW	Bandpass	Approx. 20 Å
Beam width	0·5°		

The implication of these early observations is very far-reaching. Particulate matter is present in some degree throughout the atmosphere. The possibility of mapping the effects of air motion, and in particular that associated with 'clear air turbulence' by reference to laser radar returns from particulate matter, thus appears to be realistic. Although considerable technological development will be needed and also a better understanding of the nature of clear air turbulence it is suggested this approach to the detection of clear air turbulence is more promising than any based on obtaining optical echoes from variations in the gaseous state of the atmosphere.

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Magnetic Disturbances preceding the 1964 Alaska Earthquake

Through a fortunate circumstance, a recording magnetometer was operating in the city of Kodiak, 30 km north-west of the surface trace of a fault zone along which movement occurred at the time when the earthquake occurred in Alaska on March 27, 1964. Fortunately, too, the instrument was on such high ground that it was not reached by the subsequent seismic sea wave which virtually destroyed the city. The magnetometer recorded the fact that the largest of several magnetic

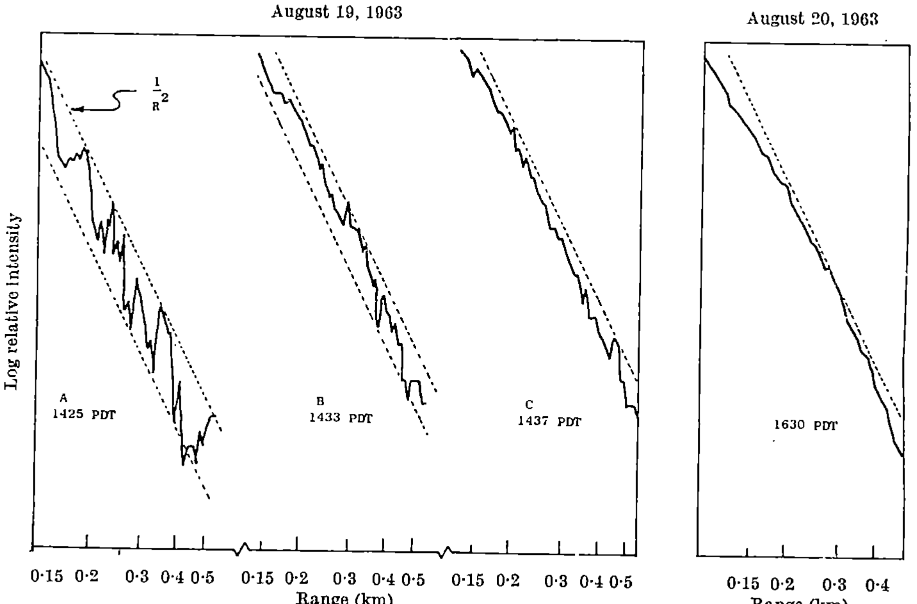

Fig. 1. Clear air observations. *SRI* ruby laser radar (*Mark I*). Bright clear afternoons with good visibility and moderate northerly and easterly winds. Azimuth 36°, elevation 15°

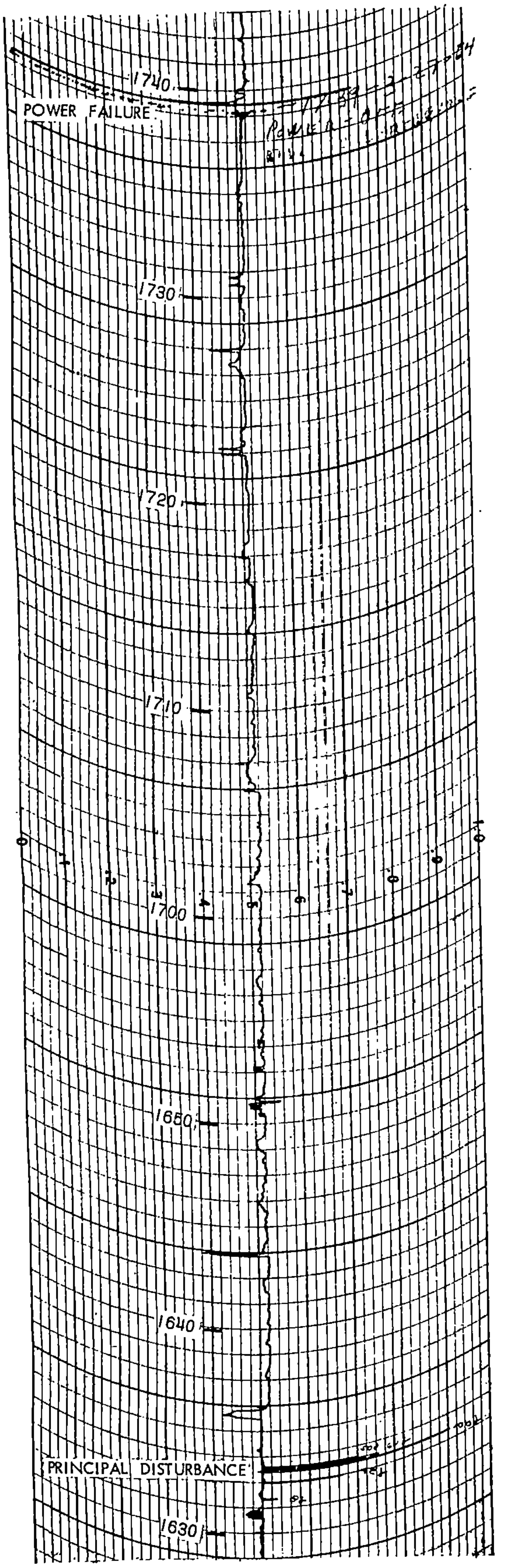

Fig. 1. Record of the positive magnetic disturbances which preceded the Alaska earthquake. Heavy time divisions equal 7·5 min. Time is Alaaks Standard Time, March 27, 1964. Full scale for the magnetic intensity, which increases toward the right, is 250 gammas

disturbances briefly increased the intensity of the Earth's magnetic field by 100γ at Kodiak, 1 h 6 min before the earthquake (Fig. 1).

The magnetometer had been installed for the Superior Oil Co. and the Pan American Petroleum Corp. by Fairchild Aerial Surveys to serve as a ground monitor for an airborne magnetic survey being made of the region. The instrument is a Gulf flux-gate magnetometer, which records the total magnetic field intensity on an Esterline Angus recorder. The sensing element had been installed on the grounds of the Bay Shore Motel in Kodiak.

The record was stopped at the beginning of the earthquake by a massive short circuit in the city power system. Automatic equipment in the Kodiak power plant made four unsuccessful attempts, in a period of about 3 sec, to reclose the circuits, and then cut off the power indefinitely[1]. Because Fairchild lost their aeroplane and one member of their team in the disaster, the magnetometer was disconnected and put in storage before the city power was turned on again. The trace that follows the record of the power failure on Fig. 1 was made approximately a month later, when the survey was resumed.

An electric clock which ran on city power in the room with the recorder stopped at 1739 Alaska Standard Time, March 27, 1964. This time reference has an estimated precision of ± 1 min (ref. 2), and according to it, the principal magnetic event occurred at 1633 A.S.T. The official time for the earthquake at its focus, 440 km to the north-east, was 1736.2 A.S.T. (0336.2 G.M.T., March 28).

The chart shows many small negative magnetic disturbances that were caused by automobiles passing on a road about 15 m south-east of the sensing element. The positive disturbances, however, which are believed to have been associated with the earthquake, began at 1632 A.S.T. with three sharp events extending over a period of about a minute, and culminated in a positive disturbance equal to 100γ. This was immediately followed by two somewhat smaller events, and a still smaller one occurred 18 min later. No other sharp positive disturbances were found on about a month of record.

One possibility is that the magnetic events which preceded the Alaska earthquake resulted from piezomagnetic effects of rocks undergoing a change in stress[3]. Why such abrupt disturbances occurred in advance of the earthquake is not known; but a causal relation is indicated by the fact that Breiner[4] has recently reported similar positive magnetic disturbances prior to minor earthquakes in Nevada and California. These observations, taken together, suggest that magnetic monitoring may provide a means of predicting a major earthquake in time to save lives and property.

Jon W. Davidson, of the Superior Oil Co., contributed to this report in helpful discussions. I thank the Superior Oil Co. for its co-operation, the Pan American Petroleum Corp., and Fairchild Aerial Surveys in permitting the release of the information. Publication was authorized by the Director, U.S. Geological Survey[5].

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GEOLOGY

Age of Labrador Anorthosites

THE age of the Nain anorthosite[1,2] on the coast of Newfoundland–Labrador is probably the same as that of the Michikamau anorthosite (1,400 ± 80 m.y.) recently reported by Emslie[3]. The Nain anorthosite is flanked on the north side by the Kiglapait layered intrusion[4,5], which shows no chill against anorthosite and is therefore thought to be barely younger than the anorthosite. The Kiglapait intrusion is invaded on its north margin by a granodiorite, possibly rheomorphic, the biotite of which gives a potassium-argon age[6] of 1,480 ± 50 m.y. This is therefore a minimum age for the Kiglapait intrusion, and also for the anorthosite.

Dikes and small stocks of subsolvus Manvers granite[5] cut the Kiglapait intrusion and anorthosite in the vicinity of Port Manvers. This granite tends to occupy flat-lying sheet joints in the Kiglapait intrusion, and is tectonically distinctly younger than the more concordant granodiorite. Its biotite gives a potassium–argon age[6] of 1,140 ± 40 m.y. The granodiorite may be equivalent to earlier stages of Wheeler's adamellite[7], and the Manvers granite may be equivalent to his later biotite facies adamellite. However, tectonically and petrographically similar granites occur up and down the coast of Labrador, so any meaningful correlation of these younger granites with adamellite will have to await further isotope age studies.

The age equivalence of the Nain and Michikamau anorthosites gives further support to the view of Emslie that these bodies in the Nain Province[8] record the true age of anorthosite emplacement in eastern North America, and that this event preceded the widespread metamorphic effects of the Grenville orogeny (950 m.y. as recently defined)[8]. Geological evidence that the Labrador anorthosites were less affected by metamorphism is mentioned by Kranck[1] as well as by Emslie. A pre-Grenville age of anorthosite emplacement in the Adirondack highlands is indicated by the stratigraphic and structural work of Walton and De Waard[9].

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Progressive Desilication of Basic Hornfelses

The progressive desilication of pelitic sediments during contact metamorphism has been known for some time[1,2]. A mineral assemblage relatively enriched in aluminium and impoverished in silicon is progressively developed with the production of such phases as spinel, corundum, enstatite, cordierite and andalusite. The initial silica content of an argillaceous sediment before metamorphism may be between 60 and 70 per cent and on contact metamorphism desilication may lower the silica value to about 45–55 per cent—a range found in some pelitic xenoliths enclosed in granite.

A similar process for basic rocks, not previously recorded, is proposed for the basic hornfelses of the Land's End granite aureole, Cornwall. Although the process involves progressive desilication, it is not strictly analogous to that seen in pelitic hornfelses as the basic hornfelses have little or no free silica as quartz and are the products of progressive additive metasomatism.

On contact metamorphism, the basic intrusives ('greenstones') of the Land's End aureole were metamorphosed to hornblende hornfelses. The allochemical addition of potassium to these rocks produced biotite-rich hornfelses or potassium-hornfelses[3,4]. Localized internal metasomatism of the hornblende hornfelses produced iron–magnesium-hornfelses[5–7] containing anthophyllite, cummingtonite and cordierite and also calcium-hornfelses[8] containing sphene, diopside, grossularite, epidote and calcite.

Two distinct desilication trends can be traced in the basic hornfelses.

The first trend is very limited in extent and concerns the hornblende hornfelses. In a few instances spinel has been found in plagioclase-hornblende hornfelses, but no other silica-poor minerals[9]. On the production of a cordierite-anthophyllite assemblage from the plagioclase-hornblende hornfels there may be some loss of silicon, although this is by no means always the case. However, marked desilication is represented when the iron–magnesium-hornfelses have developed such minerals as diaspore and spinel[5]. Such an assemblage can be considered the silica-poor equivalent of the plagioclase-hornblende or cordierite-anthophyllite assemblage (Table 1).

Table 1. Comparative Analyses of Basic Hornfelses from the Land's End Aureole, Cornwall

	1	2	3	4	5
SiO_2	44·37	49·10	48·87	47·40	33·58
TiO_2	2·96	1·20	2·71	2·25	2·96
Al_2O_3	13·64	10·54	16·71	14·74	11·79
Fe_2O_3	1·57	0·87	0·13	0·85	11·27
FeO	7·98	10·27	12·85	9·14	17·50
MnO	0·15	0·17	—	0·26	0·21
MgO	9·85	10·72	9·60	10·75	15·55
CaO	14·18	14·00	5·42	7·69	0·89
Na_2O	1·03	1·22	1·61	2·71	0·60
K_2O	0·83	0·10	0·27	1·57	0·32
P_2O_5	0·90	0·78	0·50	0·57	0·36
H_2O^+	2·51	0·90	0·99	1·97	4·60
H_2O^-	0·06	0·10	0·07	0·02	0·13
CO_2	0·03	—	0·07	0·01	0·00
Total	100·06	99·97	99·80	99·93	99·76

1 Plagioclase-hornblende hornfels, Tater-du (ref. 4).
2 Plagioclase-hornblende hornfels, Botallack (ref. 5).
3 Plagioclase-cordierite-cummingtonite-anthophyllite hornfels, Kenidjack (ref. 5).
4 Plagioclase-biotite-cummingtonite-anthophyllite hornfels, Tater-du (ref. 4).
5 Cordierite-anthophyllite-diaspore-spinel hornfels, Kenidjack (ref. 5).

The second trend involves progressive desilication during the extensive calcium metasomatism of the hornblende hornfelses. During the internal iron–magnesium metasomatism of the hornblende hornfelses, large quantities of calcium were released which effected metasomatism elsewhere[8]. The hornblende hornfelses invaded by the migrating calcium were progressively replaced by assemblages rich in calcium-bearing minerals, namely, ilmenite-sphene-diopside-hornblende, sphene-diopside-grossularite-hornblende, grossularite-clinozoisite

Table 2. Comparative Analyses of Calcium-Hornfelses from the Land's End Aureole, Cornwall

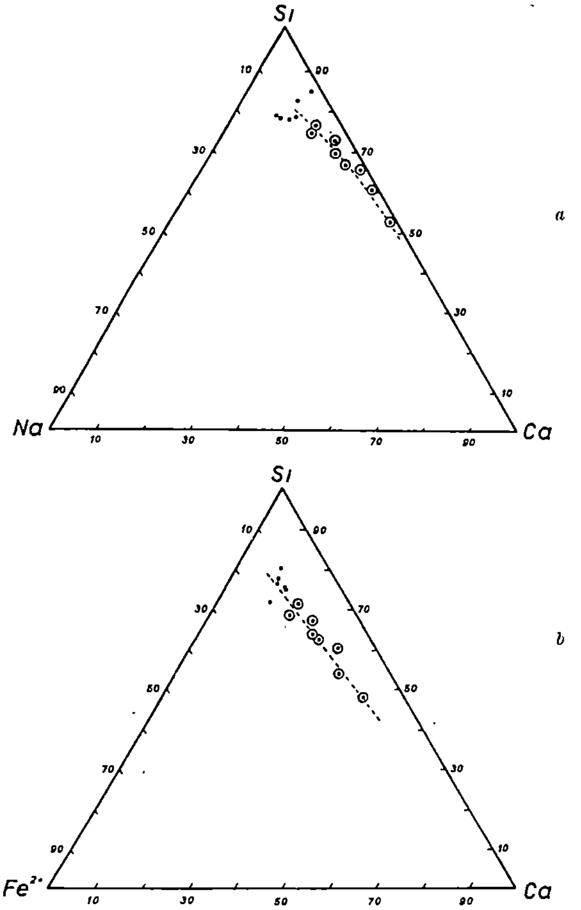
	1	2	3	4	5
SiO_2	46·74	42·05	41·69	38·67	31·19
TiO_2	2·08	3·16	2·58	2·98	2·74
Al_2O_3	12·09	12·48	13·99	10·55	12·50
Fe_2O_3	1·22	1·20	1·83	4·23	8·60
FeO	9·13	9·02	9·46	9·63	6·72
MnO	0·16	0·25	0·38	0·84	0·42
MgO	10·29	8·63	9·18	6·48	5·34
CaO	14·09	16·95	14·97	23·72	26·14
Na_2O	1·13	1·30	1·33	0·32	0·28
K_2O	0·47	1·68	1·45	0·62	0·13
P_2O_5	0·84	0·77	0·57	0·80	0·92
H_2O^+	1·71	2·05	2·00	1·35	2·63
H_2O^-	0·08	0·02	0·03	0·08	0·32
CO_2	0·02	0·02	0·01	0·01	0·12
Total	100·05	99·58	99·47	100·28	100·06*

* Total includes 2·01 per cent B_2O_3.

1 Unmetasomatized plagioclase-hornblende hornfels parent (average of analyses 1 and 2 from Table 1).
2 Hornblende-diopside-biotite-sphene hornfels, Tater-du (ref. 4).
3 Hornblende-diopside-sphene hornfels, Tater-du (ref. 4).
4 Diopside-grossularite-sphene hornfels, Tater-du (ref. 4).
5 Calcite-diopside-grossularite-sphene-axinite hornfels, Tater-du (ref. 4).

and clinozoisite-zoisite-calcite-axinite. Table 2 shows the major-element chemistry of some of the calcium-hornfelses from Tater-du, Land's End peninsula.

Figs. 1*a* and 1*b* show the antipathetic relationship of silicon and calcium for hornblende hornfelses and calcium-hornfelses (those with more than 15 calcium cations per unit cell of 160 oxygens). The trend is towards calcium enrichment, as later calcium minerals are seen to replace earlier, and also progressive desilication of the hornblende hornfelses.



Figs. 1*a* and *b*. Triangular variation diagrams showing the antipathetic relationship between calcium and silicon for hornblende hornfelses and calcium-hornfelses from the Land's End aureole, Cornwall. ●, Hornblende hornfelses; ⊙, calcium-hornfelses

It is rare in reports of metasomatic processes for the subtracted materials to be accounted for; however, in the present case, the silicon, which represents a major subtracted migrant, can probably be traced. At the Avarak, on the north-west coast of the Land's End peninsula, there is a localized area of siliceous and felspathic hornfelses, not far from the calcium-hornfels repository at Botallack, that is considered to represent the fixation of the migrating silicon cations[7].

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GEOCHEMISTRY

Possible Correlation between Classifications and Potassium–Argon Ages of Chondrites

ACCORDING to Kirsten *et al.*[1] and Hintenberger[2], the frequency distribution curves of the potassium-argon ages of stone meteorites show two distinct maxima. One occurs at ages close to 4·5 × 10^9 years, the other occurs around 1 × 10^9 years. However, if one classifies the chondrites into the Urey–Craig[3] *L*- and *H*-groups a relationship between classifications and gas retention ages is apparent (Fig. 1). The classification in *L*- and *H*-groups (that is, hypersthene-olivine and bronzite-olivine chondrites respectively) is based on the following chemical-mineralogical parameters: bulk iron[3], specific gravity[4], metallic nickel–iron[4,5], and Fe/Fe + Mg ratios in olivine and rhombic pyroxene[6,7]. Only well-classified chondrites have been included. Carbonaceous chondrites and enstatite chondrites have been excluded since they constitute separate groups[7]. Fig. 1 shows the frequency distribution of chondrites with well-known classification and well-determined age. The age values were taken from Anders[8] and Kirsten *et al.*[1]. The diagram shows that the substantial degassing, which leads to an apparent potassium–argon age of about 1 × 10^9 years, is, on the basis of the chondrites investigated so far, considerably more frequent for the *L*-group of chondrites than for the *H*-group. The uranium–helium ages are also plotted in the diagram, showing a similar effect. New potassium–argon age measurements[9] seem to yield some more *H*-group chondrites with low potassium–argon ages. However, recent uranium–helium measurements[10] again seem to support an age-classification relationship as indicated in Fig. 1. The purpose of this communication is to direct attention to the apparently more frequent, substantial degassing of *L*-group chondrites in comparison to *H*-group chondrites, and to emphasize the necessity for more potassium–argon gas retention age measurements of chondrites, for which the classification into *L*- and *H*-groups is well known.

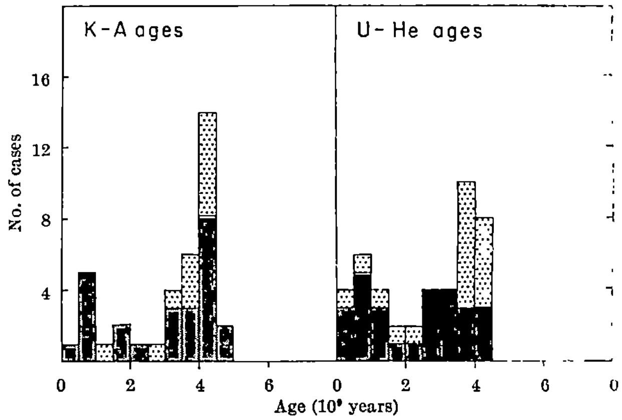

Fig. 1. Correlation between classifications and ages of chondrites. Dark shading, *L*-group chondrites; light shading, *H*-group chondrites

I thank Drs. H. E. Suess, H. C. Urey and R. Bieri for their advice.

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CRYSTALLOGRAPHY

Arsenides and Antimonides of Niobium

The phase relationships in the systems niobium–arsenic and niobium–antimony have been studied by X-ray methods. Density determinations and magnetic susceptibility measurements have been carried out.

The existence of the Nb_3Sb phase reported by Matthias *et al.*[1], Wood *et al.*[2] and Nevitt[3] has been confirmed. The NbAs phase has independently been examined by Boller and Parthé[4] and by us. The occurrence of three other previously unknown phases, the Nb_5Sb_4, $NbAs_2$ and $NbSb_2$ phases, have been established.

The lattice dimensions and observed densities are:

	a (Å)	*b* (Å)	*c* (Å)	β(°) or *c*/*a*	d_{pycn} (g cm^{-3})
NbAs	3·4517		11·680	3·3838	7·93
$NbAs_2$	9·357	3·3823	7·792	119·46	7·41
Nb_3Sb	5·2643				8·83
Nb_5Sb_4	10·314		3·556	0·3448	8·17
$NbSb_2$	10·239	3·6319	8·333	120·07	8·22

As the lattice constants are almost invariable for specimens with different initial proportions of the components no composition ranges of the phases are indicated.

The crystal structures, that is, types or space groups and parameters, are:

NbAs: Space group $I4_1$ or $I4_1md$
4 Nb and As in (*a*)
Nb: $z = 0$; As: $z = 0{\cdot}416 \pm 0{\cdot}001$

$NbAs_2$: Space group *C*2
4 Nb, As_I and As_{II} in (*c*)

	x	*y*	*z*
Nb	0·3444	½	0·3044
As_I	0·0948	0·488	0·3928
As_{II}	0·1399	0·067	0·0257

Nb_3Sb: β-*W* type
Nb_5Sb_4: Ti_5Te_4 type
$NbSb_2$: Space group *C*2
4 Nb, Sb_I and Sb_{II} in (*c*)

	x	*y*	*z*
Nb	0·343	½	0·304
Sb_I	0·096	0·5	0·393
Sb_{II}	0·142	0·0	0·027

NbAs, $NbAs_2$ and $NbSb_2$ have diamagnetic susceptibilities, whereas Nb_3Sb and Nb_5Sb_4 show weak temperature independent paramagnetism.

Details of the structures and discussion of the chemical bonding will be published elsewhere.

We thank Prof. Haakon Haraldsen for his advice.

Note added in proof. Since this communication was submitted for publication an independent confirmation of the existence of the N6As and $N6As_2$ phases has been published by Saini *et al.*[5].

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Crystal and Molecular Structure of 1,3-Dihydro-1-hydroxy-3-oxo-1,2-benziodoxole

O-IODOSOBENZOIC acid (*I*) has been recognized as an atypical compound ever since it was first synthesized[1]. Askenasy and Meyer[2] suggested that this compound existed as a 5-membered heterocyclic ring. This cyclic configuration was substantiated by the infra-red studies of Bell and Morgan[3]. Further evidence favouring the heterocyclic ring structure was offered by Wolf and Hsu[4], who synthesized homologues of (*I*) with 6- and 7-membered heterocyclic rings. In view of the lack of information concerning the chemical bonding of the iodine in these heterocyclic compounds, the present X-ray investigation of (*I*) was undertaken.

(*I*) crystallizes from water as fine monoclinic needles elongated along the *b* axis. The following crystallographic data were obtained for these crystals:

$a_0 = 12{\cdot}89 \pm 0{\cdot}01$Å
$b_0 = 4{\cdot}10 \pm 0{\cdot}02$Å
$c_0 = 14{\cdot}05 \pm 0{\cdot}01$Å
$\beta = 96{\cdot}73 \pm 0{\cdot}05°$

$Z = 4$
$\rho_{obs} = 2{\cdot}38 \pm 0{\cdot}03$ g cm^{-3}
$\rho_{cal} = 2{\cdot}33 \pm 0{\cdot}01$ g cm^{-3}
Space group $P2_1/c$

Three layers of Weissenberg intensity results were obtained with copper $K\alpha$-radiation from a crystal mounted along *b*. The intensities of 520 independent reflexions were measured from these photographs, in part with a micro-densitometer and in part visually. The intensity results were converted to F^2 in the usual manner. Absorption corrections were not made because the crystal was only 0·06 mm in diameter. The structure factors were initially scaled together by means of *hk*0 and 0*kl* precession data, and the interlayer scaling was further improved during the final stages of least-squares refinement.

The co-ordinates of the 11 atoms in this structure were readily obtained from 3-dimensional Patterson and Fourier syntheses. The refinement of these parameters was carried out isotropically by least squares, minimizing the function $\Sigma w\,(|F_0| - G\,|F_c|)^2$. The *R* value (the usual discrepancy index) was 13·7 per cent after 5 cycles of refinement.

Fig. 1 is a view of (*I*) as it appears down the *b* axis. The features of major interest in this structure are:

(1) The C(1)—I, O(3)—I and O(2)—I bond distances are 2·16, 2·00 and 2·30 ± 0·05 Å respectively. The first two of these are in agreement with the sum of the covalent radii, 2·1 and 2·0 Å (ref. 5), respectively. The greater length observed for the I—O(2) bond may possibly be due to steric strain in the molecule and/or a greater amount of ionic character in this bond over the normal covalent bond.

(2) The O(2)—I—C(1) and O(3)—I—C(1) angles are 77° and 89° ± 4° respectively. These angles are similar in magnitude to those reported by Archer and Van Schalkwyk[6]

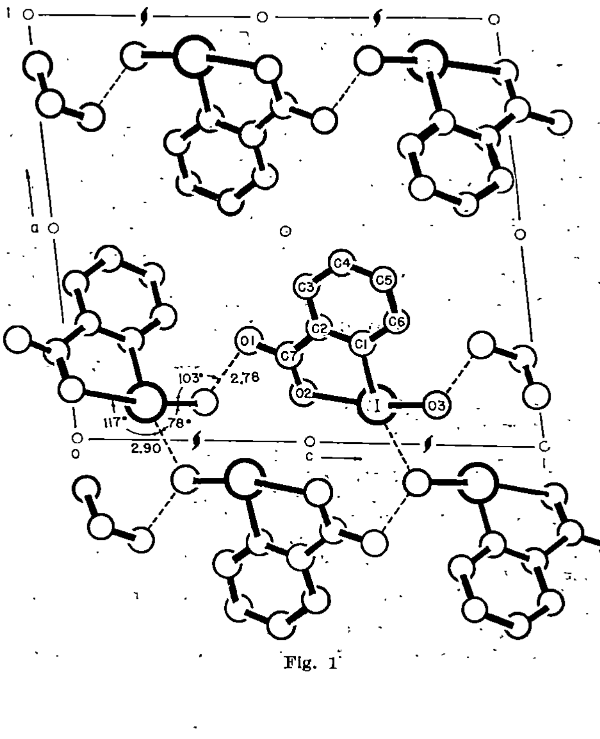

Fig. 1

for the trivalent iodine compound benzene iodo dichloride (angles of 86°). The geometry at the iodine is in accord with that found in BrF_3 (ref. 7) and ClF_3 (ref. 8).

(3) The 11 atoms in the molecule are coplanar within twice the estimated standard deviation of the individual atoms.

(4) By consideration of the angles and distances around O(3), it appears that it is hydrogen bonded to O(1) of a neighbouring molecule. The distance of this hydrogen bond was found to be 2·8 ± 0·1 Å.

(5) The contact between the iodine of one molecule and O(3) of another molecule related to the first by the screw axis is 2·90 ± 0·05 Å (Fig. 1). This distance is significantly less than the sum of the van der Waals radii of the two atoms, which is 3·6 Å. This O(3) atom is within 0·6 Å of the least squares plane of the first molecule. This type of interaction has been observed in various iodate structures[9]. Trueblood[10] has suggested that this may be another reflexion of the tendency of the iodine atom to act as a weak Lewis acid, manifested in many polyiodides and other 'complexes'.

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Unit Cell and Space Group of Co(thiourea)$_4$·(NO$_3$)$_2$

A PRELIMINARY X-ray investigation of the crystal of $Co[SC(NH_2)_2]_4(NO_3)_2$ has been made, using the method of Rossenheim and Meyer for preparing the compound. It crystallized as dark blue prisms. The unit cell dimensions of the monoclinic crystal were determined from single crystal rotation and Weissenberg photographs using copper $K\alpha$-radiation.

The values found are: $a = 20{\cdot}31$ Å; $b = 16{\cdot}80$ Å; $c = 11{\cdot}68$ Å (all ± 0·03 Å); $\beta = 91{\cdot}6 \pm 0{\cdot}2°$. $V = 3{\cdot}984$ Å^3. The number of molecules per unit cell is $N = 8$; calculated value 8·17 for a density of 1·65 g/c.c.

Observed extinctions were $h+k$ odd for hkl and k odd and l odd for $h0l$ and k odd for $0k0$ reflexions, which are consistent with the two space groups, C_s^4–C_c and C_{2h}^5–$C_{2/c}$.

Statistical determinations of the centre of symmetry by Ramachandran's method showed the presence of centre of symmetry for the projection [001] as required for the group C_{2h}^6–$C_{2/c}$.

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PHYSICS

Suggested Units for Conductivity

IN the hybrid system of units most frequently used for electrical conductivity σ the units are ohm^{-1} cm^{-1}. Often the atrocity mho/cm is used and neither form is particularly convenient if it is necessary to specify the units several times in a short space.

After giving due consideration to the disadvantages of introducing still another form of notation into the already overburdened literature, it is felt that the abbreviation 'roc' to stand for reciprocal ohm-centimetre is a worthwhile addition. The M.K.S. form follows, that is,

$$\sigma[\text{rom}] = 100\,\sigma[\text{roc}]$$

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Rapid Non-destructive Analysis of Oxygen by Neutron Activation

IT is known that in 29·4 sec oxygen-19 is formed by neutron irradiation of natural oxygen and that it emits 0·200-MeV γ-ray. Since the activation cross-section of ^{18}O (n, γ) ^{19}O and the abundance of oxygen-18 are both low (0·21 ± 0·04 m barn[1], 0·204 per cent[1], respectively), the activation analysis using oxygen-19 is only applicable to the determination of macro-amounts of oxygen.

A sample is irradiated for 30 sec in a *JRR*-1 reactor at a neutron flux of about 3×10^{11} n/cm^2/sec, cooled for 30 sec, counted for 30 sec, again cooled for 10 sec and counted for 30 sec. The counter used is a 2-in. thick × 1·75-in. diam., well-type sodium iodide crystal attached to an *RCL*-256 channel pulse height analyser. During the counting operation the sample is placed 1 cm above the crystal. An aluminium disk of 2,150-mg/cm^2 thick is used as an absorber for β-rays from the sample.

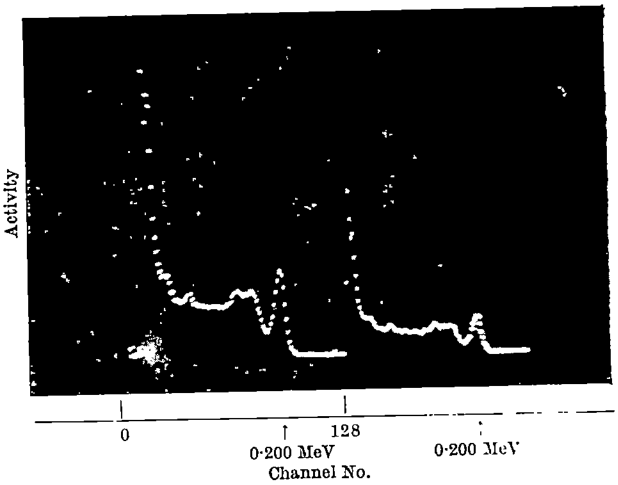

Fig. 1. γ-Ray spectrogram of oxygen-19. Sample: 5 ml. pure water

An example of successive spectra thus obtained is shown in Fig. 1. The decay curve of photopeak due to 0·200-MeV γ-ray is measured at short time-intervals and the half-life is determined as about 30 sec. From both γ-ray energy and half-life, oxygen-19 is identified.

The activation method is applied to the determination of oxygen in organic compound. Pure water is used as an oxygen standard. Oxygen is determined by comparing the peak height of 0·200-MeV γ-ray with that of standard. Some results of analyses by this method are shown in

Table 1. Results of Analyses

Sample	Oxygen content % Calculated	Neutron activation
Water	88·81	89, 87, 85
Salicylic acid	34·75	35, 36
Citric acid	60·91	61, 62
d-Mannitor	52·70	54

Table 1. The upper and lower limits of determination of oxygen are about 6 g and about 0·1 g respectively.

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CHEMISTRY

Electron Spin Resonance Spectra of γ-Irradiated Alkoxides

The free radicals formed during the irradiation of alcohols frozen at −196° C have been examined extensively by electron spin resonance and, for the lower primary and secondary alcohols, they have been identified[1] as $R_1(R_2)\dot{C}OH$. A similar investigation has been made of only one of the corresponding irradiated alkoxides, namely, sodium methoxide, for which Alger, Anderson and Webb[2] observed a three-line spectrum which was similar to that from methanol, except that the proton hyperfine splitting constant (16 œ) was slightly smaller than that in methanol (18 œ). On the assumption that the alkoxides are ionic, Symons has briefly discussed this result in terms of the $\dot{C}H_2\bar{O}$ radical[3].

If other primary and secondary alkoxides behave in a similar way it should be possible to prepare other radicals of the type $R_1(R_2)\dot{C}\bar{O}$. Of particular interest among these are the aliphatic ketyl radicals, which would also be formed by electron transfer to the corresponding ketones. The electron spin resonance spectra of two highly substituted ketyl radicals (prepared by electron transfer from an alkali metal to hexamethylacetone and pentamethylacetone, respectively) in solution have been observed[4]; but so far the spectra of the smaller ketyl radicals have not been obtained.

In a preliminary investigation we have observed the electron spin resonance spectra of γ-irradiated samples of sodium and potassium methoxides, ethoxides and isopropoxides over a temperature range from −196° C to +100° C. The alkoxides were prepared by reacting the alkali metal with an excess of the A.R. grade alcohol in an atmosphere of dry nitrogen. The excess alcohol, including the alcohol of crystallization, was removed by prolonged evacuation. (The alkali metal content was estimated by titration and found to correspond closely to $R_1(R_2)CH\bar{O}M^+$.) The samples were sealed in high-purity silica tubes under dry nitrogen and irradiated at −196° C with γ-rays from cobalt-60. The electron spin resonance spectra were recorded at −196° C and at intervals as the temperature was increased to +100° C. No appreciable decay of the radicals occurred below +20° C for any of the alkoxides.

The spectra observed from irradiated potassium methoxide, ethoxide and isopropoxide at +20° C are shown in Fig. 1. Those observed from the sodium alkoxides were similar but not so well resolved. In all cases the resolution increased as the temperature was raised from −196° C. The spectra can be interpreted logically as arising from the radicals $\dot{C}H_2\bar{O}$, $CH_3\dot{C}H\bar{O}$ and $(CH_3)_2\dot{C}\bar{O}$, respectively, the last being the ketyl radical of acetone.

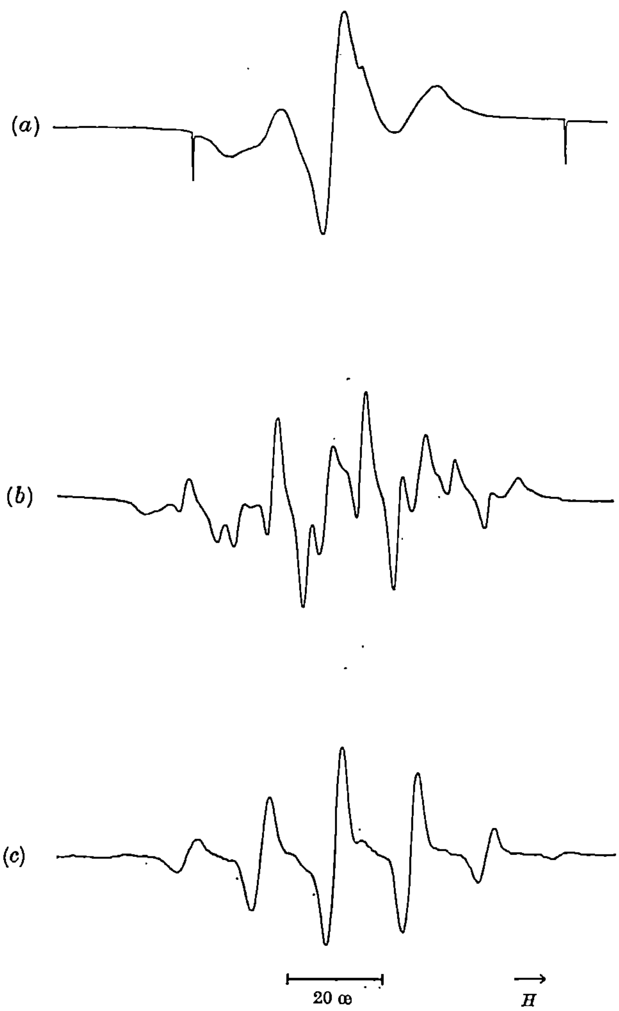

Fig. 1. First derivative spectra of γ-irradiated potassium alkoxides at +20° C: (*a*) methoxide; (*b*), ethoxide; (*c*), isopropoxide

Table 1. Hyperfine Splitting Constants for Alkoxide and Alcohol Radicals

Derivative	Hyperfine splitting constants (œ) Alkoxide* H_α	Alkoxide* H_β	Alcohol† H_α	Alcohol† H_β	Ratios $\frac{\text{Alkoxide}}{\text{Alcohol}}$ α	Ratios $\frac{\text{Alkoxide}}{\text{Alcohol}}$ β
Methyl	15	—	17·2	—	0·87	—
Ethyl	13	19·2	15·0	22·0	0·87	0·87
Isopropyl	—	16·1	—	20·0	—	0·81

* $H_\alpha \pm 1{\cdot}5$ œ; $H_\beta \pm 0{\cdot}5$œ. † Values for liquid phase, ref. 6.

The three lines observed in the spectrum of the methoxide are caused by interaction of the unpaired electron with the two α-protons. The shape of the outer lines shows that there is an anisotropic contribution to the hyperfine splitting and the overall spectrum is similar to that expected from a rotating $\dot{C}H_2$-group[5]. The spectrum of the ethoxide consists of eight asymmetric lines which can be arranged into four equally spaced pairs of lines with intensity ratios of approximately 1 : 3 : 3 : 1. This grouping can be accounted for by assuming that there is an isotropic hyperfine interaction with three equivalent β-protons and an anisotropic interaction with one α-proton. In marked contrast the seven lines in the spectrum of the isopropoxide are symmetric and well resolved. The lines are equally spaced and have relative intensities of approximately 1 : 6 : 15 : 20 : 15 : 6 : 1, showing that there is an isotropic interaction with six equivalent β-protons.

The hyperfine splitting constants based on this analysis are given in Table 1 together with those for the corresponding alcohol radicals observed in the liquid phase[6]. The isotropic β-proton splitting constants can be measured accurately, but because of the anisotropic contribution the α-proton splitting constants are less accurate. We have calculated the latter using the line shape analysis derived by Cochran, Adrian and Bowers[5]. The splitting constants for the alkoxides are consistently lower than those of the corresponding alcohol radicals (about 85 per cent), showing that the spin density on the α-carbon atom is less in the alkoxide. This reduction can be attributed to the participation of the structure $\rangle\bar{C}-\dot{O}$ in the alkoxide.

The results on the isopropoxide confirm the prediction[1] that the hyperfine splitting constant of the ketyl radical $(CH_3)_2\dot{C}\bar{O}$ should be a few œrsteds smaller than that of the isopropanol radical $(CH_3)_2\dot{C}OH$.

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Determination of the Initial Formation Temperature of Nickel Ferrite Spinel

Little information is to be found in the literature[1,2] about the merits of different techniques which may be used for the detection of initial spinel formation temperatures. This communication compares a number of methods which have been used for determining the spinel formation of nickel ferrite, $NiFe_2O_4$.

A mixture of iron and nickel oxides, both of > 99·7 per cent purity, in the proportions appropriate to the stoichiometric formation of nickel ferrite, was prepared by the usual powder metallurgy techniques[3]. Magnetic susceptibility measurements, electrical resistivity measurements and X-ray diffraction measurements were carried out on samples of the mixture that had been calcined in air for 4 h at temperatures ranging from 400° to 1,260° C. The reaction was also followed by thermal gravimetric analysis and differential thermal analysis. From the results of these measurements shown in Fig. 1 and Table 1, it is apparent that the five techniques show a spread of about 100° C in the detected initial formation temperatures.

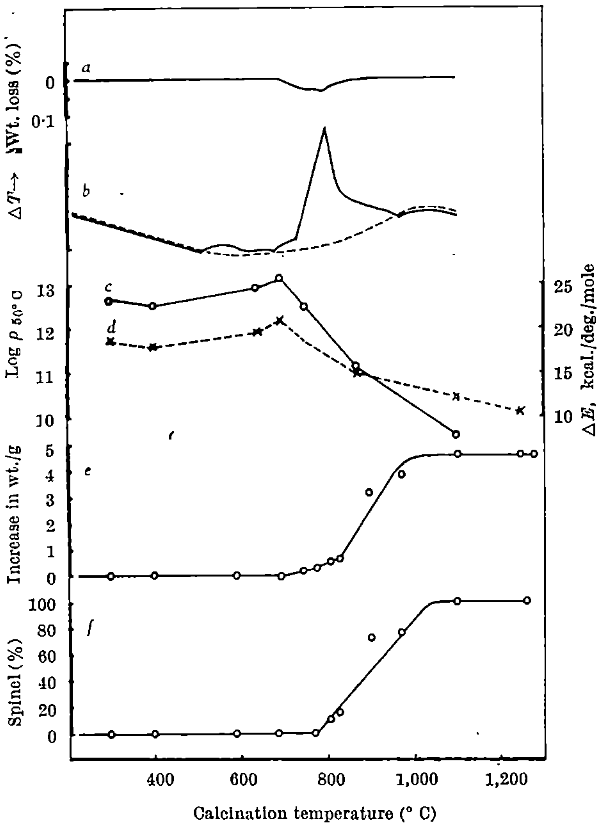

Fig. 1. Initial spinel formation temperature of nickel ferrite determined by: *a*, Thermal gravimetric analysis; *b*, differential thermal analysis (dotted line, estimated base line); *c*, electrical resistivity; *d*, activation energy (calculated from log $\rho_{50-100°\,C}$); *e*, magnetic susceptibility (Gouy method); *f*, X-ray diffractometry. Samples for *c*, *d*, *e*, *f* were calcined for 4 h in an air atmosphere

Table 1. $NiFe_2O_4$ Spinel Formation Temperatures

Method	Initial spinel formation temperature (°C)	Complete spinel formation temperature (°C)
Thermal gravimetric analysis	690	900
Differential thermal analysis	730	965
Electrical resistivity	690	—
Activation energy	690	—
Magnetic susceptibility	750	1,100
X-ray diffractometry	780	1,100

The measurement of electrical resistivity appears to be the most sensitive method; the change from the defect conductivity of NiO and Fe_2O_3 to the electron conductivity of the spinel shows clearly at 690° C. In addition to the absolute value of resistance, the activation energy of conductivity has also been calculated and plotted, indicating a similar sharp peak.

The magnetic susceptibility and X-ray diffraction techniques, while not quite so sensitive as the electrical resistivity method, enable quantitative information to be obtained about the temperature of complete ferrite formation. All three methods suffer from the disadvantage of requiring lengthy sample preparation.

Both the thermal analytical techniques—thermal gravimetric analysis and differential thermal analysis—provide a much quicker method of measurement, but the results are only of a qualitative nature. In the analysis of the formation of nickel ferrite, differential thermal analysis is much more satisfactory than thermal gravimetric analysis, but for other ferrites, where a reduction occurs in the oxide mixture (for example, MnO_2 plus Fe_2O_3), thermal gravimetric analysis can provide both qualitative and quantitative data about the spinel formation process.

This work is being continued with other transition metal spinels of the formula MFe_2O_4, where M = Cu, Co, Mg, Mn and Zn, and the complete investigation will be published shortly.

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Effect of Substituents on Electrical Conductivity of Anils

This communication reports an attempt to approach the problem of electrical conductivity in organic solids from the point of view of classical organic chemistry. A series of anils derived from salicylaldehyde and various meta- and para-substituted anilines (Fig. 1) has been prepared and the electrical conductivities of compressed silver-coated polycrystalline pellets of each species have been measured. Most of these materials showed an observable dark conductivity under vacuum even at temperatures at which they were shown to be essentially non-volatile. It seems unlikely, therefore, that the conduction process could be described at these temperatures by a surface mechanism such as has been proposed by Eley *et al.*[1] for volatile organic crystals.

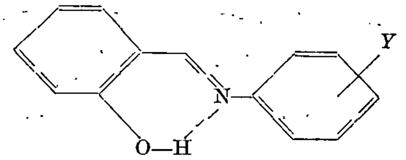

Fig. 1. Anil molecule ($Y = p$-*F*, *m*- and *p*-OCH_3, CH_3, Cl, Br, NO_2)

Materials were recrystallized three times from alcohol, dried under vacuum and finally zone refined. This last step produced no substantial change in electrical conductivity for two materials and was therefore omitted in subsequent sample preparations. In each case the relationship between conductivity and temperature could be adequately described by the equation $K = K_0 \exp(-\Delta\varepsilon/2kT)$. Ohm's law was obeyed for field strengths up to 5,000 V/cm.

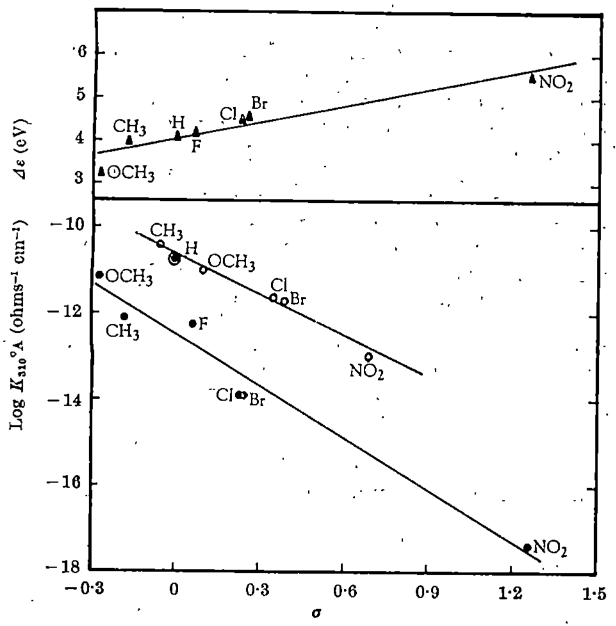

Fig. 2. Correlation of log *K* and $\Delta\varepsilon$ values and the Hammett substituent constant (σ). ▲ $\Delta\varepsilon$ versus σ for *para*-substituents; ○ log *K* versus σ for *meta*-substituents; ● log *K* versus σ for *para*-substituents

A correlation exists (Fig. 2) between log *K* and the Hammett[2] substituent constant (σ) for both the *meta*- and *para*-series of substituents, and between $\Delta\varepsilon$ and σ for the *para*-series. Thus anils containing substituents (Y) which transfer charge to the aniline ring were found to be the best conductors and in the case of *para*-substituents to have the lowest $\Delta\varepsilon$ values. This is reminiscent of the correlation found by Pullman and Pullman[3] between basic strength and ionization potential of the nitrogen lone-pair electrons for a number of biologically important compounds.

Comparing the two series, anomalies are at once apparent, for the unsubstituted salicylidene-aniline complex is out of place in the log *K* versus σ correlation for *para*-substituents, but in place in the *meta*-series. The fact that log *K* versus σ correlations exist for the two series separately but not for the overall series of substituents may indicate that closer-molecular packing occurs in crystals of the *meta*-series with consequent increase in molecular orbital overlap, carrier mobility and hence conductivity. Differences in packing arrangements among crystalline salicylidene anilines have been reported by Cohen and Schmidt[4].

Chelation of these anils with divalent metal ions produced a manifold drop in conductivity, indicating presumably that the electrons involved in the *N*-metal bond are no longer capable of contributing to the conduction process. Further evidence for this exists in that the conductivities of the metal chelates were in the order nickel > copper, whereas their stabilities, which indicate the strengths of the nitrogen-metal bonds, are in the reverse order.

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Photolysis of Nitrosyl Chloride by Ultra-violet Radiation

The photolysis of nitrosyl chloride was investigated first by Kiss[1], and later by Bowen and Sharp[2], who found that the quantum yield was 0·5 in both the spectral regions 4380–5000 Å, and 4480–5200 Å. Kistiakowsky[3] investigated the reaction more fully, using essentially monochromatic light derived either from a mercury arc, or from a tungsten filament lamp, both used in conjunction with filters. He concluded that the mean quantum yield for the decomposition over a range of wave-lengths from 6350 Å to 3650 Å was 2·1. At 3650 Å the quantum yield was 2·7, although Kistiakowsky believed that this value was incorrect because of the partial absorption of the ultra-violet radiation by the 'Pyrex' windows of the reaction cell. He suggests that the results of Bowen and Sharp disagree with his own either because they used non-monochromatic light, or because they neglected the dark reaction.

Basco and Norrish[4,5] have recently examined the flash photolysis of nitrosyl chloride under isothermal conditions. They observe that molecules of nitric oxide with up to 11 quanta of vibrational excitation are formed, which they suggest are produced in the primary process:

$$NOCl + h\nu = NO^* + Cl \qquad (1)$$

These vibrationally excited molecules of nitric oxide possess sufficient energy to allow of the reaction:

$$NO^* + NOCl \doteq 2NO + Cl \qquad (2)$$

Since the chlorine atoms formed in reactions (1) and (2) will both react with nitrosyl chloride:

$$Cl + NOCl = NO + Cl_2 \qquad (3)$$

it is possible for the quantum yield to reach a maximum value of four, rather than the limiting value of two possible when only reactions (1) and (3) take place. In the light of these considerations, Kistiakowsky's high value of 2·7 for the quantum yield at 3650 Å assumes a new significance. At that wave-length, the excess energy available from reaction (1) is only marginally sufficient to cause dissociation of the second molecule of nitrosyl chloride.

It is of considerable interest to find out whether vibrational energy possessed by a molecule can be used to provide the energy required for that molecule to enter into otherwise endothermic reactions. In order to determine whether reaction (2) does, in fact, take place, the quantum yield must be measured at shorter wave-lengths than those used by Kistiakowsky. In the present work, the quantum yield for the photochemical decomposition of nitrosyl chloride has been determined at a wave-length of 2537 Å, where there is more than 70 kcal/mole of excess energy available in reaction (1).

Mercury resonance radiation was obtained from a low-pressure lamp, which consisted of a flat quartz spiral, about 7 cm diameter, with water-cooled electrodes. It was filled with a mixture of 90 per cent neon and 10 per cent argon, to a total pressure of 1 cm mercury, and contained also a small drop of mercury. The radiation from this lamp was passed through a filter system to remove the small amount of visible and near ultraviolet radiation emitted by the lamp, and was then focused by two quartz lenses through the thermostated reaction cell on to a 1*P*28 photomultiplier tube. The quantum input to the reaction cell was determined using potassium ferrioxalate actinometer solutions according to the method of Hatchard and Parker[6]. The reaction was followed manometrically with a 'Pyrex' modified spoon gauge[7], with a sensitivity magnification of 45 : 1 compared with a mercury manometer. The resonance lamp and the optical system are more fully described elsewhere[8]. Mercury vapour was rigorously excluded from the reaction system to avoid the possibility of photosensitization.

Nitrosyl chloride was prepared by allowing a mixture of 0·2 atm. chlorine and 0·8 atm. nitric oxide to stand together in a darkened bulb for three days. The mixture was condensed in a liquid nitrogen trap, and the temperature then raised to −80° C, when the excess nitric oxide was pumped off. The crude nitrosyl chloride was redistilled under reduced pressure, and stored as a solid at liquid nitrogen temperature.

The quantum yields obtained for a number of different pressures of nitrosyl chloride, and for mixtures of nitrosyl chloride with inert gases, are shown in Table 1.

Table 1. QUANTUM YIELDS OF THE PHOTOLYSIS OF NITROSYL CHLORIDE

Run	Mixture	$T = 25°$ C quantum yield ($\lambda = 2537$ Å)
1	6 cm NOCl	1·95
2	6 cm NOCl	2·00
3	6 cm NOCl	1·95
4	6 cm NOCl	1·96
5	12 cm NOCl	1·92
6	12 cm NOCl	1·83
7	2 cm NOCl	2·34
8	1 cm NOCl	1·88
9	6 cm NOCl + 76 cm N_2	1·92
10	6 cm NOCl + 40 cm CO_2	1·88
11	6 cm NOCl + 76 cm Ar	1·90

It is seen that the quantum yield of the photolysis is approximately independent of the pressure of the nitrosyl chloride and of the presence of gases such as nitrogen, argon or carbon dioxide. The mean value for the quantum yield is 1·96 ± 0·08, so it may be concluded that reaction (2) proceeds to a negligible extent. The insensitivity of the quantum yield to the presence of carbon dioxide is further evidence that vibrationally excited nitric oxide does not enter into the reaction, since carbon dioxide might be expected to be an efficient quenching agent for vibrational excitation.

The results of Kistiakowsky may now, therefore, be extended into the ultra-violet region, the quantum yield remaining close to two.

I thank Prof. R. G. W. Norrish for providing the facilities of his laboratories.

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Structure of the β-Lactoglobulin Tetramer

DETAILED investigations of the association behaviour of the genetic variants of β-lactoglobulin have shown that the *A* variant aggregates strongly and reversibly to a tetramer[1,2] of 144,000 molecular weight in the cold between *p*H 3·7 and 5·2. The thermodynamics of this association have established the tetramer structure to be cyclic, involving the closing of four bonds[1,2], and its hydrodynamic properties[2,3] show it to be compact. A small-angle X-ray scattering examination of this association[4] has led to the conclusion that, of the various general types of possible tetramer structures, a cubic array of eight spheres was the most probable. This conclusion was based on comparison of the measured radius of gyration of the tetramer (34·4 Å) with values calculated for various models formed of four of the monomeric units deduced by Green and Aschaffenburg[5] from X-ray diffraction examination. These monomeric units have been shown to persist in solution[4]. The monomer unit consists of a combination of two identical spheres (of molecular weight 18,000) 17·9 Å in radius, impinging by 2·3 Å along their centre-to-centre axis; these two spheres are, furthermore, related by a dyad axis of symmetry through the plane of contact formed between them. Recently, we have carried out a detailed examination of compact models in the light of known structural requirements, the results of which we wish to report in this communication. The basic requirements are that all four monomer molecules must be identical with each other[2], the monomer structure must be maintained intact in the tetramer[4], and the structure must fit all known experimental information and be symmetrical in nature[6].

First, let us examine the previously proposed structure[4], that is, the cubic array of eight spheres, shown in Fig. 1*A*. In this model the four 36,000 molecular weight monomer units are aligned with polar axes vertical, and with the surfaces of contact between the spherical sub-units lying in one plane (Fig. 1*B*); the four dyad axes of symmetry are in the same plane along the diagonals of the cross-section square. Careful examination of this structure reveals that the closing of four bonds between the monomers in such manner that each spherical sub-unit is linked to one belonging to another monomer molecule, without at the same time violating the dyad axes of symmetry, requires the presence of two pairs of monomers in which the tetramerization sites are disposed as shown in Fig. 1*C*, that is, each type is the mirror image of the other[7]. Since this structure violates the requirement of identity between the four monomers, it must be ruled out. This requirement can be satisfied, however, by the second cubic structure shown in Fig. 1*D*. In this, one pair of monomers is rotated 90° with respect to the other, the surfaces of sub-unit contact (shown shaded) now lying in pairs in two mutually perpendicular planes; the dyad

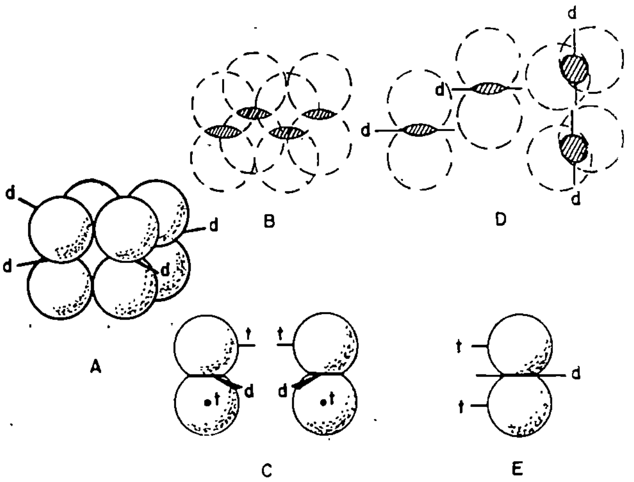

Fig. 1. Cubic models of lactoglobulin tetramer. (*A*) All four double spheres aligned vertically; (*B*) same, showing planes of sub-unit contact; (*C*) monomer structures required to close tetramer bonds in model (*A*); (*D*) monomers aligned in two pairs, one vertical, the other horizontal; (*E*) tetramer bond location on monomers in structure (*D*). In this and Fig. 2 the dyad axes of symmetry are designated by *d*, the tetramer bonds by *t*

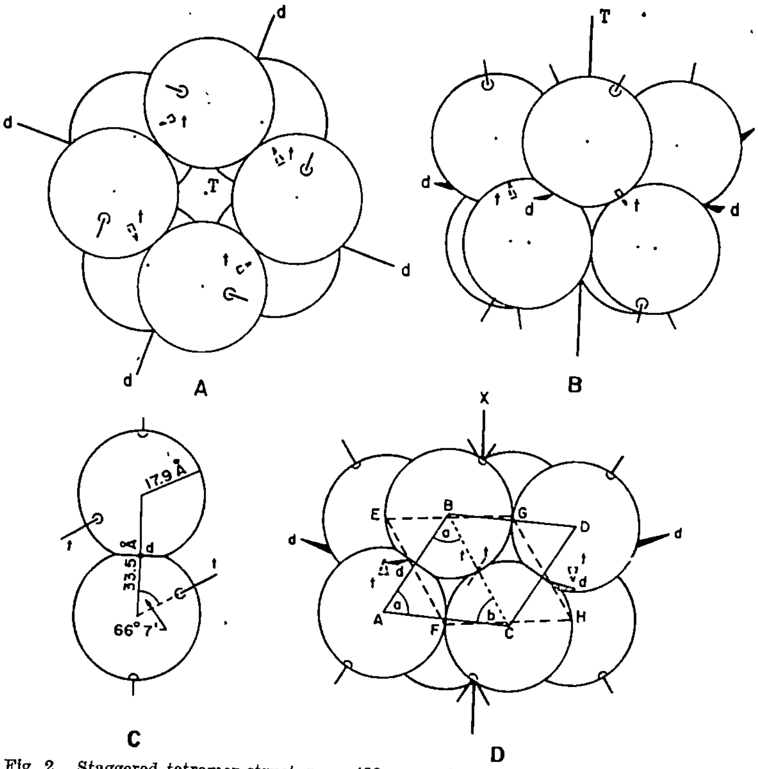

Fig. 2. Staggered tetramer structures. 422 symmetry: (*A*) top view; (*B*) side view. *T* indicates the tetrad axis of symmetry; (*C*) monomer structure required by structure (*A*, *B*); (*D*) 222 symmetry: *X* indicates overall dyad axis of symmetry; distances *AB*, *CD*, *EF*, *GH* = 33·5 Å; *AE*, *BC*, *FG*, *DH* (along tetramer bonds) = 35·8 Å. Angles *a* = 62° 8′; *b* = 55° 44′

axes are located in pairs, one pair remaining in the horizontal intersphere plane, the other pair on a vertical line going through the sub-unit contact planes of the monomers the polar axes of which are in the horizontal position. In this arrangement, the two tetramer bonds of a monomer must be located in one plane and directed at a right angle to the polar axis of the two-sphere structure, as shown in Fig. 1*E*. While this structure satisfies the requirements of structural identity, it lacks overall symmetry and, as such, is probably less likely. Furthermore, this model requires a 90° rotation of each sub-unit along the tetramer bond with respect to its tetramer partner: complementarity, if necessary for this type of bond, would not be compatible with the dyad axis of symmetry.

A symmetrical tetramer structure, satisfying all the requirements, can be constructed by arranging the monomers in an inclined array, which results in the decahedron shown in two views in Fig. 2*A* and *B*. Here, the four dyad axes of symmetry, *d*, and the four points of tetramer contact, *t*, are all in one single plane perpendicular to a central tetramer axis, *T*. The dyad axes are normal to this central axis. The tetramer bonds lie on the corners of a square; the two bonds on each monomer are located symmetrically on the sub-units and their projection on the plane of sub-unit contact forms a 90° angle. In the tetramer, they are inclined at an angle of 35° with respect to the vertical, the bond from the upper sphere pointing downward, that from the lower sphere upward. Thus, the upper sphere of each monomer forms a tetramer bond with the lower sphere of another monomer. Since the monomeric units are inclined at an angle of 31° with respect to the vertical, the inclination of the tetramer bonds with respect to the polar axis of the monomer is 66°. When viewed along the tetramer axis, *T* (Fig. 2*A*), the centre of each sphere in the upper layer is located asymmetrically between those of the two lower ones with which it is in contact. This arises because of the difference in centre to centre distances: 33·5 Å within the monomer, 35·8 Å along the tetramer bond.

The locations of the tetramer bonds and dyad axis in the monomer are shown in Fig. 2*C*. The three-sphere relationship in the tetramer structure of Fig. 2*A*–*B* is identical to the one shown by triangle *ABC* in Fig. 2*D* (in which another possible structure, to be discussed as follows, is depicted); angles *a* are 62° 8′, angle *b* is 55° 44′. The structure of Fig. 2*A* and *B* is characterized by a principal four-fold axis of symmetry, *T*, which coincides with the vertical axis through the centre of the structure. Fig. 2*A*–*B*, then, has 422 symmetry. Furthermore, in this model, intermolecular contacts are limited to the tetramer bond sites plus two further points of contact with neighbouring spheres. A cavity is present along the vertical tetrad axis, with an average diameter of about 18 Å; this permits access of water molecules, leaving some interior molecular surface in contact with solvent. Since it is quite probable that the surface of the protein molecule contains a high density of polar residues[8], this structure entails the burial of a minimal amount of polar side-chains in the low dielectric constant interior of the tetramer and, thus, the expenditure of least energy for this purpose[8–11].

One more structure which should be considered is shown in Fig. 2*D*. This is derived from the previous one by rotating alternate monomers through 45° in opposite directions about the tetramer bonds. In this structure the monomers are located in two lozenge-shaped arrays of four spheres each, the short diagonals of the two lozenges being in parallel planes and inclined in opposite directions. The lozenges are slightly folded inward along the short diagonal. The four dyad axes of symmetry, *d*, remain, as before, in one plane which also passes through the points of tetramer contact. The tetramer bonds, *t*, are present here in two conformations, two bonds lying along the short diagonals and two perpendicular. The presence of a principal dyad axis of symmetry, *X*, gives this structure 222 symmetry. In this structure, however, there is less symmetry than in the previous one. The tetramer bonds exist in two different conformations making structural complementarity across these bonds impossible. Also, the spherical sub-units are in two groups, differing by their environment: four, units *B*, *C*, *F* and *G*, make contact with five other spheres each; the others, *A*, *D*, *E* and *H*, make contact with four other sub-units each. Thus, the amount of monomer molecular surface which must be buried in the hydrophobic interior of the tetramer is considerably larger than in structure 2*A*. As a result, it is considered that structure 2*D* is less likely to occur, even though it is an isomer of the previous one formed only by rotation about the tetramer bonds. It is of interest to note that the model of Fig. 2*D* is one of the extremes allowed by the rotation of Fig. 2*A* about the tetramer bonds (assuming no complementarity); the other extreme (a mirror image) is produced by 90° rotation of 2*D* in the opposite direction.

The radii of gyration of the three structures have been calculated using the monomer molecular dimensions of Green and Aschaffenburg[5], with the results shown in Table 1. As can be seen, all three fit the small-angle X-ray scattering data[4] equally well.

On the basis of the foregoing analysis, and in particular the element of symmetry present in each model considered and the energy required to bring polar residues into the low dielectric medium in the interior of the tetramer, the structure of Fig. 2*A*–*B* (422 symmetry) is regarded as the favoured one. (The identical tetramer structure has been deduced independently by Dr. D. W. Green

Table 1. RADII (R) OF GYRATION OF TETRAMER MODELS

Model	R (Å)
Cube	33·4
422	33·4
222	33·1
Experimental	34·4 ± 4

(private communication).) Structural fluctuations between it and the two 222 structures (Fig. 2*D* and its mirror image) are not excluded, although unlikely, since they would destroy complementarity about the tetramer bonds and would involve the expenditure of extra energy in the burial of additional polar groups.

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[7] The cubic structure (Fig. 1*B*) could be satisfied also if each monomer sub-unit formed bonds with two sub-units of other monomers on tetramerization. This would require the closing of eight bonds on tetramerization and is thus rather unlikely. In this case, the symmetry requirements would be satisfied only if the eight bonds were of a heterologous, rather than homologous, nature.

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BIOCHEMISTRY

Properties of the Penicillin Deacylase Enzyme of *Escherichia coli*

THE microbial enzyme which hydrolyses the side-chain from a penicillin to give 6-aminopenicillanic acid (6-APA) and carboxylic acid, or which catalyses the synthesis of penicillin from 6-APA and a carboxylic acid or its derivative, has been termed penicillin amidase[1-6], penicillin acylase[7], benzylpenicillin acylase[8], penicillin splitting and synthesizing enzyme[9,10] or acyl transferase[11]. Investigations of the relative ease with which an enzyme from various sources will hydrolyse a series of penicillins has revealed the existence of at least two types of enzyme[9], one hydrolysing phenoxymethylpenicillin much more readily than benzylpenicillin and the other hydrolysing benzylpenicillin much more readily than phenoxymethylpenicillin. Experiments on the hydrolysis of a series of penicillins with the *Nocardia* and *Proteus* enzymes[8] have shown that the enzyme of these organisms has preference for a particular side-chain, namely, the phenylacetyl side-chain, and that all deviations from this structure cause reduction in enzyme activity. These reports indicate that substrate specificity is governed by the nature of the side-chain of the penicillin. Very little work has been done on the effect of structural modification of the acylated group on enzyme activity, but Huang, Seto and Shull[8] do report that the use of the amide or ester of benzylpenicillin and phenoxymethylpenicillin or replacement of 6-APA by 7-aminocephalosporanic acid in various penicillins has only a minor effect on enzyme activity.

The purpose of the present communication is to report that the penicillin acylase enzyme produced by a strain of *Escherichia coli* (B.R.L. 1040) is able to remove the phenylacetyl group from a wide range of phenylacetylated compounds and is thus not specific for the deacylation of penicillins and their derivatives. or for the cephalosporins.

Table 1. RATES OF HYDROLYSIS OF VARIOUS PHENYLACETYL DERIVATIVES BY THE ACYLASE ENZYME OF *E. coli* B.R.L. 1040

	Initial rate of hydrolysis* (µmoles/ml./min)
Phenylacetylglycine	0·12
Phenylacetyl D-α-aminophenylacetic acid	0·00
Phenylacetyl L-α-aminophenylacetic acid	0·092
Phenylacetamide	0·072
Benzylpenicillin	0·066
Phenylacetyl ε-aminocaproic acid	0·056
Phenylacetyl DL-leucine	0·048
Benzylpenicillin penicilloic acid	0·0144

* The reaction mixtures consisted of M/100 substrate and *E. coli* B.R.L. 1040 cells collected and resuspended at one-quarter the density at which they occurred in the growth medium. The *p*H was kept at 8·0 by automatic addition of M/50 sodium hydroxide. The reaction mixtures were stirred at 37° C.

The rate of hydrolysis for all compounds except benzylpenicillin was determined by assaying the liberated amino-acid or ammonia by the ninhydrin method using the appropriate standard curves. The rate for benzylpenicillin was determined by titrating the released phenylacetic acid with M/50 caustic soda solution. Similar results were obtained by assaying the released 6-APA using the biological assay after conversion to benzylpenicillin with phenylacetyl chloride.

The rates of hydrolysis of various compounds containing the phenylacetyl group are shown in Table 1. The rate of hydrolysis is markedly affected by the structure of the acylated compound and it will be seen that benzylpenicillin is not the best substrate. Substrate specificity experiments using *E. coli* B.R.L. 1040 have shown that there is a very close relationship between the structures of the acyl groups preferred for hydrolysis or synthesis of penicillins and those preferred for hydrolysis or synthesis of non-penicillins. From this it is inferred that the same enzyme is involved in both penicillin and non-penicillin reactions. This is supported by the fact that examination of the distribution of benzylpenicillin deacylase and phenylacetylglycine deacylase activities within a group of *E. coli* strains has shown that the two activities always occur together and in the same proportions.

In keeping with other amino-acid acylase enzymes, for example, that of pig kidney[12], the *E. coli* B.R.L. 1040 enzyme is specific for the hydrolysis of acylated L-α-amino-acids (Table 1). Phenylacetyl-L-α-aminophenylacetic acid was completely hydrolysed while the acylated D-form was unattacked. The phenylacetyl derivatives of several DL-amino-acids were hydrolysed only to the extent of 50 per cent, but phenylacetylglycine was completely hydrolysed. Benzylpenicillin, containing the acylated L-amino group, was also completely hydrolysed.

Reaction of the *E. coli* B.R.L. 1040 enzyme with a series of penicillins has shown that benzylpenicillin and substituted benzylpenicillins were the best substrates, and tests on a series of carboxylic acids for enzymatic coupling with 6-APA showed that phenylacetic acid and substituted phenylacetic acids were the best substrates. When salts of various carboxylic acids were included in media used to cultivate the *E. coli.* B.R.L. 1040 only phenylacetic acid and structurally similar compounds were found to have the good enzyme-inducing action observed by Kaufmann and Bauer[13], phenylacetic acid being the best.

The foregoing results indicate that *E. coli* B.R.L. 1040 produces an acylase enzyme which has specificity for the phenylacetyl group but which is not specific for penicillins. It is suggested that for the convenience of distinguishing this enzyme from other penicillin hydrolysing enzymes it be called a phenylacetylase.

Experiments with *Achromobacter* sp. B.R.L. 1755 (N.C.I.B. 9424) have shown that this organism produced an enzyme which hydrolyses phenoxymethylpenicillin more readily than benzylpenicillin, and the same enzyme preparation hydrolysed phenoxyacetylglycine more readily than phenylacetylglycine. Inasmuch as phenoxymethylpenicillin is hydrolysed more readily than benzylpenicillin, this enzyme is similar to those produced by moulds and some Actinomycetes[4,6,9].

The hydrolysis of various *N*-phenylacetylated compounds, including benzylpenicillin by *E. coli*, suggests that penicillin amidase is an acylase which does not differ fundamentally from other acylase enzymes and is able to hydrolyse benzylpenicillin because of its specificity for the phenylacetyl group. Liberation of 6-APA by deacylation of phenoxymethylpenicillin using preparations of animal tissue has already been reported[14]. Since then we have found that pig kidney acylase I (British Drug Houses, Ltd.), which will hydrolyse phenoxyacetylglycine, octoylglycine and hexoylglycine but not phenylacetylglycine, will hydrolyse the corresponding penicillins, phenoxymethylpenicillin, heptylpenicillin and pentylpenicillin, but not benzylpenicillin. It seems probable that the liberation of 6-APA by enzymatic splitting of penicillins by preparations from various micro-organisms[1–11], animals[14] and plants[15] is due to acylase enzymes which are specific for the acyl group present in the penicillin which is cleaved.

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Variety of Substrates for a Bacterial Benzyl Penicillin-splitting Enzyme

PREVIOUS work on a cell-bound bacterial enzyme which catalyses a reversible enzymatic cleavage of benzyl penicillin to 6-aminopenicillanic acid (6-APA) and phenylacetic acid had shown that this enzyme represents an acyl transferase which, under suitable conditions, is able to synthesize penicillins by transferring acyl- and α-aminoacyl residues from acylamino acids, acyl amides, acylated hydroxycarboxylic acids, mercaptocarboxylic acids, alcohols, phenols and thiophenols to the amino group of 6-APA (refs. 1–3). Free amino-acids, ammonia, hydroxycarboxylic acids, mercaptocarboxylic acids, alcohols phenol and thiophenol, liberated in the course of those reactions, may be considered to be hydrolysis products of the acyl compounds used.

Table 1. HYDROLYSIS OF VARIOUS ACYL-COMPOUNDS BY THE PENICILLIN-SPLITTING ENZYME AT NEUTRAL REACTION

(1) Phenylacetyl L-glutamic acid $\xrightarrow{+H_2O}$ glutamic acid + phenylacetic acid
(2) DL-α-Aminophenylacetyl glycine $\xrightarrow{+H_2O}$ α-aminophenylacetic acid + glycine
(3) DL-α-Phenoxypropionyl amide $\xrightarrow{+H_2O}$ α-phenoxypropionic acid + ammonia
(4) D-α-Aminophenylacetyl methyl ester $\xrightarrow{+H_2O}$ α-aminophenylacetic acid + methyl alcohol
(5) Phenoxyacetyl glycollic acid $\xrightarrow{+H_2O}$ phenoxyacetic acid + glycollic acid
(6) *p*-Methoxy-phenoxyacetyl thioglycollic acid $\xrightarrow{+H_2O}$ *p*-methoxy-phenoxyacetic acid + thioglycollic acid

By means of some representative reactions listed in Table 1 we wish to demonstrate that a hydrolytic cleavage of acyl- and α-aminoacyl compounds as mentioned above is not dependent on the presence of 6-APA.

On the basis of these findings it is not the penicillin nucleus which seems to be important for the substrate specificity of the enzyme, but rather the acyl residue, which may be linked to different compounds. Considering the impressive variety of enzymatic reactions including those not involving penicillins, the synonymous names 'penicillin amidase' and 'penicillin acylase' proposed for this enzyme by several authors and discussed separately by Claridge *et al.*[4] and Huang *et al.*[5] are not satisfactory.

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Influence of Extraneous Ribonucleic Acid on the Differentiation of Hæmatopoietic Tissue in Chick Embryos

EXPERIMENTAL results are given here which seem to confirm the work of several investigators[1–4] concerning the ability of phenol-extracted RNA to stimulate protein synthesis in embryonic cells.

It has, indeed, been found possible to increase somewhat the amount of hæmoglobin synthesized by the isolated vascular area of the chicken embryo by the addition of phenol-extracted chicken reticulocyte RNA, rabbit reticulocyte RNA or total 3·5-day chick embryo RNA. Moreover, isolated fragments of the extra-embryonic region of the blastodisk have also been found to increase their potential for hæmoglobin synthesis in the presence of chick embryo RNA added to their culture medium.

RNA extracted by a modification of the method described by Fraenkel-Conrat[5] was suspended in chick Ringer and added to a yolk agar medium of the type recommended by Britt and Hermann[6]. Chick embryo blastodisks were explanted, washed in Ringer and transferred to the experimental medium after removal of the embryo at stages between 6 and 11 (Hamilton–Hamburger classification) such that the explanted tissue cultivated consisted mainly of presumptive hæmatopoietic tissue, particularly favourable for examining variations in net hæmoglobin synthesis over a limited experimental period.

After culture for 24 h, the explants were homogenized separately and determinations of hæmoglobin, total RNA and residual proteins were carried out by the methods of O'Brien[7], Ogur and Rosen[8] and Lowry[9], respectively.

It has thus been possible to record, in certain experimental series, an increase in the net quantity of hæmoglobin synthesized over a 24-h period by the treated explants, cultivated on an agar yolk medium containing additional RNA. Similar effects were recorded with chicken reticulocyte RNA, rabbit reticulocyte RNA or chick 3·5-day embryo RNA. A difference, varying from 4 to 39 per cent was recorded between treated and control embryos (Table 1). It has not been found possible to detect a linear relationship between increase in concentration of added RNA and the increase in hæmoglobin synthesis. In fact, a concentration of 2 mg/ml., which

Table 1

RNA origin and concentration	Stage	Control blastodisks Hb (μg)	Control blastodisks RNA (μg)	Control blastodisks Res. prot. (μg)	Treated blastodisks Hb (μg)	Treated blastodisks % increment Hb	Treated blastodisks RNA (μg)	Treated blastodisks Res. prot. (μg)
Chicken reticulocytes 1 mg/ml.	8	7·05 ± 0·05	26·2	165	8·45 ± 0·19	19·9	30·6	166·6
	9 −	8·25 ± 0·25	26·8	155	9·4 ± 0·63		25·8	202
	9	9·0 ± 0·005	24·1	145	10·5 ± 0	24·8	35·6	230
	9 +	11·2 ± 0·81	22·1	140	13·5 ± 0·7		25·2	152·5
	10 −	11·4 ± 0	30·8	180	15·4 ± 0		38·4	250
	10	14·5 ± 0·07	31·0	240	17·05 ± 0·32	25	38·4	495
	10 +	15·5 ± 0·25	43·8	340	20·1 ± 0·44		49·2	466·6
Rabbit reticulocytes 50 μg/ml.	6	5·3 ± 0·25	21·1	143	6·3 ± 0·86	20	33·9	163
	8	8·9 ± 0·82	30·3	316	9·6 ± 0·32	7·8	34·9	332
	9	9·4 ± 0·21	40·3	369	12·1 ± 0·14	34	47·9	387·5
	10	15·6 ± 0·2	51·3	410	20·0 ± 1·0	28	48·6	445
Rabbit reticulocytes 50 μg/ml.	8	6·4 ± 1·0	35·2	225	7·0 ± 1·2	9·4	42·7	270
	9	8·2 ± 0·24	49·0	300	8·6 ± 1·74	4·8	43·0	286
	10	11·4 ± 1·6	45·0	353	13·7 ± 1·85	22	50·2	382
3-day chick embryos 50 μg/ml.	−8	7·4 ± 1·0	28·5	240	8·1 ± 1·0	8	21·0	240
	9	8·2 ± 1·5	35·0	320	10·4 ± 0·5	27	34·0	400
	10	10·6 ± 2·0	29·3	320	13·8 ± 0·9	30	35·0	400
3-day chick embryos 50 μg/ml.	8	6·3 ± 0·36	23·3	160	8·1 ± 0·44	29	28·5	180
	9	8·9 ± 0·79	31·3	250	12·1 ± 0·18	35·6	33·2	280
	10	14·1 ± 0·24	42·5	332	19·7 ± 0·25	39	49·0	452

was the highest concentration used, was found to be inhibitory in this system.

Normal variations in net synthesis in control explants may mask to a certain extent the differences between treated and control embryos, but even if this were so, the degree of stimulation of hæmoglobin synthesis is in no way spectacular. The fact that anabolism in the embryonic cell is extremely rapid may explain the limited extent to which external RNA, entering the cell, may find vacant sites at which to interfere with normal metabolism. Added RNA may also be partly degraded by intracellular RNase.

The effect of adding ribonucleic acid to the external medium may be greater in conditions creating a certain deficit in potential metabolism. It has, for example, been shown by Settle[10] that explants of the anterior region of the chick blastodisk at the primitive streak stage only produce hæmoglobin in 15 per cent of cases when grown on a rich albumen yolk agar medium. By adding 50 μg/ml. of chick embryo RNA to the medium, it was found possible to augment the number of such explants producing hæmoglobin from 18 per cent (controls) to 73 per cent in treated embryos.

Embryonic explants cultivated on purely mineral medium[11] did not respond to the presence of additional RNA; a rich agar medium containing yolk and albumen was required to obtain positive results. This may mean that ribonucleic acid molecules may only penetrate the cell in conditions favouring pinocytosis.

Some experimental results were negative, even in the conditions described, a result which seemed most easily explained by the degradation of ribonucleic acid under storage conditions or by intracellular RNase: the altered molecule may be inactive or even inhibitory.

The experiments described here used, in each case, a type of RNA the origin of which presupposed the possibility of its being used by the embryonic cell, as such, without modifying the normal metabolism of the cell. The question remains to be answered whether the embryonic cell responds more readily to RNA of homologous or heterologous origin. The relative efficiency of different types of RNA (transfer, ribosomal or messenger RNA) also needs to be established. It may be supposed that the RNA used in these experiments, extracted at room temperature, consisted mainly of cytoplasmic RNA, since further extractions with phenol at 45° and 65° C provided two further fractions differing from the first in their sedimentation characteristics, specific radioactivity after labelling with phosphorus-32, and base composition. When the extraction at 65° C is performed in the presence of sodium dodecyl sulphate, the resulting extract from chicken reticulocytes contains about 10 per cent DNA. The biological activity of the fractions extracted with hot phenol will shortly be tested.

In conclusion, it may be said that while these experimental results appear to confirm the role of ribonucleic acids in specific protein synthesis, they neither prove nor disprove the possibility of obtaining a specific response from embryonic cells by such externally supplied RNA.

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Inhibition by a Carcinogenic Hydrocarbon of Incorporation of Tritiated Cytidine into Mouse Epidermal Cells

EARLY effects of carcinogenic hydrocarbons on the cell cycle and on deoxyribonucleic acid (DNA) synthesis have been noted by several authors. McCarter and Quastler[1,2] found that 7,12-dimethylbenz[α]anthracene (DMBA) decreases the rate of incorporation of ^{3}H-thymidine into DNA and prolongs the DNA synthetic phase of the generation cycle of mouse epidermal cells. Evensen[3] found that 3-methylcholanthrene alters the proportion of epidermal cells labelled with ^{3}H-thymidine in the hairless mouse. Jensen *et al.*[4] reported a reduction in uptake of ^{3}H-thymidine in various tissues of the mouse following the administration of DMBA, including some tissues in which tumours do not usually arise after treatment with this agent. Elgjo[5] reported that 3-methylcholanthrene changes the mitotic phase of the cell cycle over an eleven-week period. We now report an early effect of DMBA on the synthesis of ribonucleic acid (RNA) in mouse skin.

The mice used in these experiments were derived from the *CFW* strain obtained from Carworth Farms, New City, and have been maintained in this laboratory by brother–sister mating for 16 generations.

The back of each mouse was shaved and 0·15 ml. of 0·05 per cent DMBA in acetone was applied to an area of 2 cm^2 (ref. 6). After varying lengths of time the mice were killed. The full thickness of treated skin and a piece of adjacent untreated skin were excised, cut into pieces 2–3 mm^2 and incubated at 37° C in a shaking water bath for varying lengths of time in Tyrode's medium containing ^{3}H-cytidine, specific activity 1·5 c./mM at a concentration of 10 μc./ml. (Schwarz Bioresearch, Inc., Mount Vernon, New York).

Pieces of skin incubated in Warburg vessels respired at a constant rate up to at least 6 h. This respiration was penicillin-resistant and totally inhibited by boiling for 10 min. The rate of respiration was not altered to a detectable degree by DMBA treatment or the presence of the radioisotope in the incubating medium. The incubation procedure was used because doses of ^{3}H-cytidine up to 500 μc. per mouse given by intraperitoneal, intracardiac or subcutaneous injection failed to produce any labelling in the skin when autoradiographs were stored for 2 months.

The pieces of skin were removed from the incubation medium at times from zero to 11 h, washed in water and fixed in formol saline for 5–7 days. Sections having a thickness of 5–7μ were cut and autoradiographs were prepared using Kodak '*AR* 10' stripping film. The slides were examined using phase contrast.

The epidermis bordering on a scissor cut, damaged by crushing, contained no grains and served for an assessment of the background count. Within a few μ of the cut edge the grain count over epidermal cells increased sharply, remained at a constant level for 100–200μ, then fell off again fairly quickly. This border of 100–200μ was the area counted. The average grain count was determined for cells sectioned through the nucleus.

Extraction of RNA with perchloric acid[7] removed approximately 90 per cent of the label from the sections. The DNA was not appreciably labelled by incubating pieces of skin in Tyrode's medium containing ^{3}H-thymidine since the mice used were in the resting phase of the hair growth cycle. With ^{3}H-cytidine the pattern of labelling was nuclear and nucleolar at early times, then later spread to include the cytoplasm. This pattern of labelling has been reported for RNA precursors by a number of authors[8,9].

Fig. 1 shows the progress with time of labelling in epidermal cells in Tyrode's medium containing ^{3}H-cytidine. Under the continuously labelled conditions, the control area took up more ^{3}H-cytidine than was taken up by the treated area. When the skin had been transferred to non-radioactive cytidine at 1 h, the percentage reduction in grain count in DMBA-treated skin compared with the control was similar to that obtained under the continuous labelling condition. The results also indicated that there is no significant cytidine pool in epidermal cells[10].

Table 1 shows the effect of DMBA on the reduction in grain count at the indicated days after DMBA application. The 'unwashed' heading refers to experiments in which DMBA was left on, and the 'washed at 1 h' heading refers to experiments in which the DMBA remaining on the surface was removed by diethyl ether at 1 h[6]. Each test was compared with its particular control. The results were expressed as a percentage reduction in grain count so that decreases at lesser and greater degrees of labelling would be additive without weighting the results in favour of the higher absolute decreases found at longer incubation times.

It can be seen that there was a decrease ($P < 0·001$) in grain count with DMBA treatment. Although the 'washed' experiment showed a smaller decrease, it was still highly significant ($P < 0·001$).

Table 1. Effect of DMBA on incorporation of ^{3}H-cytidine into RNA of epidermal cells measured by counting silver grains over sectioned nuclei of 300–600 cells from treated and adjacent control areas of mouse skin

Time after DMBA application (days)	Percentage reduction in grain count (mean ± *S.E.*)
Unwashed	
1	30·4 ± 0·8
3	36·5 ± 1·9
7	28·5 ± 1·4
Washed at 1 h	
1	22·9 ± 2·2
	16·9 ± 1·7

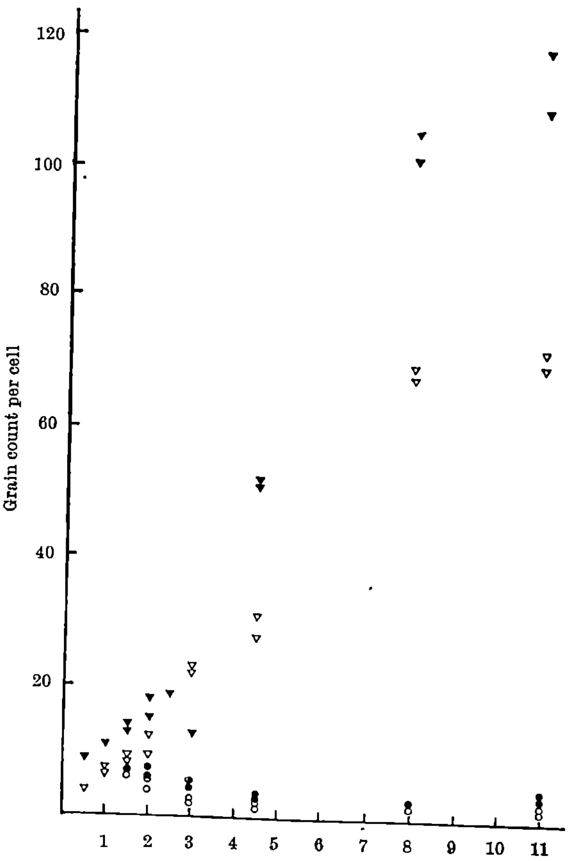

Fig. 1. No. of silver grains per cell is plotted against the time of incubation of the pieces of skin in Tyrode's medium. Triangles represent continuous incubation in Tyrode's medium containing ^{3}H-cytidine. Circles represent incubation for 1 h in Tyrode's medium containing carrier cytidine (100 times the concentration of the radioactive cytidine). Open figures represent values for skin to which DMBA was applied 24 h prior to time zero and solid figures represent values for adjacent untreated skin from the same mouse

The differences in the percentage reduction in grain count between 'washed' and 'unwashed' conditions may be explained by the fact that tissue damage was observed histologically 3–5 days after DMBA treatment in the 'unwashed' experiment, but not in the 'washed' experiment. Earlier observations[11] had suggested that the tissue damage observed after DMBA treatment may be a side-effect unrelated to the initiation of a tumour. On present evidence it is not possible to implicate tissue damage as the factor responsible for the reduction in grain count in the 'washed' experiment. The possibility remains that the effect of DMBA on RNA metabolism may be related to the tumour-initiating effect of the hydrocarbon.

In experiments in which the pieces of skin were changed from ^{3}H-cytidine to excess non-radioactive cytidine at 1 h a comparable percentage reduction in grain count in the 'unwashed' experiments was found (36·9, 44·0 and 33·8 for 1, 3 and 7 days), but not enough determinations were made in the 'washed' experiments to give a reliable answer. These results indicate that there was a reduction in incorporation of ^{3}H-cytidine into the metabolically stable RNA.

The decreased incorporation of ^{3}H-cytidine into RNA could reflect a number of changes including an absolute reduction in synthesis of RNA, increased production and utilization of endogenous precursors over an exogenous supply, more rapid degradation of the radioactive precursors into non-precursor substances, inhibition of phosphorylation of RNA precursors or the activation of an RNA exonuclease leading to breakdown of rapidly labelled RNA[12]. Alternatively, a block in the cytidine deaminase reaction at any level of phosphorylation could allow incorporation of label into the cytidine moiety only instead of into both cytidine and uridine. Because the skin is unfavourable from both a histological and biochemical point of view, this system cannot be used to resolve the possible mechanism of the effect of DMBA on ^{3}H-cytidine incorporation into RNA. Other systems are being investigated with this aim in mind.

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Carrier-free (7-^{3}H)-Testosterone

During the course of clinical investigations being carried out here the need arose for tritium-labelled testosterone of very high specific activity. Although 1,2-*tritiated* androgens are available[1,2], their use was precluded in this instance by the extensive loss of radioactivity which would occur during the metabolism of the steroid[3]. Carrier-free (7-^{3}H)-testosterone acetate was prepared by catalytic reduction in pure tritium of 17β-acetoxy-androstan-4,6-dien-3-one.

Δ^6-Testosterone acetate[4,5], m.p. 141·5°–142·5°, $\lambda_{\max}^{\text{EtOH}}$ 284 mμ ($\log\varepsilon = 4{\cdot}40$), $\lambda_{\max}^{\text{KBr}}$ 5·82μ, 6·08μ, 6·22μ, 6·38μ, 8·12μ, was prepared by dehydrogenation of testosterone acetate with chloranil in *tert*.-butanol[4]. The use of 2,3-dichloro-5,6-dicyano-*p*-benzoquinone catalysed with hydrogen chloride[6] gave the dienone more conveniently and in better yield though mixed with about 10 per cent of the 1,2-unsaturated isomer ($\lambda_{\max}^{\text{EtOH}}$ 244 mμ). This was unacceptable because chromatographic separation of testosterone acetate and its Δ^1- and Δ^6-unsaturated derivatives proved very difficult.

The reactivity with respect to catalytic hydrogenation in neutral solution of the steroidal 4,5-double bond is exceeded only marginally by that of the 6,7-double bond[7]. This difference can be increased by conducting the reaction in anhydrous methanol in the presence of a small but critical concentration of alkali[7]. In a preliminary experiment, Δ^6-testosterone acetate (42 mg) in dry methanol containing 400 mg l.$^{-1}$ of potassium hydroxide was shaken with pre-reduced 5 per cent palladium-on-charcoal catalyst (3·3 mg) under hydrogen at 1 atm. pressure, and allowed to consume 1·2 moles of gas. The solution was filtered, acidified with glacial acetic acid and evaporated. The steroid was separated from inorganic material by leaching with dry chloroform and crystallizing from methanol to yield testosterone acetate, m.p. 139°–141°, $\lambda_{\max}^{\text{EtOH}}$ 241 mμ ($\log\varepsilon = 4{\cdot}21$). The mixed melting point with an authentic sample showed no depression. Traces of the 17β-acetoxy-androstan-3-ones were detected in the mother-liquor by vapour-phase chromatography.

Using the foregoing conditions, a 60-mg sample of Δ^6-testosterone acetate was reduced in carrier-free tritium gas to give testosterone acetate showing no selective absorption at 284 mμ in its ultra-violet spectrum. This material was saponified with 0·2 N alkali in aqueous methanol and the resulting testosterone separated from saturated steroids by paper chromatography (Whatman No. 1 paper; 19 : 1 isooctane–toluene/80 per cent aqueous methanol), the zones being detected by autoradiography. The testosterone was eluted from the paper and shown by ultra-violet spectroscopy and carrier-dilution analysis to have a specific activity of 30·8 curies mmole^{-1}. This value is consistent with the formulation of the product as (7-^{3}H)-testosterone, having arisen from the initially formed (6,7-^{3}H$_2$)-testosterone by loss of the 6-tritium atom through enolization.

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Saturated Hydrocarbons from Autoxidizing Methyl Linoleate

Examination of the vapours of autoxidizing methyl linoleate by gas-liquid chromatography has revealed the presence of several compounds with retention times corresponding to methane, ethane, propane, butane and pentane. Identification of these compounds from autoxidation of a purified unsaturated ester has not, to our knowledge, been previously reported. This finding confirms a prediction made by C. D. Evans *et al.* that low-molecular-weight hydrocarbons would be produced by autoxidation of unsaturated fatty acids[1,2]. They also reported saturated and unsaturated short chain hydrocarbons had been found in products from autoxidation of soybean oil[3]. Buttery and associates identified C_1 to C_5 normal saturated hydrocarbons in dehydrated potatoes, in which linoleic acid is the principal unsaturated acid in the lipids[4]. They speculated that the hydrocarbons might have been formed in autoxidizing dehydrated potatoes by hexanal decomposition that had been catalysed by fat peroxides. However, our findings with methyl linoleate show that the saturated hydrocarbons arise early in the autoxidation process, when aldehydes are either absent or present in undetectable amounts. Therefore, the hydrocarbons are being formed either by an extremely rapid decarboxylation of aldehydes present at a low steady-state concentration or by a totally different mechanism. Japanese workers identified ethane and higher molecular weight alkanes and alkenes from air-oxidation of soybean oil at 240° C (refs. 5 and 6). Their results cannot be compared with ours, however, because our ester was relatively pure and our experiments were performed at room temperature.

We chose methyl linoleate as a simplified system for studying rancidification in parboiled wheat products (bulgur), because linoleic acid is the principal unsaturated fatty acid in the lipids of whole wheat[7]. The original purpose of the experiments was to observe the development of carbonyls produced by autoxidation of unsaturated fatty acids in a system free of the complications inherent in bulgur.

We placed 1-g samples of methyl linoleate (98 per cent purity, as analysed by gas-liquid chromatography) on purified glass wool in 50-ml. Erlenmeyer flasks, capped the flasks with aluminium foil, and exposed them to laboratory light at room temperature. Using a gas-tight syringe, we withdrew vapour samples through the foil at intervals and injected the vapour into a gas chromatograph equipped with a dual hydrogen flame detector and a 7-ft. column containing 'Apiezon *M*' on firebrick. Initial vapour samples showed only the usual air peak. As autoxidation proceeded, we observed sharp peaks possessing much lower retention times than any authentic aldehyde, ketone, or alcohol. These compounds produce wide peaks accompanied by some tailing. The sharpness of the unidentified peaks suggested that they were saturated hydrocarbons. These sharp peaks began to appear after the ester had been exposed to air less than 24 h, long before hexanal, pentanal, or higher molecular weight carbonyls appeared.

Further confirmation of the saturated nature of the compounds was obtained by shaking 10 ml. of the vapour from the autoxidizing methyl linoleate with 1 ml. of concentrated sulphuric acid, which should absorb any olefines or oxygen-containing compounds. Only three symmetrical peaks persisted in the chromatograms after the sulphuric acid treatment. Two of these peaks were tentatively identified as butane and pentane by retention times. The 'Apiezon' column, which had been chosen for its ability to separate carbonyls, failed to separate methane, ethane and propane.

In order to determine whether the third peak indeed consisted of methane ethane, and propane, the untreated vapours from autoxidizing methyl linoleate were further analysed on a molecular sieve column. Five peaks possessing retention times corresponding to hydrocarbon standards were present in these chromatograms and persisted after sulphuric acid treatment. Sulphuric acid-treated vapours of autoxidizing methyl linoleate were also run on a capillary column coated with General Electric silicone oil '*SF*–96 (50)'. Because of losses from stream-splitting, only the peak corresponding to pentane was of appreciable size.

In order to eliminate the possibility that the observed hydrocarbons were artefacts, blanks were run with each gas-liquid chromatography analysis. The blanks were prepared by withdrawing the appropriate volume of air from an aluminium foil-capped 50-ml. Erlenmeyer flask containing purified glass wool and then injecting the required volume into the gas-liquid chromatography apparatus. Chromatograms from the blanks showed only the initial air peak, whether or not the sample had been shaken with concentrated sulphuric acid.

The reference compounds, ethane, propane, and butane, were synthesized from the appropriate alkyl bromide by the Grignard reaction. Before use, alkyl bromides were purified by distillation. Methane was prepared by fusing C.P. anhydrous sodium acetate and C.P. potassium hydroxide. Pentane was obtained from a commercial source at a purity of 99 per cent and further purified by fractionation through a 20-plate 'Nichrome' helix-packed column. Each hydrocarbon gas was purified by shaking with concentrated sulphuric acid.

Table 1. IDENTIFICATION OF HYDROCARBONS IN HEADSPACE FROM AUTOXIDIZING METHYL LINOLEATE

	'Apiezon *M*' column*		Capillary column†		Sieve column‡	
	Authentic sample	Autoxidizing ester vapours	Authentic sample	Autoxidizing ester vapours	Authentic sample	Autoxidizing ester vapours
	Retention times (min)					
Methane	—	—	—	—	0·6	0·6
Ethane	—	—	—	—	1·2 4·3§	1·2 4·1§
Propane	—	—	—	—	2·2	2·2
Butane	0·7	0·7	—	—	9·4	9·2
Pentane	1·0	1·0	14·2	14·0	39·5	40·1

* Column, 7 ft. by 1/8 in. outer diam. stainless-steel, containing 10 per cent 'Apiezon *M*' on firebrick; nitrogen exit flow rate, 60 ml./min; column temperature, 100° C; chart speed, 101·6 cm/h.

† Capillary column, 600 ft. by 0·02 in. internal diam. stainless-steel, coated with General Electric silicone oil '*SF*–96 (50)'; helium exit flow rate, 12 ml./min; column temperature, 32° C; chart speed, 30·48 cm/h.

‡ Column, 2·5 ft. by 1/8 in. outer diam. stainless-steel, containing Linde molecular sieve type 5*A*; helium exit flow rate, 33 ml./min; column temperature, 150° C; chart speed, 30·48 cm/h.

§ Column temperature, 35° C.

In Table 1 the retention times of the five hydrocarbons tentatively identified in the vapours of autoxidizing methyl linoleate are compared with those of authentic hydrocarbons. Since ethane is present in the vapours of autoxidized methyl linoleate in an extremely low concentration, this compound may be an artefact produced by autoxidation of some other contaminating unsaturated acid. Pentane is the major component, representing more than 90 per cent of the hydrocarbon fraction.

Work is in progress to substantiate the identity and to determine the mechanism by which these hydrocarbons are formed during the autoxidation of methyl linoleate.

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A Second Naphthoquinone in Spinach Chloroplasts

DURING the chromatography of lipids from spinach chloroplasts we have observed a compound which appears to be a form of vitamin K which is different from the well-known vitamin K_1. The latter has previously been isolated from chloroplasts[1,2] and is widely distributed in green plants[3].

This second naphthoquinone has been isolated by extracting spinach chloroplasts (about 1,500 mg chlorophyll) with a heptane–propanol mixture[4]. The extract is chromatographed on a 'Decalso' column with heptane, 10 per cent ether in heptane, and ethanol as the eluants. The 10 per cent ether fraction is then re-chromatographed on a 3 : 1 w/w 'Supercell' silicic acid column (5 cm × 25 cm). By eluting with heptane and collecting 10 ml. aliquots, the naphthoquinone can be seen within the first 30 fractions immediately following vitamin K_1. The ultra-violet spectrum of the purified compound (Fig. 1) is similar to the vitamin K_1 and K_2 homologues except that the 269 mμ peak is absent. In this respect the new compound very much resembles the demethylated form of vitamin K_1 isolated by Baum and Dolin from *Streptococcus faecalis*[5]. Also the addition of the ethyl cyanoacetate anion gives a positive Craven's test in the presence of sodium hydroxide which would indicate a demethylated compound[6]. The new quinone shows

Table 1. CHROMATOGRAPHIC PROPERTIES OF THE NAPHTHOQUINONE AND DIFFERENT TYPES OF VITAMIN K

	Quinone	R_F in silica gel '*H*' Benzene	30% ether in isooctane
1	New naphthoquinone	0·56	0·62
2	Vitamin K_1	0·69	0·65
3	Vitamin K_2 (C10)	0·56	0·54
4	Vitamin K_2 (C20)	0·63	0·59
5	Vitamin K_2 (C30)	0·69	0·61
6	Coenzyme Q_{10}	0·39	0·37
7	Plastoquinone *B*	0·80	0·79
8	1 and 2	0·56 and 0·71	0·63 and 0·67

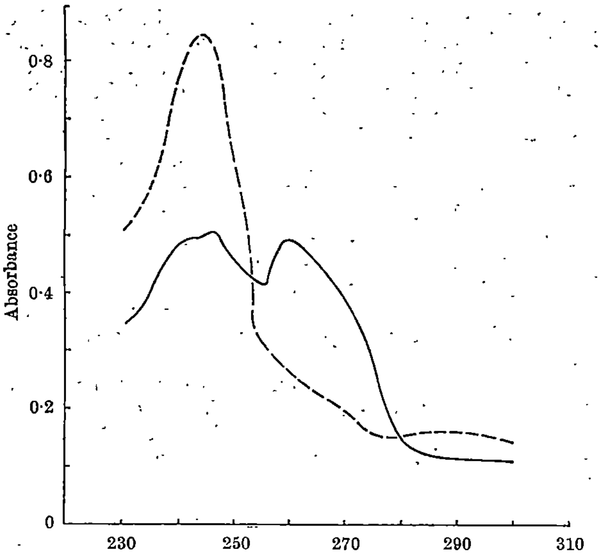

Fig. 1. Spectrum of new naphthoquinone in ethanol. ——, Oxidized form; – – –, after reduction with borohydride

different chromatographic properties from vitamin K_1 on thin-layer silica gel '*H*' in two different developing systems (Table 1), benzene and 30 per cent ethyl ether in isooctane[7]. The infra-red spectrum in carbon tetrachloride indicates a K_1 compound rather than K_2 based on the analysis of Noll[8].

Using a *millimolar* extinction coefficient of 19, which is characteristic of the vitamin K series[9], a maximum concentration of 0·002 µmoles/mg chlorophyll has been observed. From previous investigations of the specificity of naphthoquinones in assays of vitamin K activity it can be predicted that this compound would be difficult to detect in biological assays for vitamin K in plant material[10].

This is the ninth quinone to be found in spinach chloroplasts[4], and its presence should be considered in any of the investigations of restoration of biological activity after solvent extraction.

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Cellulase in Tomato Fruits

There are only a few reports of enzymes from higher plants which can hydrolyse cellulose or water-soluble cellulose derivatives. Several reports show that malt extracts are capable of hydrolysing water-soluble cellulose derivatives[1,3,11] and dispersed cellulose fibres[1,7]. A malt extract was found to hydrolyse reprecipitated cellulose more rapidly than a similar extract from barley seeds[1]. There is a single report that potato sprouts hydrolysed the cellulose in cardboard[8]. Another report[10] showed that sap from tobacco leaves and roots hydrolysed carboxymethylcellulose and reprecipitated cellulose. In addition to tobacco, cellulase was found on single occasions in bean leaves, roots of beet and woody nightshade, asparagus stems, and the flesh of vegetable marrow fruit. The enzyme was not detected in tomato fruits or rhubarb petioles which had *p*H values of 4·3 and 3·4 respectively. The author mentioned that the *p*H was greater than 5 in all the saps in which activity was found.

Although cellulase has not been reported in fleshy fruits[9], changes are known to occur in fruits during ripening which could be caused by cellulase activity. α-Cellulose decreased in ripening pears[2], and the diameter of the cellulose micelles in peaches increased during ripening[9]. The increase in diameter could have been caused by limited degradation of cellulose microfibrils in the amorphous regions.

This communication reports the presence of an enzyme in tomato fruits which depolymerizes carboxymethylcellulose. A method of extracting the enzyme, in which *p*H is particularly important, is described. Enzyme assays were carried out using Ostwald viscosimeters. The reaction mixture in the viscosimeter contained 1 per cent carboxymethylcellulose ('CMC-7M', Hercules Powder Co.), 0·1 N sodium acetate, *p*H 5·0, 0·01 per cent merthiolate, and 2 ml. enzyme in a final volume of 10 ml. Enzyme activity was expressed in cellulase units. One cellulase unit is the amount of enzyme causing an increase in $1/\eta_{sp}$ (reciprocal of the specific viscosity) of 1 per min[4]. Initial preparations were made using methods similar to those used for extracting pectic enzymes[5,6]. Ripe unblemished tomatoes were homogenized 2 min, 100 V in a Waring blender with sufficient sodium chloride to give a 10 per cent solution. Merthiolate (0·01 per cent) was added immediately after homogenizing, and the *p*H was adjusted to 8·0 with 1 N sodium hydroxide. Solids were removed by centrifuging and filtering. The filtrate reduced the viscosity of 'CMC-7M' at *p*H 5·0 from approximately 13·5 to 6 centipoises in 5 h. The relation between $1/\eta_{sp}$ and time seemed linear until the viscosity dropped below approximately 9 centipoises, so cellulase activity was calculated from the linear part of the curve. Assays were conducted at *p*H 4, 5, 6 and 7·5. *p*H 5·0 gave the highest activity, and that *p*H was used in subsequent assays. Merthiolate (0·01 per cent) did not affect activity. Complete inactivation resulted when 2·5 ml. of filtrate at *p*H 7·0 was placed in boiling water for 15 min in a 1-cm diameter test-tube. There was a marked difference in enzyme activity between mature green and ripe fruits. Filtrate from a sample of 5 mature green fruits contained 9 µunits/ml., while filtrates from 5 similar samples of ripe fruits contained 93–174 µunits/ml.

The importance of raising the *p*H and the need for sodium chloride were investigated using a homogenate from ripe fruits. A portion of the homogenate was made 10 per cent in sodium chloride. Six 70-g samples of homogenate were taken, 3 containing sodium chloride and 3 having no sodium chloride. The 3 samples containing sodium chloride were adjusted to *p*H 6·0, 7·0 and 8·0 respectively. The samples lacking sodium chloride were treated in the same way. The yield of enzyme was decreased by sodium chloride, and it was increased by each increment of *p*H (Table 1). The *p*H 7·0 filtrate, which lacked sodium chloride, was made 10 per cent in sodium chloride and assayed again. Cellulase activity was unchanged so the salt did not act as an inhibitor.

The effect of neutralization is also shown in Figs. 1 and 2. Dulpicate 70-g samples were taken from a homogenate of ripe fruits. One sample was titrated to *p*H 8·0, and the other remained at *p*H 4·2. Filtrate from the neutralized sample contained 1,130 µunits/ml. uncorrected for the addition of sodium hydroxide, while that from the unneutralized sample contained 68 µunits/ml.

Table 1. Effect of Extraction Method on Yield of Cellulase

Homogenate titrated to *p*H	Cellulase activity* (μunits/ml. filtered tomato extract) 10% NaCl	No NaCl
6·0	46	540
7·0	100	680
8·0	260	810

* Each value represents enzyme activity from a different sample.

Ripe, unblemished tomatoes were homogenized 2 min at 100 V in a Waring blender. Three samples were obtained from part of the homogenate which had been made 10 per cent in NaCl. These samples, and 3 others which lacked NaCl, were titrated to the given *p*H with 1 N NaOH. The samples were centrifuged and filtered and cellulase activity in the filtrate was determined.

Table 2. Elution of Cellulase from the Insoluble Fraction

	Treatment	Volume of supernatant (ml.)	Cellulase activity (μunits/ml.)	Recovery (% of control)
1.	Control, titrated to *p*H 8·0	62·0	805	100
2.	Sample, *p*H 4·2			
A	Supernatant, *p*H 4·2	52·0	53	12
B	Distilled water rinse	68·5	27	
C	,, ,, ,,	71·0	22	
D	0·04 M sodium citrate, *p*H 8·0	66·0	271	75
E	,, ,, ,, ,,	63·5	156	
F	,, ,, ,, ,,	61·5	143	
G	0·5 M ,, ,, ,,	34·6	23	

Firm ripe fruits were homogenized in a Waring blender at 100 V for 2 min. Two 70-g samples were taken from the homogenate, and one was titrated to *p*H 8·0. The insoluble fraction was removed by centrifugation, and the pellet from the unneutralized sample was resuspended successively in solutions *B*–*G*. Cellulase activity was measured in the supernatants.

The values were equivalent to 950 and 18 μunits/g fresh weight. Thus, in the unneutralized sample, 94 per cent of the enzyme remained adsorbed to the insoluble fraction, and activity in the filtrate was extremely low. In another experiment with ripe fruits (Table 2), a portion of unneutralized homogenate was centrifuged, and the insoluble fractions were precipitated by centrifugation. The solids were suspended in distilled water and precipitated. The procedure was repeated with a second portion of distilled water and again with portions of citrate buffer at *p*H 8·0. Most of the enzyme remained adsorbed to the insoluble fraction and was not brought into solution until extraction with the citrate buffer. The results suggest that cellulase could be concentrated by pressing the juice from a large sample of fruits and extracting the insoluble fraction with small volumes of alkaline buffer. Work is in progress to concentrate the enzyme and determine whether it can hydrolyse substrates other than carboxymethylcellulose.

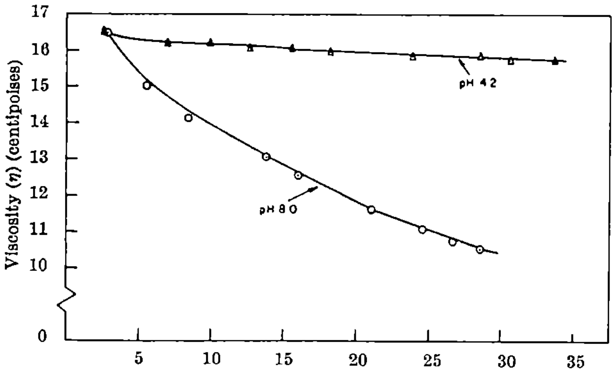

Fig. 1. Effect of tomato extracts on viscosity of carboxymethyl-cellulose ('CMC-7M'). Firm ripe fruits were homogenized 2 min, 100 V in a Waring blender. Two 70-g samples were taken and one sample was titrated to *p*H 8·0 with 5·0 ml. N NaOH. The other sample remained at *p*H 4·2. The insoluble fraction was precipitated by centrifuging, and cellulase activity was measured in the supernatant fluid

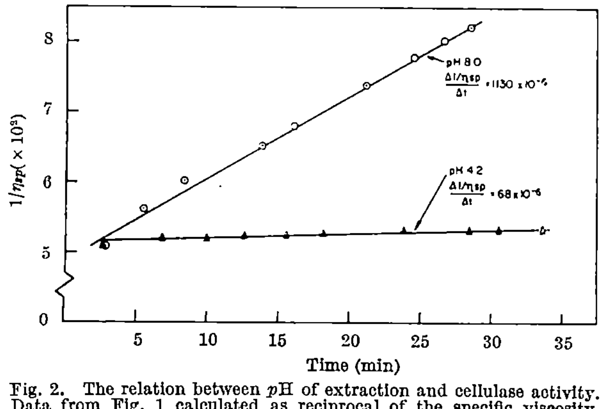

Fig. 2. The relation between *p*H of extraction and cellulase activity. Data from Fig. 1 calculated as reciprocal of the specific viscosity. One unit of enzyme activity represents a change in $1/\eta_{sp}$ of 1 per min

Neutralizing may also be necessary for efficient extraction of cellulase from other plant tissues characterized by low *p*H. The *p*H may also be important for tissues which are low in acids, since 50 per cent more enzyme was obtained by raising the *p*H of tomato homogenates from 6 to 8.

Since cellulase activity increases during ripening, the enzyme may be involved in the ripening process. Although its substrate specificity is not yet known, cellulase may act in conjunction with pectic enzymes to cause softening in ripening tomatoes.

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Prevention by 2,4-Dichlorophenoxyacetic Acid of an Increase in the Rate of Phosphate Utilization, caused by the Prolonged Treatment of Segments of Pea Stems in Maleate Buffer

While examining the influence of 2,4-dichlorophenoxyacetic acid (2,4-D) on the absorption of ions by epicotyl segments of etiolated peas, it was established that if freshly cut segments are first placed in aerated, distilled water or 0·01 M maleate buffer (*p*H 5·2) for 12 h, their ability to absorb and esterify inorganic phosphate in a given time (1 h) is increased 12-fold. This marked increase in the rate of phosphate absorption necessitated further study before the effects of 2,4-D on the uptake of ions could be further elucidated. On the basis of this work, which has been reported elsewhere[1], it is concluded that one of the basic changes occurring in the segments during the period in buffer or water is an increase in the rate of synthesis of protein, which in turn results in an increased rate of absorption of phosphate. An increased turnover of protein when pea segments were placed in water for 24 h was reported by Christiansen and Thimann[2] in 1950. Recently, Click and Hackett[3] have suggested that the increased respiratory rate and other metabolic changes occurring when potato slices are similarly treated depend on the synthesis of new RNA and protein.

It has now been demonstrated that the changes with time in the rate of absorption of phosphate can be prevented if the fresh segments are placed in maleate buffer containing 2,4-D. The results given in Fig. 1 show that the change in the capacity to absorb phosphate is dependent on the concentration of 2,4-D; it is unaffected by $1·5 \times 10^{-5}$ M, but is completely inhibited by 2×10^{-4} M. The results bring out two further points. First, that if fresh segments are placed in 2×10^4 M 2,4-D for 4 h and are then transferred to maleate buffer, their ability to absorb phosphate starts to increase. Secondly, that the transfer of segments from maleate buffer to 2×10^{-4} M 2,4-D at 8 h arrests the further absorption of phosphate, but does not bring about a significant decrease.

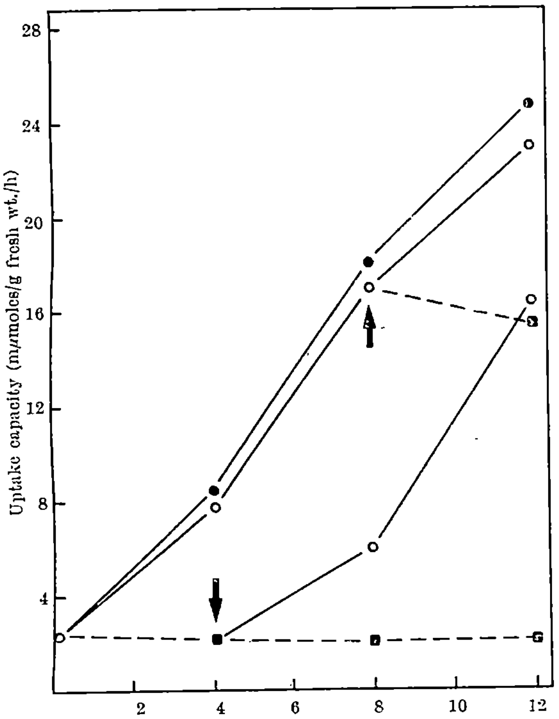

Fig. 1. The effects of placing freshly cut segments (1·0 cm) of pea epicotyls in 0·01 M maleate buffer adjusted to *p*H 5·2 (○) or buffer containing 2,4-dichlorophenoxyacetic acid (2,4-D) at (*a*) $1·5 \times 10^{-5}$ M (●) or (*b*) 2×10^{-4} M (▦ – – ■) on their subsequent capacity to absorb during 1 h phosphate from 1×10^{-3} M KH_2PO_4. The arrows indicate when segments were transferred from 2×10^{-4} M 2,4-D to maleate buffer at 4 h, or from buffer to the same concentration of 2,4-D at 8 h

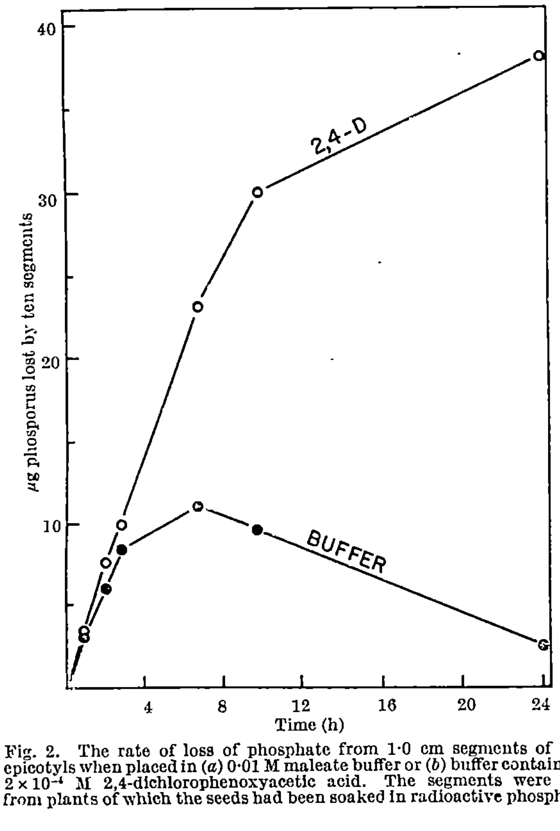

Fig. 2. The rate of loss of phosphate from 1·0 cm segments of pea epicotyls when placed in (*a*) 0·01 M maleate buffer or (*b*) buffer containing 2×10^{-4} M 2,4-dichlorophenoxyacetic acid. The segments were cut from plants of which the seeds had been soaked in radioactive phosphate

Another series of experiments was carried out to determine whether there were substantial losses of phosphorus, as inorganic phosphate, from the segments during the period in buffer, since if this were so, a large depletion of the pool of the endogenous phosphate would predispose the tissues to absorb exogenous phosphate.

From Fig. 2, it is clear that segments placed in maleate buffer lose phosphate rapidly during the first 2 h, but, afterwards, as the capacity to take up phosphate increases, much of the phosphate is re-absorbed. On the other hand, segments placed in 2×10^{-4} M 2,4-D lose phosphate progressively throughout the experimental period, and over the 24 h, the amount lost represents about 40 per cent of the total phosphorus originally contained by the segments.

Since phosphate absorption is seemingly related to an increase in the rate of protein synthesis[1] a reasonable extension is to postulate that 2,4-D prevents these changes by regulating, in some way, the rate of synthesis of proteins.

It is also evident that these significant biochemical changes must be taken into account when designing and interpreting experiments relating to the effects of auxins on the growth of excised stem tissues.

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PHYSIOLOGY

Teratogenic Risks of Drug Synergism

It is well known that the effectiveness of teratogens, measured either in incidence or in severity of resulting malformations, is frequently modified, in a plus or minus direction, by unrelated external conditions. The mechanisms by which these modifying agencies operate are largely unknown. A little, if not a great deal more, is known about the role of drug interaction in teratogenesis. There are, for example, the facts of interference with essential metabolites, such as deprivation of available vitamins by competing analogues[1] or by blockage of their utilization in co-enzyme systems[2]. Then there are the potentiating effects of lowering the available sources of utilizable carbohydrates[3] or of uncoupling oxidative phosphorylation[4]. Among the latter sources of synergism we found compounds, for example, 2-deoxy-D-glucose and chlorpromazine, which when administered alone were non-teratogenic and which even in larger (and highly toxic) amounts did not produce malformations. Other compounds of this type, however, are themselves teratogenic, in dosages higher than those required for synergism, but the range of their interference with development may be more restricted than their synergistic activity.

In the present enquiry we set out to determine if two compounds, which lead to related malformations when administered singly and in the requisite dosages, will interfere with normal development when individually harmless amounts are used in combination. The compounds selected for these experiments were sulphanilamide and 6-aminonicotinamide, both of which act as teratogens in chick development, inducing the occurrence of similar, though not altogether identical, forms of micromelia and beak defects[1,5,6]. The results of duplicate tests are given in Tables 1 and 2. It can be seen that the administration of 300 γ sulphanilamide or of 1·5 γ 6-aminonicotinamide was non-toxic and a high percentage of the treated embryos lived to hatching. The hatching percentages in the first test were, in fact, extraordinarily high; in the second experiment the relative hatches were somewhat lower, due to atmospheric conditions of the advancing season, but still very good. In the two experiments in which only sulphanilamide was used, 294 embryos survived the 13th day of incubation; 99 per cent of these were normal, none had micromelia or an abnormal beak. Among 297 embryos which after treatment with 6-aminonicotinamide reached the age of 13 days, 99·3 per cent were normal, none had micromelia and only one (0·3 per cent) had a parrot beak. To the entire harmlessness of the two compounds, in the dosages used, the data for combined administration of the same amounts form a truly dramatic contrast. The two tests yielded very similar results. Hatching was greatly reduced in both experiments. Instead of 99 per cent or more of the survivors of the 13th day being normal, only 20·7 per cent of a total of 275 embryos were without gross defects; 78·9 per cent of the embryos and chicks were micromelic, with many showing an extreme and crippling form of micromelia; 63·6 per cent of the embryos and chicks diagnosed in the two tests showed a parrot beak or a shortening of the mandible; finally, 18·0 per cent of 183 chicks, which had hatched in the two tests with combined treatment, exhibited ataxia and chorea.

Table 1. The Effects of Sulphanilamide and 6-Aminonicotinamide injected, singly or in combination and at 96 Hours of Incubation, on Development of White Leghorn Chicks. March 1964

Sulphanilamide γ/egg	300	300	—
6-Aminonicotinamide γ/egg	—	1·5	1·5
No. treated	106	108	105
Mortality (%)			
To end 6th day	2·8	7·4	4·8
7–13	1·9	2·8	1·0
14–22	5·7	18·5	1·0
Hatched (%)	89·6	71·3	93·3
Survivors of 13th day	101	97	99
Normal (%)	99·0	19·6	100
Micromelia (%)	0	80·4	0
Parrot or short lower beak (%)	0	55·7	0
Ataxia and chorea among hatched (%)	0	11·7	0
Miscellaneous defects (%)	1·0	0	0

Table 2. Repetition of Experiment recorded in Table 1, May 1964

Sulphanilamide γ/egg	300	300	—
6-Aminonicotinamide γ/egg	—	1·5	1·5
No. treated	212	206	219
Mortality (%)			
To end 6th day	8·0	11·7	7·8
7–13	0·9	1·9	1·8
14–22	12·3	35·0	9·1
Hatched (%)	78·8	51·5	81·3
Survivors of 13th day	193	178	198
Normal (%)	99·0	21·3	99·0
Micromelia (%)	0	78·1	0
Parrot or short lower beak (%)	0	68·0	0·5
Ataxia and chorea among hatched (%)	0	22·6	0
Miscellaneous defects (%)	1·0	2·8	0·5

After the administration of highly teratogenic amounts of sulphanilamide or 6-aminonicotinamide (1 mg and 10 γ/egg, respectively), the forms of expression of micromelia and beak defects tend to show subtle distinctions although their ranges overlap widely. Mean body-size is reduced after either treatment, but the dwarfing is usually more extreme following injection of 6-aminonicotinamide. In our present results, obtained by combined administration of the two compounds, the skeletal symptoms (long bones, maxilla and mandible) partake of the whole range of abnormalities observed after separate injection of the two teratogens. There was, however, little, if any, reduction in body-size even in severely micromelic chicks. The occurrence of ataxia and chorea in this material is of interest since these are symptoms of nervous dysfunction which occur following the administration of larger amounts of 6-aminonicotinamide, but have never been observed in experiments with sulphanilamide.

The relatedness of the induced defects and the possibility of complete protection with supplements of nicotinamide indicate that the intra-embryonic effects of sulphanilamide and 6-aminonicotinamide pertain to a particular chain of metabolic events and its end-product. There is also evidence, however, in addition to the slight dissimilarities of phenotype to which reference has been made, that different steps within this chain are affected by the two compounds. This is strongly suggested by the fact that 2-deoxy-D-glucose is a potent synergist of the teratogenic activity of 6-aminonicotinamide, but does not potentiate the results of injected sulphanilamide.

It is clear from the foregoing that altogether harmless amounts of potentially teratogenic substances may in combination produce powerful synergism and thereby a high incidence of severe malformations. Moreover, we have demonstrated in earlier experiments that non-teratogenic compounds may also act as highly effective synergists. The cautionary consequences of these facts should not require emphasis.

Our work was supported by a grant from the National Science Foundation.

We wish to dedicate the present observations to the memory of that wise and courageous woman—Rachel Carson.

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Blood Velocity telemetered from Untethered Animals

The magnitude of the change in frequency of sound back-scattered in moving blood is a measure of the velocity of the blood[1,2]. We have used this principle to design a system for telemetry of blood flow information from the major vessels of healthy, active, totally unrestrained animals.

Cylindrical plastic flow probes were clamped around blood vessels during an aseptic surgical procedure. A piezo-electric crystal (lead zirconate) mounted in one wall of the cylinder emitted a continuous 5-Mc/s sound beam diagonally through the blood vessel wall into the centre of the blood stream. A second piezo-electric crystal, mounted in the opposite wall of the cylinder, served as a sensor for the sound back-scattered from within the blood stream (Fig. 1). The magnitude of the difference in frequency between the emitted sound and the back-scattered sound was proportional to the velocity of the blood within which the sound was scattered, as described by the Doppler equation:

$$(F_e - F_r) = F = \frac{2\, F_e\, v \cos a}{C}$$

where: F = absolute difference in frequency between emitted signal frequency, F_e, and the received signal frequency, F_r.
v = velocity of scattering medium.
a = angle between acoustical axes and blood velocity.
C = velocity of sound in blood.

For typical application :
$F_e = 5 \times 10^6$ c/s
$C = 1{\cdot}5 \times 10^5$ cm/sec
$\cos a = 0{\cdot}7$
v ranges from 0 to 150 cm/sec so that F ranges from 0 to approximately $7{\cdot}5 \times 10^3$ c/s.

An electrical signal was developed with a frequency equal to the Doppler shift between the emitted and back-scattered signals. This audio-frequency signal modulated the frequency of a 100-Mc/s oscillator. The resultant

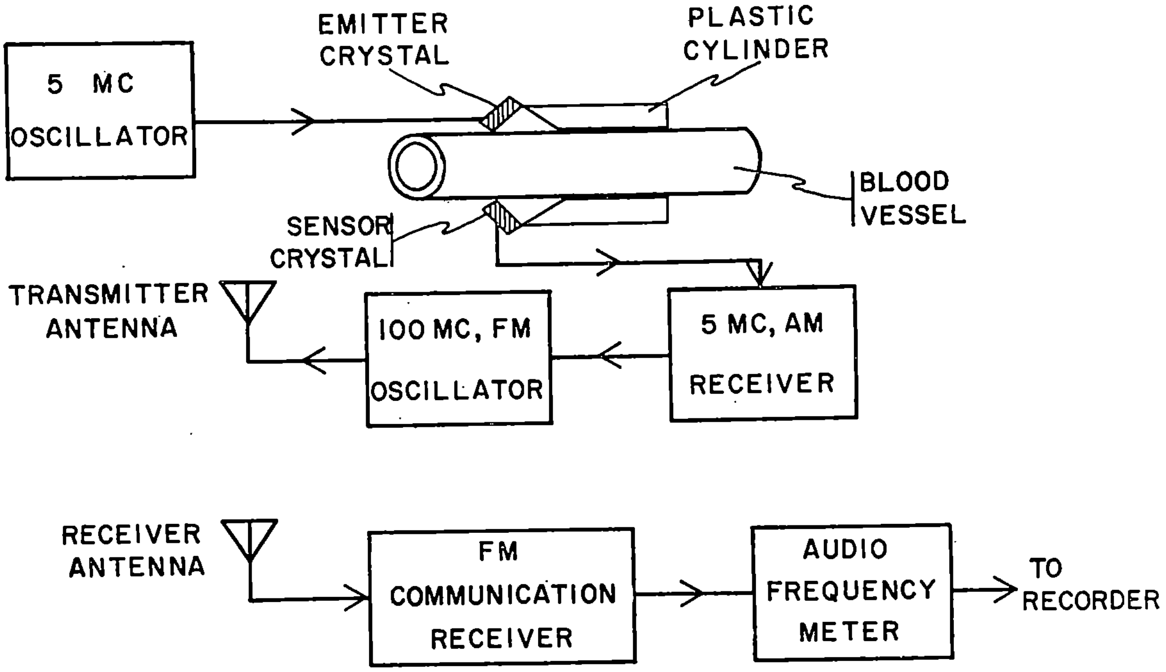

Fig. 1. Block diagram of method for telemetry of blood flow information using Doppler shift ultrasonic flowmeter

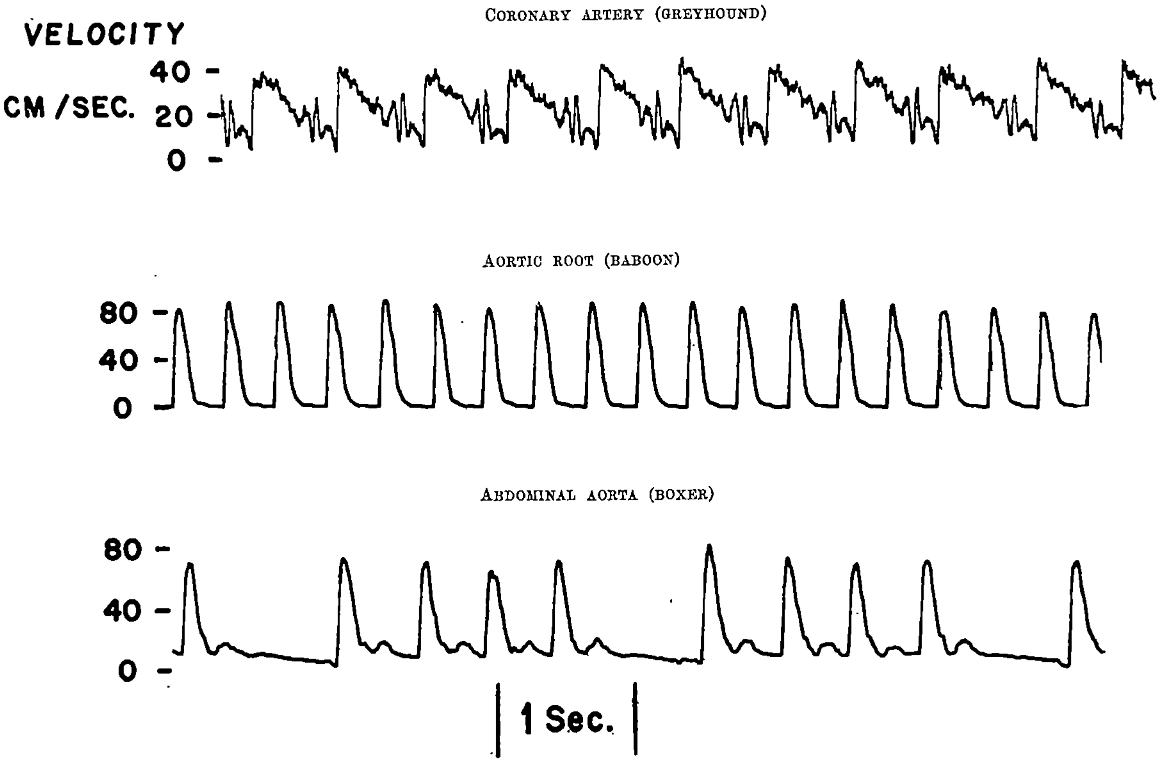

Fig. 2. Representative wave forms of flow telemetered from untethered animals: circumflex arterial flow in walking greyhound; flow through the root of the aorta in an exercising baboon; and flow through the terminal abdominal aorta in a somnolent boxer

FM/FM signal was transmitted from the animal to a remote frequency modulation tuner. The audio-frequency signal from the tuner was coupled to a frequency meter, and the voltage from the frequency meter was recorded in terms of blood velocity.

The emitter crystal was excited by a miniature (2 cm × 1·5 cm × 1·5 cm) 5-Mc/s oscillator, with a power consumption of 100 mW. The acoustical power was estimated to have been less than 200 mW/cm^2. A miniature (2 cm × 2 cm × 9 cm) low-noise, tuned radio-frequency receiver was used to amplify and detect the back-scattered signal. The power consumption of the receiver-amplifier was 30 mW. The 100-Mc/s oscillator-transmitter consumed 50-mW power. Its size was 2 cm × 2 cm × 6 cm. The entire assembly was powered by two Mallory *TR* 136 *R* batteries and had a continuous operating time of

100 h. When used in animal experiments, the components were assembled into a small packet which was carried on a harness (dogs, seals) or built into a plastic helmet (baboons). A simple loop of wire served as the transmitting antenna. The *FM* receiver was a standard 'Hi-Fi' tuner located as much as 300 m away from the experimental animal.

Zero flow-levels were obtained by opening the input to the frequency meter. This procedure has previously been demonstrated to simulate occlusion of the vessel ($F_e = F_r$, $F = 0$) and yields a reliable measure of zero flow levels[1,2]. The frequency meter was calibrated against an audio-frequency standard and the blood velocity was computed using the Doppler equation.

We have used the Doppler sonic blood flow telemetry system for measurement of blood flow through the major arteries of free ranging dogs, and the ascending aorta of baboons in an outdoor enclosure. In addition, we have measured blood flow through the carotid, iliac and flipper arteries of elephant seals in their natural habitat on Guadalupe Island off the coast of Mexico. In the latter case, blood flow was telemetered from animals on the beach to recording instruments on a research ship situated 200 m off shore. In another application, blood flow through the ventral aorta of sharks swimming in the ocean was telemetered to recording instruments on shipboard.

The Doppler shift sonic method for measurement of blood flow has proved to be well suited for telemetry of flow information from violently exercising, untethered animals. The simplicity and low-power consumption of the system are compatible with the small packaging requirements of telemetry from animals. The instrument is free of base-line drift. Drift in sensitivity is proportional to drift in the crystal excitation frequency, which can be held essentially constant, and to variations in the velocity of sound in blood which have been found to be negligible. The flow information from the 5-Mc/s receiver-amplifier is in the form of a frequency modulated, audio-frequency signal, which allows the use of standard *FM/FM* telemetry techniques without the necessity of including a separate sub-carrier oscillator.

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Hyperpolarizing Action Potentials recorded from the Œsophagus of *Ascaris lumbricoides*

THE œsophagus of *Ascaris* is a stout cylindrical organ up to 7 mm in length and more than 1 mm in diameter, which in fresh preparations is often seen to contract rhythmically. Vigorous contractions are also elicited by brief electric pulses applied directly to its surface.

Goldschmidt[1] demonstrated, sixty years ago, that although 33 cells build up the œsophagus, their boundaries are lost. Therefore, the entire organ may be regarded as a tubular, multinucleated, single cell. In cross-sections short bundles of myofibrils are seen to extend between the tri-radiate lumen and the external surface[2].

If a capillary glass microelectrode is inserted into the wall of a quiescent œsophagus, a steady potential of up to 10 mV is recorded between the cytoplasm and the external solution (cytoplasm negative). In contrast to this small resting potential the contractions of the œsophagus, both spontaneous and those which follow applied stimuli, are accompanied by slow, all-or-nothing, action potentials up to 110 mV in amplitude.

Although the shape of these action potentials resembles those recorded from many other cells their polarity is most unconventional. The main initial phase is a large negative deflexion which increases the potential difference across the surface of the œsophagus to a maximal peak value of about 100 mV.

Fig. 1 shows three such action potentials superimposed on the same frame of film. While the rising, that is, hyperpolarizing, phase is smooth and relatively fast (maximal rate of potential change about 10 V/sec) the falling phase is slower and more complex. A fairly

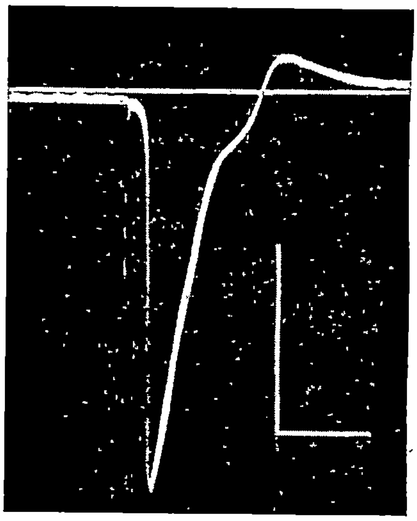

Fig. 1. Three superimposed action potentials recorded with a capillary microelectrode inserted in the wall of *Ascaris* œsophagus. The horizontal line shows the zero base-line. Downward deflexion of the trace indicates negativity of the microtip with respect to the bath electrode. The action potentials were elicited by three brief electric pulses of increasing strength, see artefacts. Vertical bar: 40 mV; horizontal bar: 0·1 sec

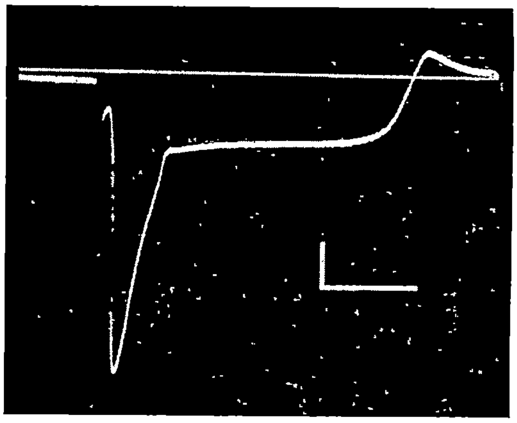

Fig. 2. Action potential recorded from *Ascaris* œsophagus. It differs from those in Fig. 1 by the shelf in the falling, that is, depolarizing, phase (see text). Vertical bar: 10 mV; horizontal bar: 0·1 sec. Horizontal line indicates the zero base-line

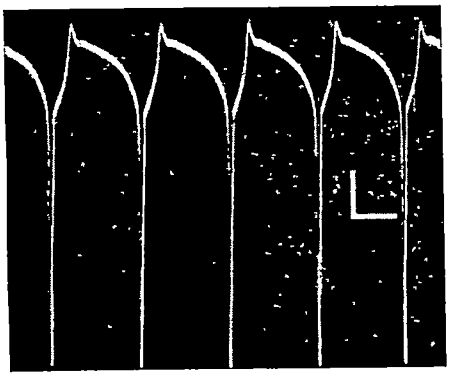

Fig. 3. Rhythmic action potentials recorded from an *Ascaris* œsophagus which exhibited spontaneous mechanical activity. Marked prepotentials are seen to precede each negative spike. Vertical bar: 10 mV; horizontal bar: 1 sec

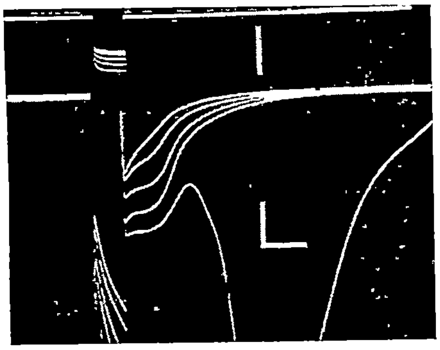

Fig. 4. Negative-going local responses following the injection of inward (that is, hyperpolarizing) membrane current into the œsophageal wall. Upper trace monitors the applied current; vertical bar: 10^{-6} amp. Lower trace shows the potential recorded between the microtip inserted into the œsophageal wall and the bath electrode. The largest local response gave rise to an action potential which flared up 30 msec after the end of the current flow. Vertical bar: 5 mV; horizontal bar: 20 msec

constant feature is the occurrence of a sudden decrease in the rate of depolarization before the potential reaches the initial base-line (Fig. 1). Frequently, the process of depolarization may come to a complete standstill at this point and, as a consequence, a plateau or shelf lasting up to 3–4 sec is observed (Fig. 2).

From this level, the potential does not return directly to the resting value but undergoes a phase of reversed polarity reminiscent of a negative after-potential. This brief positivity of the cytoplasm appears in the records as a small peak of rather constant amplitude, about 10 mV. Thereafter, the potential returns very slowly to the initial base-line; half-decay times of up to 0·8 sec have been measured.

The position of the microelectrode tip within the œsophageal wall is not very critical and it can be shifted radially over distances of 200 or 300 µ without producing appreciable changes in the amplitude of the action potential.

The spontaneous rhythmical contractions of the œsophagus are accompanied by trains of similar action potentials in which the negative spikes are often preceded by marked prepotentials; a mirror-image of those recorded from neural and muscular pacemakers (Fig. 3).

Other experiments, to be described in full elsewhere, have shown that the injection of current into the œsophageal wall with help of a micropipette gives rise to electrotonic potentials which spread decrementally over the giant cell. Action potentials are elicited by a sufficiently large flow of inward, that is, hyperpolarizing, current across the surface of the œsophagus.

A further mirror-image correspondence between the œsophageal action potentials and conventional excitation processes is the occurrence of negative sub-threshold local responses. These are particularly clear-cut following brief anelectrotonic potentials (Fig. 4).

Hyperpolarizing responses with an overall shape resembling the potentials described here have been described by several authors (cf. Grundfest[3]). However, such responses were obtained during the injection of polarizing current into cells immersed in modified ionic solutions. In contrast, the negative spikes found in the œsophagus of *Ascaris* are spontaneous and self-propagating events. When elicited by external stimuli of just threshold strength they are seen to flare up with a considerable latency after the end of the stimulus artefact (Fig. 4).

In all the experiments described here, the œsophagus was immersed in 30 per cent sea-water (v/v); a solution isotonic with *Ascaris* tissues the ionic composition of which resembles that of the perienteric fluid of the worm. Somatic muscle cells of *Ascaris* surrounded by this saline exhibit a conventional electrical behaviour[4,5].

An interesting similarity also exists between the slower œsophageal action potentials, showing a shelf (Fig. 2) and the hyperpolarizing potentials recorded by Brown and Wiesel[6], from the cat's retina.

Although one cannot exclude the possibility that the foregoing findings may be the result of bizarre structural factors yet to be revealed, all the available experimental evidence suggests that we are dealing with action potentials generated across the surface layers of the giant œsophageal cell. In spite of the inverted polarity the electrogenic properties of this cell resemble those of cells producing positive-going, that is, depolarizing, action potentials.

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Hepatic Inactivation of Angiotensin

Hypertension induced by renal artery stenosis can be cured in dogs by a reno-portal venous shunt, according to Aoki[1]. A possible explanation is that the affected kidney liberates angiotensin which is in turn inactivated when passing through the liver. The possibility of such inactivation was explored by comparing in rats the pressor response of angiotensin injected through the jugular vein and the portal system.

Twenty-nine Wistar rats, weighing 250–350 g, were anæsthetized with 20 mg/100 g body-wt. of tribromoethanol intraperitoneally and the height of the blood pressure response recorded through the carotid using a Telco strain gauge. 'Hypertensin Ciba' was made up to 2 µg/ml. of saline at *p*H 5·6 or saline adjusted to *p*H 11·7 with sodium hydroxide. Noradrenaline was dissolved in saline, 20 µg/ml. Polyethylene tubing was connected to the jugular vein, the femoral artery or the portal vein (or greater mesenteric). Either 1 µg of angiotensin or 10 µg of noradrenaline was given each time, the injection volume being kept constant at 0·5 ml. (administered within 20 sec).

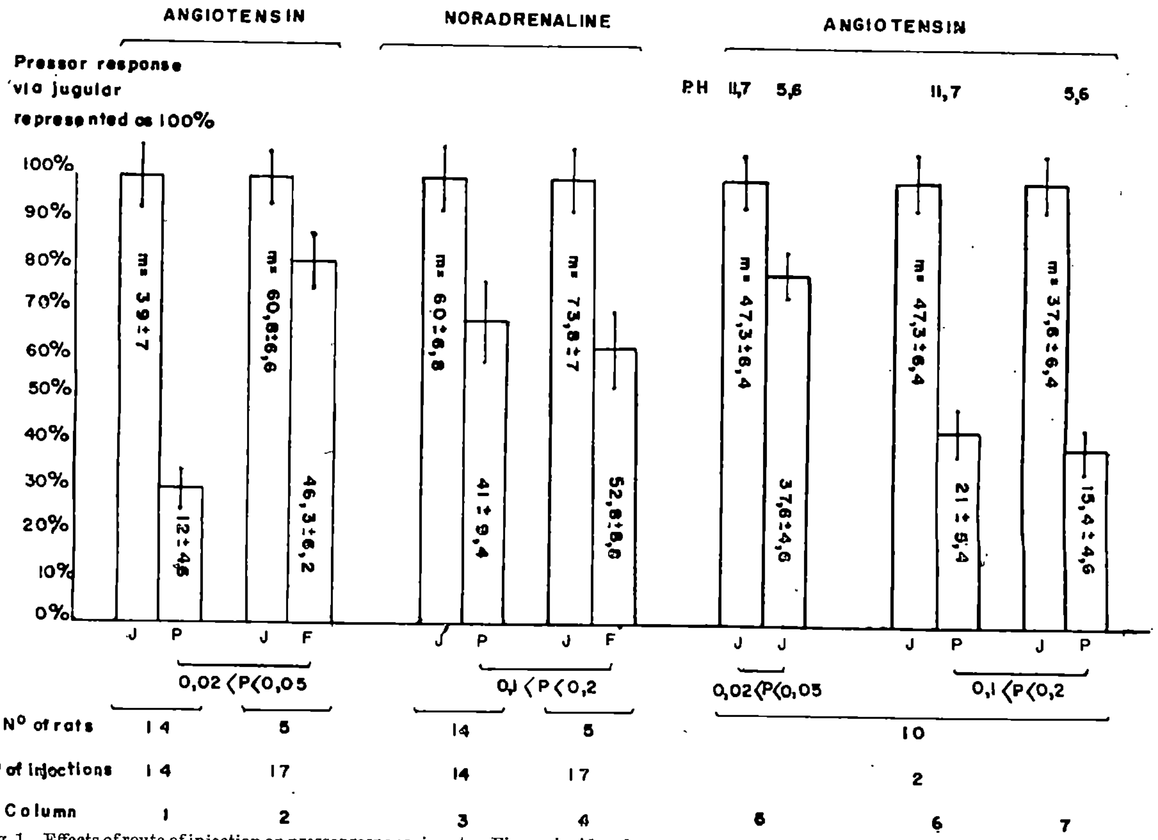

Fig. 1. Effects of route of injection on pressor response in rats. Figures inside columns are average response (mm mercury and standard deviation). *J*, jugular vein; *P*, portal vein; *F*, femoral artery

The pressor response of angiotensin via the portal system was reduced by 70 per cent in comparison with that injected into the jugular vein (col. 1). When the femoral artery was used, the extent of the reduction was significantly inferior, being only 24 per cent (col. 2). Cols. 3 and 4 show that the losses of response of noradrenaline going through the portal vein (32 per cent) and the femoral artery (37 per cent) were not significantly different from each other ($P < 0{\cdot}2$). The pressor activity of angiotensin alkalinized to *p*H 11·7 was increased by 21 per cent (col. 5); the degree of its inactivation by the liver circulation was almost identical to that of angiotensin at *p*H 5·6 (cols. 6 and 7).

The results indicate that angiotensin, but not noradrenaline, undergoes a significantly greater destruction in one passage through the liver circulation than through the peripheral capillaries. We ascribe this finding to liver angiotensinase, the role of which in angiotensin metabolism has been stressed by other data: (*a*) the *in vitro* degradation of angiotensin by liver tissue is well established[5]; (*b*) after administration of tritiated angiotensin to nephrectomized rats, more radioactivity is found in the liver than in other organs, in the form of metabolic fragments[6]; (*c*) a significant increase in plasma angiotensinase activity is found in patients with hepatic disorders such as cirrhosis, hepatitis, cholestasis[5,7] and toxæmia[8]. It is suggested that the liver may play a specific and important part in the metabolic inactivation of circulating angiotensin.

Although of smaller magnitude, the peripheral inactivation of angiotensin is nevertheless of significance and is in line with measurements of circulating angiotensin: when infused into the femoral artery of the isolated hind limb of a dog, very little of the hormone could be detected in the effluent[9]; when infused into the controlateral arm of normal subjects, much less angiotensin was recovered from the antecubital vein than from the humeral artery[2,4].

Like Paladini *et al.*[10], we found that alkalinization potentiates the pressor activity of angiotensin, but to a smaller degree (21 per cent) than that reported by those authors (66 per cent), probably because we used greater amounts of hormone per injection. Our attempt to find out whether this potentiation was partly due to an increased resistance to liver angiotensinase has shown that this is not the case.

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ANATOMY

Oligohydramnios and Cortisone-induced Cleft Palate

It has been suggested[1] that cortisone disturbs the closure of the secondary palate of mouse embryos by interfering with the development of an intrinsic motive force within the palatal processes. The force is thought to be dependent on the accumulation in the palatal mesenchyme of substances which give positive results with histochemical reactions for acid mucopolysaccharides. The marked variation in the incidence of cleft palate induced in different strains of mice by similar doses of cortisone has been attributed to differences in the rate at which the intrinsic force develops[2]. Subsequent workers[3,4] have been unable to detect histochemical differences between palatal tissues of control and cortisone-treated embryos. Although cortisone diminishes the incorporation of ^{35}S-labelled sulphate in the tissues of treated embryos, it has proved impossible to distinguish between autoradiographs of palatal tissue from strains with a high and those with a low incidence of cortisone-induced cleft palate[4]. At the time of palatal closure, there is a marked similarity between the distribution of acid mucopolysaccharides in the palatal mesenchyme of mouse and rat embryos, but cleft palate has not been observed in the progeny of cortisone-treated rats[3].

The purpose of this communication is to report that cortisone reduces the volume of amniotic fluid surrounding mouse and rat embryos during the period of palatal closure, and to offer an explanation for interstrain and interspecies variation in the incidence of cleft formation which is based on morphological criteria.

Pregnant mice of the inbred strains *A/Gr*, *C57BL/Cbi* and *CBA/Cbi* were treated with 2·5 mg of cortisone from the 11th to the 14th days of pregnancy, and the embryos were recovered both before and during the period of palatal closure. These embryos exhibited an attitude of extreme flexion when compared with control embryos at similar stages of development. Indirect estimations of the weight of amniotic fluid, obtained by subtracting the weight of the embryo and placenta from that of the whole conceptus, showed a definite reduction in the weight of fluid associated with cortisone-treated embryos. Direct measurements were therefore made of the volume of fluid associated with normal and treated embryos recovered on the 14th and 15th days of pregnancy. In each strain there was a tendency for the volume of fluid surrounding treated embryos to be less than that associated with untreated embryos of similar morphological age and weight (Fig. 1). Published criteria for assessing the morphological age of mouse embryos[5] required modification to avoid the excessive rating of treated embryos due to premature 'closure' of the external auditory meatus.

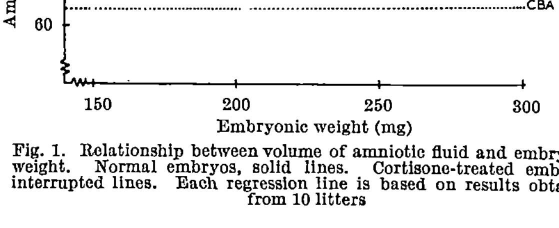

Fig. 1. Relationship between volume of amniotic fluid and embryonic weight. Normal embryos, solid lines. Cortisone-treated embryos, interrupted lines. Each regression line is based on results obtained from 10 litters

The cause of the reduction of amniotic fluid has not been established. No gross abnormalities have been observed in the heart and renal tract of treated embryos, and the possible existence of localized changes in renal glomeruli and tubules is being studied. A preliminary analysis of data obtained from the present experiments indicates that in all three strains of mice there is a tendency for the placentæ of embryos from treated litters to weigh less than those of control embryos of similar weight. Furthermore, at any given placental weight there is a tendency for placentæ from treated litters to be associated with smaller volumes of amniotic fluid.

The placentæ of treated embryos were macroscopically normal, but the membranes were invariably discoloured by a band of pigmentation which extended around the antimesometrial surface of the conceptual sac, adjacent to the decidua (Fig. 2). Much less extensive pigmentation was occasionally observed in a similar situation in the membranes of untreated embryos at earlier stages of development.

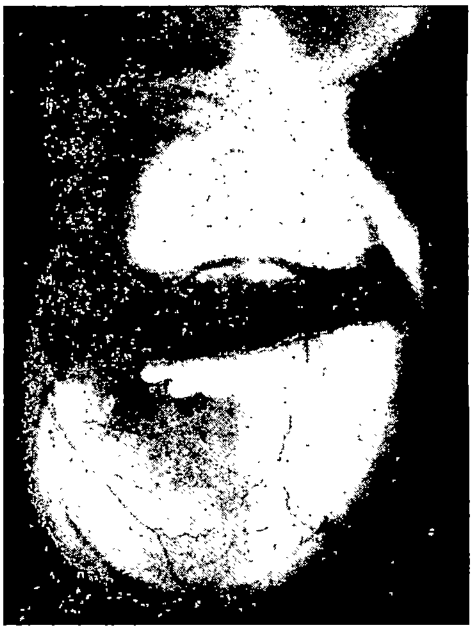

Fig. 2. Cleared preparation of part of a pregnant uterus from a cortisone-treated *A* strain mouse showing the band of pigmentation. (× 6)

The volume of amniotic fluid surrounding rat embryos during the period of palatal closure was also reduced in pregnant rats of the Chester Beatty albino and Lister Institute hooded strains which had been treated with 25 or 50 mg of cortisone from the 11th to the 15th days of gestation. There was macroscopical evidence of degeneration at the edges of the treated placentæ, but pigmentation of the membranes was seldom observed.

During morphogenesis of the secondary palate there is a change in spatial relationship between the tongue and the palatal processes. This change has been expressed quantitatively by obtaining from histological sections of embryos prepared by standardized techniques measurements of the longitudinal growth of the cranial base and Meckel's cartilage, and of the angular relationship between them. Since the cranial end of Meckel's cartilage cannot be identified on median sagittal sections, longitudinal growth of the lower jaw has been arbitrarily defined as the distance between the anterior extremity of Meckel's cartilage and the caudal end of the posterior cranial base (Fig. 3).

At the commencement of palatal closure in the rat embryos, the cranial base is flexed in the region of the craniopharyngeal canal. The point of flexion serves to

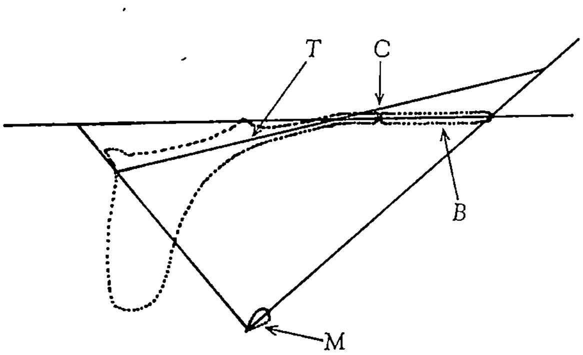

Fig. 3. Diagram illustrating portions of the chondocranium seen in median sagittal sections. Anterior extremity of Meckel's cartilage (*M*); craniopharyngeal canal (*C*); angle *T* decreases as the anterior cranial base extends; angle *B* is reduced in cortisone-treated embryos

divide the base into anterior and posterior portions. As palatal closure progresses, there is a gradual reduction in the angulation of the cranial base, and when fusion of the processes is complete, the long axes of the two portions of the base form a straight line. The amount of flexion in the cranial bases of *C57BL* and *CBA* mouse embryos at the onset of palatal closure is less than that observed in the rat embryos, but considerably greater than the amount present in embryos of the *A* strain in which the cranial base is almost straight before closure commences. There is also a definite tendency in the *A* strain embryos for the long axes of the posterior cranial base and Meckel's cartilage to be closer together. It is uncertain whether the differences noted in the *A* strain are entirely due to the greater morphological maturity of these embryos, for at the time of palatal closure there is a considerable degree of uniformity between the length of the cranial base and Meckel's cartilage in the three strains of mice.

In mouse embryos from cortisone-treated litters there is a tendency for the angle formed between the long axes of the posterior cranial base and lower jaw to be smaller than normal. The dorsum of the tongue is closer to the roof of the mouth and extension of the anterior cranial base occurs more rapidly than in untreated embryos. Extension (that is, reduction of the angle *T* in Fig. 3) is particularly rapid in treated *CBA* embryos which have the greatest reduction of amniotic fluid. The incidence of cortisone-induced cleft palate in the *CBA* and *C57BL* strains is reported to be 20–25 per cent and 17–18 per cent respectively, but in the *A* strain it approaches 100 per cent. This difference cannot be attributed to a greater reduction of amniotic fluid in the *A* strain, but it is suggested that it can be explained if the reduction of amniotic fluid is considered in conjunction with the configuration of the cranial base at the commencement of palatal closure.

The possession of a flexed cranial base may provide an embryo with a greater available range of movement in the anterior part of the head which could assist in overcoming minor degrees of malrelationship between the tongue and the palatal processes. Rat embryos possess a considerable range, and in spite of a reduction in the volume of amniotic fluid, their palatal development is not disturbed by cortisone. The range is less in *C57BL* and *CBA* embryos, but the majority appear to adjust themselves to the effects of cortisone by the rapid extension of the anterior cranial base. Embryos of the *A* strain have virtually no available range of movement because the cranial base tends to be almost fully extended before palatal closure commences. In addition this strain possesses less space between the cranial base and lower jaw. It is possible that they respond to reduction of this space by a more rapid extension of the anterior cranial base, but in this strain the available range of movement is insufficient to allow the anterior thirds of the palatal processes to clear the dorsum of the tongue before they are immobilized by extension of ossification from the maxilla.

In this explanation of the interstrain and interspecies variations in the incidence of cortisone-induced cleft palate, no reference has been made to the presumed effect of cortisone on the mesenchyme of the palatal processes. It is probable that cortisone influences the maturation of embryonic connective tissue; but in view of the morphological changes noted here, it seems unlikely that palatal closure is wholly dependent on the existence of an intrinsic force within the palatal processes.

The demonstration of a reduction in the volume of amniotic fluid provides a direct link between the palatal clefts produced by cortisone and those following the withdrawal of amniotic fluid. Since the incidence of cortisone-induced cleft palate can be influenced by genetic factors, embryos from crosses between *A* and *C57BL* mice may help to demonstrate the extent to which oligohydramnios is a contributing factor to the ætiology of this malformation.

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HÆMATOLOGY

Fibrinolysins from Fibrin Clots

THREE fibrinolytic agents have been encountered in fibrin clots. The first was described as lytic factor in an earlier communication[1]. It was extracted with borate from trypsin-digested clots. It has since been found that extraction can be carried out successfully with 0·9 per cent sodium chloride and other salt solutions. Active preparations contained a 17-ketogenic steroid and were inactivated by β-glucuronidase and by heating at 56° C for 30 min. The inhibitory effect of potent lytic factor on the clotting of fibrinogen by thrombin or aqueous clot extract[1] was very marked. An amount of 0·0004 ml. prevented fibrin formation, while with 0·0002 ml. only a partial clot was produced. With recalcified oxalated plasma different reactions were observed. One-hundredth ml. of the same preparation induced formation of a complete clot in 30–35 sec; with 0·05–0·08 ml. partial clots formed in the same time interval, while 0·15 ml. prevented clotting entirely. The lytic activity of the factor was strongly inhibited by serum, even after the serum had been treated with 0·2 N sodium hydroxide to destroy glucuronides and then neutralized with hydrochloric acid.

The second fibrinolysin was obtained from retracted fibrin clots made from 0·5 ml. portions of oxalated human plasma and kept in one-tenth volume of 0·9 per cent sodium chloride at room temperature. Such clots would dissolve in 1–5 days. The lysin titre was determined as described previously[2]. In most cases the unit was 0·05 ml. of clot supernatant. Like lytic factor, this fibrinolysin was inactivated by β-glucuronidase, but, unlike the former, it was relatively resistant to heating. Exposure to 56° C for 30 min scarcely affected it; 90 per cent of its activity was destroyed at 65° C in 10 min. Serum inhibited lysin activity but would no more do so after treatment with alkali. As the result of the action of this fibrinolysin a factor accelerating plasma recalcification was liberated, as reported previously[3], but in most preparations a clotting factor, acting directly on fibrinogen, was also found to be present. Both seemed to be identical with factors from aqueous clot extracts[1,3,4]. An apparent difference in heat stability could be explained by the far higher concentration of active material in aqueous than in saline extracts.

The third fibrinolysin was encountered in aqueous clot extracts, the supernatants of clots digested in distilled water at 5° C. At this temperature scarcely any clot decomposition would take place in saline. It was inactivated by heating at 56° C for 30 min, but not by β-glucuronidase, and was inhibited by untreated, but not by alkali-treated, serum. Potent clotting and accelerating factors were present in clot supernatants. As little as 0·0001 ml. would coagulate 0·1 ml. of recalcified oxalated plasma in 25 sec. The supernatants were greenish-yellow, resembling in appearance the substrate from which a fibrinolytic metabolite was produced by a strain of *Streptococcus faecalis*[2]. Most of the pigment was able to pass through 'Cellophane' membranes, but a fraction was non-dialysable like the bacterial metabolite, and fibrinolytic activity was associated with it. When the bacteria were allowed to act on the dialysate in the presence of gluconate, as described previously[2], fibrinolysin was produced and more pigment became non-dialysable.

It seemed, then, reasonable to assume that the pigment was also present in the culture medium, brain heart infusion, in which the bacteria had been grown, but because of the many substances making up the medium isolation of this compound was not feasible. However, some of its properties could be determined by subjecting the medium to certain procedures before the addition of bacteria and gluconate for fibrinolysin generation. Boiling or drying at 37° C did not affect it. It was partially soluble in methyl alcohol and 95 per cent ethyl alcohol, but not in acetone, chloroform, or ether. It was irreversibly destroyed by exposure to 1 N hydrochloric acid or 1 N sodium hydroxide. The chemically purer clot extract dialysates displayed the same properties except for light absorption. Both the dialysate and the culture medium showed maximum absorption at 275 mμ, but the former only had a peak at 395–400 mμ. This did not change even after boiling for 20 min, but when the dialysate was dried at 37° C, its colour became yellowish-brown and with it its spectrum identical with that of the culture medium. This did not make it unsuitable as a substrate for *S. faecalis* in fibrinolysin formation. In fact, active fibrinolytic solutions, whether obtained directly from decomposed clots following dialysis or produced by bacteria, never displayed a maximum at 400 mμ.

An attempt was made to identify the pigment. The reported anticoagulant action of bilirubin glucuronide[5] served to direct attention to the bile pigments. Yet none of the usual bile pigment reactions gave positive results. The recorded absorption spectra fitted those of dipyrrol rather than tetrapyrrol compounds. It was then noted that clots formed from the plasma of patients with myocardial infarctions decomposed more quickly and yielded more pigment. This could well be ascribed to release of pigment from heart muscle dying due to circulatory failure and would explain the presence of the same pigment in brain heart infusion medium. A pigment fluorescing green spontaneously was reported to be excreted in the fæces in conditions associated with rapid atrophy of muscle tissue and was named myobilin[6]. Fluorescence was attributed to a pigment-protein complex. From it a brown pigment, mesobilifuscin, was isolated and later identified as a dioxydipyrrylmethene. Stich[7] described various types of mesobilifuscins characterized by differential solubility in water and a number of organic solvents and tried to relate this to polymerization or association of the basic molecules. According to its spectrum, the yellowish-brown pigment mentioned above could well be a mesobilifuscin, while the greenish-yellow pigment resembles in its light absorption a monooxydipyrrylmethene, such as neoxanthobilirubic acid[8]. It is worth noting that propentdyopent, an oxidation product of both dipyrrylmethenes, appears in measurable quantities in the urine of patients following cardiac failure[9].

A new avenue in heart patient therapy may be opened if association of a larger amount of the circulating water-soluble pigment with gluconate could be induced in the body. The beneficial effects of several types of jaundice on arthritic conditions[10] might be related to the same compound. According to recent investigations[11,12], dipyrrylmethene pigments are not primarily breakdown products of hæmoglobin but are involved in its synthesis. In hæmolytic anæmias accompanied by increased erythropoiesis there is an increase of such pigments, whereas in hyporegenerative anæmias and rheumatoid disease their concentration is reduced.

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Uptake of γ-Globulin and Complement by Red Cells exposed to Serum at Low Ionic Strength

AT low ionic strength the rate of association of certain antibodies with their corresponding antigens is increased (for review see ref. 1) and the detection of human blood group antibodies may be facilitated[2,3]. In the course of testing sera which had been 'de-ionized' either by passage through 'Sephadex *G*-50' columns or by dialysis against a sucrose–saline solution of ionic strength 0·025, it was observed that the sera were giving 'false positive' reactions; that is to say, if red cells were incubated with their own 'de-ionized' serum at $I = 0{\cdot}025$, then washed and mixed with anti-human globulin serum, agglutination occurred. The red cells were only very weakly agglutinated by specific anti-7*S* γ-globulin sera but were strongly agglutinated by anti-β_{1E}-globulin serum and less strongly by anti-β_{1C}-globulin serum; thus the complement components C'_4 and C'_{3a} were both present on the red cells[4]. Positive results were also obtained with an immunoconglutinin serum ('auto-stimulation' type[5]). As expected, the addition of EDTA to the serum before incubation with red cells prevented the uptake of complement.

If red cells and serum were incubated together at low-ionic strength by simply diluting the serum with 10 to 40 volumes of low-ionic-strength medium, similar results were obtained although the reaction of the cells with anti-β_{1C} was now distinctly weaker.

The uptake of complement was found to be maximal at ionic strengths up to about 0·03; at about 0·035 less complement was taken up and none at all was taken up above an ionic strength of 0·085.

In these experiments the required amount of sodium chloride was dissolved in a solution of 6·0 per cent sucrose (0·175 M) to maintain osmolarity; it was found that similar results were obtained if glycine (0·32 M) was substituted for sucrose, although the uptake of complement was slightly less. The sucrose–saline solution was normally unbuffered and had a pH of approximately 5·0; buffering to approximately 7·0 with phosphate (0·003 M) or barbiturate (0·05 M) resulted in slightly less uptake of complement.

It was found that incubation of red cells and serum at low-ionic strength (I = 0·025) for even 1 min was enough to give detectable uptake of complement components but that the reactions increased in intensity with longer incubation periods and were maximal after about 10 min.

As Table 1 shows, red cells incubated with serum dialysed against low-ionic-strength medium reacted weakly with anti-$7S$ γ-globulin, and red cells incubated with $7S$ γ-globulin alone, at low ionic strength, reacted slightly more strongly. The following two experiments made it clear that the γ-globulin was required for the uptake of complement.

First, normal serum was fractionated on DEAE-cellulose. Two fractions were obtained; the first by elution with 0·02 M phosphate buffer pH 8·0 and the second by elution with 0·2 M phosphate buffer pH 8·0. Red cells washed in low-ionic-strength medium were incubated with the fractions separately and in combination, in each case diluted in low-ionic-strength medium (I = 0·025). Red cells exposed to the 0·02 M fraction (containing only $7S$ γ-globulin) as expected gave a negative reaction with anti-β_{1E}- and anti-β_{1C}-globulin sera; red cells exposed to the 0·2 M fraction (containing complement but very little $7S$ γ-globulin) gave weak reactions with the same antiglobulin sera but red cells exposed to a mixture of the two fractions gave strong positive reactions with anti-β_{1E}- and anti-β_{1C}-globulin sera (Table 1).

Table 1. Tests with anti-$7S$ γ- and anti-β_{1E}-globulin sera of red cells exposed at low ionic strength to: normal fresh serum, EDTA-treated serum, purified $7S$ γ-globulin, fractions of normal serum eluted from DEAE-cellulose and serum from a patient with hypogammaglobulinæmia, with and without added γ-globulin

Cells exposed to:	Reactions with antiglobulin sera Anti-$7S$ γ	Anti-β_{1E}*
Normal serum	(+)	+ + +
EDTA-treated serum	(+)	–
$7S$ γ-globulin alone (1%)	+	–
DEAE fractions†		
0·02 M	(+)	–
0·2 M	–	+
0·02 + 0·2 M	(+)	+ + +
Hypogammaglobulinæmic serum		
Alone	–	+
+ $7S$ γ	(+)	+ + +

* Similar but slightly weaker reactions were obtained with an anti-β_{1C}-globulin serum and with an immuno-conglutinin serum.

† The 0·02 M fraction contained only $7S$ γ-globulin; the 0·2 M fractio contained all other serum proteins with less than 10 per cent of the $7S$ γ-globulin present in the original serum.

The second experiment consisted in testing serum from a patient with hypogammaglobulinæmia (γ-globulin concentration about 30 per cent of normal). The patient's red cells were washed in saline and mixed with the patient's serum with and without the addition of purified $7S$ γ-globulin. The mixtures were then diluted with an excess of sucrose-saline (approximately 60 vol. of sucrose–saline to one of serum) and incubated at 37° C for 15 min; the red cells were then washed and tested with the antiglobulin sera. Red cells which had been mixed with the patient's serum alone gave only weak positive reactions but the addition of γ-globulin to the serum to bring the concentration to normal gave reactions which were as strong as those given by whole normal serum.

Tests were also made to see whether red cells which had taken up γ-globulin alone at low ionic strength would later bind complement. Red cells were incubated with purified γ-globulin at low ionic stength; after being washed in normal saline they reacted with anti-$7S$ γ-globulin but not with anti-β_{1E}-globulin (Table 1); they were now incubated at 37° for 15 min with an excess of fresh normal serum (at normal ionic strength) and were then washed and tested with anti-β_{1E}- and anti-β_{1C}-globulin sera; weak agglutination was observed, showing that a little complement had been bound.

It may be concluded that, for the uptake of substantial amounts of complement, red cells must be exposed to γ-globulin and complement together at low ionic strength.

Rapp and Borsos[6] have recently published observations of a rather similar nature. They observed that when sheep red cells were mixed with guinea pig complement, in a low-ionic-strength medium, lysis occurred. They obtained evidence of an 'auto-sensitizer' on sheep red cells which could be dissociated by washing the red cells in saline. However, even after the red cells had been washed they could still be lysed at low ionic strength if sufficient complement was supplied. One of the possibilities considered was that guinea pig serum, even after absorption with sheep cells, might contain a 'sensitizer' which activated complement at low ionic strength. The observations recorded here suggest that the 'sensitizer' in these experiments may have been normal guinea pig γ-globulin.

In our experiments only slight hæmolysis (5–10 per cent) was observed after incubation of red cells and serum at low ionic strength, presumably due to the relative resistance of human red cells to immune hæmolysis.

The fact that complement is readily taken up by red cells from fresh serum at low ionic strength can be turned to advantage to provide 'complement-coated' red cells as controls in tests with antiglobulin sera. Thus red cells which will react strongly with anti-β_{1E}-globulin and less strongly with anti-β_{1C}-globulin and only very weakly indeed with anti-$7S$ γ-globulin can be prepared by simply mixing one drop of whole fresh blood with 2 ml. of a solution containing 6 per cent sucrose and 0·15 per cent sodium chloride and incubating at 37° C for 15 min. After being washed twice, the cells are ready for testing.

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RADIOBIOLOGY

High Resistance of Mongolian Gerbils to Irradiation

IT is known that the LD_{50} of X- and γ-irradiation for mammals ranges between 300 and 800 r. (refs. 1 and 2). While maintaining a colony of mongolian gerbils (*Meriones unguiculatus*) in this laboratory, their high resistance to radiocobalt irradiation was observed in a preliminary investigation and the results are reported here.

The dosage of irradiation was monitored at each exposure with a Victoreen Radocon. Ten animals (5 males and 5 females) of a group were selected at random for various dose-levels and their ages varied from 4 months to 1 year except for Group V, in which some young and some older animals of approximately 18 months were included. They were maintained in pairs and fed as previously reported[3]. The body-weight of the animals was recorded daily throughout the experiment. Blood samples for red and white blood cell counts were obtained by clipping the tail without anæsthesia. In the animals where a testis or ovary was removed at various times after irradiation, the anæsthetic 'Nembutal' was injected subcutaneously at a dosage of 6 mg/100 g body-weight. The gonads were fixed, sectioned and stained with hæmatoxylin and eosin.

Table 1. MORTALITY OF MONGOLIAN GERBILS FOLLOWING RADIOCOBALT IRRADIATION

Group	Irrad.	Dose (r.)	Interval between exposures (days)	No. of animals irrad.	Death		Remarks
					No.	Days after irrad.	
I (6 unilaterally castrated)	1st	1,000	64	10	2	33, 35	1 died 1 day after castration 1 died 3 days after ovariectomy
	2nd	1,000	41	8	1	16	Died 35 days after ovariectomy
	3rd	1,000	30	7	1	18	Died 62 days after ovariectomy
	4th	1,500		6	6	7, 7, 8, 9, 14, 26	1 died 64 days after castration 1 died 131 days after castration
II (5 unilaterally castrated)	1st	1,000	78	10	2	12, 19	1 died 1 day after ovariectomy 1 died 8 days after castration
	2nd	1,000	37	8	1	13	Unoperated
	3rd	1,000	33	7	2	15, 21	1 was unoperated 1 died 9 days after ovariectomy
	4th	1,000		5	0		
V	1st	1,000	31	10	1	26	
	2nd	1,000	31	9	4	10, 12, 13, 14	
	3rd	1,000		5	5	9, 9, 10, 11, 14	
IV	1st	1,500		10	9	9–19	The survivor gained 21 g and his hair turned white
III	1st	2,000		10	10	9–12	

From Table 1 it can be seen that un-operated animals were able to withstand 1,000 r. in repeated doses except for Group V in which older animals were included and the interval between exposures was short. These factors may be attributed to their mortality. The mortality following irradiation at 1,000 r. was chiefly due to operation because, among 9 animals that died in Groups I and II, 7 were operated animals. The gerbils, however, were not able to withstand a dose of 1,500 or 2,000 r. and all except one succumbed between 9 and 19 days after the exposure. Previous irradiations did not seem to increase or decrease the resistance of the gerbil to a subsequent exposure as shown by the death of 6 animals in Group I following irradiation at 1,500 r. in spite of being irradiated at 1,000 r. 3 times before. The survivors of 5 out of 10 animals in Group II following the 4th irradiation at 1,000 r. is strong evidence of their high resistance. In the majority of cases, death occurred between 9–15 days after irradiation and death of females occurred sooner than males.

In Groups III (at 2,000 r.) and IV (at 1,500 r.) there was a constant decrease in body-weight until death. The average weight loss was 22·6 per cent with a range of 15·6–27·2 per cent (Group III) and 16·7 per cent with a range of 4·4–25·2 per cent (Group IV) of the body-weight before irradiation. In the groups irradiated at 1,000 r. there was an increase of body-weight in the unoperated animals throughout the experiment. An increase of body-weight of the only survival of Group IV was remarkable, from 117 to 138 g, and the colour of its hair changed from dark-grey to white-grey about 3 weeks after irradiation at 1,500 r.

Blood counts were made on Group II before and two days after the initial irradiation. Subsequent counts were made on animals of various groups before death. In all cases there was a significant decrease in both red and white blood cell counts after irradiation. In animals where counts were made before death a severe leucopenia was noted and a significant increase in clotting time of the blood occurred.

The ovaries displayed a complete disappearance of large and small follicles 32 days after irradiation. Some medium-sized follicles were present, but the ova were either in a degenerate state or missing in the majority of these follicles. The seminiferous tubules of the testes appeared to be normal 19 day post-irradiation but by the 32nd day spermatogenetic activity ceased and no sperm was present in the small tubules of the cauda epididymis. By the 57th day post-irradiation few tubules contained germ cells and the sperm appeared to be abnormal. Thirty-nine days following first irradiation the animals in Group I were paired in an attempt to breed them but no sign of pregnancy was observed.

On autopsy the intestines of all animals appeared hæmorrhagic and the peritoneal fat displayed petechial hæmorrhages in many instances. The pyloric region of the stomach was usually hæmorrhagic but the stomach contained food in all cases.

The mongolian gerbils do not drink water, their cholesterol metabolism differs from other animals[4] as does the lipoprotein pattern[5], their water content in the body is rather low, and their adrenal glands synthesize different corticosteroids[6]. Whether or not these and other factors are correlated to their high radio-resistance is under investigation.

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Increase in the Cæsium-137 Content of Man due to Radioactive Fall-out, 1962–64

THE presence of cæsium-137 in human beings was established in the United States in 1955 (ref. 1) and measurements of the average levels in a small group of unexposed subjects have been made at this Establishment since early 1956. The results of these measurements showed that there was a slow, irregular increase in the cæsium-137 content to a maximum in the summer of 1959, after which the level declined to a lower value in April 1962 than at any time since our measurements began[2,3]. The resumption in September 1961 of large-scale atmospheric testing of high-yield nuclear weapons did not result in any detectable increase in cæsium-137 contents until after April 1962, although the presence of short-lived zirconium-95 in human beings was demonstrated prior to this[3]. In this communication we are

Table 1. AVERAGE CÆSIUM-137/POTASSIUM RATIOS AND CÆSIUM-137 CONTENTS IN A CONTROL GROUP OF SUBJECTS RESIDENT IN BERKSHIRE AND OXFORDSHIRE

Date	No. of subjects	^{137}Cs/K (pc./g)	Approximate average ^{137}Cs content (nc.)	Average radiation dose-rate (μrad/h)
April 1962	15	25	3·5	0·05
July 1962	12	35	4·9	0·07
Oct. 1962	15	50	7·0	0·10
Jan. 1963	10	54	7·6	0·11
April 1963	12	64	9·0	0·13
July 1963	11	90	12·6	0·18
Oct. 1963	13	115	16·1	0·23
Jan. 1964	13	138	19·3	0·28
April 1964	13	150	21·0	0·30

The values for the average radiation dose-rates in the final column of Table 1 were calculated with the assumptions that the cæsium-137 was uniformly distributed throughout the body, and that the average weight was 70 kg. The dose-rates refer to a point at the approximate centre of the body.

reporting the results of our measurements of cæsium-137 in human beings during April 1962–April 1964; we have used throughout the technique described previously[3], which was intended primarily for estimating the lung contents of zirconium-95/niobium-95. The results of serial measurements of the latter radionuclides will be reported elsewhere.

Table 1 summarizes our findings on cæsium-137; as explained previously[3] the geometrical arrangement of detector and subject does not lend itself to accurate calibration for cæsium-137 and potassium contents, but we have abundant evidence that the cæsium-137/potassium ratios are directly comparable with those obtained by our more usual method of total body radioactivity measurement[2]. The cæsium-137 concentration (which is the quantity of interest from the point of view of radiation dosimetry) may be obtained in pc./kg by multiplying the cæsium-137/potassium ratios by 2. The average cæsium-137 content was estimated approximately by assuming an average potassium content of 140 g.

The average values for the cæsium-137/potassium ratio are plotted as a function of time in Fig. 1, together with the previously reported[2,3] values from 1956. Also plotted are the results for subjects *R* and *C*, who have consistently shown the lowest and highest values of the ratio. The difference between the two subjects can be attributed to a combination of the facts that the biological half-life of cæsium in subject *R* is known to be about 60 days, whereas that in subject *C* is at least 140 days[4,5], and that subject *R* drinks much less milk than subject *C*. Milk is an important source of cæsium-137 in the diet, but its influence in controlling the levels in different individuals is less than we originally thought[2]. Variability in the biological half-life among the individuals is probably the biggest single factor in determining the levels[5].

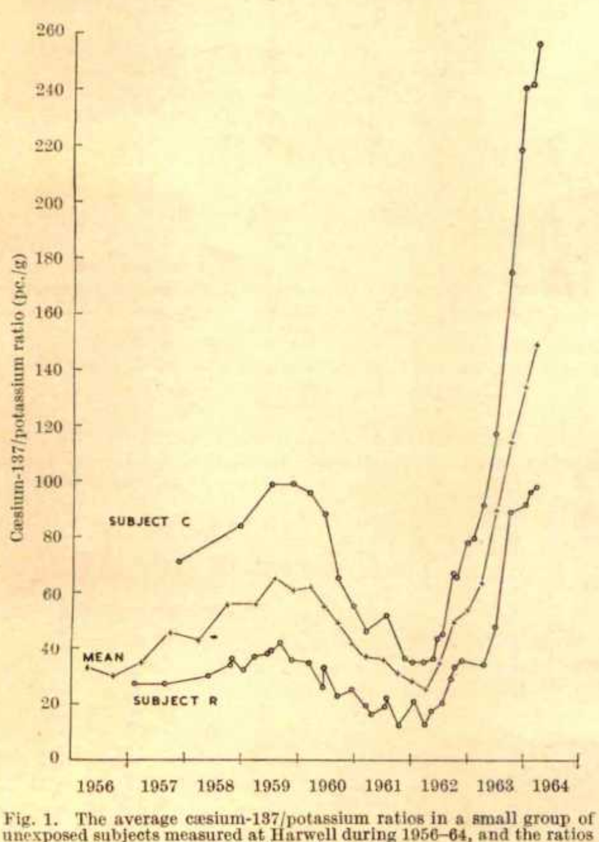

Fig. 1. The average cæsium-137/potassium ratios in a small group of unexposed subjects measured at Harwell during 1956–64, and the ratios for subjects *R* and *C*

Finally, we may note that the radiation dose-rates from the cæsium-137 are still small compared with the dose-rate from natural sources. Thus the contribution from potassium-40 amounts to 2·2 µrads/h, which is seven times the average dose-rate from the cæsium-137 in April 1964. The latter contribution amounts only to 2·6 per cent of the total dose-rate from natural sources (about 100 mrads/yr. or 11·4 µrads/h).

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BIOLOGY

Splenic Regeneration

IN recent investigations in experimental carcinogenesis in the rat, following implantation of a benzopyrene tablet into the devascularized spleen[1], an incidental finding was the presence of nodules of tissue situated in the dorsal mesogastrium at six weeks after operation (Fig. 1).

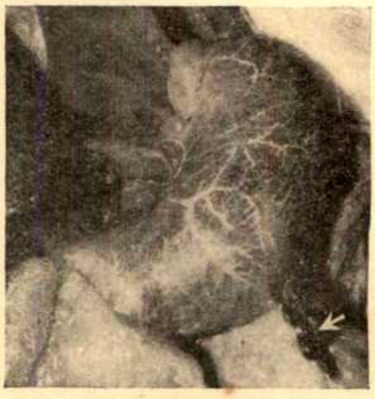

Fig. 1. Photograph of regenerating nodules in the dorsal mesogastrium indicated by the arrow (same size)

The operative procedure consisted of ligating each vascular pedicle of the organ with a double-thread ligature close to the splenic hilus. Confirmation that the blood supply was cut off was afforded by the affected splenic segments, which became a dusky black colour. In animals killed three weeks after operation no sign of the infarcted organ seen at operation could be observed.

At six weeks after operation and periods later than this, a cluster of nodules resembling splenic tissue was present in the dorsal mesogastrium. The larger nodules lay close to the greater curvature of the stomach and their size decreased in a caudal direction; septa of connective tissue were interspersed between separate nodules.

Prior injection of the arteries through the thoracic aorta and the venous system (either through the portal vein or one of its larger tributaries) showed that the main features of vascularization were: additional drainage into systemic veins, probably due to adhesions, and an arterial supply derived from nearby vessels such as the gastro-epiploic and the left spermatic arteries.

Fig. 2 illustrates the arterial supply to a cluster of nodules, injected on death with 'Micropaque' and radiographed after removal. It can be seen from Fig. 1 that the nodules are discrete and circumscribed, and that they have a rich blood supply is evidenced by the filling of their finer branches with the injection medium (Fig. 2).

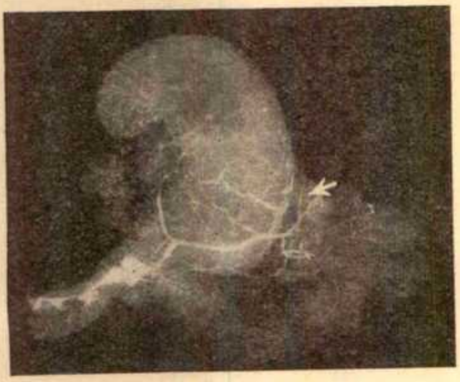

Fig. 2. Positive radiograph of the arteries to the regenerating nodules indicated by the arrow (same size)

Three possible explanations may account for the presence of these newly formed nodules. (1) They have developed *de novo* either from embryonic cell rests in the dorsal mesogastrium, or from the fully differentiated cells of this part of the mesentery. (2) They represent hypertrophy of splenic tissue which was not completely deprived of its blood supply at the time of operation. (3) They consist of tissue other than spleen, such as hæmolymphatic nodes.

The third possibility can be discarded on histological grounds, and the available evidence supports the first explanation for the following reasons: (*a*) The complete infarction of the organ, which can be seen with the naked eye, at operation. (*b*) The apparent absence of the necrotic organ in animals killed 3-4 weeks after operation. (*c*) The arrangement of the nodules, which are larger near the greater curvature of the stomach and decrease in a caudal direction. (*d*) The separation of individual nodules by connective tissue septa. The findings in (*c*) and (*d*) appear to retrace the development of the spleen in the embryo. (*e*) The arterial supply is derived from different sources other than the splenic artery, and the venous drainage occurs in part or in whole into systemic veins. (*f*) At periods later than six weeks after operation the nodules coalesce into a lobulated organ which later undergoes re-modelling to a shape comparable to that of the adult spleen in the normal rat.

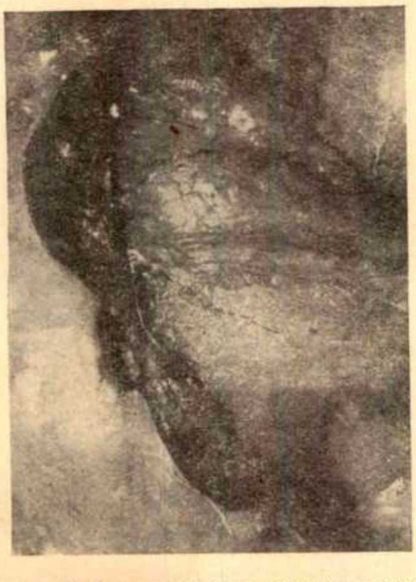

Fig. 3. Photograph of a regenerated spleen which has undergone almost complete re-modelling (× 6)

Final proof of the accuracy of this explanation, however, can only be afforded by removal of the spleen though leaving the dorsal mesogastrium intact, supported by histological confirmation of the absence or presence of mitoses in the regenerating nodules. These problems are now under investigation.

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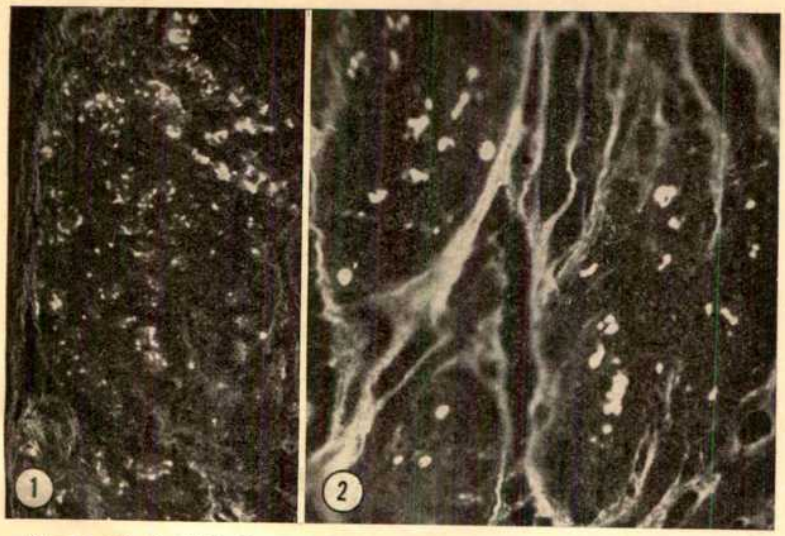
Cytochemical Demonstration of Catecholamines in the Human Carotid Body

During embryonic development, accumulation of neural components in the carotid body of human and animal subjects has been demonstrated[1]. However, efforts to demonstrate the presence of possible transmitter substances in the carotid body of adult animals have produced controversial results. While some have succeeded in showing a positive chromaffin reaction in the glomus cells of animals[2,3], others have reported negative results with the human carotid body[2,4]. There is, however, some direct chemical evidence of the presence of noradrenaline in the carotid body of the calf[5], and recently Pryse-Davies *et al.* have demonstrated in a single fluorimetric assay of a fresh human carotid body obtained at autopsy a high level of catecholamines[4].

We have recently performed a comprehensive cytochemical investigation on a number of human carotid bodies, which have been excised at operation for the treatment of bronchial asthma. The work now also includes five carotid bodies where the presence of monoamines have been histochemically examined by formaldehyde-induced fluorescence, a method originally described by Eränkö[6] and first adapted by Falck[7] to the examination of nervous tissue. The extirpated tissue was immediately frozen on a microtome tissue holder with liquid air. It was sectioned in a cryostat, in which the sections were then allowed to dry[8]. The dry sections were treated with formaldehyde gas for 1 h at 80° C, mounted in a non-fluorescent medium and examined with a fluorescence microscope.

In all the carotid bodies so far examined the formaldehyde treatment resulted in the appearance of intensely fluorescent cells in the lobules of the bodies (Fig. 1). The colour of the fluorescence was always green, never showing yellowish components. The fluorescent cells were relatively few in number (Fig. 2) and they were usually of an even size and situated separately. They were also evenly distributed among the alveoli of the lobules and did not show any clear dependence on the capillary spaces of the bodies. No fluorescing nerve fibres were visible, but the connective tissue fibres showed a non-specific fluorescence, which did not disappear after the dried sections had been exposed to moist air as did the specific fluorescence of the glomus cells.

The specificity of the cytochemical fluorescence method for monoamines is high, and the constant green colour of the omitted light can be taken as indicative of the presence of catecholamines rather than tryptamines[9]. These compounds have not been directly demonstrated before, although many indirect staining techniques have given positive indication for the presence of phenolic amines in



Figs. 1 and 2. Carotid bodies of patients suffering from bronchial asthma. Some of the glomus cells show intense green fluorescence after treatment with formaldehyde gas. The connective tissue fibres are also fluorescing, but no parenchymatous adrenergic nerves are visible (Fig. 1 × 32, Fig. 2 × 80)

the carotid body[3]. Electron microscopically membrane-bound granular bodies have been observed in some of the glomus cells; these granules could be depleted by the administration of reserpine[10].

The work recorded here shows that the human carotid body probably lacks adrenergic parenchymatous innervation. In interpreting the possible functional role of the catecholamines in the glomus cells one has, however, to remember that the carotid bodies which we have examined cannot be considered as normal because the bronchial asthma might well have altered the composition of the glomus cells from that of a normal individual.

We thank Dr. E. Vaheri, of the Department of Oto-Laryngology, University of Helsinki, for the material.

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Frequency of Spontaneous Arousal from Hibernation in Bats

ALL species of mammalian hibernators 'spontaneously' raise their body temperatures to or near the normal homeothermic level at intervals during a winter's hibernation. The frequency with which such arousals occur is in part at least a species-specific characteristic ranging from an arousal every 2–7 days in hamsters[1] to maximal periods of 12–20 days in various species of the genus *Citellus*, and 20–33 days in *Glis glis*[2]. Folk[3] has reported that 4 bats (*Eptesicus fuscus*) remained without movement for 64–66 days in the dark at ambient temperatures of 4°–8° C. However, reliable information about the maximal duration of continuous hibernation (or conversely the frequency of spontaneous arousal) is available for only a few species. Reports of long periods of continuous hibernation based on daily visual observations of the postures of hibernating animals must be interpreted with great caution as there is no well-documented correlation between postural adjustment and change in body temperature in many species of hibernators.

Arousals from hibernation represent a considerable expenditure of energy and no obvious and consistent function can at present be seen to be served by them. Several theories have been advanced in an effort to explain the function of arousal from hibernation[2–4], but very little evidence is available which bears on any of them.

Results during the work recorded here were obtained by continuously recording the outputs of copper–constantan thermocouples sewn under the skin in the scapular region of hibernating *Myotis lucifugus*. Animals were maintained individually in 1-pint glass jars with 0·25 in. of water on the bottom and wire screening around the inside of the jar from which they could hang. These jars were placed in constant darkness, well insulated from noise and vibration, in a constant-temperature room which was held between 3° and 5° C. The experiments reported here were all performed during January–June. Four animals were used in 1960, 9 in 1961, and 11 in 1962. Of these 24 animals, 11 either chewed through their leads, aroused only once before dying, or otherwise produced unusable records. Results from the remaining 13 animals are considered here.

Temperatures of individual bats were monitored continuously for periods ranging from 27 to 142 days, with 79 as the mean number of days of continuous recording for the group of 13 animals. In all, 69 arousals were recorded from the 13 animals during a period of 1,023 'bat hibernation days'. Under the foregoing conditions, the duration of individual arousals was quite short (an average of 3 h 10 min during which the body temperature was more than 1° C above the ambient temperature). Thus, of the 1,023 'bat hibernation days', there were about 1,014 days of actual hibernation and 9 days of arousal.

Fig. 1 is a histogram showing the distribution of the intervals between arousals of all 13 bats. Thus, the abscissa represents the number of days of continuous hibernation with less than 1° C difference between the animal's body temperature and the environment, and the ordinate represents the number of such intervals in the entire group of animals. The short 1–5 day intervals form by far the largest group (27) and it is of some interest to note that 7 of these occurred during the 10 days immediately following the beginning of experiments, 14 occurred during the 10 days immediately preceding death, and the remaining 6 all come from one animal. The most interesting feature of the data, however, is the fact that bats are capable of very long periods of continuous hibernation, uninterrupted by arousal or in fact by any significant deviation of body temperature from ambient temperature. The two intervals of more than 80 days of continuous hibernation are from two animals, one in 1960 (which lived a total of 133 days in the experimental chamber) and one in 1961 (which lived 142 days). Both these animals had several arousals subsequent to that which terminated the long period of hibernation. Long periods of continuous hibernation account for more of the hibernation than is readily apparent in Fig. 1. Of the 1,023 bat hibernation days, almost half (490) occur in the 10 intervals of more than 30 days each.

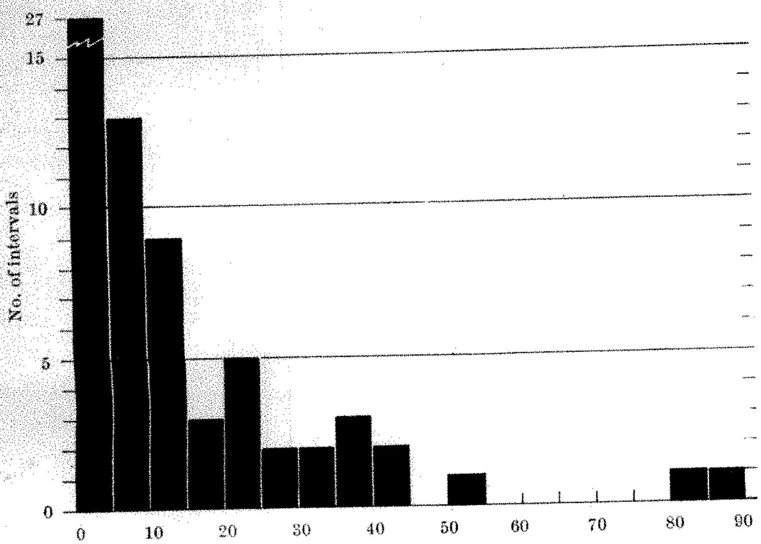

Fig. 1. The 69 intervals (periods of continuous hibernation) obtained from 13 bats have been grouped according to their durations as indicated on the abscissa. The ordinate indicates the total number of intervals in each group. Note the 2 extremely long intervals (82 and 86 days) during which the bats' body temperatures did not rise more than 1° C above the ambient temperature of 3°–5° C

It seems probable from the foregoing results that bats can hibernate without arousing for much longer periods than can any other mammalian species thus far examined. If there is to be a single interpretation of the need of all mammalian hibernators to interrupt their hibernation with periodic arousals, it must account not only for the very frequent arousals of many rodent species but also for the very infrequent arousals of bats.

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Regulation of Acid Invertase-levels in Sugar Cane Stalks by Auxin- and Metabolite-mediated Control Systems

IMMATURE storage parenchyma cells of sugar-cane stalks contain an acid invertase located in the vacuoles and the outer space (cell walls). The physical properties of the enzyme at both loci appear to be identical. The enzyme is readily made soluble simply by grinding the tissue. Measurements of the rate of internode elongation and total acid invertase show that these two parameters are linearly correlated. This particular acid invertase is absent from the mature storage tissue of sugar cane, but persists in the mature tissue of two closely related species which do not store sucrose in high concentrations. However, in mature tissue of sugar cane other invertases occur but not in the vacuole[1-3].

To explain the correlation between internode elongation and acid invertase-level, we have postulated that the outer space and vacuolar acid invertase of young, rapidly expanding cells co-operate to maintain a constant high level of hexose available in the cytoplasm. The regulatory role of this enzyme depends largely on the fact that sucrose must be hydrolysed prior to entry into metabolic pathways in the storage parenchyma cells. The outer space enzyme hydrolyses sucrose originating from the conducting tissue. The vacuolar enzyme enables sucrose in storage to become available for use in metabolic processes when the supply from photosynthesis is inadequate. The level of the vacuolar enzyme may change rapidly in the course of a few hours. We have no evidence for similar rapid changes for the outer space enzyme, although the amount present varies considerably in different batches of tissue.

The postulate outlined here assumes precise regulation of the activity of the vacuolar acid invertase. A search conducted for control systems revealed two different classes of regulatory substances which affected enzyme levels in tissue slices. One class includes indolyl-3-acetic acid (IAA) and synthetic auxins. Low concentrations of IAA (10^{-5}–10^{-7} M) increased the invertase-level when incubated with tissues over 6–10 h periods, while higher concentrations decreased the level[4]. In some batches of tissue, decreased invertase activity was observed for all levels at which auxins were applied, and was taken to indicate that the endogenous auxin-level was near optimal This latter response is similar to that reported for the invertase bound to the cell walls of carrot root and artichoke stem tubers[5].

The other class of substances included certain sugars and derivatives and were effective only at relatively high concentrations (about 10^{-2} M). Some increased the enzyme-level; others decreased it. Among this class only glucose and fructose are known to occur in sufficiently high concentrations *in vivo* to have any marked effect on the enzyme content of the tissue. Both sugars always decrease the invertase-level. However, since they are readily interconvertible following phosphorylation, and are also rapidly metabolized it was not possible to conclude that either sugar acted as such.

Investigations using radioactive glucosamine provided the evidence that glucose is an endogenous regulator of invertase-levels. Glucose and glucosamine gave identical effects at different *p*H values and over a large range of concentrations. After treatment with ^{14}C-glucosamine, the unaltered sugar plus a phosphorylated compound and *N*-acetyl glucosamine were recovered from the tissue. *N*-acetyl glucosamine is not produced from glucose. On hydrolysis, the phosphorylated compound yielded free glucosamine. Kinetic considerations lead us to exclude phosphorylated sugars as possible control substances. We, therefore, conclude that free glucosamine and free glucose act directly to lower the enzyme level.

Mechanisms by which glucose might affect acid invertase content have been considered and experiments devised to test the several possibilities. When included in assay solutions at concentrations which were effective on tissue slices glucose had no effect on the activity of the enzyme. Hence it is not a competitive or allosteric inhibitor. The possibility that low molecular weight inhibitors accumulated in the tissue following glucose treatment is unlikely as exhaustive dialysis at low temperatures made no difference to the enzyme activity of crude extracts. Control by means of changes in levels of high molecular weight inhibitors or proteolytic enzymes was virtually excluded by experiments which showed that the total enzyme activity in mixed extracts was equal to the sum of the activities of extracts taken from tissue high in invertase and from tissue which had been treated with glucose to give a very low enzyme-level. Another possible regulatory mechanism would be through *p*H control as the isolated enzyme loses activity rapidly at *p*H values lower than about 5·0. However, there was no measurable difference in the *p*H of tissues treated for 10 h in glucose or water. By a process of elimination we were left with reduction of the rate of invertase synthesis as the most plausible explanation of the glucose effect[6].

The specificity of the glucose effect was shown by the observation that large changes in acid invertase could be induced without a concomitant change in the total protein content of the tissue. Its reversibility was readily demonstrated by transferring tissues incubated in glucose and containing low invertase-levels to a glucose-free medium, whereupon a rapid increase in the enzyme-level occurred. In one experiment this amounted to an 11-fold increase in 6 h.

That enzyme synthesis is involved in the increase in invertase activity following transfer of glucose-repressed tissues to a glucose-free medium has now been shown by use of chloramphenicol (CAP), a potent inhibitor of protein synthesis. Synthesis of invertase commenced in these tissues after a 2-h lag period, but no synthesis occurred if transfer was to CAP (Fig. 1). Actinomycin *D* was also effective in preventing synthesis of acid invertase, a concentration of 5 μg/ml. being required to reduce the enzyme-level by 50 per cent compared with tissues in water. Recent evidence indicates that both CAP and actinomycin owe their effects on protein synthesis to interference with the production or utilization of messenger ribonucleic acid[7,8]. A consistent interpretation of the results shown in Fig. 1 is that the glucose effect on invertase production is by repression of synthesis of the messenger RNA which specifies the amino-acid sequence for acid invertase. If so, the system has many features in common with metabolite-controlled end-product repression of enzyme synthesis as found in micro-organisms[9].

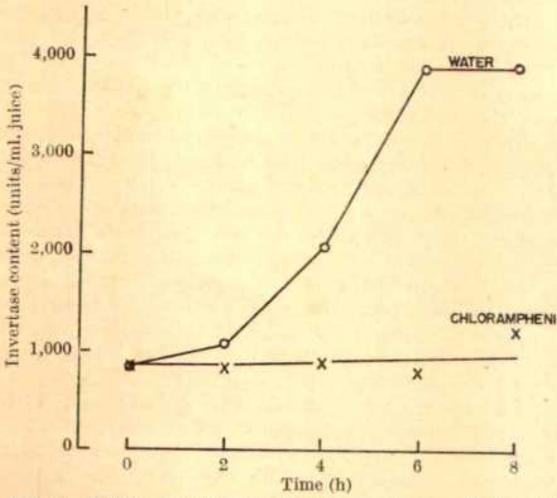

Fig. 1. Effects of chloramphenicol on invertase synthesis following release of repression by glucose. Tissues were incubated for 16 h in 0·1 M glucose, then washed under running tap-water and transferred to water or chloramphenicol solution (0·75 mg/ml.). Samples were harvested at two-hourly intervals, ground finely, the juice dialysed at 3° C, and the invertase content of the dialysate determined

From examination of the steric requirements for activity on invertase synthesis, we have learned that conversion of a sugar, active as a repressor, to its methyl glycoside virtually destroys its activity, while reduction to the polyhydric alcohol confers inducer properties on the molecule. However, the role of inducers of this type in the intact plant is uncertain as we know of no compound present at sufficiently high concentrations to be effective. It seems likely that in tissue slice investigations these inducers compete with endogenous glucose at its site of action as a repressor. Since the auxins are chemically quite different from the metabolite-type inducers and repressors and are effective at concentrations which are three to five orders of magnitude lower, we conclude that it is unlikely that two classes of regulatory substances act in the same way.

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Enzyme Activation in Germinating *Petunia* Pollen

WHEN pollen and maternal tissue come into contact, the rate and sequence of enzyme activation control the growth of pollen and the nature of the compatibility reaction. Intraspecific pollination in certain plants results in an incompatibility reaction in the maternal tissue. In *Petunia*, one of the few species examined in detail, pollen growth is characterized by formation of a new protein fraction in the style and inhibition of pollen tube growth[1]. It has been postulated that this new protein forms in response to an inducer: a chemical moiety, such as a protein, which diffuses from the pollen[2].

This communication reports the effect of time and varying external osmotic environment on sucrose metabolic activity in initially germinating *Petunia* pollen. It also considers the possible relationship of enzyme protein activities to the incompatibility reaction.

More than seventy years ago Green demonstrated that *Lilium* pollen could metabolize starch *in vitro*[3]. Histochemical investigations have since shown that germinating pollen secretes many enzymes[4]. Recent investigations with *Oenothera* pollen indicate that a protein and starch digesting enzymes diffuse out of the pollen from 1 to 15 h after it is set out on an agar surface[5]. These *Oenothera* proteins reacted with antiserum prepared from whole pollen with related S genes[6]. When *Petunia* pollen is placed on a sucrose media, a number of enzymes are activated and metabolize this substrate to carbon dioxide. Assay of $^{14}CO_2$ released from carbon-14 uniformly labelled sucrose by *Petunia* pollen provides a direct means of studying the activity of these enzymes *in vitro*.

Air-dried pollen of *Petunia hybrida*, clone *W*166*K* with self-incompatibility alleles S_1S_2 from plants grown in greenhouses with supplemental lights at Nijmegen[7], was germinated in Warburg flasks with carbon-14 uniform by labelled sucrose (Radiochemical Centre, Amersham). At various time-intervals of 5–45 min the pollen was placed into the liquid media; sulphuric acid was added to terminate growth and to release all carbon dioxide. The carbon dioxide was collected in potassium hydroxide, and the amount of radioactivity in the barium carbonate precipitate of the potassium hydroxide was determined. These experiments showed that sucrose was rapidly metabolized early in the germination of the pollen (Table 1).

Concomitant growth investigations, made under the light microscope with pollen from the same sample, showed that tubes were not visible until approximately 30-min germination at 26° C in optimal concentrations of sucrose and boron, that is, 0·2 M sucrose

Table 1. $^{14}CO_2$ RECOVERED FROM *Petunia* POLLEN METABOLIZING SUCROSE-UC-^{14}C*

Pollen (mg)	Time metabolized at 26° C (minutes)	Counts per minute Total recovered	Counts per minute Increase per 5 min
7	5	124	104
7	10	254	130
7	15	640	386
7	30	1,910	424
7	45	2,960	350
0	5	23	
0	15	19	

* Each Warburg flask contained $1·9 \times 10^5$ c.p.m. sucrose-UL-^{14}C in 0·9-ml. germinating media of 0·2 M sucrose and 20 p.p.m. boron as H_3BO_3. Growth was stopped by the addition of 0·4 ml. of 4 per cent sulphuric acid from the side-arm. Centre well contained 0·2 ml. 5 M potassium hydroxide. The experiment was repeated three times with the same trends and results apparent in all experiments, although absolute counts differed.

and 20 p.p.m. boron. After half an hour at 26° C, about 25 per cent of the pollen showed tube enations; after 45 min this increased to about 60 per cent. When the $^{14}CO_2$ released at 5-min intervals in the initial 45 min of germination (Table 1) was related to tube growth, it was apparent that high levels of sucrose metabolism occurred before tubes were apparent. The rate of $^{14}CO_2$ released was relatively constant from 10 min to the termination of these studies at 45 min, except for a slight increase during intervals corresponding to times of maximum tube enation.

The effect of varying the osmotic environment on activation of this sucrose-metabolizing system was examined by substituting from 0·0 to 0·9 M mannitol for 0·2 M non-radioactive sucrose (Fig. 1). Sucrose-UL-^{14}C, 2×10^{-8} moles containing $1·9 \times 10^5$ c.p.m., was added for enzyme assay. Since mannitol has been reported to be a substrate in some plant tissues[8]; its effect on *Petunia* pollen germination first was ascertained. Visual assays showed that germination during a 3-h period at 26° C in all boron–mannitol solutions and in the controls without sucrose was less than 2 per cent.

Differences in $^{14}CO_2$ recovered from germination using mannitol from 0·0–0·33 M and from 0·66–0·9 M (Fig. 1) were not statistically significant. Differences between $^{14}CO_2$ recovered at 0·33 and 0·66 M, however, were highly significant. The increase in the external osmotic pressure apparently inhibited the release of carbon dioxide from the pollen. This observation and the fact that nearly maximum sucrose metabolizing activity—measured by release of carbon dioxide—occurs under approximately zero osmotic pressure (Fig. 1) are interesting in view of previous related investigations.

Investigations have shown that germinating pollen can incorporate free $^{14}CO_2$ into carbohydrates, amino-acids, and even proteins[9]. Inhibition of pollen growth usually occurs in sucrose solutions above 0·6 M, or with osmotic values greatly different from the pollen[10]. Thus it is unlikely that decreased levels of $^{14}CO_2$ released at increasing osmotic pressure (Fig. 1) were the result of increased activity of enzyme pathways leading to $^{14}CO_2$ incorporation. Both growth inhibition and decreased $^{14}CO_2$ release at high osmotic pressures probably result from inactivation of the enzymes involved in growth and metabolism.

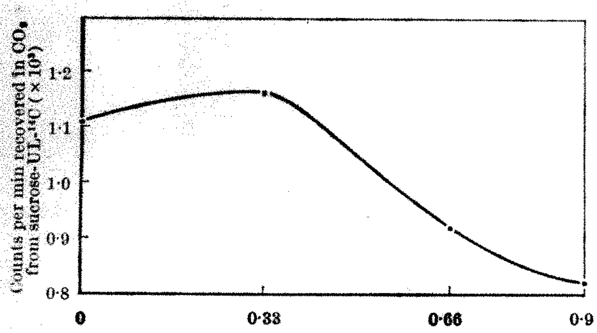

Fig. 1. Effect of osmotic pressure on $^{14}CO_2$ recovered from *Petunia hybrida* pollen during initial 15 min at 26° C. Each Warburg flask contained 7 mg pollen in 0·9 ml. solution containing mannitol at the molarity indicated plus 2×10^{-8} moles sucrose-UL-^{14}C containing $1·9 \times 10^5$ c.p.m. After being shaken 15 min at 26° C, 0·4 ml. 4 per cent sulphuric acid was added to the germinating media. Each point represents the mean of three replicate experiments

The high rates of $^{14}CO_2$ production at nearly zero osmotic pressure may be explained by one of several mechanisms. (1) At zero osmotic concentration essential enzyme proteins may be activated on the pollen surface-binding sites. (2) Pollen membranes may be 'leaky sieves' in an environment of low osmotic pressure and freely release active enzymes. (3) Uptake of water and micro-levels of radioactive sucrose may be the same in the initial 15-min period used in this study over the range 0·0 to about 0·4 molar; however, mannitol at higher concentrations would diminish water and sucrose absorption since the mannitol would dilute the water and compete with entering radioactive sucrose. (4) *In vitro* studies with enzyme preparations showed that the orientation and available charged groups decreased as concentrations of polar compounds increased[11]. Thus another hypothesis is that the rate of activation and stereo-orientation of charged groups of these pollen enzymes are normal at low molarities.

Assuming considerable amounts of non-radioactive sucrose were metabolized with the 2×10^{-8} M sucrose-UL-^{14}C leads to the conclusion that enzyme activity was lower in mannitol than in sucrose. Release of $^{14}CO_2$ from sucrose-^{14}C in mannitol was higher (Fig. 1) than from sucrose-^{14}C in sucrose (Table 1), that is, 1,150–640 c.p.m. Thus counts per min activity recovered in the all-sucrose media after 15 min was only about 60 per cent that recovered in mannitol at 0·2 M. Although dilution of radioactive sucrose was the same in 0·2 M mannitol as in 0·2 M sucrose, there was no competition for active enzyme sites in the mannitol. Non-radioactive sucrose is metabolized along with the 2×10^{-8} M sucrose-UL-^{14}C. Therefore, considering the virtual swamping with unlabelled sucrose (Table 1), enzyme activity metabolizing sucrose in mannitol is probably considerably less than that in the all-sucrose medium.

Growth of pollen tubes was not observed in 0·2 M mannitol with 2×10^{-8} M sucrose-UL-^{14}C. Such lack of growth was generally accepted as evidence that the external carbon source was not being metabolized. Although no growth occurs in mannitol, sucrose is metabolized. Lack of tube growth may only indicate that energy released from sucrose oxidation is not utilized in growth processes, or that 2×10^{-8} M of sucrose supplies too little energy or building moieties to facilitate tube growth. This result also shows that the assumed relationship between metabolism and growth may be in error in certain cell systems.

This study evaluated the ability of mannitol to support pollen tube growth, that is, germination. Hrabětová and Tupý[12] observed a slight growth of apple pollen in mannitol. Thus only by use of labelled mannitol and an analysis of the products can it be clearly demonstrated that mannitol is or is not metabolized—even though tube growth does not occur.

Detailed observations of the site of sucrose-metabolizing activity during the initial stages of pollen germination indicated that the enzymes were localized on or in the pollen, or that the enzymes were too unstable to be recovered in the surrounding solution[7]. The kind of enzyme proteins activated and diffusing from the pollen and whether they are the same as the protein inducing the incompatibility reaction must still be determined.

Antiserum prepared from whole pollen homogenates[8] or homogenates of whole styles[2] have been shown to react with diffusates from pollen with related incompatibility *S* genes. This serological test confirmed the presence in the initial pollen cells of proteins similar to those in the pollen diffusate. However, serological type reactions have never been shown to occur in stylar tissue. In fact, such simple, direct antigenic reactions are at present not thought to occur in the style[13]. Since different proteins occur after pollination of incompatible styles than after pollination of compatible styles[1], a messenger-RNA type reaction is still a valid hypothesis[2], as is the interpretation of a general protein-complexing type of reaction[14].

It is apparent from spectrophotometric investigations that other types of compounds frequently associated with proteins, such as flavanols, also diffuse into solution during the first few minutes of germination[7]. It is possible that these or related lipo-carotinoids on the pollen surface, already shown to stimulate stigma cell metabolism[15], facilitate induction of constitutive changes in the maternal tissue. The initial high stylar respiration during incompatible pollination[1] may be the result of stimulation by such components. The component that diffuses from pollen is known to be gene specific. This diffusate could exert its effect on growth by inducing, releasing, or complexing stylar proteins to activate or form a protein moiety with a different molecular weight than is normally present in compatible pollinations[14,16].

The incompatibility reaction may involve only enzyme protein, or a non-enzymatic moiety. Serological and electrophoretic methods provide a promising approach to understanding this type of incompatibility. Genotypes characterized by pollen with high initial protein and chemical activities at the surface, or in the diffusate, can be readily adapted for such investigation.

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Cytoplasm Exudate from Cut Phloem

CYTOPLASM structure in sieve elements and particularly at sieve pores is of critical importance in any consideration of the translocation mechanism. The mass flow theory[1] assumes a degenerate sieve element protoplast in which vacuolar fluid fills the sieve element and is continuous through open sieve pores. A fluid flow of sucrose solution is thought to occur through sieve tubes due to an osmotic pressure gradient. Other theories propose that metabolic energy provides the motive force required to move nutrients over long distances through plants. These 'active' theories assume that the sieve elements have cytoplasmic contents similar to parenchyma cells but with exceptionally large end wall pores which are filled with cytoplasm. This interpretation is consistent with de Vries protoplasmic streaming theory[2], with Mason and Maskell's activated diffusion theory[3], with Kursanov's molecular transfer theory[4] and Spanner's electrokinetic theory[5].

My own view of cytoplasm structure in sieve elements provides another interpretation which can be linked with an active transport mechanism. In earlier papers[6,7], I put forward the proposal that transcellular strands of cytoplasm are in parallel arrangement with the long axis of the sieve elements and fill sieve pores to form a continuum in the sieve tubes. The outer membrane of the transcellular strand forms a microtubule which provides an unobstructed pathway for enclosed fluid and particulate cytoplasm. The translocation mechanism is thought to be limited to the streaming cytoplasm[6], and in this concept concentration gradients take a subsidiary role, as part of a mechanism regulating movement in and out of the translocation system. Canny[8] has put forward an alternative model in which translocation is explained as diffusive movement, initiated by concentration gradients and accelerated by transcellular streaming.

If the sieve element protoplast consists of normal amounts of physiologically active cytoplasm, as proposed by both the 'parenchyma cell' and 'transcellular strand' interpretations, it might be expected that the release of phloem pressure in cutting would result in the exudation of cytoplasm from the cut phloem. Alternatively, if sieve tube elements are degenerate only trace amounts of cytoplasm would be expected in the phloem exudate.

In this communication I wish to report the exudation of fluid endoplasm, together with two distinct kinds of cytoplasmic particles, from the cut ends of fine veins in soybean leaflets. The plants from which leaflets were taken were grown in a greenhouse under very favourable light and temperature conditions. Pieces (cm^2) were cut from the leaflets of trifoliate leaves, mounted immediately in tap water between slide and coverslip (abaxial surface uppermost), and observed with a times 40 achromatic objective. A flow of cytoplasm from the cut ends of fine veins has been recorded on 100 ft. of 16-mm ciné film. Figs. 1 and 2 are photomicrographs of the cut ends of fine veins and reveal ribbons of cytoplasm, containing particles. The smaller of the two kinds of particles are comparable in size to plant mitochondria (diam. 0·5μ) while the larger particles have the appearance of unusually small, rod-shaped chloroplasts (diam. 1·0μ, length 3·0μ). The larger particles are green and stain intensely blue-black with Lugol's iodine solution. Particles like these were previously reported[6] in the exudate from cut soybean

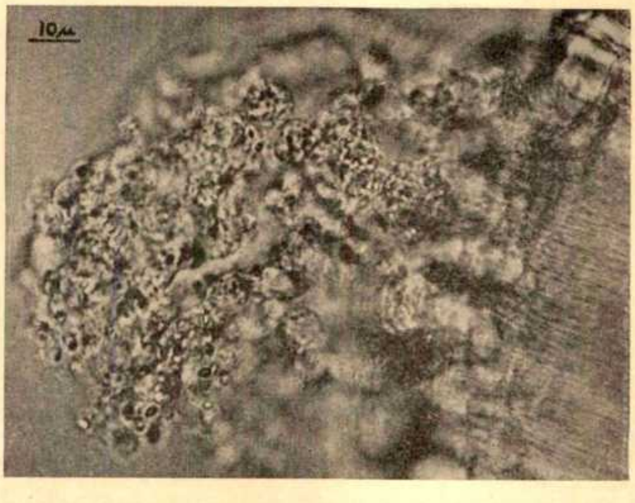

Fig. 1. Cut end of a fine vein in a cm^2 of soybean leaflet. Mitochondria-like particles and small chloroplasts are abundant in a mass of precipitated cytoplasm exuded from the cut phloem. Mounted in tap water, abaxial surface uppermost

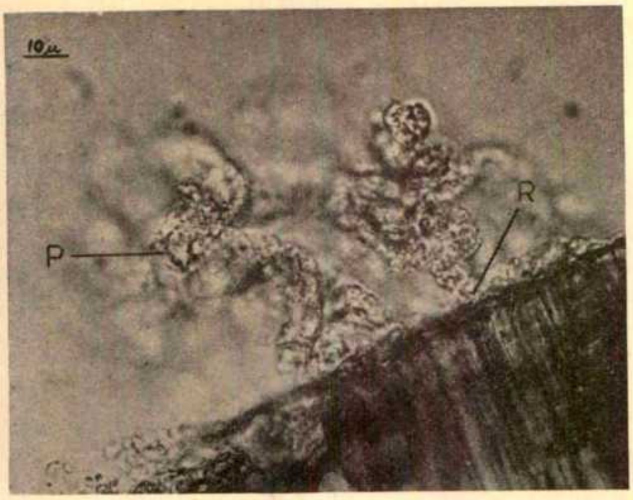

Fig. 2. A preparation similar to Fig. 1. Exuded cytoplasm has a ribbon-like form (*R*) attached to the cut ends of phloem cells. One of these ribbons shows particles (*P*) arranged in single file

stems. Some of the ribbon-shaped forms in the exuded cytoplasm can be traced back to an attachment with the cut vein (Fig. 2). In the ciné film the movement of particles can be followed in the vein and afterwards the same particles can be identified in the exudate. Measurements of vein dimensions in transverse sections were related to the planes of focus in which exuding cytoplasm was seen and, in this way, it was established that cytoplasm was exuded only by the cells of the phloem, between the sub-epidermal parenchyma and the xylem. Unfortunately, due to the interference of overlying cells, the phloem cells which gave rise to the exuded cytoplasm were not clearly defined. In most preparations, exudation continued for about 10 min, and the longest time recorded for any vein was 12 min. There was no cytoplasm exuded from cut border parenchyma cells, epidermal cells, or mesophyll cells.

The flow of cytoplasm from the cut soybean leaflet vein, and the occurrence of significant amounts of cytoplasm in soybean stem exudates[6], supports the view that sieve elements contain normal amounts of cytoplasm. Consequently, this evidence is consistent with both the 'parenchyma cell' and 'transcellular strand' interpretations of protoplast structure in sieve tube elements. Particles have already been observed moving from cell to cell in single files in mature sieve tube elements from *Primula obconica* petiole phloem[9], and in Fig. 2 it can be seen that some of the smaller particles are arranged linearly in single file, which may be a reflexion of their structural arrangement before exudation. It is therefore conceivable that fluid endoplasm and cytoplasmic particles exuded from fine veins are the contents of sieve tubes which flow from cut transcellular strands when phloem pressure is released by cutting.

I thank Prof. R. D. Preston and Dr. E. K. Woodford for their advice.

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A Factor controlling Sexual Reproduction in *Phytophthora*

AN understanding of the physiology of reproduction in the fungus *Phytophthora*, one of the most important plant pathogenic genera, is hampered by there being no defined culture medium on which the sexual cycle can be completed. The fusion organs are always obtained in culture by growing it on a variety of organic media the precise compositions of which are not known; oatmeal is perhaps used the most. The only attempt to extract from such media substances which are active in promoting production of oogonia and antheridia in *Phytophthora* appears to have been by Leonian and Lilly[1]. They obtained from peas an unidentified fraction active down to 0·1 p.p.m. Zentmyer[2] reported stimulation of fusion organs in *P. cinnamomi* Rands by a non-protein extract from roots of avocado pear.

Temperate and tropical isolates which have been used are: *P. cactorum* (Leb. and Cohn) Schroet., and *P. heveae* Thompson; these reproduce sexually as single isolates. *P. cryptogea* Pethyb. and Lafferty paired with *P. cambivora* (Petri) Buis, and *P. cinnamomi*; also *P. palmivora* (Butl.) Butl. paired with *P. cinnamomi* (see Waterhouse[3]). The basic culture medium was: sucrose, 30 g; $Ca(NO_3)_2$, 0·05 g; KH_2PO_4, 0·6 g; $MgSO_4.7H_2O$, 0·3 g; L-asparagine, 2·0 g; L-tyrosine, 0·5 g; thiamine, 1 mg; agar, 15 g. in 1 l. distilled water. To 1 l. was added 1 ml. from the following solution in 100 ml.: $FeSO_4.7H_2O$, 50 mg; $ZnSO_4.7H_2O$, 50 mg; $CuSO_4.5H_2O$, 2 mg; $MnCl_2.4H_2O$, 2 mg; $NaMO_4.2H_2O$, 2 mg. The sugar and calcium were autoclaved separately from the other constituents, *p*H was 5·5 before autoclaving at 15 lb./10 min, and the two parts were mixed after cooling to 50° C. All media were used in 9-cm Petri dishes.

Vegetative growth on the basic medium was good, and comparable to that on one prepared from oatmeal; but fusion organs, as expected, formed only on oatmeal. When oatmeal was extracted with ether and the extract added to the basic medium fusion organs were formed; but they were absent on the medium prepared from oatmeal after extraction. The ether extract was then saponified[4] and only the non-saponifiable fraction was found to be active. This active material was chromatographed on a column of silicic acid (Mallinckrodt) and acid-washed 'Celite' (1 : 1 w/w) and fractions eluted with light petroleum, 1 and 25 per cent diethyl ether in light petroleum, ether and methanol. After removal of solvent by distillation the residual material was tested for biological activity. This was present only in the fraction eluted with 25 per cent ether in petrol.

This fraction, as indicated by thin-layer chromatography, contained several compounds. It was re-chromatographed on a second column of silicic acid/'Celite', and the column eluted with 5, 10, 15, 20 and 25 per cent ether in petrol. Only the 10 and 15 per cent ether fractions contained active material. The residue from these two fractions was dissolved in a minimum of hot ethanol and formed crystals on cooling; the crystals were separated and recrystallized twice. These gave a single spot on a thin-layer chromatogram, which, after spraying with 50 per cent sulphuric acid followed by heating at 105° C for 10 min, turned red.

Apparently normal oogonia and antheridia were always abundant in the basic medium to which the crystals had been added. The sterol nature of the active material, suggested by the spray reaction, was confirmed by precipitation of sterol as digitonide from the combined 10 and 15 per cent ether fractions. The sterol was regenerated[5] and shown to be active. A similar sterol was prepared from dried garden peas by digitonin treatment of the active fraction from the first chromatography.

An indication of the degree of activity of these extracts is shown in Table 1. This suggests that the extracted substances will prove active at concentrations considerably

Table 1. Formation of Fusion Organs in *Phytophthora* on a Medium of Known Composition with a Purified Extract from Oats and Peas

Days after plate inoculation		7				9				10			
Concentration active substance (p.p.m.)		10	20	40	80	10	20	40	80	10	20	40	80
Peameal:	*P. heveae*	*2	1	2	3	3	4	4	4	4	4	4	4
	P. cactorum	0	1	1	2	2	2	3	3	3	3	3	3
	Control	No fusion organs											
Oatmeal:	*P. heveae*	0	1	1	2	1	3	4	4	2	4	4	4
	P. cactorum	0	0	1	1	2	1	2	2	2	2	3	3
	Control	No fusion organs											

* Scoring (number of fusion organs in one microscope field 1 mm. diam.). 0, none; 1, up to 10; 2, 11–50; 3, 51–100; 4, >100.

lower than 10 p.p.m. The following sterols have been examined: β-sitosterol C.P., stigmasterol and cholesterol U.S.P. (Nutritional Biochemicals Corp., Ohio). These three known sterols all promote the formation of fusion organs when added at 10 p.p.m. to the basic synthetic medium. Thin-layer chromatography showed these substances to have similar R_F values, and colour reactions with sulphuric acid, as the substances from the oat and pea.

Gas-liquid chromatographic analysis of the sterol fractions (kindly performed by Dr. E. I. Mercer, Department of Agricultural Biochemistry, University College of Wales, Aberystwyth) has shown that the oat sterol mixture contains β-sitosterol and stigmasterol as the main components of the six separated. The pea sterol contains mainly β-sitosterol together with stigmasterol.

This work is being done while one of us (J. A. L.) holds a Ramsay Memorial fellowship awarded to him at the University of Madrid.

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Preliminary Results of a Comparison between Tetraploid and Diploid Ryegrass

Much interest has been aroused by the introduction from Holland of bred varieties of tetraploid ryegrasses. Herbage production experiments[1] have not shown any remarkable differences in dry matter yield between these tetraploid and good diploid varieties; it was noted, however, that during the winter the tetraploids were singled out by hares for very hard grazing. The moisture content of the tetraploids is higher and in experiments at the West of Scotland Agricultural College[2] Italian tetraploid outyielded *S.*22 on a green weight basis. Dent and Aldrich[3] found that the soluble carbohydrate content of both perennial and Italian tetraploids was higher than that of comparable diploid varieties throughout the summer. In view of these findings, diploid and tetraploid varieties of both Italian and perennial ryegrass were sown in an animal production experiment in 1963; further sowings will be made in 1964. The preliminary results are presented here.

Table 1

Plot No.	Plot size (acres)	Sowing date	Seeds used (lb./acre)	Utilized herbage (D.M. 100 lb./acre) Silage	Hay	Grazing
1*a*	1·89	March 22	28 *S.*22	16·1	12·0	19·4
1*b*	1·89	March 22	30 'Tetila Tetrone'	16·5	13·1	20·4
2*a*	1·49	March 23	20 *S.*24 + 87 barley	*		23·7
2*b*	1·49	March 23	20 'Reveille' + 87 barley	*		24·9
3*a*	1·83	May 13	30 *S.*22			26·7
3*b*	1·83	May 13	30 'Tetila Tetrone'			28·0

* Not given as mainly composed of barley.

The varieties were sown in paired plots (Table 1). Fertilizer was applied either in the seed bed or as a top-dressing in July or August, the same amount being used on each of the paired plots. The plots were either cut for conservation or grazed. All grazing was done by thirteen February-born calves per plot. The yields of conserved herbage were higher from the 'Tetila Tetrone' than from *S.*22. All grass cut for silage was weighed, a sample of grass from each load being used to determine the dry matter content. The quantities of hay produced were assessed by weighing the hay bales and determining their dry matter content. The amounts of grass utilized at each grazing were estimated on the basis of a herbage intake study carried out in September and the live weight of calves. As the estimated intake was higher on the tetraploid, and average live weights were similar, the estimates of herbage utilized by grazing were higher from the tetraploid swards. The calves grazed these plots during August 13–October 28 except for a period of fourteen days. During this period the average live-weight gain of the thirteen on the diploid varieties was 121 lb. and of the thirteen on the tetraploid varieties 133 lb.

In this first year a detailed comparison was made of herbage intake of calves grazing *S.*22 Italian ryegrass and 'Tetila Tetrone' during September 15–26 on plots 1*a* and *b*. Each plot was split into three sections and each section was grazed for 5–6 days. Fæcal output was measured by dosing with chromic oxide and sampling fæces from the sward[4]. Digestibility was determined *in vitro*[5] on herbage samples grazed by an œsophageal-fistulated calf and on samples taken with hand shears. During the intake study herbage samples from both plots contained nearly 100 per cent of sown species of which 85 per cent was green leaf and sheath, 6 per cent stem and 9 per cent dead material on 'Tetila Tetrone'; the respective percentages on *S.*22 were 79, 7 and 14. Percentage ash, dry matter (D.M.) and normal acid fibre (N.A.F.) in the herbages of *S.*22 were all higher and soluble carbohydrate (S.C.) lower than in the 'Tetila Tetrone' (Table 2). The organic matter digestibility of the tetraploid was on average 3 units higher than that of *S.*22, by either sampling method. The digestibilities of samples taken *via* the fistula were higher than those of cut herbage. Furthermore, N.A.F. content of samples taken through the fistula was higher than that of cut herbage and soluble carbonate content lower. These results are undoubtedly due to the action of saliva, and so the digestibility determinations on cut herbage were used for the determination of intake[4].

The fæces of animals grazing 'Tetila Tetrone' were darker and there was also more scouring in this group, but dry matter content of the fæces was similar (12·2 per cent *S.*22 and 12·1 per cent 'T. Tetrone'). Fæcal output was

Table 2

		% O.M. dig.	% ash	% D.M.	% N.A.F.	% S.C.	Fæcal output (lb. O.M./calf/day)	O.M. intake* (lb./calf/day)
*S.*22	Cut herbage samples	75·5	12·6	18·6	29·0	19·2	2·18 ± 0·044	8·9 ± 0·18
	Fistula samples	76·1	13·8	11·9	35·9	9·3		
'Tetila Tetrone'	Cut herbage samples	78·4	11·8	15·8	28·3	21·9	2·03 ± 0·036	9·4 ± 0·17
	Fistula samples	79·9	14·0	11·2	34·8	10·8		

* Calculated from the equation: wt. organic matter (O.M.) eaten $= \dfrac{100 \times \text{fæcal output lb. O.M.}}{100 - \text{O.M. dig. } \textit{in vitro} \text{ of cut herbage}}$

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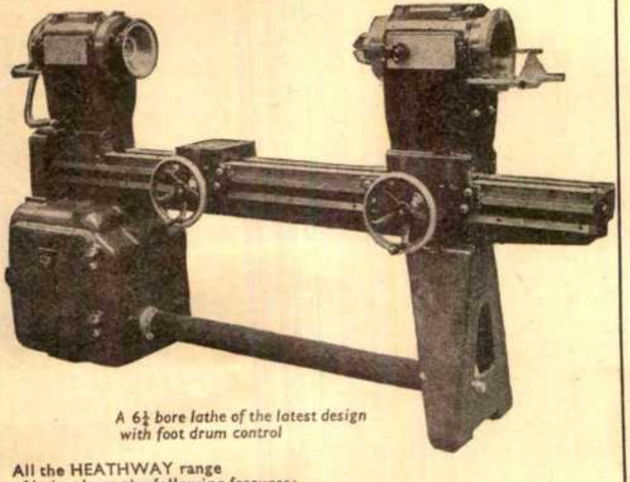

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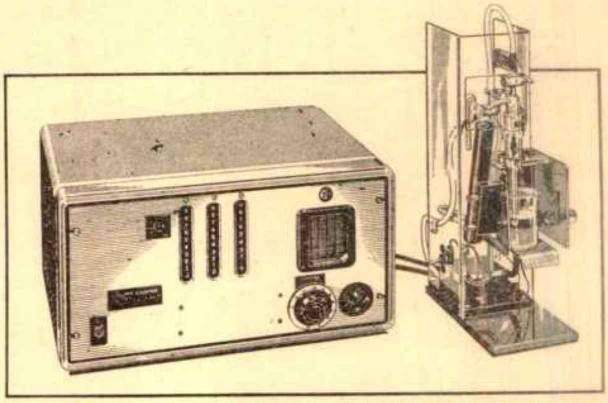

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highest on *S*.22 but, with the higher digestibility of 'Tetila Tetrone', intake was estimated to be higher on the latter. The average weight of the two groups of calves was similar at this time.

The hay made from plots 1*a* and *b* was fed to one pair of dizygous twin cattle housed in individual pens during a four-month period. The hays fed to the animals were changed over at intervals. The mean intakes, lb. of organic matter per 100 lb. live weight, were 1·7 for *S*.22 and 1·8 for 'Tetila Tetrone'. The O.M. digestibility of the hays measured *in vitro* was 73·8 for *S*.22 and 76·7 for 'Tetila Tetrone'. When an animal was fed 'Tetila Tetrone' hay it appeared to gain weight at a faster rate, but with the complication of gut fill in the short periods of feeding the validity of this measurement is doubtful. The fæces of the animal fed *S*.22 always appeared to be firmer but the dry matter contents were again similar (*S*.22 13·2 per cent, 'T. Tetrone' 13·6 per cent).

All the results favour the tetraploid varieties. The differences between *S*.22 and 'Tetila Tetrone', if confirmed, could be sufficient to have a significant effect on animal performance during a grazing season.

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Wax and the Water Vapour Permeability of Apple Cuticle

THE storage characteristics of apples depend on the variety. In particular some varieties have a tendency to shrivel or 'wilt' because they have lost excessive water by transpiration. The transpiration rate of an apple will clearly depend on the resistance to water vapour movement of the apple cuticle.

If an apple cuticle is separated from its adjacent tissues and is then immersed in warm ether, the ether soluble fraction, referred to here as 'wax', amounts to some 50 per cent of the total cuticular matter[1]. It has been shown that transpiration from leaf surfaces[2] and insects[3] is affected if the cuticular wax is disturbed. The question arises, therefore, as to what influence wax has on the permeability of apple cuticles.

The varieties selected for study were the relatively wilt resistant 'Granny Smith', and the relatively wilt susceptible 'Golden Delicious'. Eight disks of apple skin were carefully peeled from each of these varieties, which were of normal maturity and had been in cool store. To prevent the disks curling, they were mounted on a polythene annulus. The underlying cellular matter was removed using a gentle enzyme method[4]. Cuticular wax was removed from half the disks by soaking them for half an hour in two successive solutions of hot chloroform. The central area of each cuticle (5 cm^2) was then sealed with micro-crystalline wax over an aluminium pot containing distilled water. The pots were placed in an air stream circulating at 70 ft./min and controlled at 25° C and 50 per cent relative humidity. The rate of loss of water was determined gravimetrically. The method was so designed that it was not necessary to handle the cuticle surface.

The results thus obtained are shown in Table 1. The limits quoted are the standard error of the means.

To check the reliability of the method, the transpiration of whole apples under the same conditions was measured. The permeabilities of the apple cuticles were then calculated on the assumption that the vapour inside the apple was saturated.

Table 1. PERMEABILITIES WITH AND WITHOUT WAX

	Permeability (mg day^{-1} cm^{-2})		
	Wax intact	Wax removed (uncorrected)	Wax removed (corrected*)
Granny Smith	1·5 ± 0·2	67 ± 7	107
Golden Delicious	3·2 ± 0·4	64 ± 4	98

* It is necessary to apply a small correction for the impedance of the dead air between the water surface and the cuticle, and the stagnant air film adhering to the outer surface. To determine this impedance, a thin porous paper of negligible impedance was sealed across a pot in place of the cuticle. The measured permeability was 180 mg/day^{-1} cm^{-2}. This corresponds to an air layer of total thickness 1·8 cm. The correction is negligible for the low permeability cuticle with intact wax.

The results shown in Table 2 are believed to be in error by not more than 10 per cent. They show reasonable agreement with the more direct measurements.

Table 2. PERMEABILITIES MEASURED *in situ*

	Permeability (mg/day^{-1} cm^{-2})
Granny Smith	2·5
Golden Delicious	4·0

The difference in permeability between cuticles with and without wax is striking. For 'Granny Smith' the ratio is 1 : 70 and for 'Golden Delicious' 1 : 30.

These figures demonstrate that the cuticular wax is a prime factor determining the impedance to water vapour of an apple cuticle. The reason for the difference between the varieties is not known. It is possible that the density or geometry of the lenticles is important or it may be that the distribution or type of wax plays the dominant part. Further work aimed at resolving this point is in progress.

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Promotion of Cell Division in Tissue Cultures by Gibberellic Acid

IT is well established that gibberellic acid (GA) stimulates not only cell extension but also cell division in intact plants[1,2]. It has been shown that cambial division is stimulated when GA is applied to disbudded woody shoots (including those of *Acer pseudoplatanus*)[2], but that the derivative cells do not undergo further expansion and differentiation unless indolyl-3-acetic acid is also supplied; moreover, the rate of cambial division is greatly enhanced when both hormones are applied simultaneously. Reports on the effects of GA on the growth of tissue cultures are more contradictory. Netien[3] states that GA inhibits callus tissue growth while Schroeder and Specta[4] obtained an increase in callus tissue growth in the presence of GA. Nickell *et al.*[5] investigated a large number of tissues grown in callus culture and found that GA (10 p.p.m.) in some cases caused promotion and in other cases caused inhibition of growth. These experiments were carried out on callus cultures grown on agar and in all cases the results were expressed in terms of fresh weight increase. It cannot be said from this whether GA is effecting cell division or cell enlargement, though Schroeder *et al.* suggest from anatomical observations that there was an increase in cell number in their experiments.

A series of experiments has been carried out using tissue derived from the cambium of sycamore (*Acer pseudoplatanus*), growing both on agar as a callus, and in liquid suspension culture. In both cases a modified Heller salt medium with added vitamins was used as the culture medium. No coconut milk was added to the medium.

In the case of cells grown in liquid suspension culture GA was found to promote cell division at concentrations from 15 to 50 p.p.m. but not below 15 p.p.m. (Fig. 1). At

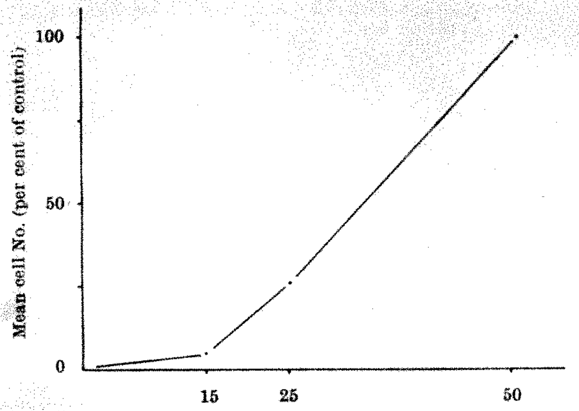

Fig. 1. Effect of increasing GA concentration on cell number. Control (no hormones) is zero

50 p.p.m. GA there was a 100 per cent increase in cell number as compared with the controls to which no hormones had been added. This stimulation of cell division was slightly enhanced by adding IAA (1·0 p.p.m.). Callus cultures grown on agar showed no increase in fresh weight when treated with 50 p.p.m. GA and in certain cases there was slight inhibition of growth as compared with the controls.

Observations on the size of the cells of the suspension cultures treated with GA show that with increase in cell number there is a corresponding decrease in mean cell size, indicating that though GA treatment is promoting cell division it is not promoting cell expansion to the same extent (Fig. 2). It is suggested that the reason for the failure to observe an increase in fresh weight in the callus cultures in response to GA treatment is due to the fact that, although cell division is promoted, there is little cell expansion and so the overall fresh weight does not increase.

It was noted in suspension cultures that there was a significant increase in cell number only at relatively high GA concentrations (about 15 p.p.m.). This indicates that the cultures may be relatively self-sufficient for gibberellins, and it has been shown previously[6] that callus cultures are capable of synthesizing gibberellins. The medium in which a suspension culture had been growing was bioassayed using a dwarf pea assay, and small amounts of gibberellins were detected. No gibberellin activity was found in medium which had not supported the growth of a cell suspension.

It has also been found that cell suspensions grown in a Heller salt medium containing 20 per cent coconut milk show increased cell division in response to GA. Again, 50 p.p.m. GA gives about 100 per cent increase in ce number as compared with the control.

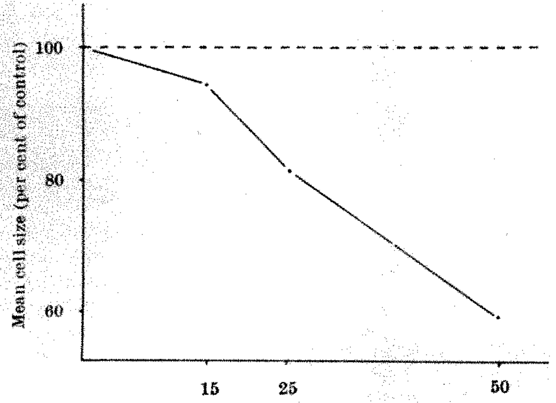

Fig. 2. Effect of increasing GA concentration on mean cell size. Control (no hormones) is 100

Thus it has been shown that GA promotes cell division of the cambium of sycamore both in the intact shoot and when the cambial cells are cultivated in liquid suspension tissue culture, suggesting that gibberellins are importan in the stimulation of normal cambial activity.

We thank Dr. D. H. Northcote, Department of Biochemistry, University of Cambridge, for providing the sycamore cambial cultures and for his assistance in the early stages of this work. This work was carried out during the tenure of a Department of Scientific and Industrial Research studentship by one of us (J. D.).

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MICROBIOLOGY

Development *in vitro* of Bacterial Cross-resistance involving Penicillins, Chloramphenicol and Tetracycline

The selection of antibiotic-resistant variants of bacteria can readily be demonstrated *in vitro* by repeated transfer of the culture in the presence of the particular antibiotic in question. Resistant variants isolated in this way show cross-resistance to other antibiotics of similar chemical structure to the antibiotic in which the original organism was cultured. Cross-resistance among unrelated antibiotics has been reported on occasion[1–5] with strains of bacteria selected from *in vitro* experiments, but the phenomenon has not become well established and it is usually accepted that cross-resistance is not seen among chemically dissimilar antibiotics. We now wish to report the development of cross-resistance between certain penicillins, chloramphenicol and tetracycline, with bacteria cultured in the presence of any of these antibiotics.

The results of experiments with a strain of *Salmonella paratyphi B* (B.R.L. 1824) are shown in Table 1. This culture was sensitive to ampicillin, benzylpenicillin, chloramphenicol, streptomycin and tetracycline before repeated transfer in sub-inhibitory concentrations of these antibiotics. After resistance had developed to the particular antibiotic in which the organism was transferred, the resistant culture was tested for sensitivity to the other antibiotics. It was found that there was cross-resistance between the penicillins, chloramphenicol and tetracycline, such that the increase in resistance which developed as a result of culture in the presence of one of these antibiotics was accompanied by a lesser, but nevertheless significant, increase in resistance to the other,

Table 1. SELECTION *in vitro* OF ANTIBIOTIC-RESISTANT VARIANTS OF *Salmonella paratyphi* B (B.R.L. 1824) SHOWING CROSS-RESISTANCE INVOLVING PENICILLINS, CHLORAMPHENICOL, AND TETRACYCLINE

Antibiotic in which culture was transferred	No. of transfers	Minimum inhibitory concentration (μg/ml.)				
		Ampicillin	Benzyl-penicillin	Chloram-phenicol	Tetra-cycline	Strepto-mycin
None (parent culture)	0	1·25	12·5	5·0	1·25	12·5
Ampicillin	12	250	> 500	50	25	25
Benzylpenicillin	12	125	> 500	50	25	12·5
Chloramphenicol	10	25	125	125	50	5·0
Tetracycline	10	25	125	25	125	25
Streptomycin	4	1·25	5·0	5·0	1·25	> 500

unrelated, antibiotics. The development of this multiple resistance occurred in a gradual stepwise manner, and there was no evidence that polyresistant variants were present in the original antibiotic-sensitive cultures.

In contrast to these findings, the multiple-resistant variants described here were, in general, as sensitive to streptomycin as was the parent strain, and streptomycin-resistant variants of the original culture of *Salm. paratyphi B* (B.R.L. 1824) remained sensitive to the penicillins, chloramphenicol and tetracycline. Likewise, a culture of this organism repeatedly transferred in the presence of ampicillin showed no change in sensitivity to cycloserine, kanamycin, neomycin or polymyxin.

The development of cross-resistance involving penicillins, chloramphenicol and tetracycline was also demonstrated with a strain of *Escherichia coli*, a strain of *Klebsiella pneumoniae* and several *Salmonella* species. Cross-resistance was not seen with two strains of *Proteus mirabilis*, and one strain each of *Shigella flexneri* and *Shigella sonnei*.

It would seem, therefore, on the basis of the findings reported here, that the emergence of antibiotic-resistance *in vitro* should not be regarded as a phenomenon which is highly specific for the antibiotic in which the bacterial culture is transferred. On the contrary, it is evident that cross-resistance among dissimilar antibiotics can readily be demonstrated *in vitro* with certain bacteria, particularly species of *Salmonella*, although the clinical significance of this is as yet not known.

These results will be reported in detail elsewhere.

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Role of Cobalt in Nitrogen Fixation by *Azotobacter chroococcum*

COBALT is known to accelerate nitrogen fixation by the blue-green algae, and the lowest cobalt requirement of *Nostoc muscorum* was observed to be 0·05 p.p.m.[1]. Its influence on *Azotobacter* does not seem to have been examined critically. The experiment recorded here was therefore planned to examine the effect of cobalt at different concentrations varying from 0·02 to 5·0 p.p.m. on nitrogen fixation by *Azotobacter chroococcum* in Jensen's medium[2]. The results obtained were compared with those observed with molybdenum, which was already known to be essential for *Azotobacter*. The basal medium was tested for cobalt and molybdenum by 'Nitroso-*R*-salt' method[3] and *Aspergillus niger* method respectively. While cobalt could not be detected in the medium, molybdenum of the order of 0·00028 p.p.m. was found to be present. The cobalt salt $CoCl_2{\cdot}6H_2O$ used was also tested for molybdenum by the same method as mentioned here, and it contained 0·0001 p.p.m. of molybdenum at 0·1 p.p.m. concentration of cobalt, at which nitrogen fixation was found to be enhanced.

The results indicated that cobalt at a dose as small as 0·1 p.p.m. increased the amount of nitrogen fixed. At 1·0 p.p.m. and higher concentrations, however, the response was less. Molybdenum was found to accelerate nitrogen fixation at and above a concentration of 1·0 p.p.m. examined. Higher concentration of molybdenum up to 5·0 p.p.m. examined did not result in any further increase in this value.

Table 1. EFFECT OF COBALT AND MOLYBDENUM IN NITROGEN FIXATION BY *Azotobacter chroococcum* IN JENSEN'S MEDIUM

Conc. (p.p.m.)	Nitrogen fixed in mg/g of sucrose oxidized (average of 4 replications) Molybdenum	Cobalt
0·00	6·06	6·06
0·02	6·47	6·22
0·10	6·35	9·00
1·00	13·10	8·35
2·00	13·82	8·22
3·00	13·25	8·20
4·00	13·25	7·90
5·00	13·88	7·95
S.E.	0·069	*S.E.* 0·090
C.D. at 5% level for Mo	0·21	*C.D.* at 5% level for Co 0·27

In order to find out the extent to which molybdenum as impurity in the cobalt salt would influence nitrogen fixation, another experiment was conducted with a control, 0·0001 p.p.m. of molybdenum and cobalt (0·1 p.p.m.), before and after removal of traces of molybdenum from the culture medium following the method of Nicholas[4].

Table 2. NITROGEN FIXED IN MG/G OF SUCROSE OXIDIZED (average of 4 replications)

Before removal of Mo			After removal of Mo		
Control	Mo 0·0001 p.p.m.	Co 0·1 p.p.m.	Control	Mo 0·0001 p.p.m.	Co 0·1 p.p.m.
6·74	6·9	9·66	7·21	6·83	9·73
S.E.	0·3276		*S.E.*	0·2148	
C.D. at 5% level	1·05		*C.D.* at 5% level	0·69	

From the foregoing it can be seen that enhanced nitrogen fixation observed was only due to cobalt and not to molybdenum present as impurity in traces.

Cobalt, being the centre of a complex in vitamin B_{12}, is likely to play an important part in plant and animal metabolism as vitamin B_{12} is known to have a definite role in cell synthesis.

We thank Dr. B. P. Pal, director, Indian Agricultural Research Institute, New Delhi, and Dr. R. V. Tamhane, the then head of the Division of Soil Science and Agricultural Chemistry, for their advice.

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Deoxyribonuclease Activity from 'Lactic Acid Pleuropneumonia-like Organisms'

THE relationship of the micro-organisms of the pleuropneumonia group (PPLO, Mycoplasmatales) to the true bacteria is uncertain. Two general explanations have been advanced to explain the nature of this group and its relation to other micro-organisms[1]: one holds that pleuropneumonia organisms are a natural biological class; the other that PPLO are an assemblage of the stable *L* forms of various bacteria.

Previously we have emphasized the basic biochemical heterogeneity of the organisms making up the group[2] and proposed that primarily these organisms could be divided into sets consisting of fermentative and non-fermentative strains. These fermentative strains were characterized as catalase negative, nitrate negative aerobes that accumulate lactic acid. This description is highly reminiscent of certain lactic acid bacteria, particularly the *Streptococci*. I would term this set of organisms 'lactic acid pleuropneumonia-like organisms'.

Either the resemblance between this set of pleuropneumonia organisms and the lactic acid bacteria occurs because these *Mycoplasma* and the Lactobacillaceae are homologous groups that have arisen coincidentally in Nature or these PPLO are in fact stable *L* forms of certain members of the Lactobacillaceae.

To characterize further these fermentative PPLO and also aid in the selection of possible bacterial parents, a search for enzymes common to the armory of the *Streptococci* was made. DNase activity was sought preferentially because the DNases are well characterized and because of the possibility of finding and characterizing a specific cellular enzyme inhibitor. Also, in particular, a knowledge of DNase activity would be important in preparing DNA for characterization.

This communication describes deoxyribonuclease activity found in three fermentative strains[2] of PPLO: Sewage *A* (*M. laidlawii* A), California calf, and kid strain of goat arthritis PPLO. Organisms were grown at 37° C in Difco trypticase soy broth adjusted to pH 7·8–8 and (except sewage *A*) supplemented with 10 per cent horse serum. Cells were gathered at 36–48 h, washed once in 0·89 per cent saline, disrupted by three or more cycles of freezing and thawing, and taken up in *tris*–hydrochloric acid buffer (0·05 M, pH 7·5); a supernatant (15,000g, 30 min) was examined for activity. Enzyme activity in culture supernatants of sewage *A* was partially purified by ammonium sulphate fractionation.

Initially activity was assayed by the alcohol precipitation method of McCarty[3]; quantitative assays were by viscometry[4]. Enzyme activity was calculated from the slope obtained by plotting, on semi-logarithmic paper, the difference between flow-time at intervals during the reaction and the flow-time of solvent, against time. Usually five points were obtained and gave straight lines for at least 12–15 min. The activity of PPLO DNase was compared with a crystallized standard DNase I (Worthington, 1 methyl green unit per mg[5]).

The pH optimum is 7–8 in *tris*–hydrochloric acid or barbital–hydrochloric acid buffer. A divalent cation requirement is satisfied by Mg^{++} or Mn^{++} (0·0075 M) while disodium ethylenediamine tetraacetate completely blocks activation; activity is restored by addition of Mg^{++}.

Activity requires protection (provided by 0·01 per cent gelatin) and is destroyed by heating for 5 min at 60° C. Dialysed ammonium sulphate fractions are stable for at least 4 months at $-10°$ C.

The deoxyribonuclease from sewage *A* has been examined more extensively. Activity of culture supernatants is equivalent to approximately 0·3 μg of crystalline pancreatic DNase per ml. Calf thymus and salmon sperm DNA are attacked at the same rate without a lag period.

Inhibition of DNase activity with boiled cell extracts of sewage *A* and *E. coli* has been obtained, but only to a level of 30 per cent so far; yeast RNA (0·3 mg/ml.) inhibits 50 per cent of the activity. The inhibitory effect is abolished by digestion (37° C for 30 min) with RNase (50 μg/ml., heated 80° C for 10 min). Treatment of crude culture supernatants with RNase does not increase activity.

Because of the rapid loss of viscosity, the enzyme would appear to be an endonuclease, and resembles the RNA inhibitable DNases of *Streptococci* and *E. coli*.

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PSYCHOLOGY

Sex Differences in Hemispheric Asymmetries of the Human Brain

THE minor and seemingly random morphologic differences between the two hemispheres stand in contras to the marked and consistent differences in their function as reflected, for example, in the specialization of the le hemisphere for speech[1]. Some evidence that unilater removals of cerebral tissue have different effects in th two sexes[2] suggests that relationships between morpholog and function might become apparent if observations o cerebral asymmetry were analysed for each sex separatel Of interest in this respect is a recent investigation b Conel of a series of eight brains from 4-year-old children no consistent feature was apparent in the difference between the hemispheres. However, if the sex of th children is taken into consideration, two noteworth differences emerge. Conel's Table IX shows that in 4 ou of the 5 female brains the amount of myelination is great in the left FA_γ-hand area than in the corresponding are on the right, while in the 3 male brains this difference i reversed. In Table X the number of exogenous fibr in layer I of areas FA_γ and PB is greater on the rig in the 4 female brains for which data are provide but greater on the left in 2 of the 3 male brains. Neith of these comparisons reaches acceptable levels of statistic significance, but the importance of, and the difficulty i obtaining, these limited data may warrant a conjectur Could these anatomical differences be related to th finding that side differences in the tactual thresholds o the thumbs of young children are not the same for th two sexes[4] ?

Some earlier research may also be used in speculatin about sex differences in cerebral asymmetry. An invest gation of variations in cerebral venous drainage sugges that the right vein of Trolard is larger than the left i girls, but not in boys[5]. Since this is often the major vei in the hemisphere opposite to that used in speech[6], it possible that the differences in venous drainage a related to the superiority of girls over boys in certai verbal skills[7] ?

These questions are not presented as being derive from relationships which have been reliably demo strated, but are posed as questions deserving systemat research. The observations indicate that the sex patients is a factor which should be heeded in investig tions of the laterality of cerebral function.

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Formation of Higher Habits

ALMOST since the beginning of the systematic stud of performance experimenters had noticed the tenden for subjects to combine 'elements' into 'groups' f perceptual and motor purposes. One of the class studies in human learning is the series of experimen on morse-learning by Bryan and Harter[1], who show some sixty-five years ago that the process of learni was arrested periodically and that 'plateaux' appear in the learning curve for receiving. This they interpreted by supposing that during the plateaux subjects consolidated what they had learned previously and reorganized elements into higher order groups: from dealing with inputs letter by letter one proceeds to words, phrases and whole sentences. A similar progression was postulated for learning to send, even though no plateaux were found. Their interpretation has generally been accepted but so far as we know it has never been put to the test. We have gone a little way towards remedying this defect, and wish to communicate a first set of results.

If one could generate word vocabularies made up from the same set of letters and train subjects on one vocabulary, transfer to another vocabulary of the same size could indicate whether subjects were still dealing with letters or had learned to deal with words: if there were no decrement it would be fair to assume that subjects were still dealing with letters only; but the presence of a decrement would suggest that subjects had started to deal with the words of the first learned vocabulary and had now to relearn a new set of words. The course of the process could then be studied by varying the vocabulary sizes and moments of transfer.

Our input consisted of meaningful five-letter words made up from the letters ADEHLNORST. The words were displayed on printed lists and subjects had to copy them into a ten-finger keyboard. Time and accuracy scores were obtained and subjects were trained for ten days, the first day's training being on single letters. Two pairs of vocabularies of size 100 and 10 words respectively were made up with certain constraints and separate groups of subjects trained first on one member of a pair and afterwards transferred to the other.

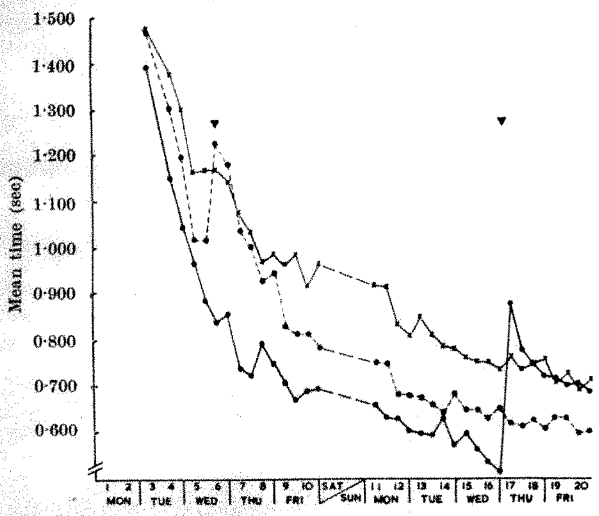

Fig. 1. Showing learning curves for three groups of subjects in terms of mean time per response for successive blocks of 5 × 50 responses. There were 6, 5 and 9 subjects in each group. Arrows mark the points of transfer from vocabulary 1 to vocabulary 2. —●— Small vocabulary late transfer; --●--, small vocabulary early transfer; ×, large vocabulary late transfer

Fig. 1 shows what happened to the mean time per response for three groups of subjects: there were two groups trained on a small vocabulary of ten words, and one which was trained on a large vocabulary of 100 words. When the subjects in the large vocabulary group were transferred to another large vocabulary after 7 days of training there was no decrement. When the group trained on the small vocabulary was transferred to another small vocabulary after 7 days' training there was a large decrement, while transfer after only one and a half days' training was followed by a smaller decrement for the remaining group. We concluded that subjects had not yet started to generate words in the case of the 100-word vocabulary, but that with the 10-word vocabulary word generating appears at a very early stage.

We are continuing to develop this technique in order to be able to examine the conditions which facilitate or hinder the formation of 'higher habits' in a keyboard skill.

We thank Dr. R. Conrad for ADEHLNORST.

J. A. Leonard
R. C. Newman

Medical Research Council,
Applied Psychology Research Unit,
Cambridge.

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[1] Woodworth, R. S., *Experimental Psychology* (Holt and Co., New York, 1938).

MISCELLANEOUS

Metric to Inch Conversion

The readers of the article by Mrs. Anderton and P. H. Bigg on 'The Yard Unit of Length'[1] may be interested in the results of an investigation following a discussion on metric to inch conversion. The definition of the yard, 0·9144 m exactly, legalized by the Weights and Measures Act, 1963, is equivalent to 1 in. = 25·4 mm exactly. The reciprocal, 1 mm = 1/25·4 in. equals 0·039 370 078 740 157... and we were interested in the way that the first group of figures 3937 was doubled to form the next group 7874 and looked as if it would be doubled again to give 157....

In the case of 1/25·4 it is simple to predict the exact form that the worked out decimal will take. For a reason given later, since 254 is not a multiple of 4, the number will not divide out exactly. If we carry out the long division sum, the remainder after the division taking us as far as 0·03937 is 2. Since we 'started with' 1 it follows that it will continue 007874 when the remainder will be 4 and so on, each cycle of six figures being double the previous six, until we reach a remainder of not 256, of course, but 2. The previous six figures will be 003937 × $2^7 + 1$, which equals 503937, and we see that the number has started to recur a few places before the remainder 2 arrives for the second time. Here is the worked out decimal: it starts to recur after 42 places.

It starts to recur here

0·039 370 078 740 157 480 314 960 629 921 259 842 519 685 ↓ 039

It is interesting to examine the general case of a number of the form $\frac{1}{N}$ where N is an integer. In common with other fractions, it must, if it does not divide out exactly, yield a recurring decimal. If we think of ourselves working out the long division sum and continuing long enough, the remainder must ultimately be repeated after which the number will recur.

When N is an even number the remainder must be an even number and therefore the maximum possible number of stages of division before the number starts to recur is $\frac{N}{2} - 1$. When N is even, but not a multiple of 4, $\frac{1}{N}$ will not divide out exactly. The only number ending in nought which might occur which is exactly divisible by N is $5N$. This can only occur when the remainder from the previous stage of division is $\frac{N}{2}$. However, when N is even but not divisible by 4, $\frac{N}{2}$ will be odd and an odd number cannot occur as a remainder.

The manner in which the cycle of numbers progressively doubles as the decimal value of a reciprocal is worked out is by no means peculiar to 1/25·4. The reciprocal of any rational number, A, can be given by:

$$\frac{1}{A} = \frac{B}{10^K - 2}$$

where K is a positive integer and B is a fraction or an integer.

Expanding by the binomial theorem:

$$\frac{1}{A}=\frac{B}{10^K}\left[1+\frac{2}{10^K}+\frac{2^2}{10^{2K}}+\frac{2^3}{10^{3K}}+\ldots\ldots\right]$$

When calculating $\frac{1}{A}$ in decimals, B must first be expressed as a decimal and it depends on the number of figures in B whether the progressive doubling is immediately apparent or not. In the case of 1/25·4, if we let K equal 6 then $10^K - 2$ equals 999, 998 and $B = 39370$. Thus:

$$\frac{1}{25{\cdot}4}=\frac{39370}{10^6}\left[1+\frac{2}{10^6}+\frac{4}{10^{12}}+\frac{8}{10^{18}}+\ldots\ldots\right]$$
$$= 0{\cdot}03937 + 0{\cdot}000\ 000\ 078\ 74 + \ldots\ldots$$

W. M. Anderson
B. E. Robinson

Mullard Radio Valve Co.,
New Road, Mitcham Junction,
Surrey.

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[1] Anderton, Mrs. P., and Bigg, P. H., *Nature*, **200**, 730 (1963).

Dissemination and Storage of Scientific Information in the United Kingdom

The storage and retrieval of scientific information is a subject the study of which has received scant official support in Great Britain; indeed until recently the small volume of research in this field was mostly financed from abroad. Likewise, as stated in the leading article in *Nature* of May 23, Government support for non-conventional means of dissemination and storage of scientific information is very disappointing. The fact that this information is the basic raw material of a research 'industry' with an annual turn-over of several hundred million pounds allows one to sense the seriousness of this lack.

It may be of interest to put on record what has already been done—thanks to the generous help of a number of industrial organizations and a local authority—to build up the nucleus of an organization to provide the information needs of modern research. In late 1962, a number of persons, bringing together experience of academic and industrial research and industrial and governmental administration, formed themselves into a Council and thereby founded the Scientific Documentation Centre, Ltd. This organization was charged *inter alia* with the purposes of compiling central collections of scientific data, of supplying scientific information services and of carrying out research aimed at improving the means of handling scientific information and data.

Substantial progress has already been made with these tasks. Research has shown that papers germane to each of a wide range of subjects are widely scattered throughout the literature—important papers often appearing in the most unexpected journals. As a result of this intelligence, the Centre has built up a series of 'Current Awareness Services' which bring to the individual scientist and to libraries up-to-date notification of papers on subjects of interest to them. This covers more than 1,100 journals, and includes the U.S. Government research reports and a large number of Russian journals. 'Current Awareness Services' are already operating on such subjects as: astronomy; arsenic; carbohydrate chemistry; carbon; carcinogens and carcinostats; cell membranes; ceramics and glass; chromatography; chemical processing; computer applications; cryogenics and superconductors; digitization; electroencephalography; fluorimetry; gas chromatography; geophysics; infra-red of natural phenomena; integrated circuits; interferometers; lasers; management and personnel; marine biology; mass spectroscopy; molluscicides; non-conventional aromatic systems; nuclear magnetic resonance; optical rotary dispersion; packaging; paper technology; pattern recognition; plastics—materials and techniques; polarography and related techniques; polymer analysis; spectrometers (manufacture); spectroscopy (applications); tobacco; terpenes, perfumes and flavours; triazines; town gas; ultra-high pressure; ultra-high vacuum; ultrasonics; water desalination. The Centre has been able to provide material for all 'Current Awareness' requests so far received.

Progress has also been made in the compilation of central collections of scientific data. The Centre's *Spectra Index*, which we regard as a pilot experiment for larger indexes, now contains some 20,000 spectra, the majority not being available in searchable form elsewhere. The *Spectra Index* now comprises the largest generally available collection of spectra or spectral data originating in Europe.

Though the Centre has made substantial progress since its foundation, despite the lack of official financial support, given greater resources, the nucleus that has been set up could very rapidly expand to give more complete and more numerous services to British research.

P. S. Davison

Scientific Documentation Centre, Ltd.,
Halbeath House,
Dunfermline,
Scotland.

Early Recognition of Galileo's Work in England

This year, the four hundredth anniversary of the birth of Galileo, has been the occasion of widespread consideration of all aspects of the work of the master, among which the contemporary reception of his work in England is of particular interest to us*. Poets and philosophers, men of general learning and men of science have been cited[1], but among them I have never seen Robert Burton.

Burton's *Anatomy of Melancholy*, which contains many references to Galileo, is a sage and extremely individual consideration of the problems of life, characterized by pertinent quotations from, perhaps, every author accessible at the time. As an example, we may take "every schoolboy hath the famous testament of Grunnius Corocotta Porcellus at his finger ends". Keats, Sterne and Samuel Johnson, men of very different temperament, were, among other celebrities, devoted to the *Anatomy*, the first edition of which was published in 1621.

In his book, Burton, who died just two years before Galileo, refers to Galileo's observation of the Medicean stars and of the ill-resolved ring of Saturn—". . . and that those severall Planets, have their severall Moones about them, as the Earth hath hers, as Galileo hath already evinced by his glasses, foure about *Juppiter*, two about *Saturne* (though *Sittius* the *Florentine* cavill at it)": to his observations on the Moon—". . . hils and dales, and such like concavities, if we may subscribe to, and beleeve Galilies observations": and in the second edition, 1624, to the discovery of the nature of the Milky Way—". . . and that *via lactea*, a confused light of small starres". In later editions he also refers to Galileo's observations of the sun-spots and consideration of the tides. There are also many passing allusions to his work.

The great scholar clearly had an understanding of the significance of Galileo's work and must have been responsible for bringing it to the notice of a great part of the world of learning in Britain.

E. N. da C. Andrade

The Athenæum,
Pall Mall,
London, S.W.1.

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* See also *Nature*, **201**, 653 (1964).—Editor.

[1] See in particular the excellent detailed discussion by Marie Boas Hall, to be published in the Notre Dame Galileo volume.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer or Lecturer (graduate in fuel science or technology, or in physics or physical chemistry) in the Department of Fuel Science, Houldsworth School of Applied Science—The Registrar, The University, Leeds, 2 (August 10).

Assistant Lecturer (with a degree in economics or agricultural economics, preferably with postgraduate experience in agricultural economics) in Agricultural Economics—The Registrar, The University, Manchester, 13 (August 10).

Lecturer or Assistant Lecturer (graduate in ceramics, glass technology, physics or physical chemistry) in Ceramics—The Registrar, The University, Leeds, 2 (August 10).

Lecturers (2) in Pure Mathematics—The Registrar, The University, Manchester, 13 (August 10).

Lecturer or Assistant Lecturer (with a special interest and qualifications in climatology and/or bio-geography) in Physical Geography—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (August 12).

Lecturer or Assistant Lecturer (with special qualifications in physical chemistry, and preferably some teaching and research experience) in the Department of Chemistry—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (August 14).

Scientific Officer, Grade III or IV (with a first- or second-class honours degree in zoology, physiology, or biochemistry, or a Ph.D. in one of these disciplines) in the Cytochemistry Section, to work with a group on the metabolism of human leucocytes using micro-techniques—The Secretary (B), Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2 (August 14).

Senior Research Associate or Research Associate (with a good honours degree in physics or an allied subject, and some research experience for appointment in the senior grade) in the Applied Nuclear Science Group in the Department of Physics, for research in the field of neutron radiography—The Administrative Officer, Department of Physics, The University of Birmingham, Birmingham, 15 (August 14).

Assistant Lecturer (preferably interested in problems of embryogenesis and cyto-differentiation) in Zoology—The Registrar, University College of Wales, Aberystwyth (August 15).

College Lecturer (with an honours primary degree in horticulture; a higher degree in a field of work related to horticulture; teaching experience and some practical experience in research, applied or basic) in the Faculty of General Agriculture—Department of Horticulture—The Secretary and Bursar, University College, Dublin, Republic of Ireland (August 15).

Demonstrator (preferably with an interest in parasitology) in Zoology—The Registrar, University College of Wales, Aberystwyth (August 15).

Experimental Officer (with a degree or equivalent qualifications) in the Instrument Laboratory for duties which will include the maintenance and operation of infra-red, ultra-violet and N.M.R. spectrometers and gas chromatographic equipment—Prof. Pauson, University of Strathclyde, George Street, Glasgow, C.1 (August 15).

Head (with a pharmacy or chemistry qualification, experience of pharmacopoeial work and a good knowledge of French) of the Technical Secretariat, European Pharmacopoeia Commission, Strasbourg, for duties covering all aspects of the preparation of the European Pharmacopoeia—The Secretariat, Establishments Division, Council of Europe, Strasbourg, France (August 15).

Lecturer or Assistant Lecturer (preferably with an interest in petrology) in Geology in Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee (August 15).

Lecturer or Assistant Lecturer (with interests in soil mechanics) in Engineering—The Registrar, The University, Manchester, 13 (August 15).

Research Associate (normally with some post-doctoral experience) in the Physics Department, to assist in a programme of work on optical masers—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (August 15).

Research Engineer or Physicist (with a good honours degree in physics or engineering and a Ph.D. or equivalent experience) in the Building Science Laboratories, to work under the direction of Dr. G. H. Tattersall, on the compaction of concrete by vibration—The Registrar, The University, Sheffield (August 15).

Senior Lecturer or Lecturer (with a special interest in at least two of the following: economic or political geography, regional geography of the Middle East or Africa (especially north of the equator), and preferably a knowledge of Arabic or a West African language and experience in tropical conditions) in Geography at Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (August 15).

Lecturer or Assistant Lecturer (with a good honours degree in civil engineering and good general training and experience) in the Department of Civil Engineering—The Registrar, The University, Leeds, 2 (August 17).

Graduate (with an honours degree or equivalent in horticulture, agricultural science or science) in the Horticulture Division, for mushroom research—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland (August 20).

Lecturer or Assistant Lecturer in Psychology at Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee (August 21).

Second Chair of Physics at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 21).

Lecturer or Assistant Lecturer (with a first- or second-class honours degree, preferably in biochemistry or chemistry, with suitable postgraduate experience) in the Procter Department of Food and Leather Science—The Registrar, The University, Leeds, 2 (August 24).

Assistant Lecturer (preferably interested in plant biochemistry, physiology or cytology) in Botany—The Secretary, The Queen's University, Belfast, Northern Ireland (August 28).

Senior Lecturer/Lecturer (with an honours degree with research qualifications in botany or a related field of biology, equivalent to the Ph.D., and preferably qualifications in genetics, including cytogenetics, biometrical genetics or fungal genetics) in the Department of Botany, School of Biological Sciences, University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (August 31).

Lecturer or Assistant Lecturer in the Department of Moral Philosophy—The Secretary, The University, Edinburgh (September 1).

X-ray Crystallographer (with previous research experience of X-ray crystallography) in the Physics Department for crystallographic studies of materials at high pressures—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1 (September 7).

Lecturers (specialists in one of the following subjects: (a) theory of machines, (b) strength of materials or (c) thermodynamics) in the Department of Mechanical Engineering at University College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 10).

Lecturer or Senior Lecturer in Geochemistry at the University of Tasmania—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 11).

Lecturer, Senior Lecturer or Reader in Statistics at the University of Tasmania—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 11).

Reader in Human Genetics—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne 2 (September 26).

Lecturer (with a postgraduate degree, and preferably some experience of teaching biochemistry at university level) in Biochemistry at Lincoln College (University of Canterbury), New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

Director (with high scientific attainments in a relevant science, and preferably some experience in horticulture and administration) of the Scottish Horticultural Research Institute—The Secretary, Scottish Horticultural Research Institute, Mylnefield, Invergowrie, Dundee (October 12).

Professor of Organic Chemistry—The Registrar (Room 22, O.R.B.), The University, Reading (October 24).

Assistant Lecturer or Lecturer (or, in special cases, Senior Lecturer) in Mathematics—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, marking envelope "Application".

Chair of Sociological Studies—The Registrar, The University, Senate House, Bristol 2.

Chair of Zoology; a Lecturer in Zoology; and a Lecturer or Assistant Lecturer in Inorganic Chemistry or in Chemistry with a leaning towards Inorganic Chemistry at the University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1.

Experimental Officer for the maintenance and development of complex electro-mechanical and electronic measuring devices for use in high energy nuclear research; a Senior Technician to develop new experiments for undergraduate teaching laboratories; a Senior Technician for an ionic physics research laboratory; and Junior Technicians to be trained for a variety of posts in teaching and research laboratories—The Secretary, University College, Gower Street, London, W.C.1.

Graduate Assistants (2) (preferably with experience in tissue culture methods, microscopy or serology) in a research group working on respiratory infections—The Administrative Secretary, School of Veterinary Medicine, Madingley Road, Cambridge.

Graduate Scientific Assistant, and Technicians (preferably with previous experience in the handling of laboratory animals) for interesting and varied work in the field of biological research, principally the assessment of drug safety—The Administrative Officer, Huntingdon Research Centre, Huntingdon, quoting Ref. PRN/3.

Lecturer or Senior Lecturer (with a good honours degree) in Pharmacology—The Registrar, City of Leicester College of Technology, Leicester.

Master (graduate) to be responsible for the organization and teaching of geography throughout the school—The Head Master, St. John's School, Leatherhead, Surrey.

Mathematician (with a good honours degree and a knowledge of computing) with the British Railways Board, to lead a team devising new approaches to railway control and systems analysis within the Electrical Research Division of the Board's Research Department in London (studies relating to other research within the Division will also be involved)—Headquarters Staff Manager (R.B.26/5), British Railways Board, 222 Marylebone Road, London, N.W.1.

Medical Laboratory Technician (I.M.L.T.) for Cardiological research in the Departments of Cardiology and Clinical Measurement—The Assistant House Governor, Westminster Hospital, London, S.W.1.

Nematologist (male, national of the United Kingdom or the Republic of Ireland, with an honours degree in zoology or entomology and at least one year's relevant postgraduate experience) in Uganda, to undertake a survey of the occurrence of harmful nematodes on crops, to develop techniques for testing host reactions and for controlling nematodes of economic importance—Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC213/183/010.

Physicist (graduate prepared to take an interest in rheology and flow measurement) to join a team engaged on research in problems of chocolate manufacture—The Director of Research, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey.

Post-Doctoral Research Worker to investigate regulation of enzyme synthesis particularly in bacterial extracts—The Chairman, Department of Chemistry, Washington State University, Pullman, Washington, U.S.A.

Postgraduate Student to work on some aspects of porphyrin chemistry—Dr. U. Eisner, Department of Chemistry, Sir John Cass College, London, E.C.3.

Research Student (with a good honours degree in botany or agricultural botany) for work under the direction of Dr. R. K. S. Wood, on the control of plant diseases with fungicides—Dr. R. K. S. Wood, Department of Botany, Imperial College of Science and Technology, London, S.W.7.

Research Assistant in the Department of Psychology to work on human decision processes—The Registrar, The University, Nottingham.

Science Graduate to assist in developing techniques used in separating proteins in human plasma, particularly anti-hæmophilic globulin—The Secretary, The Lister Institute of Preventive Medicine, Elstree, Hertfordshire.

Senior Physicist (with experience in medical physics and electronics techniques) to be in charge of a group concerned with the application of electronics in medicine—Chief Physicist, Regional Medical Physics Department, Sheffield Regional Hospital Board, 21 Claremont Crescent, Sheffield, 10.

Senior Research Assistant in Physics to be an important member of a new group of five members and will be expected to make a lively contribution to work on electron spin resonance and relaxation in compounds which exhibit co-operative magnetism—The Registrar, The University, Sheffield.

Senior Research Associate (with a good honours degree in either biochemistry or chemistry, and have carried out postgraduate work in either subject) in the Department of Organic Chemistry, for work on the biosynthesis and structure of substances in bacterial cell walls and membranes—Prof. J. Baddiley, F.R.S., Department of Organic Chemistry, The University, Newcastle upon Tyne.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Scientists' Responsibility in the Atomic Age. By Prof. J. Rotblat. (The Essex Hall Lecture, 1964). Pp. 28. (London: The Lindsey Press, 1964.) 2*s*. 6*d*. [16

Ambassade de France, Service de Presse et d'Information. A Survey of Political and Economic Affairs in France, 1959–1964. Pp. 26. (London: Ambassade de France, Service de Presse et d'Information, 1964.) [16

Steel Castings Development Committee. Data Sheet No. 10: Hardenability of Steel Castings. Pp. 11. Data Sheet No. 11: Modulus of Elasticity of Steel Castings. Pp. 4. (Sheffield, 10: Steel Castings Development Committee, 13 Broomsgrove Road, 1964.) [16

The Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme. Tenth Annual Report. Pp. 8. (London: The Royal Society and the Nuffield Foundation, 1964.) [16

The Enigma of Stonehenge and Its Astronomical and Geometrical Significance. By C. A. Newham. Pp. 42. (Tadcaster: C. A. Newham, 5 Sedge Rise, 1964.) [16

Borough of Lewisham Library Service. Scientific and Technical Periodicals: a Catalogue of the Holdings of the Science and Technical Reference Library. Pp. 40. (Lewisham, London, S.E.6: Scientific and Technical Reference Library, 1964.) [16

The British Hydromechanics Research Association. Sixteenth Annual Report, October 1962 to September 1963. Pp. 40. (Harlow: The British Hydromechanics Research Association, 1964.) [16

British Institute of Management. British Business Schools: Report by The Rt. Hon. Lord Franks, P.C., G.C.M.G., K.C.B., C.B.E. Pp. 20. 5*s*. British Business Schools—The Cost: Report by a Working Party under the Rt. Hon. Lord Normanbrook, P.C., G.C.B. Pp. 10. (London: British Institute of Management, 1964.) [16

Department of Technical Co-operation. Report of the Committee on Technical Assistance for Overseas Geology and Mining. Pp. 32. (Cmnd. 2351.) (London: H.M. Stationery Office, 1964.) 2*s*. net. [16

Department of Technical Co-operation. Technical Assistance for Overseas Geology and Mining: Policy on the Recommendations of the Committee. Pp. 7. (Cmnd. 2352.) (London: H.M. Stationery Office, 1964.) 8*d*. net. [16

Overseas Development Institute, Ltd. Not by Governments Alone: The Role of British Non-Government Organisations in the Development Decade. By Peter Williams and Adrian Moyes. Pp. 51. (London: Overseas Development Institute, Ltd., 1964.) 3*s*. 6*d*. [16

International Indian Ocean Expedition. R.R.S. *Discovery* Cruise 2 Report: Geology and Geophysics in N.W. Indian Ocean, 23 August to 4 December 1963. Pp. 60. (London: The Royal Society, 1964.) [16

The British Food Manufacturing Industries Research Association. Annual Report 1963. Pp. 68. (Leatherhead: The British Food Manufacturing Industries Research Association, 1964.) [16

An Approach to the Rapid Description and Mapping of Biological Habitats. By M. E. D. Poore and V. C. Robertson. (Based on a Survey of the Hashemite Kingdom of Jordan by Hunting Technical Services, Ltd., and on the work of the British Jordan Expedition, 1963.) Pp. xiii + 68. (London: Sub-Commission on Conservation of Terrestrial Biological Communities of the International Biological Programme, c/o The Nature Conservancy, 1964.) 10*s*. [16

The British Council: Science Department. Nuclear Studies, List No. 8. Pp. 31. (London: The British Council, 1964.) [16

The Finance of Medical Research. Pp. 32. (London: Office of Health Economics, 1964.) 2*s*. [106

Lawes Agricultural Trust. Rothamsted Experimental Station—Report for 1963. Pp. 318. (Harpenden: Rothamsted Experimental Station, 1964.) [106

The Londoner and his Library. By Brian Groombridge. Pp. viii + 124. (London: The Research Institute for Consumer Affairs, 1964.) 15*s*. [106

Department of Scientific and Industrial Research. Report of the Tropical Products Institute 1963: The Report of the Tropical Products Institute Committee with the Report of the Director of the Tropical Products Institute. Pp. vi + 33. (London: H.M. Stationery Office, 1964.) 3*s*. net. [106

Fishery Limits Bill. Pp. 7. (London: H.M. Stationery Office, 1964.) 1*s*. net. [106

British Palaeozoic Fossils. Pp. vi + 208 (69 plates). (London: British Museum (Natural History), 1964.) 12*s*. 6*d*. [106

Department of Scientific and Industrial Research. Report of the National Physical Laboratory for the year 1963. Pp. viii + 262 + 18 plates. (London: H.M. Stationery Office, 1964.) 17*s*. net. [106

National Vegetable Research Station. Fourteenth Annual Report, October 1962—September 1963. Pp. x + 93. (Wellesbourne, Warwick: National Vegetable Research Station, 1964.) 8*s*. 6*d*. [106

Ambassade de France, Service de Presse et d'Information. French Foreign Policy—Statement made by the Minister of Foreign Affairs in the National Assembly. Pp. 12. A Survey of Political and Economic Affairs in France, 1959–1964. Pp. 26. (London: Ambassade de France, Service de Presse et d'Information, 1964.) [106

On Human Wisdom. By K. W. Monsarrat. Pp. 24. (Liverpool: Henry Young and Sons, Ltd., 1964.) 6*s*. [106

Other Countries

The Soils and Geography of the Boliland Region of Sierra Leone. By A. R. Stobbs. Vegetation. By Dr. T. S. Bakshi. Pp. 45 + 12 plates. (Freetown, Sierra Leone: Government Bookshop; London: Crown Agents for Oversea Governments and Administrations, 1963.) 28*s*. [16

World Health Organization. Monograph Series, No. 49: Operation and Control of Water Treatment Processes. By Charles R. Cox. (Prepared in consultation with twenty-four specialists in various countries.) Pp. 390. (Geneva: World Health Organization; London: H.M. Stationery Office, 1964.). 22 Sw. francs; 37*s*. 6*d*.; 7.25 dollars. [16

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PROBLEMS OF STATECRAFT AND THE CIVIL SERVICE

LORD BRIDGES modestly describes his account of the Treasury and its functions*, as well as the way it works, as rather prosaic, and it would be unfair to expect a book planned to give a factual account, including the reorganization of November 1962, of such a large subject to possess the liveliness of Lord Bridges's earlier lecture, "Portrait of a Profession". Nevertheless, the book has something of the charm of that lecture. It is fascinating throughout and some of its chapters, notably those on the co-ordination of economic policy, on Treasury control of the Civil Service, and on how staff control at the Treasury works, are highly pertinent to present-day discussions alike on Parliamentary reform and on the relation of the expert to the making of policy. Lord Bridges's own reflexions are limited to a short chapter of ten pages, but with the historical material, the sections on expenditure and its control and on home and overseas finance, the book is likely to remain an invaluable reference work for those concerned with the control of public expenditure or the organization of Government for years to come.

Lord Bridges's own reflexions are, as has been said, brief. Nevertheless, they are much to the point and deal with issues of the first importance at the present time. He lends the weight of his authority and experience to greater planning. This he regards as inevitable within limits, and accompanied with a transition from a yearly budget and annual estimates to a system based on a longer time unit. However, much has yet to be done before we can hope to achieve the right balance between a long enough view to permit sensible planning and sufficient flexibility to allow important changes in policy to be made quickly. On economic co-ordination, he recognizes the implications of the establishment, following earlier measures which met with varying degrees of success, of the National Economic Development Council. Here again we are very far from being able to assess the precise role that Council will occupy in our affairs and the way it will affect the work of the Treasury. Lord Bridges believes that the assumption by the Treasury of the duties of economic co-ordination means a more professional approach to budgetary problems as well as a better understanding and more effective methods of communication between the Treasury and the world of business, industry and commerce.

This in itself may lead to big changes in the way in which the Treasury works. However, while Lord Bridges welcomes this as a healthy development on the issue of management, he is directly opposed to some other proposals for reform. He regards the Plowden Report (*Nature*, **192**, 485; 1961), on the recommendations of which many of the changes of 1962 were based, as a most important and helpful document. In particular, he thinks it would have been a false step to take the management side out of the Treasury and to make it into a separate department. This, he holds, would have greatly weakened the effectiveness of both the management and the financial functions, and would have been inconsistent with the essential feature of British organization, whereby in every Department of State the responsibility for advice to the Minister on policy, finance and administration rests in the same person. It would also inevitably have led to the administration being treated as something different from, and less important than, finance. Incidentally, while accepting that some increase in specialization in Treasury work is inescapable, Lord Bridges insists that this should not be introduced in a way that will lessen the close knowledge which many Treasury officers acquire of the work done in other departments or hinder the development of relations of understanding and confidence.

In contrast to this view of Lord Bridges, one of the key proposals from a group of Fabians† for the reform of the Civil Service, and especially the administrative class, is to transfer the responsibility for personnel management and the structure of the Civil Service from the Treasury to the Civil Service Commission. This would be enlarged and include representatives from other Government departments besides the Treasury, from large employers and from the universities. It would be made responsible for all questions of conditions of service definition, of grades and categories (including pay), avenues of promotion, career planning and the making of higher appointments for which central control was desirable, the maintenance of common standard and training. One of its first tasks would be to institute a searching and independent investigation into the selection methods used for candidates to settle whether they are as unbiased and efficient as they can be made.

The Fabian group's proposals should, however, be considered against the background of the report itself. That report is the first major Fabian report on the Civil Service since the report of 1947, *The Reform of the Higher Civil Service*, most of the recommendations of which have yet to be implemented. The present report challenges the continued acceptance of the difference between management and administration, the latter traditionally embracing advice on policy, implementation of policy, Parliamentary work, co-ordination, negotiation and personnel management. While the changes since the Plowden Report are welcomed as a step in the right direction, the Fabian group does not regard them as anything like adequate. It looks for a change in the character of the staff to perform the new tasks and considers that the Service is still insufficiently prepared to provide men and resources for long-term investigations, with the result that the available expertise is not sufficiently used.

Three main characteristics of the present system attract the group's chief criticism. The system is amateur, resulting from the 'all-rounder' tradition and the belief that the administrator on the job is the best adviser on policy. Civil Servants tend to be too negative in their approach, concentrating on procedure rather than on the substance of problems and being too ready to seek compromises. Again, the system tends to be too closed and secretive in formulating policy. These weaknesses are regarded as stemming largely from the present methods of recruitment and training, the immobility, the inadequate articulation of careers and the lack of specialists at a senior level and their non-assimilation.

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* The Treasury. By The Right Hon. Lord Bridges. (The New Whitehall Series, No. 12.) Pp. 248. (London: George Allen and Unwin, Ltd.; New York: Oxford University Press, Inc., 1964.) 30*s*. net.

† *The Administrators: The Reform of the Civil Service.* Pp. 45. (Fabian Tract No. 355.) (London: The Fabian Society, 1964.) 4*d*.

Some of these points—notably those regarding movement in and out of the Civil Service and in respect of secrecy—have been made repeatedly in various connexions as regards the organization of Government and the place of the expert in making policy. It is to meet these weaknesses that the Fabian group advances its proposals for wider recruitment, better training and more movement in and out of the Service; the fuller use of experts; the improvement of the status of professional Civil Servants as against general administrators, and some increase in their numbers; and the explicit acceptance of the need for some political appointments. On the recruitment to the Service, the group attributes the failure to recruit a larger proportion of scientists to the fact that scientists and mathematicians are attracted to posts where they can make more direct use of their subjects and where the opportunities to rise are greater, and also to the belief among graduates outside Oxbridge that there is a bias against them.

To encourage movement into and out of the Service the group advocates the institution of unified contributory pensions, with a corresponding adjustment of salaries; and secondment of staff during their careers to outside posts in local government, nationalized industry, or, subject to safeguards, private industry. It would establish for training purposes a school of administration, providing a course of two years or so, partly of work in departments and in the field, partly of study, which, combined with the greater openness of the Service, would give an attractive, first-class qualification for Civil Servants and provide some staff for nationalized industries, other public agencies and possibly private industry. With this would go the better articulation of careers so that experience was progressively widened and the provision of better and fuller courses for training in mid-career.

The Fabian group would also abolish the grading or 'class' distinction between specialists and administrators from the rank of assistant secretary upwards, giving a Senior Civil Service with equal ranks and pay, increased scope for interchange, and competition on a more equal footing for the highest posts. More specialists would be required and greater openness, so that outside study and discussion of long-range issues of policy would be encouraged, and not discouraged by excessive secrecy, and fuller use would be made of outside research. The group would also provide explicitly for two types of political appointment: experts to help implement the particular policies of the Government of the day, and personal aides to provide general help to Ministers in their private office—a proposal which Lord Bridges, in a later talk, thought most unwise.

Support for some of the Fabian group's ideas is to be found in a talk, "Intelligent Laymen in Whitehall", broadcast by Prof. Ely Devons and published in *The Listener* (**71**, 819; 1964). Prof. Devons, discussing Lord Bridges's book, questioned the apparent assumption in it that professional knowledge nearly always implied biased enthusiasm, and that, accordingly, it was dangerous to allow such experts the final say in the advice on policy given to Ministers. Prof. Devons doubts whether, without professional knowledge, the Treasury officer is competent to ask the relevant probing questions and to know whether the answers he gets are revealing and significant. He does not suggest any ideal solution to the problem of controlling and evaluating the worth-whileness of alternative projects of public expenditure, or of securing efficiency in spending. However, this plea for a closer look at the ideas of amateurism in Whitehall is one to be taken seriously and is highly relevant to the debate on science and public policy.

Prof. Devons, too, would like to see more interchange of staff between the administrative and professional, and especially scientific, branches of the Civil Service, and he reminds us of how long and persistently since the Second World War this idea has been pressed with authority. He instances, as what is possible, the increasing use now being made of economists and, pointing out that politics and public administration are not simply the pursuit of power and the reconciliation of pressure groups, but also the art of taking decisions in a state of relative ignorance, he suggests that if scientists understood this and had administrative experience, they might approach problems of policy and politics with much more sympathy than at present. It might also be added that the need to diminish this area of relative ignorance in taking decisions gives urgency to the present-day discussion on the place of science and scientists in policy making, to which Mr. S. P. Chambers's recent Chuter Ede Lecture to the National Union of Teachers (*Nature*, **203**, 227; 1964) is a notable contribution.

Prof. Devons's idea of a Senior Civil Servant as a man who, whatever his subject when he entered the Civil Service, has had experience during his career in an administrative division, a scientific or technical division, a statistics division, and perhaps even a technical costs or contracts division, clearly has much in common with the views advocated by the Fabian group as well as those which Sir Henry Tizard consistently urged. On the economics side, however, Prof. Devons has reservations to which he gave expression in a further talk broadcast on the B.B.C. Third Programme and published in *The Listener* (3, 72; 1964). In this talk, "Economic Management in Whitehall", returning to Lord Bridges's book, as well as to views expressed by Mr. Harold Wilson, Mr J. Grimond, Mr. J. Enoch Powell and by Lord Bridges in other broadcast talks, "Whitehall and Beyond", Prof. Devons suggested that we should now recognize that the major task of the Senior Civil Service is no longer administrative but managerial. He makes the distinction that the administrative type is primarily interested in seeing that the organization functions, whereas the managerial type has his eye on the trouble spots, seeking to take action in anticipation of trouble, and to see that the crucial issues of policy are faced and handled. It is, in Prof. Devons's view, the managerial approach that is needed to-day.

Prof. Devons is aware that the importance of the managerial function is already appreciated in Whitehall, and Lord Bridges's book could also be cited in evidence. While Lord Bridges refers to the advantages which new methods and techniques could bring, he comments that, in the end, men matter more than measures. While rebutting the view that managers in Whitehall should come primarily from the administrative class, Prof. Devons does not hold that professionalism and technical expertise by themselves make good managers. Both in public and private business good managers may come from those with an academic background and actual experience in arts, science, technology, economics and any other social science. The essential feature of a rising and successful manager is that he should have experience of more than one branch of the business.

It is on this that Prof. Devons bases his argument for a single-headed service beyond a certain level and would reject the Fabian proposal. He points out too that the management side of the Treasury is also the focal point for considering whether departments are appropriately organized for their functions, and that this responsibility increases in importance as the Treasury exercises less control over detail and gives more responsibility to departments. From this angle the Fabian proposal to entrust such responsibility to a reorganized Civil Service Commission seems dubious. However, Prof. Devons is no more dogmatic on such issues than is Lord Bridges, and his view that the management side of Whitehall, especially the Treasury, has much to learn from more dialogue with business on problems of organization is undoubtedly right.

The most valuable contribution of this whole series of broadcast talks is the way in which they direct attention to the crucial importance of the whole issue of management and structure to the successful development of Government in Britain. Certainly the series disposed of any tendency to rest content with the recommendations of the Plowden Report, *The Control of Public Expenditure*, and they should help to ensure that this aspect receives adequate attention in discussions on science and public policy. Moreover, Prof. Devons directs attention to an important question which was not discussed by either Mr. Harold Wilson or Lord Bridges in considering the management responsibilities of the Treasury. Those could scarcely be assessed without first considering what aspect of economic co-ordination the Treasury is competent to handle. Prof. Devons believes that the Treasury can and should look after the general level of economic activity and the balance of payments. However, he feels that there are strong arguments against expanding the Treasury and concentrating power there to deal with other important aspects of general economic policy and economic co-ordination which it does not adequately handle.

On the contrary, Prof. Devons would encourage the handling of these matters in all the other main economic departments. His suggestion that what is needed is some ordered investigation of what is taking place in the economy, as a necessary condition for initiating policy, instead of waiting until troubles become acutely obvious before dealing with them, is essentially the recommendation of the Haldane Committee almost half a century ago. Prof. Devons recognizes that this development has already started and he is convinced that this is what is needed rather than a new Ministry of Expansion or Production. He would indeed place the Economic Section and the Economic Adviser to the Government in the Cabinet Office, as they were during the Second World War and for a time afterwards, and not in the Treasury. So placed they would serve the Cabinet as a whole, forming part of the Prime Minister's staff and acting as the focal point for all central economic initiative and co-ordination.

What is particularly welcome is the whole tone of this debate. It indicates an open-mindedness and a recognition of the importance of hard, critical thinking which should be as welcome to the scientist and technologist as the unqualified support given to their claims to be considered on their own merits for the highest posts in the Civil Service. There is indeed much yet to be done, but the essential questions are being asked and the answers are being sought independently of party or prejudice. Is it too much to hope that the reform of Parliament and the relations between the Executive and Parliament and the Civil Service may be considered in just as critical and impartial a way? Certainly Mr. Harold Wilson's proposals at Stowmarket on July 3 afford some grounds for that hope. His suggestions for relaxation of the Parliamentary voting system to allow more 'free' votes and to eliminate the rigid doctrine of each vote involving an issue of confidence should receive more support from Conservatives as well as from his own supporters. Moreover, the proposal for all-party committees on the lines of the present Estimates Committee and Public Accounts Committee has something in common with what has already been recommended independently. Taken together, his proposals should go far toward restoring the authority of Parliament and easing the difficulty of attracting members of outstanding character and ability. Behind all these difficult problems of structure and organization, of advice and responsibility lies that ultimate issue stirred by Lord Bridges of men rather than measures, and that need we disregard at our peril.

IDEAS IN MOLECULAR BIOLOGY

Progress in Biophysics and Molecular Biology

Vol. 13. Edited by Prof. J. A. V. Butler, Dr. H. E. Huxley and Prof. R. E. Zirkle. Pp. vii + 328. (London and New York: Pergamon Press, 1963.) 90*s*. net.

WORKERS in the most rapidly advancing fields of research often complain of the impossibility of reading all the relevant published papers. In fact, good connexions with other laboratories, conferences and preprint circulations would often seem to reduce the published papers in one's own field to a record of priority, but there remains the problem of reading sufficiently around one's immediate subject to keep up to date with present-day ideas. The review article can be a great help here, but as the need for this type of article grows, so does the number of annual review volumes, and the more specialized become their contents. Reversing this trend, the editors of *Progress in Biophysics* have added molecular biology to their title and state that they also intend to include articles on related biochemical topics. Although it seems a formidable task to cover even briefly such wide and rapidly developing fields of research, and probably just as difficult to persuade the right people to produce the right type of review, there is no doubt that the product aimed at would be very useful.

In Volume 13, one's dependence on reviews is clearly illustrated by the thorough and valuable article of M. Grunberg-Manago on the enzymatic synthesis of nucleic acids. The number of references listed approaches 400, although this field of research only began with the discovery of polynucleotide phosphorylase in 1955. Since then, many other enzymatic systems have been reported for synthesis of RNA and polyribonucleotides, and the article considers their various roles in the *in vivo* production of the different types of cellular RNA, and the possible control mechanisms that might favour, at various stages, the production of a particular RNA, or of DNA.

The article by O. Kratky on low-angle X-ray scattering from dilute solutions of biological specimens unfortunately reproduces much that can be found in text-books—the theory of the method; ways of correcting for finite collimation; absolute intensity methods for determining molecular weights; and experimental techniques. The remainder of the article deals with some of the more recent results of the method applied to determine the molecular weights and radii of gyration of several proteins, the mass per unit length and cross-section areas of DNA and RNA, and the apparent adoption of the 3_{10} helical structure by some polypeptides in certain solvents. With one exception, all these results come from the three laboratories specializing in low-angle X-ray scattering, those of Luzzati, Beeman and Kratky. This probably reflects the caution with which many people regard the results of X-ray diffraction investigations, and a more critical appraisal of these results would perhaps have helped to stimulate other laboratories into using this very powerful technique as an available routine procedure. The article suffers greatly from a lack of sub-editing, both in readability and in misprints (a combination introducing some strange notions into Fig. 12).

A very critical review of *in vivo* investigations into the mechano-chemistry of muscular contraction by F. D. Carlson considers the published results in this field in the light of recent investigations. Whether or not one accepts the proposed scheme of events, the article is clearly useful in resolving the apparent conflict of the results of many earlier experiments by rejecting those which do not satisfy conditions which should now be applied.

Two articles review some of the recent work and ideas on the interaction of radiation with biological specimens. The first, by L. G. Augenstein, considers the various mechanisms by which energy from X-rays and γ-rays is absorbed by biological material. Recent observations indicate that energy transfer occurs at specific sites only and possible mechanisms involving excitation energy transfer and charge migration in biological systems are discussed. The second article, by G. Scholes, is concerned with the radiation chemistry of aqueous solutions of nucleic acids, their components and the nucleoproteins nucleohistone and nucleoprotamine.

The volume also includes an account by R. D. Preston of the problem of phloem transport in flowering plants, with results culled from a meeting of the British Biophysics Society in 1961. The conclusion drawn from the meeting is that organic solutes are translocated along plant stems, not by a simple hydrostatic mechanism, but by a transfer mechanism active along the whole length of a sieve tube.

J. T. Finch

CLASSIFICATION OF THE ACANTHOCEPHALA

Systema Helminthum

Vol. 5: Acanthocephala. By Satyu Yamaguti. Pp. vii+423 (85 plates). (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1963.) 450*s*.

VOLUME 5 of Satyu Yamaguti's *Systema Helminthum* concerns the classification of the thorny headed worms, a group of somewhat uncertain affinities. The division of the body into three regions, a retractile proboscis beset with spines, an often inconspicuous 'neck' and a cylindrical 'trunk', and the pseudocœlomic pattern of its structure give little indication of relationship with the round worms. Conversely, the absence of an alimentary system at all stages of development is a feature shared with tapeworms, some of which have developed the spiny proboscis as an organ of attachment. Moreover, the first larva of acanthocephalans, the acanthor, bears some resemblance to the hexacanth of the cestodes, and there are serological indications of affinity between these two groups.

However, the great systematic problem has always been to decide whether to relate the thorny headed worms with the round worms or with the flat worms, although Van Cleave proposed the setting up of an independent phylum and other writers have indicated alternative affinities with the Priapuloidea. Yamaguti takes the conservative view, defining the Acanthocephala as gonochoristic Nemathelminthes and arranging them in a single class. He has followed the scheme of Petroschenko, who divided the class into three sub-classes, but has promoted these groups to the status of orders named in 1925 by Southwell and Macfie, adding an order erected by Thapar for a single family two years later. In Yamaguti's scheme, the four orders of Acanthocephala are the Opororhynchidea (with one family and one genus), the Echinorhynchidea (with eleven families and fifty-five genera), the Neoechinorhynchidea (with four families and twenty genera) and the Gigantorhynchidea (with six families and twenty-one genera). The twenty-two families together contain about one hundred genera and more than nine hundred species, including many synonymous names. Seven families are monotypic and four have only two genera each, but six are divided into sub-families with up to fifteen genera each.

The Acanthocephala are unevenly distributed among their vertebrate hosts. The Neoechinorhynchidea occur almost exclusively in fishes, only one genus (*Neoechinorhynchus*) occurring in other hosts (an amphibian and chelonians) as well. The Echinorhynchidea are mainly parasitic in fishes, forty genera occurring in such hosts, but ten genera occur in birds, one genus in reptiles, one in mammals and three genera in various combinations of two or three classes of hosts. The larvæ of these two orders are to be found in crustaceans. The Gigantorhynchidea are mainly parasites of birds (six genera) and mammals (twelve genera) but one genus occurs in amphibians and one in reptiles, two genera in birds and mammals, and one genus in four classes of hosts (amphibians, reptiles, birds and mammals). In this group, the larvæ utilize insects as intermediate hosts. In all, therefore, fifty-nine genera occur exclusively in fishes, sixteen in birds and thirteen in mammals, about a dozen genera sharing various classes of vertebrate hosts. The only representatives of the Opororhynchidea are three species of the aberrant genus *Opororhynchus*, which are no more than 0·4 mm long and occur in American and Brazilian birds. At the other end of the size range, however, there is the cosmopolitan species which infects pigs and occasionally man, *Macracanthorhynchus hirudinaceus* (Gigantorhynchidea), the females of which may be up to twenty-six inches in length.

Some genera of acanthocephalans have been very productive of species, and a few examples serve to indicate the diversity of hosts which arises out of a predator–prey relationship. Thirty-nine species of *Neoechinorhynchus* (Neoechinorhynchidea) occur in fishes, one species in amphibians and seven species in chelonians. In the Echinorhynchidea, *Echinorhynchus* has thirty-two species in fishes, *Acanthocephalus* has a score of species in fishes and a dozen in amphibians and reptiles, *Arhythmorhynchus* has twenty-five species in piscivorous birds, *Polymorphus* twenty-six species in birds, occasionally mammals, and *Corynosoma* has thirteen species in aquatic birds and twenty-two species in mammals. In the Gigantorhynchidea, *Centrorhynchus* has fifty-seven species in birds, mainly raptatorial forms, and five in mammals, *Prosthenorchis* has only one species in birds but nineteen in mammals, especially monkeys, and *Prosthorhynchus* has only two species in mammals but a score in birds. The nine genera together contain more than three hundred species, and at least one-third of known species.

These and many other interesting features concerning the spread of acanthocephalans in the animal world can be gleaned from Yamaguti's most recent survey. Nearly one thousand references will guide investigators through the maze of available information on the group, and more than eight hundred clear line diagrams (the best which have appeared in the *Systema Helminthum*) will also serve. Information concerning size has sometimes been omitted from the volumes of this, series but this criticism no longer applies; size criteria are given liberally for adults, eggs and other characteristics and for the majority of species, even when these are numerous in a genus. There are many references concerning experimental infections and information concerning larval forms and intermediate hosts in excess of what could be expected in a systematic essay, and a good general account of structure by way of introduction. In short, the blemishes noticed in preceding volumes have been avoided in this volume, although a few typographical errors are evident. This is not surprising in view of the plethora of phantasmagorial names such as *Acanthocephalorhynchoides ussuriense*, and an occasional lapse, such as in '*Arythmorhynchus*' (p. 395) and '*Gigantorhynchus*' (p. 404), is pardonable. The names of authors provide their own typographical problem, but it is odd to find, closely following a tribute to dedicated bibliographers working in the Catalogue Room at the Beltsville Parasitological Laboratory, that Yamaguti is indecisive and recalcitrant about the spelling of the name Petroschenko, which appears thus in the Index-Catalogue. At the same time, we have to thank Yamaguti for a sustained attempt to present scientific names accurately and a determined effort to provide a reliable taxonomic scheme, which at best can only be provisional. It is to be hoped that readers will realize how often and with what magisterial serenity Yamaguti had adjudicated in the face of difficulties created by writers who produced imperfect and defective work. It is a lamentable fact that much bad workmanship is evident in the original literature on the systematics of helminths and perhaps the greatest value of Yamaguti's great efforts will lie in the direction of better descriptions of species and genera in the future than have often been forthcoming, with more emphasis on the range of their variability. A taxonomic scheme will be as reliable as the work of the taxonomist and no more reliable, and with this admonition to the would-be systematist in the field of parasitology we can leave Yamaguti's *Systema Helminthum* in what we hope will be good hands.

BEN DAWES

CALCULATIONS IN COMPLEXOMETRIC ANALYSIS

Complexation in Analytical Chemistry

A Guide for the Critical Selection of Analytical Methods Based on Complexation Reactions. By Anders Ringbom. (Chemical Analysis: a series of Monographs on Analytical Chemistry and Its Applications, Vol. 16.) Pp. x + 395. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1963.) 113*s*.

COMPLEXATION reactions are now widely used in analytical chemistry and it is not unusual for the analyst to have to choose between several complexometric methods of analysis and for him to have difficulty in selecting the method which will give the most accurate result. Alternatively a method may need modification to suit the particular circumstances of the analysis. Such problems are frequently resolved as the result of a series of inconclusive experiments, or by the personal prejudice of the analyst, as insufficient time is available for a more complete investigation. A rapid, less empirical, approach has been long overdue.

In *Complexation in Analytical Chemistry* Ringbom shows that an increased application of theoretical principles to chemical analysis—in particular to complexation reactions—can greatly assist the analyst in making rapid and accurate decisions concerning the choice of analytical procedures. A simple mathematical treatment of equilibria is presented and this enables the analyst to establish the optimum experimental conditions for complexation reactions. Existing methods of analysis can be compared for determining their relative accuracies. Errors caused by various side reactions can be estimated and ways of reducing or eliminating the interferences can be quantitatively studied. There are also interesting possibilities of developing new methods for solving special analytical problems.

The mathematical treatment is lucidly explained and illustrated by more than a hundred examples. Analysts, once they understand the basic principles on which the calculations are based, should have no difficulty in making such calculations, as may be necessary, to assist in solving particular problems.

Complexation reactions are treated in their broadest sense, chapters being devoted to titrations, masking, acid-base reactions, ion-exchange, metal extraction, electrochemical reactions and photometric techniques. Actual methods for particular analyses are not given as the object of the book is to enable the analyst to devise these for himself and no attempt is made at reviewing the literature on complexation reactions in analytical chemistry. An extensive appendix, more than one-fifth of the book, contains details of equilibrium constants and other useful values abstracted from the literature. Notwithstanding the extent of the appendix, the main limitation to this simple mathematical approach to complexation reactions is the lack of the relevant equilibrium data for many reactions of interest.

The book is well produced and the text is clearly arranged; but it seems unfortunate that a work which is intended to assist the analyst in solving everyday analytical problems could not have been more attractively priced.

J. R. COOKE

AN ENCYCLOPÆDIA OF INORGANIC ANALYTICAL CHEMISTRY

Methods of Quantitative Inorganic Analysis

An Encyclopedia of Gravimetric, Titrimetric and Colorimetric Methods. By Kazunobu Kodama. Pp. xiv + 507. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1963.) 160*s*.

A NUMBER of works on inorganic analytical chemistry, often running to several volumes, have appeared in recent years to meet the demand of analysts for organized compilations from the original literature. This present treatise, *Methods of Quantitative Inorganic Analysis*, represents an interesting and generally successful attempt at compressing a wide range of detailed material within the compass of a single volume. Dr. Kodama has achieved this difficult task by efficient arrangement of material and by adoption of an abbreviated style reminiscent of that of *Chemical Abstracts*. Theoretical discussions and diagrams of apparatus have been omitted and there has been some sacrifice of typographical clarity. However, there are some fifty-nine tables where a great wealth of varied and analytical data is clearly and concisely presented.

Originally, the author intended this treatise to cover a wider field than that now presented but limitations of space have precluded any treatment of purely physical techniques such as spectrographic, X-ray fluorescent, radiometric methods, etc. The scope of *Methods of Quantitative Inorganic Analysis* is exactly described by its sub-title: *An Encyclopedia of Gravimetric, Titrimetric and Colorimetric Methods*. However, where flame photometry is an important method of analysis references are given to the pertinent literature, but polarographic methods which are valuable in metal alloy analysis are given no mention.

The book is divided into three parts, each representing a different and complementary aspect of inorganic analysis. For a particular problem it will often be necessary for the analyst to consult and combine material from each part.

Part 1 covers the field of general analytical techniques. An informative chapter on the decomposition of solid samples includes a table showing the percentage loss of volatile elements during acid attack. Another chapter comprehensively surveys the fields of complexometric, iodometric, acid-base and redox titrimetry; the properties of a wide range of indicators are presented in several tables. There are brief chapters on gravimetry, electrodeposition and optical methods.

Part 2 includes a detailed and comprehensive survey of the many organic reagents now in use in gravimetric and colorimetric analysis. Structural formulæ of many reagents and metal complexes are given which will be of assistance to the analyst not well versed in organic chemistry. In addition, there are useful chapters on ion-exchange, chromatographic and solvent extraction techniques. As is the case throughout this work the text is augmented by many tabulated data.

The arrangement of Part 3, the main section of this volume, follows the classical scheme of group analysis and each chapter is devoted to one element or group of similar elements. Chapters are subdivided into sections on attack, separation and determination. Treatment is comprehensive but selective, as methods are enumerated in order of analytical importance, preferred methods often being indicated; this is a useful feature for the analyst who has little time for method investigation. Systematic schemes of analysis are not neglected and many are presented in lucid diagrammatic form and include the analysis of silicate and fluorosilicate materials, the platinum metals, rare earth and uranium minerals. The needs of an analyst confronted with a wide range of analysis are fully met, only the analytical chemistry of the transuranic elements being neglected.

Numerous references both to standard works and to original papers are given at the end of each chapter and with many of the tables. There is an extensive index, and clarity has been enhanced by separation into two sections—one general and one devoted entirely to organic reagents.

The author has drawn heavily on such authorities as Hillebrand and Lundell, Schoeller and Powell, Fresenius-Jander, Sandell, Welcher, etc., and this book can be regarded as an omnibus digest of these works supplemented by methods from the original chemical literature up to 1957. This treatise is therefore comprehensive and authoritative, but there has been considerable delay in publication due to checking and correction. There are a few minor errors in the text.

This work will undoubtedly appeal both to the research and routine analytical chemist. The encyclopædic nature of this work makes it useful for reference, but, above all, this is a book for the inorganic analyst to have on his bench as a practical working manual.

A. D. WILSON

TEXT-BOOK OF ACOUSTICS

A Course of Lectures on the Theory of Sound

By S. N. Rschevkin. Translated from the Russian by O. M. Blunn. Pp. xv+464. (Oxford, London, New York and Paris: Pergamon Press, 1963.) 84*s*.

AT a time when there is a tendency to play down acoustics in physics departments of universities, it is interesting to observe the material presented in this subject to students specializing in acoustics in the Faculty of Physics of the University of Moscow. The impression is gained that the presentation is much more extensive and detailed, and therefore better drill, than in Britain. Thus the 450 pages of *A Course of Lectures on the Theory of Sound*, as compared with existing fundamental books on acoustics (for example, P. M. Morse's now classic *Vibration and Sound*), are easy to read through and form a good introductory text-book on the theory of sound. There are also occasional practical touches to the book which will endear it to applied acousticians; numerical examples are worked into the text from time to time to show the physical orders of magnitude involved, and the treatment of lumped acoustic impedances and acoustic waveguides is carried through in enough detail to make the methods and results readily accessible to those not primarily interested in the theory for its own sake.

The book is not, however, a comprehensive text-book on all aspects of acoustics, being confined almost entirely to problems of sound transmission and radiation. Nothing is said about such broad subjects as room acoustics, aerodynamic noise (apart from some reference to Gutin's propeller noise theory), nor about physiological acoustics. These omissions make it incomplete as a technological text-book. Regrettably, therefore, it is not the answer to the often heard prayer for a complete modern text-book of acoustics.

There are several things about the treatment which might well be taken up in British presentations. For example, the confusion that can arise from following Lord Rayleigh's tradition of commencing with the vibrations of strings and membranes is overcome by eradication of these topics, and the whole subject is developed immediately from the hydrodynamic equations. The traditional method is dear to the hearts of most physicists, of course, but in those unfamiliar with wave motion it can easily create the impression that standing wave motion, rather than travelling wave motion, is the basic ingredient of sound.

The book is well written and translated, the adherence to the symbolic notation used in the Russian edition doing surprisingly little to detract from its value. To sum up, it is a good book for those desiring an extensive treatment of the basic elements of acoustic transmission and radiation theory and, as such, it will undoubtedly be useful to students of acoustics, filling the gap between existing elementary and advanced works. It fits into present engineering courses in Britain on acoustics as a reference book, however, rather than as a principal text-book.

E. J. RICHARDS

POLITICAL TRANSGRESSION OF THE PACIFIC

The Western Invasions of the Pacific and Its Continents

A Study of Moving Frontiers and Changing Landscapes, 1513–1958. By Dr. A. Grenfell Price. Pp. viii+236+11 plates. (Oxford: Clarendon Press; London: Oxford University Press, 1963.) 35*s*. net.

SIR GRENFELL PRICE has chosen a huge canvas—the largest and deepest of oceans and its contiguous continents, the land-mass of Asia, North and South America and Australasia and peoples as remote from each other as the Eskimos and the Maoris. He spans four centuries, from the invention of the lateen sail which enabled European invaders "to sail close to the wind and avoid the dangers of lee shores" to the present redrawing of the political map.

It would have been a big enough task for any historical geographer to reassess the motivations and impact of the Portuguese, the Spaniards, the Dutch, the British, the French and the Russians as conquerors, sojourners and settlers. This Sir Grenfell Price has done with an insight, illumined by the political events of our times, and with an objectivity which will not make it 'recommended reading' in emergent countries which do not count themselves as beneficiaries. That is a pity because, as the heirs of the empires, it might help them to evaluate their inheritance.

But *The Western Invasions of the Pacific and its Continents* goes much further. It deals with the landscape, which was changed by the colonial transfers of exotic plants for industrial crops and plantation agriculture (as well as the ravaging of flora and fauna); with the transfers of people, not only of the European settlers but also of the alien labour whom they imported and who, in many places and with present embarrassment, are now outnumbering the indigenous population; with the geography of disease and with the population explosion.

When we speak to-day of the great benefits which modern (Western) medical services and medical sciences have conferred on Pacific peoples, it is well to remind ourselves (as the author does) that the most dangerous exports of the Western world were small-pox, measles, pulmonary diseases, malaria, filariasis, hookworm, leprosy, maybe syphilis and certainly gonorrhœa, which decimated indigenous peoples who had no immunity.

On population distribution and redistribution, the book is less convincing. Australia, with the swarming millions of Asia too close for comfort, is compared with Canada (6 per square mile) and Asian Russia (2 per square mile). By deducting the arid lands and the tropics, Australia has 720,000 remaining out of 2,900,000 square miles, which gives 14 (whites) per square mile. But there can be argument even about that subtraction. "Unsuitable for settlers" depends on what sort of settlers and whether lands can be made suitable. After all, the whole story of man is one of mastering his environment. Nor is it better than a debating point to say that Australia is taking the majority of migrants from some of the most crowded countries on the Earth—Britain with 540 per square mile, compared with India with 293; the Netherlands with 839 per square mile, compared with Japan with 610; and Italy with 413 per square mile, compared with Indonesia with 139. He makes the point that the 'white policy' of the U.S.S.R. in its Asian territories and the inviting sparseness of Siberia, just north of expanding China, "will require a solution one day": *verbum satis sapienti*.

He has a case, however, for arguing that countries now looking enviously at open spaces might look at their own. Indonesia, with Soekarno clamouring for *lebensraum*, has a population of 700 per square mile in Java, 70 in Sumatra and 7 in South Borneo. The sensible plans operated by the Dutch for resettlement have been conspicuously unsuccessful under his régime.

This is an invaluable book for those who want to see the imperial past, without romance or recrimination; who want to understand what is behind the crisis headlines and who want to be sensible about the future.

RITCHIE CALDER

TROPICAL METEOROLOGY IN AFRICA

Tropical Meteorology in Africa

Proceedings of the Symposium Jointly Sponsored by the World Meteorological Organization and the Munitalp Foundation. Edited by D. J. Bargman. Pp. xv + 446. (Nairobi: Munitalp Foundation, 1960. Available from the World Meteorological Organization, Geneva.) 20 Sw. francs; 33*s*. 3*d*.; 4.70 dollars.

WHILE the *Proceedings* of a symposium, published some years after the event, may sometimes represent an unnecessary record of material which is either ephemeral or already on record elsewhere, this is certainly not true of the Nairobi symposium on tropical meteorology in Africa. Under the joint sponsorship of a United Nations specialized agency and an independent research foundation, tropical meteorologists attended from twenty-one African countries, as well as from Belgium, Britain, France, Germany, Spain and the United States; and the record of proceedings, despite a few typographical and linguistic lapses, reflects part of a unique background of collective professional experience, much of which has still to reach the literature.

There were a number of authoritative and valuable reviews of the problems and methods of tropical meteorology in general, but special attention was naturally directed to new findings specifically concerning Africa. These may perhaps complement some of the earlier post-war progress in tropical meteorology, which was based predominantly on data 'climatically insular' rather than continental. In particular, while fully recognizing the continuing need for better observational cover, the symposium unanimously accorded a high priority to further work on new synoptic models. This need was also the main theme of the 1964 presidential address to the Royal Meteorological Society, which posed, as the central problem of meteorological analysis in general, the fact "that the rapidly increasing amount of meteorological data which is being collected will not be properly put to use—indeed part of it may not be used at all—unless an adequate system exists to collate and present it in a form which can be readily understood and digested". At the Nairobi symposium, special interest centred on the progress in this respect reported by Johnson and Mörth, establishing in eastern Africa relationships between equatorial weather and typical flow patterns in the middle troposphere; and visiting meteorologists were particularly impressed to see these ideas already incorporated into present-day forecasting practice. Furthermore, this work, utilizing the additional upper-air data which became available during the International Geophysical Year (and incidentally supported by one of the few Colonial Development and Welfare schemes in tropical meteorology), demonstrated for the first time a series of systematic relationships between winds and contours of isobaric surfaces in the equatorial belt. This is a long-standing problem of the general circulation, to which attention was also directed by the work of Cochemé in the same region.

Another subject on which potentially classical results were first presented in detail at this symposium was the energy-balance of tropical land surfaces. Pereira and McCulloch described catchment-area experiments in which continuous direct measurements were made of all the more important factors in the heat and water balance over complete valleys, varying in extent from sixteen hectares to eight square kilometres. From these results, together with heat-flux analyses over bare soil and water-use studies of grasses and crops, measured values were presented for a representative sample of land-surface conditions, ranging from the warm and semi-arid plains of central Tanganyika at an altitude of 1,000 m to bamboo mist-forest up to 3,600 m in the Aberdare mountains of Kenya. The importance of these data, as a contribution not only to applied meteorology but also to the physical basis of the science, was emphasized by Prof. Flohn—whose presentation of his own analyses of climatological data from Africa and the adjoining oceans, undertaken in such a manner as to provide significant information on synoptic-scale phenomena, was strongly approved by synoptic meteorologists present.

Space does not permit mention of other significant lines of work reported, on meteorology and on its applications to African problems. A final point, which is perhaps of some interest in relation to the organization of such symposia in general, is the effectiveness with which technical discussion was stimulated by a relevant and extensive travel programme, including, for example, the agricultural resettlement schemes of the Aberdare catchments; the Murchison Falls, with their fantastic associated wild-life probably still much as Samuel Baker saw it a hundred years ago; and the Owen Falls dam, where the scale of the combined uncertainties both of the total rainfall over Lake Victoria, and of evaporation losses from its surface, is still of the same order of magnitude as the resulting flow of the Nile.

R. C. RAINEY

Clinical Toxicology of Commercial Products

Acute Poisoning (Home and Farm). By Marion N. Gleason, Robert E. Gosselin and Harold C. Hodge. Second edition. Pp. xv+1211. (London: Baillière, Tindall and Cox, 1963.) 176*s*.

Clinical Toxicology of Commercial Products consists of eight sections, separately paginated and each printed on differently tinted paper, but bound together as a single book. Section 1 (pp. 15) relates to general first-aid and emergency treatment in the event of acute chemical poisoning, both before and when medical aid becomes available. Section 2 (pp. 141) is an index to the active ingredients of some 3,000 proprietary and other substances; toxicity ratings from 1 to 6 (practically non-toxic to 'super-toxic') are ascribed to the principal chemicals included, while broad classes of compounds are identified with one of 77 specific congeners, these being separately indexed with toxicological details, symptoms and treatment in Section 3 (therapeutic index, pp. 157). General remedial measures, from respiratory aids to nutritive support, are considered in Section 4 (pp. 76), which is followed by a large index (Section 5, pp. 674) of more than 14,000 trade names of products which might be ingested, either accidentally or suicidally. In this the principal toxic constituents are identified by an asterisk, though in practice such might number as many as (say) five of a total of seven listed ingredients. Cleaning fluids, pesticide formulations and sedative tablets all feature prominently; entries, most of which are American proprietaries, range widely, from 'Lifebuoy Soap' to 'Kitty Chaperone' (keeps cats off furniture). Formulations are indexed by function in Section 6 (pp. 113) and by manufacturer in Section 7 (pp. 22). The final section (pp. 13) is a provisional classification of chemicals which either have proved, or may prove to be, poisonous in man, with a numerical ætiological assignation; the latter is an extension of an existing classification and, as such, a contribution to the rationalization of the subject, despite many necessary compromises.

There is no suggestion that this large volume is an emergency ready-reference volume. It could be used as such only after familiarity with the basic plan has been acquired, when (as in other circumstances) the initial logical sequence of consultation might be to Sections 5 (or, failing this, 6), 2 and 3. Such an approach, with a somewhat extended explanation, is set out diagrammatically as the frontispiece. As a reference source-book there is no doubt of its value, the main limitation to which in Britain lies in the fact that the proprietaries are mainly of North American origin. H. Egan

Progress in Chemical Toxicology

Vol. 1. Edited by Abraham Stolman. Pp. xii+436. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 14 dollars.

This book, the first of a continuing series of volumes covering developments in chemical toxicology, is divided into eleven somewhat unevenly balanced chapters. For example, the chapter by Rieders on "The Determination of Antiarthritics, Antihistamines and Thymoleptics" occupies 7 pages, whereas "Narcotics and Related Bases" by Farmilo and Genest covers 87 pages. I should have thought that with a more consistent classification (either pharmacological or analytical classification, but not both) of chapters, the 7-page chapter could have been incorporated in one of the other manuscripts.

Chapter 1, by Freimuth, briefly describes isolation and separation techniques for identification of poisons. Then follows a chapter by Goldbaum, Schloegel and Dominguez on the application of gas chromatography to toxicology. Aliphatic alcohols are thoroughly covered by Harger and Forney, and Curry, in a well-written and informative chapter, discusses the analysis of acidic and neutral poisons. There is a useful chapter by McBay and Algeri on ataraxics and non-barbiturate sedatives. Feldstein deals effectively with the toxicity of, and analytical methods for, air pollutants. Tyler contributes 43 pages on poisonous mushrooms, and Schwarting concludes the volume with a chapter on poisonous seeds and fruits.

It is a pity that this volume should have taken such a long time before its final publication. I am aware that one of the contributors sent his manuscript to the editor early in 1962. The editor's preface is dated August 1963. The book appeared in 1964. I feel that neither the editor nor the publishers are to be blamed for the delay, and that this is due to the lack of a sense of urgency and complete co-operation on the part of one or some of the contributors. A book of this type is of greatest value when the date of its appearance is as close as possible to the date of publication of the papers on which the various chapters are based. Some way of dealing effectively with procrastinating contributors should be devised.

Otherwise, part of the volume is a useful compilation of some of the advances which have taken place in chemical toxicology in recent years. H. V. Street

Meristems, Growth and Development in Woody Plants

An Analytical Review of Anatomical, Physiological and Morphogenic Aspects. By J. A. Romberger. (U.S. Department of Agriculture Technical Bulletin, No. 1293.) Pp. vi+214. (Washington, D.C.: Government Printing Office, 1963.) 1.75 dollars.

In this monograph the author, as his sub-title indicates, has attempted to give an account of aspects of the morphogenesis, anatomy and physiology of selected gymnosperms and woody angiosperms. His approach thus combines in a useful manner the classical methods of anatomists of an earlier period with those of contemporary investigators of plant morphogenesis. It is valuable to have the several accessions of information brought together, briefly but in sufficient detail, in one volume—the book is primarily intended for students of physiology, horticulture and forestry—though, unavoidably, there are repetitions of facts and concepts that have received wide publicity in recent years. The general objective, maintained throughout, has been to relate growth, development and the functional activities of organs to the physiological processes in main and lateral shoot meristems (and also in other meristems, for example, roots) from which they all originate.

In the section dealing with anatomy and 'physiological morphology', a useful range of information, some familiar, but some less familiar, is clearly and fairly presented and discussed. In other sections, the episodic growth and dormancy of shoots, and the physiological processes that are, or may be, involved, are discussed in some detail, with reference to *Pinus sylvestris* and selected woody dicotyledons. In this section the phenomena of photomorphogenesis, Circadian rhythms, endogenous growth regulators, etc., are critically examined. A final section provides a useful account of episodic growth and dormancy in roots. The author, whose attitude is one of moderated scepticism, has properly been the critic of his own efforts and of the contemporary state of botanical science as he sees it. As he notes in his retrospective survey: "The reader who expects a pithy summary, replete with sweeping truths about the behaviour of meristems, the control of growth and development, and the physiological basis of dormancy, will be disappointed". Quite simply, botanical science has not yet reached that point. But, as the present volume indicates, many avenues of approach to the central problems are now being explored, and progress, though often fragmentary and highly incomplete, is being made.

The book, which is clearly and succinctly written, sometimes in a provocative but agreeable style, is modestly illustrated and well produced. C. W. Wardlaw

MONEY FOR RESEARCH AND RESEARCH FOR MONEY*

By Dr. ALAN T. WATERMAN

Chairman of the Board of Directors, American Association for the Advancement of Science, and former Director of the U.S. National Science Foundation

THE Michigan Association of the Professions appears to be a unique organization. Out of curiosity I looked up the word 'profession' in Webster's dictionary and was very amused. I find there are two major meanings: a profession may be either that which one professes, or that about which one professes to have knowledge. (Incidentally, one meaning of 'profess' is to pretend to have knowledge.) I conclude that, according to the dictionary, membership in a profession may have a very broad base. But, curiously, of all the professions there is only one which entitles a person to be a professor. (Again incidentally, the dictionary defines a professor as one who publicly teaches any science or branch of knowledge—which puts science in its place!)

However, I was finally reassured to find that a profession is a collective body of persons engaged in a calling, which serves the present purpose.

As has often been said, money is not everything but it surely helps. Its importance is attested in many of our sayings. In fact we might make an intriguing combination of a couple of these: 'the root of evil is that which makes the world go ' round'.

Money is certainly essential for modern research, and it is no secret that research can be a money-getter. For my part I have found that the progress of research has been chiefly retarded by two groups: (*a*) those who want to withhold money from research, as a foolish and unnecessary expenditure; (*b*) those who believe research can do anything if enough money is put into it. Curiously, it is the latter group which is frequently the chief source of ammunition for the former. If outlandish claims were not so often made in the name of science, there would be fewer arguments against the progress of research. All of which should indicate that one of the most constructive moves we can make is to bring about better public understanding of science.

When one separates the topic as I have done into money for research and research for money one does two things—one separates two important and different objectives of research and at the same time one can resolve much of the argument which occurs in justifying research for budgetary purposes.

The first of these objectives for performing research states that research is important in its own right. This is a point of view found especially in academic environment. In its pure form it holds that 'to seek the truth' is one of the highest pursuits man can follow, and no further justification is required. Essentially basic research does that; it is merely a modern and highly sophisticated technique for seeking the truth, namely, knowledge and understanding of man and the world he lives in. Why is that important? Well, research, and especially research in science, has been largely responsible for progress in human thought and philosophy. It has had much to do with exploding superstitions and removing that greatest fear of all, fear of the unknown, and has made noteworthy advances in our thinking, to say nothing of broadening our outlook on the world we live in. It puts emphasis on the higher things in life, much like a religion. But one can scarcely put a precise money value on it, nor are its advocates inclined to do so.

The second objective takes a practical and realistic view. Essentially it says of the first: 'For those who like that sort of thing, it is just the sort of thing they like', but so what? There is no particular harm in carrying out research for its own sake. If anyone wants to spend his life collecting butterflies or cosmic rays or satisfying his curiosity by looking through a microscope or telescope, that is his business. After all this is a free country where we allow such activities to go on, which is more than some other countries do. But no sensible citizen would expect to contribute funds for such a purpose, except as we do for symphony orchestras, art museums, etc., perhaps as a matter of civic pride, or as we provide toys for children. Public money? Certainly not. Hard-earned dollars should go in quantity only to programmes and projects where the practical need has been demonstrated, where there is every likelihood of a financial return, or, failing that, where some practical accomplishment can be made, such as the cure of a disease, the production of a superior military weapon, or the improvement of traffic conditions. Occasionally there are other practically important projects, too, such as desalination of sea-water or a remedy for air pollution.

According to this school of thought all research should be planned for, and judged by, its practical value. It may originate from awareness of needs—national, commercial or private—and materialize in the form of proposals for research to meet these needs. Or it may go a bit further, as occurs more and more in our technology, and support research which might open a new market, directly useful then for making money, and indirectly for raising standards of living.

But let us pause for a minute and see how much money is now going into what kinds of research, and who is providing it.

This is a subject which has received an increasing amount of attention, and properly so, on the part of industry, the U.S. Federal Government and economic analysts. In its entirety it has come to be called science and technology. Science enters as the discipline or study—the profession if you like—which seeks to explore the unknown, to discover new facts, new phenomena, or new techniques either to make further advances in knowledge or to show the possibility of useful application. Thus science is commonly understood to include all basic and some applied research. Technology seems to be the best term we can find to take in the entire gamut of what it takes, from the technical point of view, to bring about economic strength, prosperity, military security and general welfare. Thus it commonly starts from science as its technical base and includes, especially, applied research, engineering development, test and evaluation, production, distribution and use.

For present purposes we need merely note that the analyses on the subject are chiefly concerned with research and development and with man-power.

The national total for research and development in the United States is at present estimated at about 15 billion dollars†. This is about 2·5 per cent of the gross national product, an increase from 1·4 per cent 10 years ago. Of this the Federal Government provides about 65 per cent, while about 32 per cent is provided by industry. Thus industry and the U.S Federal Government are paying practically the entire research and development costs in the ratio of 1 to 2.

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* Substance of an address before the fifth Annual Congress of the Professions held at Lansing, Michigan, during February 7–8.

† 'Billion', a thousand millions in the U.S.A., France, etc.; in Britain, Germany, etc., a million millions.—Editor.

In this national effort, industry is doing most of the work. Seventy-four per cent of the total funds are used by industry in performance of research and development, 14 per cent by the Federal Government in its own laboratories, and 12 per cent by non-profit institutions.

The distribution of scientific and technical man-power among these economic sectors is similar. Thus of the total number of scientists and engineers employed in research and development activities in 1960, 68 per cent were in industry, 15 per cent in the Federal Government, 12 per cent in academic institutions, and the rest in other, non-profit institutions.

Although the total national funds for research and development have about tripled since 1953, this distribution of funds and scientific and engineering man-power has changed very little, and practically not at all since 1957. During this period the labour force has increased by 15 per cent, and scientific and technical man-power by 57 per cent. As a percentage of the labour force, scientific and technical man-power now stands at 3·6 per cent; it was 2·5 per cent in 1953.

The distribution of efforts in basic research is somewhat different but it is similar in its stability. National funds for basic research at present amount to nearly 1·5 billion dollars, which again is about three times the amount in 1953. As a percentage of the total funds for research and development, basic research funds remained nearly constant at 8 per cent until the past two years, when they rose to about 10 per cent. This largely reflects costly new projects in such subjects as oceanography, high-energy accelerators, and space research, where research facilities are especially expensive. The Federal Government is the source of less than 60 per cent of national funds for basic research, industry about 25 per cent, while academic and other non-profit institutions provide the remaining 15 per cent, in the ratio of about 2 to 1.

Colleges and universities have consistently performed nearly one half the nation's basic research, industry about one-third, and government about one-sixth.

What is especially important to note is that the American nation's effort in research and development has been a remarkably consistent one in its distribution of funds and technical man-power for the past decade. In recent years, increasing apprehension has been voiced over the large increases in the Federal budget for scientific research and development. While these increases have indeed been large, it should be noted that they have been quite effectively matched proportionally by the increases in contributions from the other sectors of the economy. Of particular significance is the fact that the Federal Government is not acquiring a monopoly or control over scientific and technological activities any more than it has been exercising for the past decade. In view of this stability in distribution of effort, it would seem that one should proceed with caution in the formulation of any radical change. Obviously such could lead to serious dislocations and loss of valuable time and effort in the process. This we cannot afford, for we must never forget that we live in a highly competitive world, and the modern key to successful competition lies in science and technology.

Now how does this analysis relate to my topic? In the first place, the figures I have given cover research, development and man-power. Since we are concerned here with money, it is logical to include development, since it is development which bears most of the cost and not research. In fact, research, both basic and applied, accounts for only 30 per cent of the total effort; basic research for only 10 per cent.

At this point I am somewhat in a quandary, since the theme here is research, not development, whereas most of the money involved goes to development and not research. The two are not the same, and the failure to make this distinction is responsible for much confusion and concern. Generally speaking, research is relatively inexpensive; it is at the point of decision to undertake engineering development that one has to face the commitment of large sums of money. The distinction is simple enough in principle: research, whether basic or applied, is exploratory in nature. It tries to find something new scientifically or to establish the feasibility of a development in principle, not in practice. Development starts from the premise that a practical undertaking may be feasible and attempts to work out and perfect a device or process suitable for production. Since the particular objective of development is known and understood, the decision whether to initiate development or not, though often difficult, is more clear-cut, and since it involves large sums of money and extensive employment the decision must be examined with great care. The whole affair may be likened to the opening of a new territory. The comprehensive exploration of the territory corresponds to basic research, the search for mineral or other resources corresponds to applied research, and the examination of how these resources may be exploited corresponds to development.

In proceeding further with this matter I shall touch briefly on considerations relating to development and then go into the subject of research somewhat more thoroughly.

For the most part, development has money as its fundamental objective. It is carried on in typical fashion by the technical industries in the hands of which it has reached high standards of quality and efficiency. Development financed by Government cannot, of course, be said to have a profit motive in the same sense, namely, profit to the financing agency. But it may carry a reasonable return to a profit organization under contract. Apart from that, as in the case of military development, it may be said to provide a form of national insurance. In any event, if conducted wisely it has value in strengthening the national economy. Because of its relatively high cost, special attention should be directed to the criteria for undertaking developmental projects. Thus in making a decision regarding a particular developmental project, one could consider: (1) What are its objectives and what are their relative priorities as compared with other objectives? (2) Will the programme if successful meet these objectives? (3) Is the programme technically feasible? (4) Is the estimated cost of the effort justifiable in money, man-power and facilities? The operating agency should be held strictly responsible for making these decisions wisely.

On the other hand, most basic research belongs in the first category—it requires money rather than produces it. Occasionally basic research is required in association with development to remove bottlenecks or to provide essential background. Some applied research and development belongs in this category, too, like the engineering development of elaborate instruments or installations for scientific purposes such as high-energy accelerators, radio telescopes and instruments for the study of outer space. These are not primarily concerned with making money, but money is required for their prosecution. As research in science becomes more complex and sophisticated the financial outlay begins to assume larger and larger proportions. As a result, money for science and basic research in particular begins to compete with money-making research, with research aimed directly at meeting pressing needs and resolving critical problems.

Dr. Vannevar Bush pointed out a consequence of this situation in the terse observation that applied research drives out basic—a sort of Gresham's law. Thus, in the face of the inevitable budget limitations, available funds go to programmes which are immediate and critical and to research projects the usefulness of which can be clearly foreseen and justified. Basic research does not fall in this class since it works in the unknown and cannot predict with any certainty whether its findings will be significant, much less whether they will be useful. In fact, one definition of basic research is research for which one cannot secure money!

In view of this competition for funds and of the extraordinary increase in research and development activity, what should our policy be?

If we are forced to economize in science and technology then we should give maximum care and attention to where most of the money is going—namely, development. As for research, we should encourage applied research and expect that industry and government will support basic research which underlies specific important practical objectives. But this is not enough. In fact, to adopt this criterion exclusively is most short-sighted and dangerous. As history amply records, most of the epoch-making discoveries have come from research with no other aim than free exploration of the unknown. How could one have planned for discoveries such as X-rays, radioactivity and antibiotics when they were not known to exist?

As a matter of fact, this type of pioneering research should be regarded and justified as an investment. It should be comprehensive in scope and cover all areas of science. It should include all degrees of promise—from projects of slow but certain progress to difficult and speculative projects which if successful would yield a high return, scientifically or practically. In planning such a programme one must count heavily on the experience and competence of individuals and groups to be supported, since they are the experts in this type of exploration and are thus best able to decide on promising leads. The investment when planned and executed in this way is statistically certain to yield results; in any event results of scientific value, but also practical results which more than pay for the cost of the investment, as the technical industries well know. It may be that the individual who makes a scientific discovery does not see a practical application or does not choose to proceed with one. Perhaps a series of basic research findings are necessary before an application emerges. But, as history also abundantly proves, the findings of science generally find their way into useful application.

The conclusion I would draw concerning the major question I have raised is that there should be no real conflict between the pursuit of free basic research and that of applied research, aimed at development. We must actively support both—basic research as an investment to explore the scientific and engineering frontiers and open new potentialities for technology, and applied research to investigate the feasibility of these findings and to search for answers to our pressing technical problems. The latter will often bring a direct money return. It also paves the way for accomplishments which are of great value in other ways, solving critical problems, maintaining military security, strengthening the economy, and improving the general health and welfare.

In the long run basic research does the same thing. It provides the knowledge and understanding of the world we live in which leads to power—power to adjust in an optimum way to our environment, to make maximum use of natural resources, and even to consider substantially altering our environment. Above all, it will uncover vast possibilities which we do not yet even know. In all this, research cannot fail to have a profound effect on our thinking, our culture and our philosophy of life. The degree to which we succeed in these endeavours does not depend primarily on money. It depends on people. For one thing it depends obviously on the number of competent scientists and engineers we have and can train. Fortunately, careful investigations seem to indicate that we can indeed train the numbers we need for the next decade, but we shall have to work at it much harder than we have been doing, and it will take a lot more money than we are now investing. A severe burden is coming on our colleges and universities, and especially our graduate schools. For this latter purpose we need to provide more funds and facilities to universities for basic research as an essential factor in advanced training. We must also direct attention to other professional training such as that of technicians and we badly need to improve the quality of instruction in education all along the line.

A country such as the United States should be able to provide, from public and private sources, the funds needed for the technological progress one wishes to make. This is a matter of priorities and continued prosperity. But the decision as to priorities lies ultimately with the people. Hence the progress we will make depends on the degree to which our citizens have a general understanding of science and technology, their potentialities and their limitations. This in turn depends on a solid background in education for our children and a live forum for information and discussion among the public generally.

Thus, in the last analysis, the degree of success we shall have in attaining our goals which involve science and technology will be directly dependent on the degree of understanding by our citizens of these objectives and their accomplishment. This is a subject which can well repay thought and effort on the part of those in the professions which are concerned.

AMERICAN SOCIETY FOR TESTING AND MATERIALS

By Dr. ROBERT F. LEGGET

Director of Building Research, National Research Council of Canada, and Vice-President of the Society

ON May 11 the American Society for Testing and Materials dedicated its impressive new headquarters building in Philadelphia with suitable ceremonies, international in character in keeping with the international scope of the Society and its work. The President of the United States sent his greetings in a personal letter that was read by Dr. A. Allan Bates of the U.S. National Bureau of Standards. Diplomatic representatives of Canada and Mexico presented appropriate greetings from their respective countries. The good wishes of the International Organization for Standardization (I.S.O.) were presented by Mr. Olle Sturén, director of the Swedish Institute of Standards, who brought with him, as a gift to the Society from his own Institute, a beautiful example of Swedish glass.

The principal address was delivered by Mr. Stewart S. Cort, president of the Bethlehem Steel Co., who illustrated the rapid growth of standards in the American economy and their increasing importance in the world-wide trade of to-day with telling examples from the steel industry. Reminding his audience how James Watt's work had been advanced by Wilkinson's improved boring machine, Mr. Cort went on to say that "the most important single development of those early industrial days was the system of the interchangeable-part manufacture—individual parts made so nearly identical that the complete device could be put together without the hard work and the high cost of shaping and fitting. A number of different inventors can claim a share of the credit for this big step forward. It was an American,

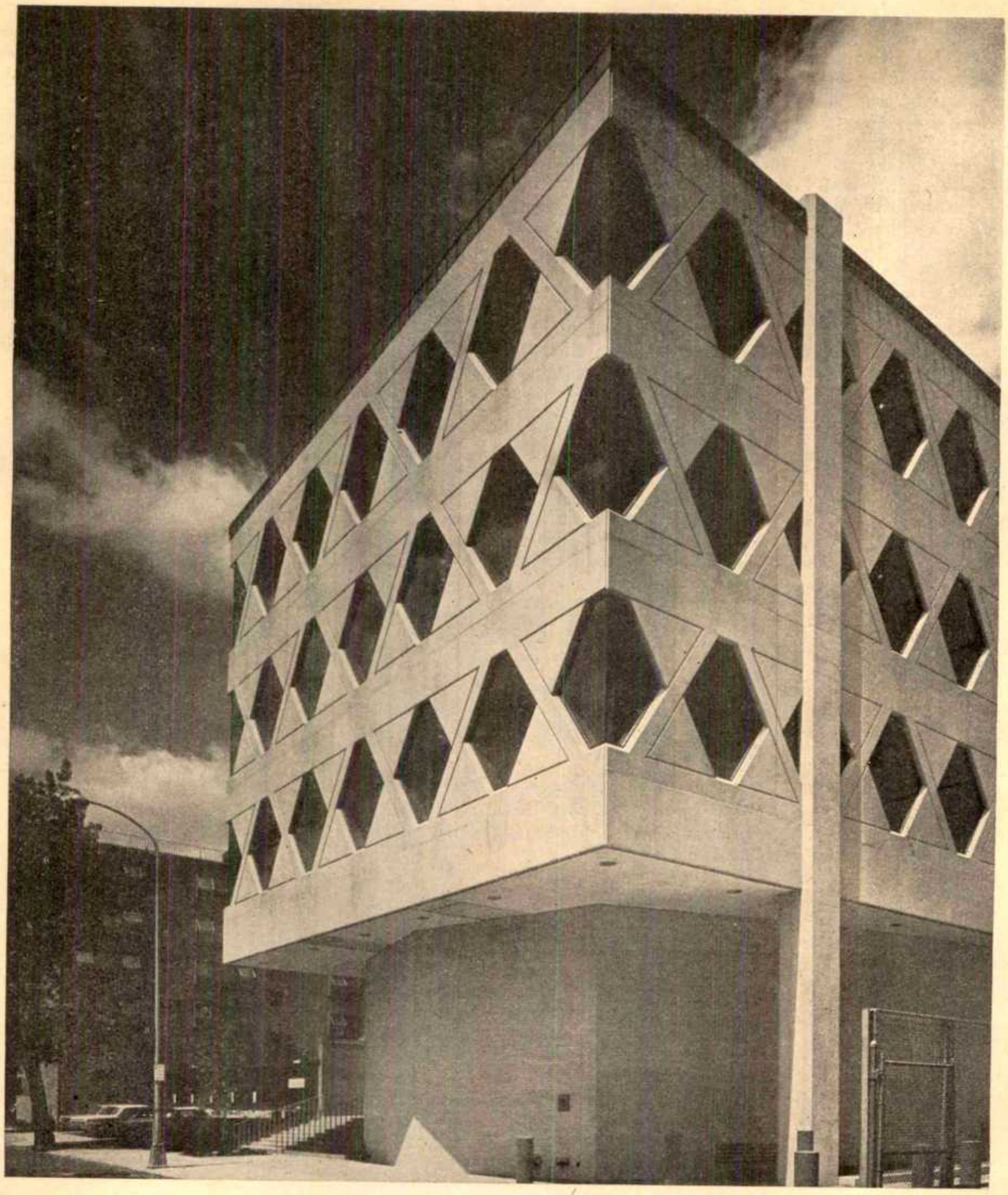

Fig. 1. New headquarters of the American Society for Testing and Materials, Philadelphia

Eli Whitney, who first developed the system on a commercial basis. . . . His secret was the jig, which he had invented to produce interchangeable parts, plus the use of power-operated tools. . . . It might seem surprising that almost one hundred years went by before the idea of standardized parts led to the idea that properties of materials from which those parts were made could also be standardized . . . the first organized meeting to discuss the subject of uniform methods of testing was held in Europe in 1882". Tribute was paid to the pioneer work of Albert L. Colby, a founding member of the American Society for Testing and Materials in 1902 and a leading chemist of the steel industry.

The new building (Fig. 1) replaces a smaller and somewhat inconvenient building on the same site, facing on Logan Square, an almost European feature of downtown Philadelphia with its impressive large fountain and park-like flower beds. The Franklin Institute and the Free Library of Philadelphia are neighbours. The architects, Carroll, Grisdale and Van Alen of Philadelphia, have achieved a masterly design in fitting so large, so convenient and so beautiful a building on so difficult a site. It is L-shaped with only a 40-ft. frontage on the square, and is 284 ft. deep and 93 ft. wide for its rear half. Sacrificing part of the 40-ft. strip has permitted the inclusion of a courtyard and garden behind the glass entrance doors (Fig. 2), giving an interesting view from the Square of the main part of the building in the rear. This is a four-storey structure which can eventually be extended to eight storeys when the need arises. On the

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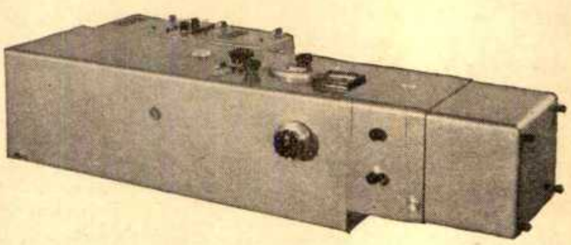

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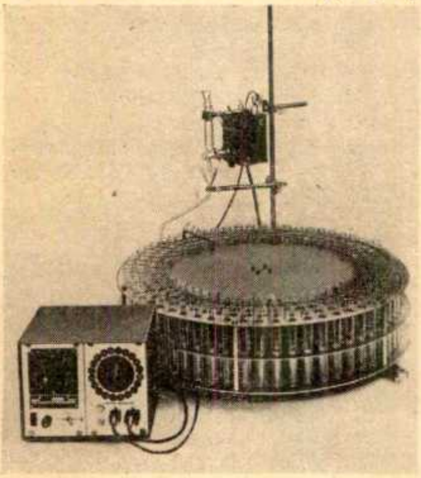

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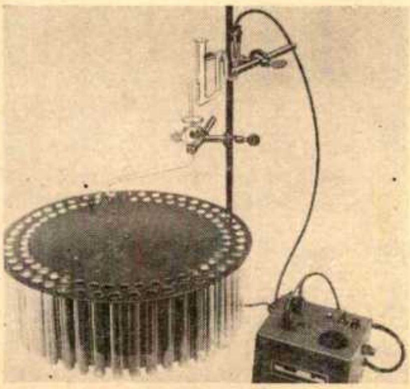

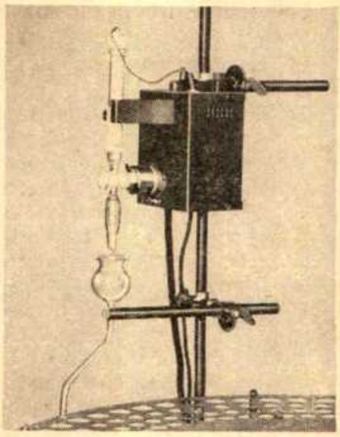

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ground floor is a 350-seat auditorium accessible from both directions. The remaining floors accommodate all the office functions of the Society, movable partitions for all subdivisions giving a flexible floor arrangement. The Society is now elegantly and efficiently housed for many years to come.

The new facilities are already being used almost to the full, so great have been the demands on the Society during recent years. It is a unique organization, entirely voluntary in nature, self-supporting financially, and yet producing standard material specifications and test methods which are extensively used by American Government and industry, and to a somewhat lesser extent in every English-speaking country of the world. In other countries the Society's standards are frequently used as reference documents or as the basis for the preparation of a number of recommendations of I.S.O. The Society's work with I.S.O. is channelled through the American Standards Association, which also presented its greetings at the dedication ceremony through its Executive Director.

Research has always played an important part in the work of the Society and is one of the foundations on which its standards are based As a means of emphasizing its research function the Society recently made a small but significant change in its name by inserting the word 'and' between 'Testing' and 'Materials'. This was in keeping with its original charter wherein the function of the Society is stated to be "the promotion of knowledge of materials of engineering, and the standardization of specifications and methods of testing".

As further evidence of its research interests, there was established in 1960 a Division of Material Sciences, the function of which is still further to stimulate research in the field of materials. The Division has already been responsible for some outstanding symposia at annual meetings of the Society on such subjects as: "Properties of Crystalline Solids" (1960); "Major Effects of Minor Constituents on the Properties of Materials" (1961); "Stress-Strain-Temperature Relationships in Materials (1962); "Properties of Surfaces" (1963); "Irreversible Effects of High Pressure and High Temperature on the Properties of Materials" (1964); "Internal Friction, Damping and Cyclic Plasticity Phenomena in Materials" (1964). All have been published in the Society's series of *Special Technical Publications*.

The international aspect of the Society's activities is shown by the fact that of its 12,000 members no less than 1,700 come from countries other than the United States. Surprisingly these now number 73. Copies of the Society's publications are now mailed to every part of the world. A Canadian is not infrequently a member of the Board of Directors. One Canadian has already served as president. Seven Canadians hold leading offices in the technical committees through which the Society carries out its important work in the field of standardization. Mr. H. A. R. Binney, director of the British Standards Institution, was made an honorary member at the Chicago annual meeting of the Society in June and at the same meeting Dr. F. M. Lea, director of the British Building Research Station, received the Walter Voss Award.

These international links, which the Society has always enjoyed to some degree, are in keeping with its international origins, for it started in 1898 as the American branch of the International Union for the Testing of Materials. It was soon found that a separate American organization would be desirable, and so the Society was chartered, as such, in 1902. From this small start has developed the Society of to-day, with its new three-million-dollar building in Philadelphia. The headquarters of the Society have always been in the 'City of Brotherly Love', initially because the first secretary was dean of civil engineering in the University of Pennsylvania, but more recently by choice. Four men only have

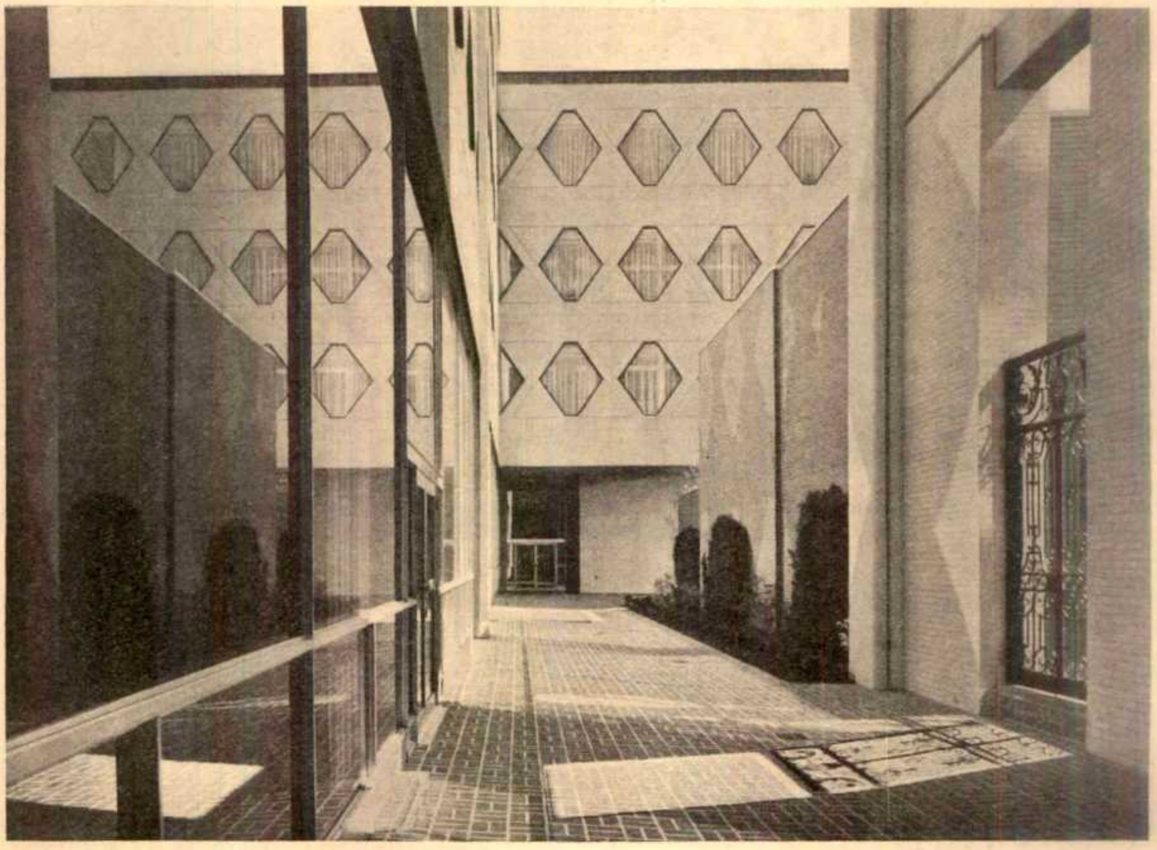

Fig. 2

served the Society as its top executive throughout its 62-year history—E. Marburg (1902–18), C. L. Warwick (1919–52), R. J. Painter (1952–60), the present incumbent being T. A. Marshall, jun.

Most scientific societies fulfil two main functions—providing a specialized service to their own members and publishing scholarly papers and books. The American Society for Testing and Materials performs these functions well but in addition it develops and publishes standards for materials and test methods that are, in effect, public documents. They now total more than 3,200. Commencing in 1964 they are being issued on an annual basis in a series of 32 volumes, having a total pagination of almost 20,000. All these documents are initially prepared and kept continually under review by 99 main technical committees aided by (one might almost say) innumerable sub-committees and working groups. Subjects range all the way from steel (Committee *A*–1) to peat (Committee *D*–29). Especially significant has been the recent establishment of Committee *F*–4 on surgical implant materials, the medical profession having solicited the aid of the Society in applying well-established engineering procedures to the standardization of the materials now used for so many and varied surgical implants.

This development is another clear indication of present-day awakening to the vital importance of standardization in fields far removed from engineering where this discipline naturally started. It confirms the timeliness and importance of the Society's new building, whence comes most of the secretarial and administrative support for all the Society's technical committees. All committee work is entirely voluntary. Some committees are now so large (one has more than 200 sub-committees) that their own separate meetings are minor scientific conferences. The focal point for committee activity is, however, the Society's annual meeting, a unique gathering. More than 3,000 men (with some women) meet throughout a whole week in order to attend working meetings of more than 1,100 technical groups. The physical arrangements for this remarkable schedule, enlivened by some general technical sessions and two invited lectures but by a minimum of social diversions, always evoke the admiration of the newcomer.

It is in the workings of the individual committees that there can be seen in a quite remarkable way the application of science to the needs of man. Here the scientist, the research worker, the university professor sit down with the engineer to pool their information in the drawing up of a standard document which can be put to public use. Compromise is essential but the very process of reaching agreement on some controversial point will often point the way to needed research. It is impossible to estimate what stimulation has thus been given to research in North America but it is certain that the influence of the American Association for Testing and Materials has been very great indeed. Although the new structure in Philadelphia, therefore, appears to be 'only an office building', it is in fact a research building and as such appears to be worthy of this brief record in the pages of *Nature*.

PLUTONIUM IN THE MARINE ENVIRONMENT

By K. C. PILLAI*, DR. R. C. SMITH and DR. T. R. FOLSOM

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PLUTONIUM-239 and -240 have been separated from sea water by radiochemical techniques and measured by use of an alpha spectrometer; ^{239,240}Pu has also been found to be highly concentrated in marine organisms. In addition, plutonium-238 has been detected in some of these organisms. The significance of plutonium in the marine environment and the application of concentrating organisms for trace-element detection is discussed here.

Naturally occurring plutonium has been shown to exist only in extremely small amounts, about one part in 10^{14}, in uranium-rich minerals like pitchblende and carnotite[1]. This plutonium is produced by neutron absorption of uranium-238, the neutrons necessary for this reaction arising from various sources[2]: from the spontaneous fission of uranium-238, from reactions of the type (α, n), by neutron multiplication in uranium-235 and neutrons produced by cosmic rays. On the other hand, man-made plutonium has found wide use in nuclear bombs and reactors. In nuclear explosions, plutonium isotopes can be formed by neutron capture by uranium-238 atoms or by the escape of fissile nuclides which have not undergone fission. It has been reported[3] that a total fission yield of 193 megatons was put into the atmosphere as of January 1, 1963. This amount corresponds to $2 \cdot 8 \times 10^{28}$ fissions. If we assume that fall-out particles from an explosion contain one plutonium atom per fission, this gives 11×10^{6} g or 0·68 Mc. of plutonium-239 put into the atmosphere. The analysis of air-borne debris in the first large-scale thermonuclear explosion[4] showed that the major isotope of plutonium formed was 239, with other isotopes like 240, 241, 242, and 244 being formed in much smaller quantities. Curium-242 (half-life = 162 days), the parent of plutonium-238, was also detected in minute amounts.

Plutonium isotopes are far less abundant in fall-out than strontium-90 and cæsium-137 and, relative to those of strontium-90 and cæsium-137, the half-life of plutonium-239 is very long—more than 24,000 years. Plutonium is one of the most toxic elements. It can exist in a multiplicity of oxidation states which exhibit significant differences in chemical properties, and it is thought to be relatively unrelated chemically to the essential constituents of plants and organisms. Since it is artificial, and possesses peculiar chemical properties, the presence and distribution of this element in the environment are unique and its investigation is of great interest.

Fall-out plutonium has been measured in air, soil, various human organs and in commercially available meat products[5]. However, until now, no results have been available on the levels of plutonium present in the marine environment. Concern over plutonium in the marine environment has increased since the development of SNAP (Systems for Nuclear Auxiliary Power) generators for space-vehicles. In some of these systems, plutonium-238 has been used as a source of auxiliary power[6]. It is of some importance, therefore, to acquire a general knowledge of environmental concentration-levels, distribution patterns, and the concentrating ability of marine organisms so that the consequences of a space craft failure can be asssessed[7]. Examination of the marine environment is important in this respect since the likelihood of this plutonium being deposited in the ocean's large area is high. This article summarizes preliminary findings concerning the plutonium content in sea water, and the concentration factors for this element in certain selected marine organisms.

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The radiochemical separation of minute traces of plutonium from the whole spectrum of elements in sea water is difficult. In addition, relatively large samples are needed to provide counting statistics sufficient for accurate measurements. The preliminary concentration of plutonium from sea water (45 l.) was done by co-precipitating reduced plutonium with bismuth phosphate. This was successful when high concentrations of bismuth and phosphoric acid were used and additions of reagents were made to the sample at about 80° C in a reversed sequence[8]. In the case of marine organisms, the samples were dried and ashed in a muffle furnace at 600° C. The ash was dissolved in nitric acid, and plutonium was co-precipitated with bismuth phosphate. Plutonium was separated from the bulk of bismuth phosphate by carrying it on lanthanum fluoride. Finally, plutonium was isolated using an anion-exchange column and was then electroplated on a platinum planchet[9]. Since the radiochemical separation involved a number of steps, it was necessary to demonstrate the recovery of plutonium in each case. For this purpose, a known amount of plutonium-236 was added as a spike to all the samples before they were processed. This isotope is convenient because it is not a fall-out constituent and because its α-spectrum contains peaks in a suitable energy region. It was possible to carry out the assay without interference from other nuclides present in sea water which may have escaped chemical separation and have energies close to that of plutonium-239 (for example, 5·017-MeV α of protactinium-231, 5·305-MeV α of polonium-210) by using an α-ray spectrometer having high resolution and low background.

Since the amount of plutonium-239 measured was extremely small, it was necessary to check reagents for possible contaminants by making blank assays. Separate reagent blanks were carried out for sea water and for marine organisms because different amounts of reagents were used in each case. The blank assays were carried out in the same way as the sample; the same amount of spike was added and distilled water was used in place of the sample.

The α-counter system consists of an *ORTEC* silicon surface-barrier detector in a vacuum system, a pre-amplifier, a biased linear amplifier, and a pulse-height analyser served by a read-out system which includes a punch tape-recorder and a chart-recorder. Sensitive meters are also provided in order to monitor the behaviour of the detector. The amplifier and analyser system was set to record α-particles from 4 to 6 MeV in 200 channels. This energy range was calibrated using the 4·195-MeV α of uranium-238 and the 5·763-MeV plutonium-236. The results stored in the pulse-height analyser were read into a strip chart recorder and counting evaluations were then made from the recorded histograms.

It has been our experience that selection of a good detector is necessary in order to make reliable measurements at very low levels of radioactivity. With a selected detector having 450 mm² area, the background from the detector and amplifier system was maintained at 0·025 counts per hour per 200-keV energy-interval. The energy resolution of the detector-amplifier system was 70 keV full width at half maximum for 5·763-MeV α-particles, and the drift of this system was less than 10 keV during counting periods of 140 h.

Figs. 1 and 2 are photographic reproductions of histograms displayed by the chart recorder. Fig. 1 is an α-spectrum of 45 l. of coastal sea water and shows the small plutonium-239 peak and also the plutonium-236 standard peak (reduced to half-scale). No other α-particle peaks were detected. The resolution of the detector system is insufficient to distinguish between the α-spectra of plutonium-239 and plutonium-240. Since fall-out is expected to contain predominantly plutonium-239, and since no attempt in these measurements has been made to distinguish between plutonium-239 and plutonium-240, reference will be made to these as plutonium-239. Because the spectra of plutonium-236 and plutonium-239 contain multiple energy peaks[10], and because resolution was 70 keV (full width at half maximum), an energy interval of roughly 200 keV was considered to encompass the total number of counts (area under the peaks) in either of these α-spectra.

The activity of plutonium-239 was determined directly by comparing its peak area with the peak area of the known amount of standard plutonium-236. Using this technique, the accuracy of measurement is high since the measurement is largely independent of the chemical yield and the efficiency of the detector. The final plutonium-239 activity of the sample was calculated by subtracting the reagent blank background activity. This background, measured by counting a blank sample containing all chemical reagents and including the plutonium-236 spike, had an average value of 0·016 d.p.m. per 200-keV energy-interval. The sea-water sample with the lowest activity has more than three times this background activity. A sample of plankton obtained in 1950 prior to extensive nuclear testing was measured for plutonium-239 and this also provided background information. The background measured in this way was found to be 0·019 d.p.m. per 200-keV energy-interval. As shown in Table 2, this is zero within the accuracy of the measurement.

Table 1 summarizes the analyses of sea-water samples. The errors given are one standard deviation of the counting error. All the water analyses were made on surface samples and the work recorded here has not included the distribution of plutonium-239 in deep waters. Four samples of coastal surface water taken at the Scripps Institution of Oceanography pier at La Jolla, California,

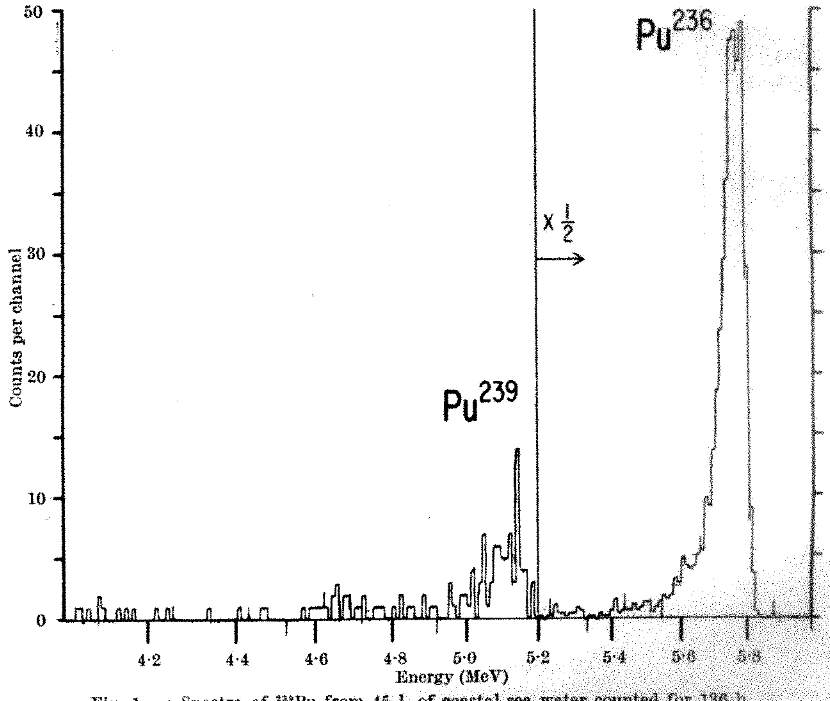

Fig. 1. α-Spectra of ^{239}Pu from 45 l. of coastal sea water counted for 136 h

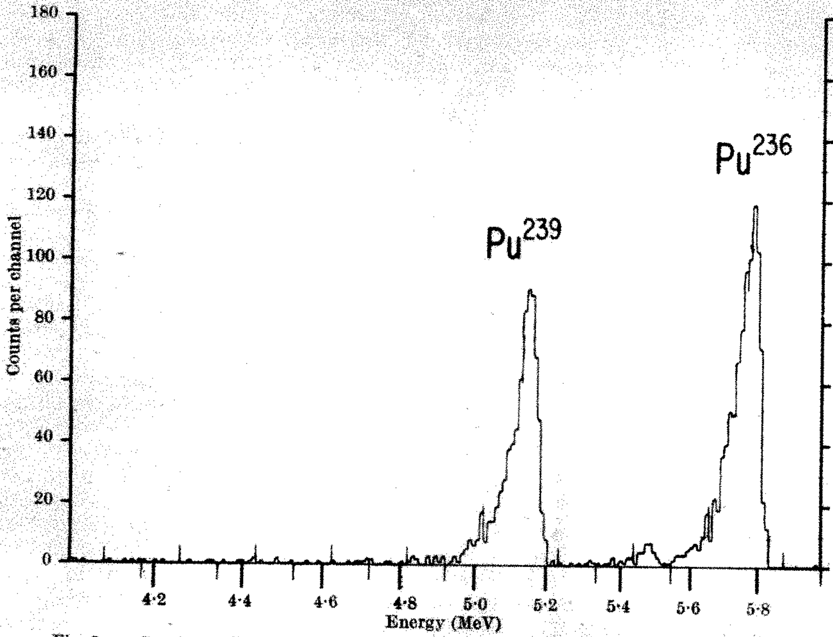

Fig. 2. α-Spectra of ^{239}Pu (and ^{236}Pu) from 760 g of palm kelp (*Eisenia arborea*) counted for 94 h

are compared with samples taken about 1,500 km and 2,500 km from the coast. It should be noted that the coastal samples are not replicates. Some variability in the concentrations in the coastal samples is to be expected when collections span several months. Large variations were observed in concentrations of cæsium-137 over similar periods during 1959–61 (ref. 11). It should be noted that the concentrations of plutonium-239 in water far off the coast are much higher than the average for the coast. These were samples at points believed to be in the gyral lying west of the major southward flow of the California Current. On the other hand, water reaching the coast at La Jolla generally comes from a flow from the south where the average precipitation and consequent fall-out are relatively low[12]. This water may also include a considerable up-welled constituent which would be expected to be low in fall-out. This pattern of relatively low fall-out in these coastal waters has also been observed for cæsium-137.

Three surface sea-water samples were taken simultaneously at one station 2,500 km from the coast. These samples were analysed for plutonium-239 (as reported in Table 1), cæsium-137 and strontium-90. The cæsium-137 content was found to be 0·46 μμc./l. and that[13] of strontium-90, 0·37 μμc./l. The plutonium-239 activity amounts to 0·7 per cent of the cæsium-137 activity, and 0·8 per cent of the strontium-90 activity in Pacific Ocean surface water as compared to 0·8 and 1·0 per cent respectively as calculated from the Richmond, California, fall-out results[14]. In spite of its very different chemical nature, plutonium apparently has not appreciably fractionated from strontium and cæsium in this oceanic surface water.

Table 1. Plutonium Activity in Surface Sea Water

Location	Counting time (h)	Total ^{239}Pu activity (d.p.m.)	^{239}Pu 10^{-4} (μμc./l.)	Date of sampling (1964)
S.I.O.* Pier				
32° 52′ N. 117° 15′ W.	136·0	0·035 ± 0·006	3·5 ± 0·6	January
,, ,,	118·5	0·047 ± 0·008	4·7 ± 0·8	January
,, ,,	115·5	0·045 ± 0·009	4·5 ± 0·9	February
,, ,,	140·0	0·039 ± 0·007	3·9 ± 0·7	April
North Pacific (1,500 km from coast)				
27° 49·5′ N. 129° 15′ W.	115·0	0·194 ± 0·024	19·4 ± 2·4	February
North Pacific (2,500 km from coast)				
30° N. 140° W.	100·75	0·303 ± 0·024	30·4 ± 2·4	March

*S.I.O., Scripps Institution of Oceanography.

Table 2 summarizes the results of analyses of certain representative marine organisms. These have been selected as possible indicators of environmental changes due to fall-out. The total activity of plutonium-239 in each sample is given along with the activity per kilogram of the sample's wet weight. In addition, a concentration factor, taken as the ratio of the sample activity to that of the average coastal water, is given. The average of coastal waters is used as reference in this ratio because the samples of marine organisms were taken near the southern California coast. The concentration factors are given to show that plutonium is indeed concentrated by marine organisms and to give some indication of the variability of this build-up. It should be noted, for example, that marine plants have large concentration factors whereas the negligible concentration by the fish sample indicates that discrimination against plutonium can take place at higher trophic levels. However, these concentration factors should be considered as only a relatively coarse measure of the extent to which plutonium is concentrated in marine organisms from sea water. This is because the comparison of various organisms by wet weight is somewhat arbitrary, and it is to be expected that the concentration of plutonium in organisms will depend to some extent on numerous complex variations in environmental conditions and sample collection techniques.

An intense bloom 'red tide', which occurred recently in local waters, was used to obtain a sample of phytoplankton large enough for analyses of minute amounts of fall-out activity. Surface water was pumped through a suspended standard phytoplankton net (35μ mesh) which was repeatedly back-washed with a sharp spray of sea water. The organisms caught were largely dinoflagellates, but no determination was made of what sort of organisms escaped or how much of the cell content was lost from the organisms that were caught. The zooplankton samples largely consisted of small copepods and euphausiids and were the only samples preserved in formalin. The wet weight given in Table 2 for plankton samples is the weight obtained after draining through Whatman No. 1 filters. The green algae were taken from rocks at the intertidal zone and the kelp specimens from coastal waters. For the mussel sample only the soft parts were used, and for the fish sample only the edible portion and the bones were analysed. Thus, the wet weight describes the size of the sample only roughly since it depends on the treatment given to the specimen.

Fig. 2 is an α-spectrum of 760 g of palm kelp and shows the much higher level of plutonium-239. The small peak in the region of 5·5 MeV is due to plutonium-238 and corresponds to an activity of 0·015 ± 0·005 μμc./kg. This is an example of how the concentrating property of organisms can be used to detect isotopes which could not be found in the direct analyses of sea water. The ratio of the peaks in Fig. 2, after correction for background, gives the isotopic ratio of plutonium-239 to plutonium-238 as 30 : 1 in the kelp. Assuming this same isotopic ratio for sea water, and by using the average of the coastal sea water samples for the concentration of plutonium-239, the concentration of plutonium-238 in coastal sea water is found to be 1·3 × 10^{-5} μμc./l. Traces of plutonium-238 were also evident in mussels, and quantitative measurement of this isotope should be possible in all organisms

Table 2. PLUTONIUM ACTIVITY IN MARINE ORGANISMS

Sample	Date of sampling	Wet weight (g)	Counting time (h)	Total ^{239}Pu activity (d.p.m.)	^{239}Pu ($\mu\mu$c./kg)	Concentration factor
'Red tide' (dinoflagellates)	May 1964	127	95	0·078 ± 0·010	0·276 ± 0·037	660
Green alga (*Enteromorpha* spp.)	April 1964	218	112·75	0·316 ± 0·021	0·653 ± 0·042	1,570
Giant kelp (*Macrocystis pyrifera*)	April 1964	740	47·0	0·528 ± 0·041	0·321 ± 0·025	770
Palm kelp (*Eisenia arborea*)	April 1964	760	94·0	0·759 ± 0·038	0·450 ± 0·023	1,080
Plankton (mixed coastal zooplankton)	January 1950	71	122·0	0·0036 ± 0·0047	0·023 ± 0·030	—
Plankton (mixed coastal zooplankton)	March 1964	48	88·0	0·114 ± 0·015	1·08 ± 0·14	2,590
Mussels (*Mytilus californianus*)	January 1964	1,000	122·0	0·214 ± 0·016	0·096 ± 0·007	230
Mussels (*Mytilus californianus*)	April 1964	528	98·5	0·140 ± 0·014	0·120 ± 0·012	290
Fish (bonito) (*Sarda lineolata*)	May 1964	1,185	119·5	0·0037 ± 0·0048	0·0014 ± 0·0018	3

which have high concentration factors by analysing larger quantities of these samples. Thus, while this work was principally concerned with measurement of plutonium-239 it is clear that plutonium-238 can also be detected by a suitable choice of concentrating organism.

It is of some interest to compare our results in sea water with fall-out measurements reported for land. Plutonium-239 fall-out has been measured at Richmond, California (38° N.), from 1960 until the middle of 1963[14]. Using this report, about 0·25 mc./km^2 of plutonium-239 fall-out was estimated to have accumulated at Richmond as of January 1, 1964. Assuming this amount fell in the ocean and mixed uniformly to 100 metres, a concentration of about 25×10^{-4} $\mu\mu$c./l. would be expected. This concentration is of the same order of magnitude as that indicated by our open ocean-water measurement. Further, the plutonium-239/plutonium-238 ratio of 30 : 1 found in kelp is of the same order as the average ratio reported for fall-out monitored in the United States for 1961–63.

The immediate aim of this work was to show that plutonium could be measured in the marine environment. This has been accomplished, and, in addition, a modest beginning has been made toward establishing contemporary concentration levels of plutonium-239 in marine organisms. In certain marine organisms plutonium-238 as well as plutonium-239 have been measured. Future investigation, using the techniques outlined here, could be directed to the problem of the distribution of plutonium in the oceans.

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ANALYSES OF RADIONUCLIDES OF BIOLOGICAL INTEREST IN PACIFIC WATERS

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INVESTIGATIONS of the distribution of radionuclides in marine environments have been made by the Laboratory of Radiation Biology, University of Washington, since 1946. These investigations have included radiochemical analyses of marine samples from the western and central Pacific, the north-eastern Pacific and the Arctic Ocean (Chukchi Sea). The analyses discussed here are those of samples collected recently in the Pacific Ocean. One group of samples was collected in the vicinity of Christmas Island during the 1962 tests of nuclear devices by the United States; another group was taken from near the mouth of the Columbia River where the effluent, carrying radioactive wastes from the Hanford reactors, mixes with the Pacific Ocean; and one sample was taken 250 miles off the Oregon coast.

Radionuclides from both natural and artificial sources occur in these waters. The most abundant and easily detected naturally occurring radionuclide is potassium-40. Other natural radionuclides to be expected in sea-water are radium, uranium, thorium and their decay products. Most of the artificial radionuclides generally present in the oceans are added as fall-out from nuclear detonations. The fall-out nuclides of biological interest include the fission products: strontium-89, 90, zirconium-95–niobium-95, ruthenium-103, 106, cæsium-137 and cerium-141, 144; and the induced radionuclides: manganese-54, iron-55, 59, cobalt-57, 58, 60 and zinc-65. The induced radionuclides phosphorus-32, chromium-51 and zinc-65, carried to the sea by the Columbia River, are produced by neutron activation of elements in the water used to cool the Hanford reactors.

Radionuclides in sea-water may be concentrated many thousand-fold by plankton. However, plankton constitutes only a minute fraction of the total water mass, hence the amount of radioactivity in the total water mass is greater than in the total plankton.

The concentration of radioactivity by plankton provides a convenient method for determining the presence, but not the amounts, of radionuclides in sea-water. A quantitative measurement can be made only by water analysis. The measurement of radionuclides in sea-water is difficult because of the necessity of separating and concentrating atom quantities of the nuclides from gram quantities of sea salts. β-emitting radionuclides, such as phosphorus-32 and strontium-90, require complete separation of the nuclides from the salts to reduce the absorption of β-particles by the sample. Photons from γ-emitting radionuclides, such as chromium-51, zinc-65 and zirconium-95–niobium-95, are not absorbed in the sample; but the amount of salt in a sample large enough to contain a measurable quantity of a radionuclide usually is so great that the sample geometry is reduced to an unacceptable value. Therefore, radionuclides for γ-spectrum analysis also are separated from the salt.

Our purpose here is to discuss methods used at this Laboratory for the analyses of radionuclides in sea-water and to present the results of these analyses. The methods and results of analyses of Christmas Island samples have been reported by Welander and Palumbo[1], and those of the Columbia River samples are reported by Seymour and Lewis[2]; however, they are reviewed here to present a summary of the Laboratory's methods and results. In addition, values for strontium-90, as determined by other investigators, have been included to indicate the world-wide distribution of this radionuclide in the surface waters of the oceans.

Collection and preparation procedures. Surface sea-water samples were collected from three areas in the Pacific Ocean: in the vicinity of Christmas Island (approximately 2° S.–2° N. latitude and 153° W.–168° W. longitude); within 20 miles of the Columbia River (approximately 46° N. and 124° W.); and 250 miles off the Oregon Coast (approximately 45° N. and 130° W.).

The Christmas Island samples were collected during the summer of 1962 and the samples taken near the Columbia River were collected during January 1961–June 1962. 10 l. of sea-water were filtered using 47-mm *HA*-type 'Millipore' filters with 0·45μ pore size. After filtering and chemical separation, the samples were analysed for γ-emitting radionuclides. The Christmas Island samples were analysed also for strontium-90.

On March 20, 1963, a 100-l. sample was collected off the Oregon coast. This sample was divided into 50-l. aliquots which were analysed by different methods for strontium-90. The particulate matter was separated from the water by centrifugation, rather than by filtration, using an *SS*-4 Servall enclosed superspeed centrifuge with the Szent-Györgyi continuous-flow system. At 17,000 r.p.m., the flow-rate was 200 ml./min. This system was about 30 per cent as effective as the 'Millipore' filter in removing the particulate matter.

Chemical separation. A hydroxide precipitation method was used for the samples taken from near the Columbia River mouth, and a sulphide precipitation method adapted from Sood, Golden and Velten[3] was used for the Christmas Island samples. The sulphide method was preferred because it proved to be simpler and equally reliable.

Hydroxide precipitation method. Carriers of manganese, cobalt, iron, zinc and chromium (200 mg each) in their chloride form were added to 10 l. of filtered sea-water sample. Under constant stirring, a predetermined amount of concentrated ammonium hydroxide was added drop-wise and the precipitate was allowed to settle for 15 min. Then a few more drops of ammonium hydroxide were added to check the completeness of the precipitation. The amount of concentrated ammonium hydroxide required for complete precipitation of a 10-l. sample varied with the salinity according to the equation:

$$V = 100 + 3{\cdot}7S$$

where V is the volume of reagent in ml. and S is the salinity of the sample in parts per thousand. This relationship is based on observations in the range of 10–32 per cent. The precipitate was allowed to settle overnight or longer, after which time most of the clear supernate liquid was siphoned off. The remaining mixture was filtered through a Whatman-42 filter paper under vacuum. The precipitate was wet-ashed with concentrated nitric acid and 30 per cent hydrogen peroxide, dried in a 250-ml. 'Pyrex' beaker and counted. The elements present in greatest quantity in sea-water remain in the supernate. This fraction also was dried and counted. The chemical yields by the hydroxide precipitation method for manganese-54, cobalt-60, iron-59, zinc-65 and chromium-51 ranged from 89 to 100 per cent.

Sulphide precipitation method. Carriers of lanthanum, iron, cobalt, zinc, nickel, zirconium, ruthenium, cerium, cæsium and strontium were added to 'Millipore'-filtered sea-water samples, with cæsium and strontium serving as hold-back carriers. The sample was acidified to dissolve any suspended matter, heated to 80° C and the *p*H then adjusted to 11 with ammonium hydroxide. Hydrogen sulphide was bubbled slowly into the solution under constant stirring. After the solution had been saturated with hydrogen sulphide, it was taken off the heat and allowed to settle overnight. After separation from the supernate by filtering, the precipitate, containing ruthenium, cerium, cobalt, chromium, zinc, iron, zirconium and lanthanum, was placed in an eight-ounce plastic jar and counted. The percentage chemical yields by the sulphide precipitation method for zinc-65, cobalt-60, zirconium-95–niobium-95, cerium-144 and ruthenium-103 tags were in agreement with those of Sood *et al.*[3] and ranged from 90 to 97 per cent. Strontium was retained in the supernate, as evidenced by less than 1 per cent strontium-85 activity in the precipitate. As in the hydroxide method, potassium and cæsium remained in the supernate with the bulk of the sea salts and no further chemical separation was made.

Strontium-90. The supernate from the foregoing procedure was acidified, hydrogen sulphide was driven off by heat and the *p*H was adjusted to 5 with ammonium hydroxide and acetic acid. Strontium was co-precipitated with calcium as the oxalate using ammonium oxalate. The precipitate, after filtering, was wet-ashed with nitric acid and hydrogen peroxide. The ash residue was re-precipitated in 80 per cent nitric acid to reduce the calcium–strontium ratio. The strontium nitrate thus obtained was dissolved in 0·2 N hydrochloric acid and the resulting solution was passed on to a 'Dowex 50*W X*-8' (100–200 mesh) ion-exchange resin column. After rinsing with 0·2 N hydrochloric acid to remove any unadsorbed contaminants, 0·5 per cent oxalic acid was added to remove radioiron. The column was stripped off yttrium-90 as yttrium citrate complex with 5 per cent ammonium citrate at *p*H 3·5. Storage time for 2 weeks was allowed for the secular equilibrium between strontium-90 and yttrium-90 to be established. The column then was re-eluted with a minimum volume of ammonium citrate, the eluate acidified to break up the yttrium citrate complex and the resulting solution was passed on to a short-stemmed eyedropper column containing a 2-cm resin bed of 'Dowex 50*W X*-8' (100–200 mesh). When the yttrium-90 was adsorbed to the resin bed the citrate mass was removed with dilute acid (0·2 N nitric acid) wash. The yttrium-90 was stripped off the column with about 5 ml. of 6 N nitric acid on to a stainless steel planchet, dried and the relatively mass-free residue counted. This strontium-90 method was adapted from a procedure developed by Kawabata and Held[4].

Strontium-90 was determined in the sea-water from off the Oregon coast by this method and also by the method of Miyake, Saruhashi and Katsuragi[5]. In the latter method an oxalate precipitation of calcium and strontium is used, followed by EDTA-masking of calcium and recovery of yttrium-90 as yttrium hydroxide after secular equilibrium is reached.

Phosphorus-32. The method of extracting phosphorus-32 from sea-water was suggested by the work of Proctor and Hood[6] and Murphy and Riley[7]. Sea-water was placed in a separatory funnel and allowed to react with a composite reagent containing ammonium molybdate, sulphuric acid, ascorbic acid and potassium antimony tartrate[7]. Seven parts of *N*-butanol were added to 150 parts of sample, and the solution was made more acidic to favour extraction of the molybdenum blue. After extraction the organic layer was backwashed with dilute sulphuric acid and dried under low heat. The residue was dissolved in a minimum amount of aqua regia and again evaporated to dryness. The residue was dissolved in 50 ml. of 15 per cent hydrogen peroxide and made up to 2 N in hydrochloric acid. This mixture was added to a separatory funnel and the molybdate extracted with an equal volume of tri-butyl phosphate. The organic layer was discarded and the aqueous layer extracted again with an equal volume of tri-butyl phosphate. The aqueous layer was then dried on a steel planchet and counted for phosphorus-32. (A more complete description of the method will be reported by Chakravarti, Tuell and Larson[8].)

Counting systems. The γ-counting system included a 3-in. solid crystal of sodium iodide, thallium activated, used in conjunction with a 512-Nuclear Data 120 analyser with an *X–Y* recorder and a digital print-out. Calculations were carried out on an IBM 709 digital computer programmed to analyse for nine isotopes and to express results in picocuries (μμc.) per g or per l. at time of collection. Only values which were greater than the background plus the 95 per cent counting error were considered significant; all other values were considered to be zero.

Two types of low-background Geiger-Müller counters were used, one for yttrium-90, another for phosphorus-32. The systems included sufficient massive shielding and cosmic-ray guard tubes to reduce the background to approximately 1·5 and 1·0 counts per min, respectively. The counting efficiencies were approximately 25 per cent for yttrium-90 and 40 per cent for phosphorus-32. All counts were standardized and the results are expressed in picocuries per litre (pc./l.) at the time of collection.

Table 1. RADIONUCLIDES IN FILTERED SURFACE SEA-WATER FROM TWO AREAS OF THE PACIFIC OCEAN (VALUES IN pc./l.)

Radio-nuclide	Mouth of Columbia River *N′/n*†	Average	Maximum	Christmas Island* *N′/n*†	Average	Maximum
	January–September 1961					
^{51}Cr	10/25	150	770			
^{65}Zn	15/25	21	350			
	November 1961–June 1962			May–July 1962		
^{51}Cr	7/9	580	1,300			
^{65}Zn	8/9	5·1	12	4/24	1·3	13
^{95}Zr–^{95}Nb	7/9	4·5	16	11/24	16	71
^{103}Ru	5/9	0·79	2·5	4/24	3·7	32
^{141,144}Ce	9/9	9·4	27	13/24	10	46
^{137}Cs				3/24	0·62	11
^{60}Co				2/24	0·38	7·0

* From ref. 1.

† *N′/n*, Number of samples with radioactivity greater than background plus the 95 per cent counting error/No. of samples analysed.

The average and maximum values of radionuclides, except those of strontium-90, potassium-40 and phosphorus-32, in sea-water samples are given in Table 1. This Table is a summary of the results of analyses of samples collected at various times during the period indicated. The appearance of mixed fission products after September 1961 in the samples from the mouth of the Columbia River is the result of fall-out, presumably from the nuclear tests by the U.S.S.R. in October 1961.

Chromium-51, produced in the Hanford reactors, was the most abundant radionuclide in the coastal waters near the mouth of the Columbia River, where it is of interest to the oceanographer as a tag of Columbia River water and sediments. Chromium-51 was neither expected nor detected by visual inspection of the γ-spectra of the Christmas Island water samples.

The two values for strontium-90 from the single sample of sea-water from off the Oregon coast analysed by both the method of Miyake, Saruhashi and Katsuragi[9] and the modified Kawabata–Held method[4] described here were 0·037 ± 0·003 (95 per cent counting error) and 0·041 ± 0·009 pc./l., respectively. The latter was the faster of the two methods and produced a sample of lesser mass. The reduction of mass was from several grams to a few milligrams. The values for strontium-90 in surface sea-water from off the Oregon coast and from other areas round the world ranged from 0·01 to 3·1 pc./l. and are presented in Table 2. This radionuclide seems to be ubiquitous in surface waters since no zero values are given in these reports.

Table 2. STRONTIUM-90 IN SURFACE SEA-WATER (VALUES IN pc./l.)

Ref.	Location and dates	No. of samples analysed	Range of values
—	250 miles off Oregon coast, January 1962	1	0·04
1	Christmas Island, January 1962	5	0·03–0·2
10	Atlantic Ocean and Carribbean Sea, 1956–60	60	0·031–0·30
11	Chukchi Sea, 1959–62	7	0·005–0·27
12	Pacific and Indian Oceans, 1960–61		
	0°–40° N	25	0·1–0·9
	0°–40° S	6	0·06–0·1
	60° S (Antarctic)	2	0·01–0·02
9	Western North Pacific Ocean, 1958–60	15	0·6–3·1

Table 3. PHOSPHORUS-32 IN FILTERED SURFACE SEA-WATER FROM NEAR THE MOUTH OF THE COLUMBIA RIVER, JULY 1963 (VALUES IN pc./l. ± S.D.)

Station	Miles from mouth	Salinity (parts per thousand)	No. of determinations	^{32}P
63	30	31·76	3	3·57 ± 0·60
69	24	31·63	1	5·15
74	18	27·80	3	8·03 ± 0·58
75	9	27·74	3	4·68 ± 1·63

Values for phosphorus-32 in the sea-water near the Columbia River mouth are given in Table 3. These values ranged from 3·6 to 8·0 pc./l. To our knowledge no other values for phosphorus-32 in sea-water have been reported.

In order to compare the radioactivity from naturally occurring and artificial radionuclides in sea-water, the values for potassium-40 were determined as well as the radionuclides discussed here. For samples collected near the mouth of the Columbia River, these values ranged from 190 to 370 pc./l. and were related to the salinity values. The calculated value for potassium-40 in sea-water of 35 parts per thousand salinity is 330 pc./l.

This work was carried out under contract *AT* (45–1) 1385 with the U.S. Atomic Energy Commission.

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REORGANIZATION OF CIVIL SCIENCE IN BRITAIN

In the House of Commons on July 28, Sir Harry Legge-Bourne asked the Secretary of State for Education and Science if he would make a further statement on the measures to be taken in consequence of the Trend Report on the organization of civil science. Mr. Quintin Hogg replied that, as foreshadowed in the statement issued by the Prime Minister on February 6, it was proposed to implement the recommendations of the Report with some modification. These changes would necessitate legislation which was now being prepared for introduction at an early opportunity. This legislation would include provision for the increased responsibilities of the Secretary of State.

The present Advisory Council for Scientific Policy will be replaced by a body on the lines recommended by the Committee, to be called the Council for Scientific Policy. The Council will advise the Secretary of State in the formulation and execution of general scientific policy. Specifically, the Council will advise the Minister on national scientific needs as a whole, including the fostering of new growing points; on the broad allocation of resources; on scientific man-power; on international scientific policy; and on the administrative machinery for the promotion of scientific research. As recommended by the Committee, the Council will consist entirely of independent members. Assessors appointed by interested bodies, including the Board of Trade, the Ministry of Aviation, the Atomic Energy Authority, the University Grants Committee and the Research Councils, will see the papers and attend by invitation. The Council will have its own staff and secretariat which will form part of the Department of Education and Science.

It also is proposed to form three new bodies as follows:

(1) *The Science Research Council.* The Council will take over from the Department of Scientific and Industrial Research the function of supporting scientific and technological research at universities through ear-marked grants in fields other than those for which other Research Councils are responsible. It will also take over the Department's function of making training awards to postgraduate students in science and technology, other than those who are supported by the more specialized Research Councils. The Science Research Council will take over responsibility for the National Institute for Research in Nuclear Science and the Royal Observatories at Herstmonceux and Edinburgh. It will also assume responsibility for the scientific space programme and, in particular, the work of the Space Research Management Unit of the Department of Education and Science and the Department of Scientific and Industrial Research Radio Research Station. The Ministry of Aviation and the University Grants Committee will be represented on the Council by assessors.

(2) *The Natural Environment Research Council.* This Council is to be formed, following the recommendation of the Committee on the Organization of Research into Natural Resources and the Committee on the Organization of Civil Science, to support research in the field of environmental sciences and natural resources. Specifically, the Council will support research in geophysics, geology, oceanography, fisheries, hydrology, forestry, terrestrial ecology and nature conservation. It will provide research grants to universities and make training awards to postgraduate students in these fields. The Council will assume responsibility for: (*a*) the Geological Survey and Museum; (*b*) the Soil Surveys; (*c*) the Nature Conservancy; (*d*) the Hydrology Research Unit and the Committee on Hydrological Research.

The Council will finance and co-ordinate the work of these organizations. It will take over from the Navy Department and the Development Commission responsibility for supporting the National Institute of Oceanography. The Council will establish a Fisheries Advisory Committee on the same lines as the present Advisory Committee on Fishery Research of the Development Commission. On hydrology, the Council will maintain close contact with the Water Resources Board, which also has research functions. It will support research in geomagnetism and seismology undertaken by the Meteorological Office and will be associated with its general programme of research. Under these arrangements, the Council will be in a position to foster and co-ordinate

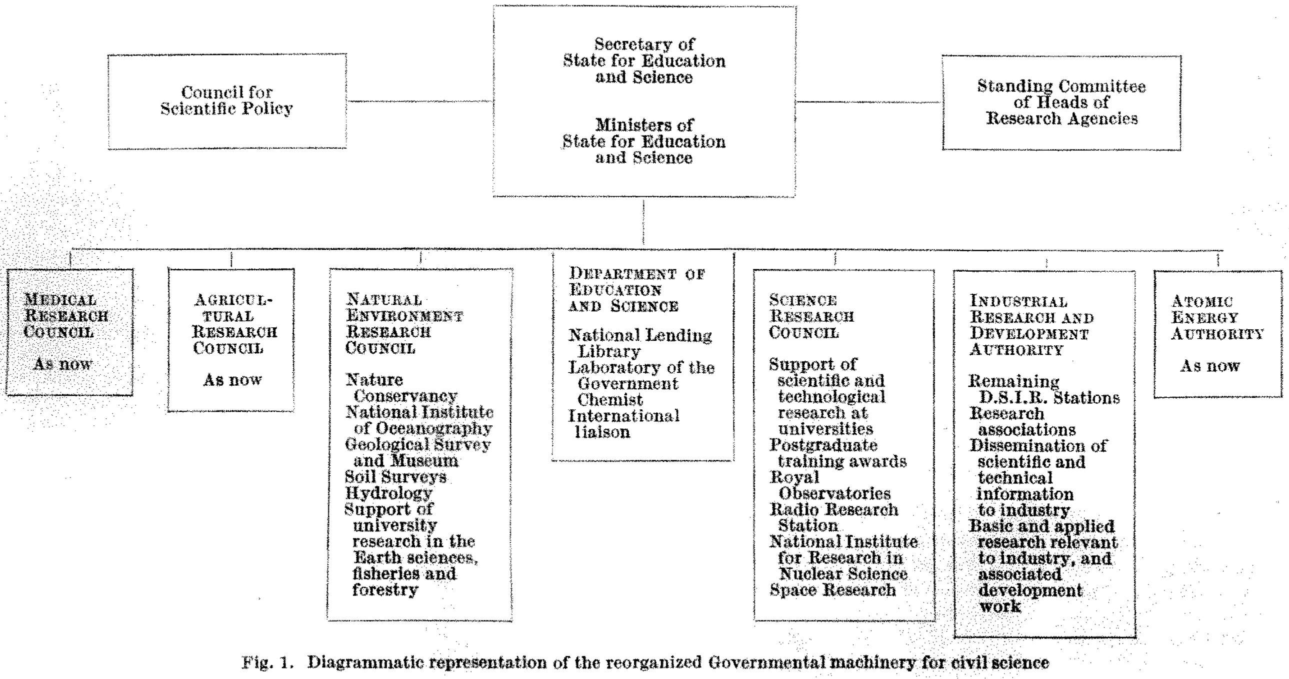
Council for Scientific Policy
Secretary of State for Education and Science
Ministers of State for Education and Science
Standing Committee of Heads of Research Agencies
Medical Research Council
As now
Agricultural Research Council
As now
Natural Environment Research Council
Nature Conservancy
National Institute of Oceanography
Geological Survey and Museum
Soil Surveys
Hydrology
Support of university research in the Earth sciences, fisheries and forestry
Department of Education and Science
National Lending Library
Laboratory of the Government Chemist
International liaison
Science Research Council
Support of scientific and technological research at universities
Postgraduate training awards
Royal Observatories
Radio Research Station
National Institute for Research in Nuclear Science
Space Research
Industrial Research and Development Authority
Remaining D.S.I.R. Stations
Research associations
Dissemination of scientific and technical information to industry
Basic and applied research relevant to industry, and associated development work
Atomic Energy Authority
As now

Fig. 1. Diagrammatic representation of the reorganized Governmental machinery for civil science

investigations associated with the planetary environment of the Earth, air and water.

(3) *The Industrial Research and Development Authority.* This body will be formed to continue and extend the work hitherto carried on by the Department of Scientific and Industrial Research in relation to industrial research and development. It will carry out research in its own establishments; support co-operative industrial research associations; encourage and support advanced development work arising out of these activities and disseminate scientific and technical information to industry. In development contract work, it will act in consultation with the National Research Development Corporation and use it as its agent when this is appropriate. It will not be concerned solely with applied research and development. It will be an important part of its functions to support basic research which may have important implications for industry. The Authority will consist of a full-time chairman and part-time members from industry, science and technology. It will also include representatives from the National Research Development Corporation, the Board of Trade, the Ministry of Aviation and the National Economic Development Council.

The Authority will take over the responsibility for all stations hitherto belonging to the Department of Scientific and Industrial Research except the Radio Research Station, the Geological Survey and Museum, and the Hydrology Research Unit, and, as mentioned later, the Laboratory of the Government Chemist. The future position of the National Physical Laboratory is still under consideration. It is, however, intended that a close link with the Royal Society of London will be maintained.

There will be no change in the present functions of the Medical Research Council and the Agricultural Research Council.

The Department of Education and Science will assume responsibility for the National Lending Library for Science and Technology and for the Laboratory of the Government Chemist.

Important questions arise about the position of staff which will be transferred, under these proposals, to the Industrial Research and Development Authority and to the new Research Councils from the Department of Scientific and Industrial Research and other organizations. These questions are under consideration. There will be full consultation with the appropriate staff organizations before decisions are taken on these matters.

As recommended by the Committee on the Organization of Civil Science, the Overseas Research Council will be dissolved. Its functions have now passed in the main to the Department of Technical Co-operation and its advisory bodies.

Under these measures of reorganization, all the functions of the Department of Scientific and Industrial Research will pass to the new research agencies referred to here, and to a minor extent, to the Department of Education and Science. They will thus bring to an end the Department of Scientific and Industrial Research in its present form, and create a wider group of research organizations, operating under the general supervision of the responsible Minister, acting with appropriate advice. It will be necessary for the research agencies to work closely together in certain fields of scientific work and marginal adjustments in their functions may be necessary from time to time. It is the Government's intention that these arrangements should be kept under review, with the advice of the Council for Scientific Policy, in order to meet the needs of developments in science and technology. The Government considers that these measures of reorganization, which are being undertaken after a full study of the whole field by the Trend Committee, will provide both a substantial measure of autonomy for the Research Councils, and also the means of developing a coherent national scientific policy. In particular, it believes that these measures will enable the distinguished work of the Department of Scientific and Industrial Research during the past fifty years to be developed and extended on an expanding scale, and with increasingly rewarding results.

NEWS and VIEWS

Director of the Microbiological Research Establishment, Porton: Dr. D. W. W. Henderson, C.B., F.R.S.

Dr. D. W. W. Henderson was appointed director of the Microbiological Research Establishment at Porton in 1946, at which time the Establishment was housed in cramped huts associated with the Chemical Defence Establishment. Plans for a new building were, however, under discussion. The first few years of Dr. Henderson's directorship were largely devoted to completing these plans and seeing the building built and equipped—a period of hard work and struggles with authority in which the new director received great help and support from the late Lord Hankey. The magnificent laboratories that now house the Microbiological Research Establishment are a standing tribute to the foresight and acumen of Dr. Henderson, Lord Hankey and the band of distinguished microbiologists who stood behind the enterprise. Dr. Henderson had worked at Porton during the Second World War as a seconded member of the scientific staff of the Lister Institute, which he had joined in 1935. The Lister Institute has always been a leading centre of microbiological research; the work of Sir Paul Fildes and of Dr. Muriel Robertson, from the days of the First World War onwards, was pre-eminent: it was fitting that the director of the Microbiological Research Establishment should be chosen from the Lister Institute.

Before 1935, Dr. Henderson had been a lecturer in bacteriology in the University of Durham (1926–31) and had held Carnegie (1931–32) and Beit Memorial (1932–35) research fellowships. In 1946 he was awarded the Medal of Freedom, Bronze Palm, of the United States. He was made a Commander of the Bath in 1957, and elected Fellow of the Royal Society in 1959. He became president of the Society for General Microbiology in 1963. In spite of the many and exacting tasks that fall to the director of such an establishment as the Microbiological Research Establishment, Dr. Henderson has maintained a steady programme of his own research, ranging over various immunological and bacteriological topics. He has been a most successful stimulator of the younger people of his staff. Under his direction, the Establishment has developed into one of the leading centres of microbiological research in the world. Dr. Henderson retired from the directorship on August 1. However, he will continue to undertake research at the Establishment.

Dr. C. E. G. Smith

Dr. C. E. G. Smith has been appointed director of the Microbiological Research Establishment, at Porton, Wiltshire, in succession to Dr. D. W. W. Henderson, as from August 1. Dr. Smith is an honours graduate of the University of St. Andrews. After holding appointments in the Cumberland Infirmary, Carlisle, from 1947 until 1948, he joined H.M. Colonial Medical Service in 1948. He then held various appointments in Malaya, and while at the Institute of Medical Research at Kuala Lumpur did research on virus diseases. In 1957 Dr. Smith was appointed senior lecturer in bacteriology at the London School of Hygiene and Tropical Medicine. In 1960 he became a member of the World Health Organization's study group on arthropod-borne viruses. Since 1961 Dr. Smith has been reader in virology in the University of London at the London School of Hygiene and Tropical Medicine. He is also head of the Arthropod-borne Virus Research Unit financed by the Department of Technical Co-operation.

Human Environment in the Institute of Archæology: Prof. G. W. Dimbleby

DR. G. W. DIMBLEBY, who has recently been appointed to the chair of human environment in the Institute of Archæology, London, took an honours degree in botany at Oxford in 1939. He later worked on soil problems—particularly on oxidation-reduction potentials as measures of soil activity. During the Second World War, he was in the Royal Air Force and applied his powers of observation and his ecological training to the interpretation of air photographs and was largely instrumental in developing methods for the detection of decoy sites in enemy-occupied territory designed to lead Allied bombers astray. After the War, Dr. Dimbleby was appointed as ecologist in the Forestry Department of the University of Oxford. His early work on heathlands was concerned with the natural succession back to deciduous woodland if the heaths were protected from fire. He found that this was accompanied by progressive regeneration of the soil which implied that the poor state of the soil under heathland did not reflect its full potential. To find out what this might have been, Dr. Dimbleby set out to investigate soils buried beneath earthworks of different ages, using pollen analysis and comparative soil typology as his main tools. It became apparent that the process of deterioration had its origin in the initial forest clearance by Bronze-Age (or even earlier) man, and that it was not determined purely by climate or parent material. The original forest was a mixed deciduous one on a brown forest soil, but traces of podzolization appeared during the farming phase which followed clearance and the process of degradation has continued ever since. On the face of it, it may seem a far cry from forestry to archæology, but Dr. Dimbleby's botanical and pedological investigations have revealed such a close connexion between the activities of early man and the state of the land to-day (and therefore its use) that his work has been fundamental to both disciplines.

Deputy Director of Mullard Research Laboratories: Dr. K. Hoselitz

DR. K. HOSELITZ has been appointed deputy director of Mullard Research Laboratories. He will continue as head of the Laboratories' Solid State Physics Division. Dr. Hoselitz was educated at the Universities of Vienna and Bristol. He gained his Ph.D. at Bristol in 1942 and afterwards joined the Permanent Magnet Association as a research scientist. He was promoted director of the Association's Central Research Laboratories in 1947. In 1952 he joined Mullard Research Laboratories to establish the Solid State Physics Division. The Division has since grown to a strength of more than 100 and has made notable contributions in the fields of semiconductors, magnetic materials and low-temperature physics. Dr. Hoselitz, whose special subjects are magnetism and the properties of metals and alloys, is the author of a textbook on those subjects and has contributed to several other books. Mullard Research Laboratories are near Redhill, Surrey, and employ about 700 people, of whom more than 200 are graduate scientists.

Post Office Research Station

IT has been announced that the Post Office Research Station at Dollis Hill is to be moved to the vicinity of Martlesham, in Suffolk. The aim is to start the move about 1968. The Research Station, which employs some 1,400 staff, has occupied its present site at Dollis Hill, London, since 1912. It has been recognized for some time that the Station would have to be moved because the present site is severely congested and there is not enough space to allow for development. The area chosen is less than 2-h travelling time from London, and is near the University of Essex.

Agricultural Research Council Unit of Nitrogen Fixation

THE Agricultural Research Council has announced, in consultation with the authorities of Queen Mary College, University of London, that its Unit of Nitrogen Fixation will be leaving the College owing to difficulty of accommodation there. It is being transferred to the University of Sussex, where new laboratories will be erected to accommodate it. The Unit will continue to be directed by Prof. J. Chatt, who is resigning the chair of inorganic chemistry at Queen Mary College. The Unit was established to investigate the mechanism of nitrogen fixation. Dr. J. R. Postgate, formerly of the Microbiological Research Station, Porton, is in charge of the biological aspects of the research.

Steroid Reference Collection

A Steroid Reference Collection, established by the Medical Research Council, on the advice of their Clinical Endocrinology Committee and with the aid of generous gifts of steroids from the pharmaceutical industry, can supply to laboratories working on steroid metabolism, small quantities (about 2 mg) of reference steroids. These steroids, which are mainly hormone metabolites, are intended as standards for such procedures as paper chromatography, colour reactions, infra-red measurements and mixed melting-point determinations; larger quantities (5–20 mg) can, on occasion, be supplied for studies of new analytical procedures. Applications for samples, with a brief statement of the purpose for which they are required, should be sent to Prof. W. Klyne, Chemistry Department, Westfield College, London, N.W.3, from whom a list of available compounds can also be obtained.

Water Pollution Research

ANYONE revolted by sewage solids on beaches will welcome the investigations described in the recent report of the Department of Scientific and Industrial Research Water Pollution Research Board (*Water Pollution Research, 1963*. Pp. vi + 142 + 4 plates. London: H.M.S.O., 1964. 8*s*. 6*d*. net). The results indicate that maceration and screening can prevent visual offence without increasing the concentration of coliform bacteria in the sea. It should be possible to site discharges so that the concentrations of coliform bacteria (and hence pathogens) encountered by bathers signify a negligible risk of infection. Better treatment of industrial and domestic wastes is an important part of the work of the Laboratory, leading to cleaner rivers. In particular, the analysis made of the conditions favouring the oxidation of ammonia to nitrate in the activated sludge process should enable more works to attain consistent nitrification. Work on rivers continues to concentrate on the toxicity of various effluent materials and the oxygen balance. Investigations of individual toxins enable predictions to be made of the toxicities of mixtures of toxins. These agree well with experimental observations and can be used to show the dilution needed for an effluent, or if the removal of one toxic factor would allow fish to inhabit the river. It is important for all aquatic biologists to note that a significant source of error in the standard Winkler procedures for oxygen has been found, which means that the oxygen solubility values in a number of recent publications are incorrect.

Zoology in South-west Africa

Cimbebasia, issued by the State Museum, Windhoek, South-west Africa, records present zoological research in that country. No. 8, December 1963, is an interesting account by R. F. Lawrence of the Solifugae of South-west Africa, a country that seems to offer a particularly favourable ecology to these creatures, for no less than 10 per cent of the 800 known species are found there. Although the paper is primarily systematic, the preliminary sections on the biology of these peculiar arachnids is of special value. *Cimbebasia* (No. 6; November 1963) contains four papers on various reptiles and one each on mites and diatoms, mostly by members of the Museum's staff.

Boliland Region of Sierra Leone

A 'BOLI' is the term for a flat treeless grassland that is flooded during the rainy season, and a survey of the bolilands was called for in order to delineate areas suitable for rice cultivation. The work was carried out under the Colonial Development and Welfare Scheme, and the results are described by A. R. Stobbs in *The Soils and Geography of the Boliland Region of Sierra Leone* (Pp. 45 + 12 plates. Freetown, Sierra Leone: Government Bookshop, London; Crown Agents for Overseas Governments and Administrations, 1963. 28*s*.). The area concerned consists of a strip about 140 miles long and 25 miles wide, with an annual rainfall of up to 130 in., mainly in a six-month period. The soils of the undulating upland are derived from sandstones and sandy shales; mudstones and shales, weathering into puddled clay subsoils, underlie most of the bolis, which are poor in mineral nutrients and have not attracted settlement as have the more fertile adjacent soils. The mapping of the soils was based on a geomorphological interpretation of aerial photographs, extended by the identification of soil series, which is a method of great value in a large area with poor communications but with uniform climate and geological pattern; six individual typical riverain and individual bolis were also mapped on a large scale to show the effects of variations in height on the flooding régime and to provide a basis for experimental work. The soils are classified, on the system that has already proved of value in West Africa, into the soil groups: oxysols, groundwater laterites, acid gleisols and alluviosols. Typical profiles are described and analytical results are appended, and comparisons are made for planning future development. There is a preliminary description of the existing plant communities by T. S. Bakshi. The land use in the uplands is based on shifting cultivation with bush fallow; there is a wide range of crops, but it is just subsistence farming and there is room for considerable improvement. In the flood plains, water control would enhance the possibility of swamp rice and dry-season pasture.

Witchcraft and Sorcery in Oceania

IN his presidential address to the Anthropology Section of the Australian and New Zealand Association for the Advancement of Science, Prof. M. G. Marwick, of Monash University, examined some aspects of the sociology of witchcraft and sorcery (*Australian Journal of Science*, **26**, No. 9; 1964). In Oceania, one of the well-founded propositions of the sociology of sorcery and witchcraft does not seem to apply. Elsewhere, especially in Africa, it has been repeatedly recorded that both believed attacks and accusations of witchcraft and sorcery occur only between persons already linked by close social bonds, but in Oceania it is more often reported that the sorcerer, who seems to be commoner than the witch, is believed to direct his destructive magic outside his own group. A corollary to the generalization supported by the African material is that the relationship between alleged witch and sorcerer and believed victim is not only close but also strained; and this fact gives a means of detecting the tension-points of a social structure by the frequency with which attacks of witchcraft and sorcery are believed to occur between persons standing in various relationships. Prof. Marwick suggests that the person who carries out field-work on the sociology of Oceanian sorcery or witchcraft might bear the following points in mind. First, terms such as sorcery and witchcraft must be clearly defined and the differentiation between destructive magic socially approved and that socially condemned should be maintained; for the difference between them is, for clear reasons, material to sociological analysis. Secondly, the well-established principle of comparing the ideal with the real applies in this field of enquiry as much as in any other. Thirdly, beliefs in sorcery and witchcraft invariably have a social setting in a sense that they mediate, though they sometimes complicate, the living together of people in the on-going process we call a society. These considerations mean that, fourthly, the field-worker, in collecting his material, has to direct particular attention to the three central characters, the accuser, the alleged sorcerer or witch and the believed victim, and to the social relationships, the rivalries and the alliances between them. Finally, the ethnographer should again apply a well-established canon of field-work to his particular topic, that is, when he cannot observe the relationships believed to be involved in a particular instance of misfortune, and even when he can, he should not rely on only one informant for each case, but should record the interpretations of different persons differently placed in relation to the central characters.

Spiral Structure in the Andromeda Nebula

THE Great Nebula in Andromeda has played an important part in the development of our knowledge of galaxies. The late Dr. Walter Baade initiated many investigations of the Nebula which are now being completed by his colleagues. The results of an examination of the emission nebulæ in the Nebula have just been published (Baade, W., and Arp, H., *Astrophys. J.*, 139, 1027; 1964). At one time it had been thought that no gaseous emission nebulæ were present in the Andromeda Nebula. Baade doubted this, and accordingly photographed the Nebula with the 100-in. telescope at Mount Wilson, using various specially selected combinations of photographic plates and filters. Photographs taken in different wave-length regions were compared, and in all 688 emission nebulæ were catalogued. These objects at first appeared to outline the spiral structure very well, but later work has shown that their interpretation is not easy. Arp (*Astrophys. J.*, 139, 1045; 1964) has made a detailed discussion of the problem. The distribution of the brightest stars must also be considered. Direct photographs have shown that the best representation of the spiral structure is obtained if it is assumed that the Nebula is tilted at an angle of 16° to the line of sight. When attempts are made to fit the emission nebulæ to this picture difficulties arise. There does not seem to be any tilt angle which, when allowed for, will give a picture of the emission nebulæ looking like a continuous spiral curve. A much better fit is obtained if the angle of tilt is 11° to the line of sight. Dr. Arp suggests that the whole plane of the Nebula is warped, with a tilt of 16° for the side more distant from us, and for the central regions, and a tilt of 11° for the near-side regions. On this picture the emission nebulæ form a complete spiral. There is evidence that the warping is caused by the companion elliptical galaxy, *M* 32.

Research Scholarship in Environmental Engineering

APPLICATIONS are invited from graduates in engineering or physics who wish to undertake research on problems associated with the study and production of comfortable environmental conditions in buildings. The award is made by the Heating and Ventilating Contractors Association and has a value of £800 per annum. The award would be made, in the first instance, for a period of one academic year, but is renewable subject to satisfactory progress. Holders of the scholarship would be permitted to submit their work for a high degree where university regulations permit. Further information and a form of application can be obtained from the Secretary and Clerk to the Governors, National College for Heating, Ventilating, Refrigeration, and Fan Engineering, Borough Polytechnic, Borough Road, London, S.E.1.

Chelsea College of Science and Technology

In accordance with the decision of the University Grants Committee, the Governing Body of Chelsea College of Science and Technology, with the approval of the Academic Advisory Committee, has in anticipation of the grant of University Status to the College conferred the title of professor on the following heads of Departments: Dr. A. H. Beckett (pharmacy), Dr. S. E. Dicker (physiology and pharmacology), Dr. J. F. J. Dippy (chemistry), Dr. K. W. Keohane (physics), Mr. A. E. Ludlam (mathematics), Dr. R. D. Purchon (botany and zoology). In addition, the title of reader has been conferred on Dr. W. E. Smith (geology).

University News: East Anglia

Dr. D. C. Champeney, at present lecturer in physics in the University of Birmingham, has been appointed lecturer in physics.

London

Dr. Jack Tizard, of the Social Psychiatry Research Unit of the Medical Research Council at the Institute of Psychiatry, has been appointed to the chair of child development at the Institute of Education. The following have been appointed to readerships: Dr. P. W. M. Jacobs (physical chemistry at Imperial College of Science and Technology); Dr. K. B. Roberts (physiology at the London Hospital Medical College); Dr. A. M. James (physical chemistry at Queen Elizabeth College); Dr. M. H. Quenouille (statistics at the Imperial College of Science and Technology); Dr. E. A. Ash (electrical engineering at University College). The following titles have been conferred: professor of pædiatrics, on Dr. J. P. M. Tizard, in respect of his post at the Institute of Child Health; professor of social institutions, on Mr. O. R. McGregor, in respect of his post at Bedford College; reader in physics, on Dr. D. W. O. Heddle, Dr. C. Henderson and Dr. R. E. Jennings, in respect of their posts at University College; reader in biochemistry, on Dr. M. W. Neil, in respect of his post at the London Hospital Medical College; reader in human environment, on Dr. I. W. Cornwall, in respect of his post at the Institute of Archæology.

Southampton

Prof. G. M. Lilley, professor of fluid dynamics at the College of Aeronautics, Cranfield, has been appointed to the chair of aeronautics and astronautics. Mr. P. Hammond, Fellow and director of studies in electrical sciences in Pembroke College, Cambridge, has been appointed to the chair of electrical power engineering. Dr. H. B. Griffiths, senior lecturer in mathematics in the University of Birmingham, has been appointed to the second chair of pure mathematics. Mr. J. H. Smith, lecturer in social science in the London School of Economics, has been appointed to the chair of sociology. Prof. E. J. Richards, professor of aeronautics in the University, has been appointed to the new chair of applied acoustics and the directorship of the Institute of Sound and Vibration Research. The following appointments to lectureships have been made: Dr. R. A. East (aeronautics and astronautics); Dr. R. Baker, Dr. M. C. Flowers, Dr. N. B. H. Jonathan, Mr. J. M. Mellor and Dr. J. S. Wood (chemistry); Dr. M. E. Barton and Dr. B. S. Smith (civil engineering); Mr. J. D. E. Beynon (electronics); Mr. E. W. Haddon (mathematics); Dr. J. G. McEwen (physics); Dr. A. Crowe (physiology and biochemistry); Dr. T. Priede and Mr. P. L. Tanner (sound and vibration research); Miss D. M. Shepherd (sociology and social studies).

Announcements

Dr. Frank Dollin, chief turbine engineer, C. A. Parsons and Co., Ltd., has been awarded a Thomas Hawksley Gold Medal by the Institution of Mechanical Engineers, for his paper, "Some Design Problems Arising in the Development of Very Large High-speed Turbines". The award, established in 1908, commemorates the eminent engineer, Thomas Hawksley, who was president of the Institution in 1876 and 1877, and is given for the best paper published by the Institution in the year.

The Bulletin *Science East to West*, formerly known as *Russian Technical Literature*, issued by the Directorate for Scientific Affairs of the Organization for Economic Co-operation and Development, covers scientific and technical literature from East Europe, the U.S.S.R., and China and Japan. The January 1964 issue (No. 13) includes an article on the production and utilization of energy in the U.S.S.R., a list of recently published translations of books from Russian and details of courses available in the Russian language.

A discussion meeting on "The Future of Chemical Publications" will be held by the Chemical Society in the University of Nottingham during September 22–24. Further information can be obtained from the General Secretary, the Chemical Society, Burlington House, Piccadilly, London, W.1.

A symposium on "Hydroxycinnamic Acids and Related Compounds", arranged by the Phytochemical Group (formerly the Plant Phenolics Group), will be held at the University of Sheffield during September 22–23. Further information can be obtained from A. H. Williams, Research Station, Long Ashton, Bristol.

A scientific meeting of the Hospital Physicists' Association will be held, to celebrate the twenty-first anniversary of its foundation, at St. Bartholomew's Hospital during September 24–26. Further information can be obtained from Miss K. Smith, Hospital Physicists' Association, 6 Paddington Street, Marylebone High Street, London, W.1.

A conference on "Many-body Problems", arranged by the Institute of Physics and the Physical Society, will be held in the University of Manchester during September 22–24. The programme will include sessions on many-body problems in: atomic physics; molecular physics; nuclear physics; solid-state physics; polymers; liquids. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A short course on "Practical Noise Control", arranged by the Institute of Sound and Vibration Research, will be held in the University of Southampton during September 16–18. The course will consist of ten lectures, supported by laboratory demonstrations, and will cover acoustics, noise measurements, subjective assessments, technical principles of noise control and medical and legal implications. Forms of application and further information can be obtained from Dr. T. Priede, Institute of Sound and Vibration Research, the University, Southampton.

Erratum. The author of the book *Planetary, Lunar, and Solar Positions* A.D. *2 to* A.D. *1649 at Five-day and Ten-day Intervals*, which was reviewed by Dr. R. H. Garstang on p. 943 of the June 6 issue of *Nature*, is Bryant Tuckerman and not Bryant Zuckerman as published.

THE NATIONAL PHYSICAL LABORATORY

THE National Physical Laboratory held its annual open week during May 25–29, and 175 items were on display in the nine Divisions of the Laboratory. Some 3,000 visitors attended during the two main open days, while on the final day visits were made by more than 1,000 sixth-form pupils from more than fifty schools. No lectures were given this year but two new films were shown to visitors: one, "Ship Shape", dealt with the work of the Ship Division; and the other, "Precise Measurement in Engineering", dealt with the work of the Standards Division.

The visitors were able to inspect for the first time two new buildings in which have been rehoused the two newest Divisions, Autonomics and Basic Physics, which previously were scattered in a number of small, often temporary, buildings within the grounds of the Laboratory.

An exhibit which attracted much interest in the Basic Physics Division concerned the behaviour of solid carbon dioxide when subjected to very high pressure. Some time ago the group working on the behaviour of materials when subjected to very high pressures discovered that carbonyl sulphide can, like carbon disulphide, be transformed into a black, stable, semi-conducting polymer at high pressures. As a preliminary to an attempt to produce this polymer from solid carbon dioxide and carbon disulphide it was decided to investigate the behaviour of solid carbon dioxide under high pressure. It was then found that above a certain pressure, in the neighbourhood of 35 kilobars, the carbon dioxide underwent a change of phase and its specific resistance dropped by several orders of magnitude. The exact values of the pressure at which this change takes place have yet to be determined and little is yet known about the properties of the resulting substance. This high-pressure form, however, is not stable, and when the pressure is removed the carbon dioxide reverts to its normal state. Subsequent experiments with carbon disulphide at lower temperatures than those used to produce the stable polymer have shown that a change to an unstable conducting phase can be produced by high pressures.

The Basic Physics Division was also exhibiting the results of its latest work on sub-millimetre stimulated emission sources. The apparatus used for this work consists essentially of a 50-kV, 2·5-μsec pulsed gas discharge between two plane mirrors, and power is extracted by a dielectric film (thin compared with the wave-length of the emission) set at 45° to the optical axis of the mirror system. This arrangement provides a resonator of reasonably high '*Q*' and a Fabry–Perot interferometer of high resolving power; the addition of a third mirror provides a Michelson interferometer of lower resolving power for wide-band spectroscopy.

Strong, narrow lines with peak powers of the order of watts and wave-lengths 20–120μ were observed to be emitted by laser action when water vapour was introduced into the resonant cavity provided by the mirror system of the interferometer. The introduction of vapour containing the cyanide radical CN (from hydrogen cyanide, methyl cyanide or ethyl cyanide) gave rise to a strong stimulated emission at 336·5μ with a peak power of the order of 10 W. This discovery has interesting possibilities for the long-standing problem of generating sub-millimetre waves of reasonable power. A possible practical importance arises from the closeness of the new line to the most favourable wave-length in the sub-millimetre range to minimize absorption attenuation due to atmospheric water vapour, which is in the region of 345μ.

In the Autonomics Division building, visitors were able to see work on character recognition, which is concentrating on two main problems. One is the recognition of poorly printed numerals, such as may be produced by typewriters or cash registers, and the other is the recognition of printed text.

The systems of character recognition being investigated are based on the premise that characters consist of a limited number of features (for example, straight lines, curves, intersections and line endings) grouped and joined together in various spatial arrangements to form the characters of a set. Two-dimensional autocorrelation techniques are used to deduce the orientation and relative positions of these features and groups of features, and these in turn determine the identity of the characters.

For the recognition of numerals the output from a conventional flying-spot scanner is fed to three serially connected delay lines, and tapping points along the delay lines are used to sample the stored wave-forms. The output of a multiplier associated with each group of sampling points is integrated; the outputs from the integrators indicate the extent to which each feature is contained in the scanned character, which is then identified by a likelihood comparison with similar sets of characters using a linear decision network. The apparatus demonstrated is the result of a two-year design investigation which has shown the feasibility of constructing a practical system of character reading. Its main advantages are a high reading rate—more than 1,000 characters per second—and tolerance of a wide range of print quality and of the orientation of the character within the field being scanned. Its print quality tolerance is largely due to the use of analogue signals.

The recognition of text presents additional problems, one of the most important being the difficulty of segmenting words into individual characters, particularly if the quality of the printing is poor. An experimental system for the reading of text without prior segmentation, at present restricted to the ten most frequent letters of the alphabet (e t a o n r i s h d) in one size and style, but with variations in the quality of printing, is being simulated on the *ACE* computer. This system is shown schematically in Fig. 1. A line of text is scanned and stored in the form of a binary pattern, on which are then performed auto-correlation transformations similar to those made in the character reading described above. However, in this case the output is not integrated but the positional information is retained in two-dimensional form. This information about the position of features is then, by means of further auto-correlation transformations, searched for the presence of groups describing the familiar alphabetic characters. This results in a sequence of probability scores which are then scanned by a decision aperture to produce a sequence of identifications.

Another new building on view contains the mechanical working laboratory of the Metallurgy Division. The object of this laboratory is to facilitate metallurgical work for the expected requirements of engineering developments during the next twenty years, and to this end work will be directed towards three primary objectives. The first of these is the examination of the large group of body-centred cubic metals in Groups IV, V and VI. These include vanadium, niobium and tantalum, chromium, molybdenum and tungsten, and the body-centred cubic forms of titanium, zirconium and hafnium. They are all of high melting-point and easily contaminated, and special equipment has therefore had to be installed for their proper handling.

The second objective is to exploit the new knowledge of the structure of metals which is arising from the direct observation of dislocations. Earlier work in the Division on the dislocation patterns in iron, and their relation to mechanical behaviour, showed that these patterns may be controlled partly through the purity of the material and partly through the working operations applied during

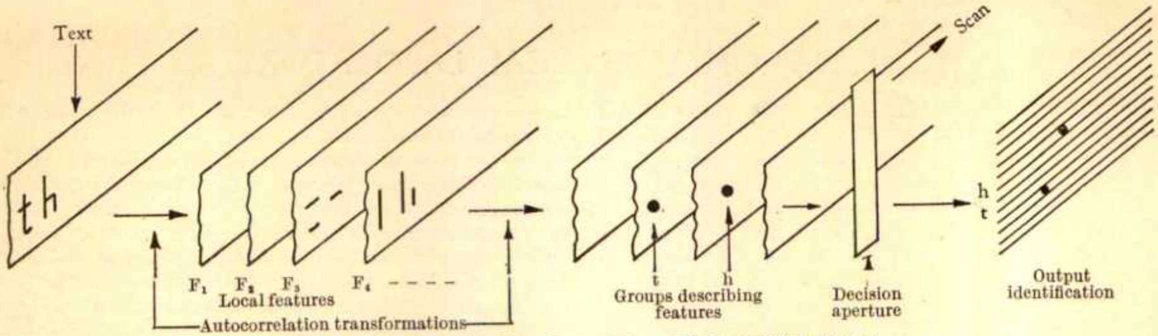
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Fig. 1. Schematic diagram of processing and recognition of text

the course of manufacture, and that the patterns will control many of the more subtle mechanical characteristics. It is therefore planned to examine these effects in more detail using iron and its simpler alloys, since iron and steel seem likely to remain the basis of engineering practice.

The third type of work for which the laboratory is suitable, and which it may be desirable to do in the future, is the preparation and study of bodies composed of mixtures of metallic and non-metallic phases. Important developments have taken place in the production of powders in which the non-metallic phase is uniformly coated by the metallic phase, and these considerably simplify the making of the desired composites.

The equipment on view consists of two rolling mills, a 700-ton extrusion press (Fig. 2), swaging machines, a forging hammer, a wire-drawing bench, together with saws, slitting wheels and similar ancillary equipment. There are two vacuum furnaces of novel design. The larger of these enables a billet for extrusion to be heated to temperatures up to 1,700° C and quickly removed from the furnace without breaking the vacuum in the heating chamber. Small specimens can be heated to temperatures up to 2,400° C in the smaller furnace and quenched into water, again without breaking the vacuum.

Also to be seen in the Metallurgy Division was a universal electron probe microanalyser. The electron probe microanalyser has many applications in metallography since it can be used to explore fluctuations of composition in inhomogeneous specimens and to reveal the differences in composition of phases seen in the microstructure. As developed by Castaing some years ago, it cannot detect elements with atomic numbers below 12 because there are no crystals capable of diffracting the long-wave X-rays characteristic of the lighter elements. Metallurgically important constituents found in industrial alloys (such as oxides, carbides or nitrides) therefore fall outside the range of the analyser.

The foregoing limitation may be overcome by the use of optical line gratings to analyse the radiations from the light elements, but hitherto the diffraction efficiency of these gratings for X-rays has been low because of imperfections in the grating surface produced by the action of the ruling tool. A joint programme of research between the Light and Metallurgy Divisions has produced a development of the Siegbahn-type grating which consists of an optically worked surface interrupted by grooves. The National Physical Laboratory gratings, which have 7,500 lines per in., are manufactured by a process involving the replication and etching of a ruled grating and have a very high diffraction efficiency. They have been used in microprobe analyses and all elements down to lithium have been detected by attaching a grating spectrometer to a commercial microprobe analyser. In addition to metallurgical applications, the new X-ray gratings should be of value for the precise measurement of X-ray wave-lengths, the examination of solar and stellar X-ray emissions observed in outer space, and the elucidation of processes taking place in thermo-nuclear devices.

The Aerodynamics Division had an exhibit dealing with the maintenance of laminar boundary layers in order to reduce the drag of an aircraft, a subject which has for many years been investigated at the Laboratory within the Reynolds number limitation of its low-speed wind-tunnels.

One problem associated with swept-back leading edges is that wedges of turbulent flow originating at the wing root, or from insects accidentally impacted on the leading edge, may cause unlimited span-wise contamination of the outboard laminar boundary layer. Results were shown of a recent Laboratory experiment which has established roughness criteria for this occurrence for comparison with the existing two-dimensional criteria. Ways of limiting such span-wise contamination, and of raising the Reynolds number at which it occurs, have been found and are jointly being investigated at the Laboratory and the College of Aeronautics. The latter work forms part of a flight-research programme, sponsored by the Ministry of Aviation, on a swept fin in which boundary-layer suction is applied through a slitted surface, and aimed at possible applications to the next generation of high-speed swept-wing transport aircraft.

A long-term application of boundary layer control, to a supersonic aircraft of slender planform, could be seen in the 13 ft. × 9 ft. wind-tunnel. A delta wing, with leading edge swept back 76° and with a wholly porous upper surface of sintered steel, is being tested at low wind

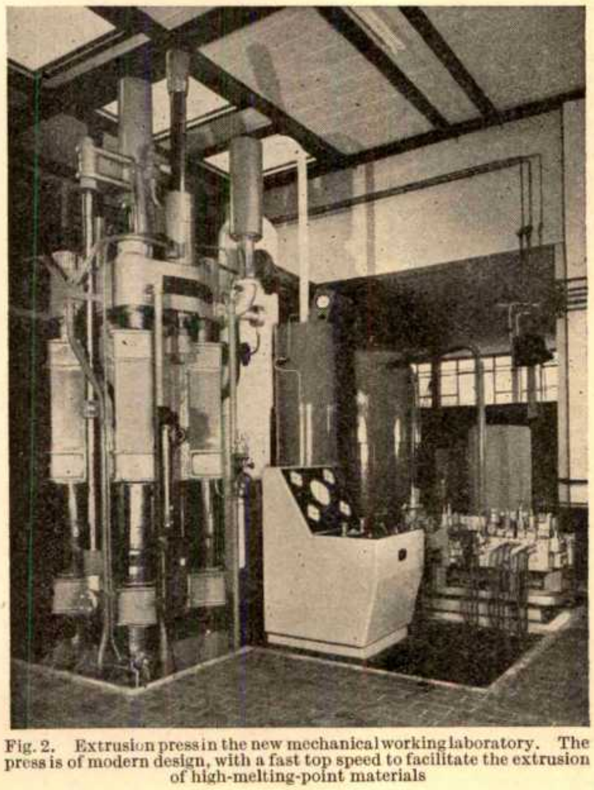

Fig. 2. Extrusion press in the new mechanical working laboratory. The press is of modern design, with a fast top speed to facilitate the extrusion of high-melting-point materials

speeds in order to determine the distributed suction needed to maintain laminar boundary layers over the upper surface in the presence of the three-dimensional flow associated with the very high leading-edge sweep. The interior of the wing is divided into six compartments, and the flow through the surface over each compartment can be controlled independently. The work includes the examination both of attached flow at the leading edge and the leading-edge vortex type of flow which produces a swept attachment line inboard of the leading edge in the vicinity of the highly turbulent vortex. Distributed suction is expected to affect the position of the vortices by eliminating secondary separation.

An interesting exhibit in the Ship Division illustrated the effect of a contaminant on turbulent boundary layers in water. In the apparatus used for this demonstration two identical revolving flanged disks were immersed in identical cylindrical casings, containing equal volumes of water, and the torque exerted by the water on each casing was shown. The addition to one casing of a small quantity of 'Polyox', sufficient to give a concentration of about 30 parts per million by weight, reduced the torque by about 30 per cent. Another contaminant which causes the same effect is guar gum.

Investigations have shown that this reduction in torque is not associated with any delay in transition from laminar to turbulent flow, but the thickness of the turbulent boundary layer is appreciably reduced by the addition of the contaminant. The exact mechanism producing this change has yet to be determined, but it is thought that it is possibly associated with a weakening of the larger eddies in the boundary layer. This effect may explain some anomalous results which have been obtained from time to time in ship hydrodynamic tests, the supposition being that certain algae in the water can act in a similar manner to 'Polyox' or guar gum. These additives can be used to reduce the power required to pump liquids over long distances.

The Ship Division also showed, in its larger water-tunnel, an impressive demonstration of the flow from a fully cavitating propeller (Fig. 3). The conventional marine propeller is designed to minimize the amount of cavitation that occurs at high forward speeds. However, propellers in high-speed craft often cavitate heavily, and under such conditions the mode of operation of the propeller is different from that of the non-cavitating propeller. The growth of large cavities in the wake restricts the mass of water that can effectively be accelerated astern and, in consequence, the thrust and torque of the fully cavitating propeller are reduced in comparison with the equivalent non-cavitating propeller.

Existing design methods developed for propellers which operate with cavities completely covering the suction side of the blade surfaces are now recognized to be inadequate, and attempts are being made to produce an improved design procedure. This improved design method requires, at least, knowledge of the size and shapes of the cavities in the wake downstream from the propeller and attempts are being made to obtain such information from experiments with fully cavitating propellers. It is hoped to be able to correlate this experimental information with a simple theory which will allow future predictions of cavity dimensions to be made. Further progress is thus dependent on the results of experiments, such as that demonstrated, with models designed using approximate estimates for some of the essential factors. In this experiment, the circumferential thickness of the cavities in the wake is measured by using a number of probes spaced in the radial direction and held by a stationary strut in the wake of the propeller.

One of the exhibits in the Radiology Group of the Applied Physics Division was a solid-state spectrometer for α- and β-rays. The requirements of a spectrometer for detecting and measuring the energy of charged particles are: good resolution, that is, the ability to distinguish between particles of closely similar energies; an output proportional to the energy deposited in the detector and independent of the nature of the radiation; a rapid response, allowing high counting rates; compactness; and simplicity. Gas-filled ionization chambers are simple, have good resolution and a linear output; scintillation counters are compact and possess a rapid response. However, solid-state devices possess all the required properties and are replacing ionization chambers and scintillation counters in many applications.

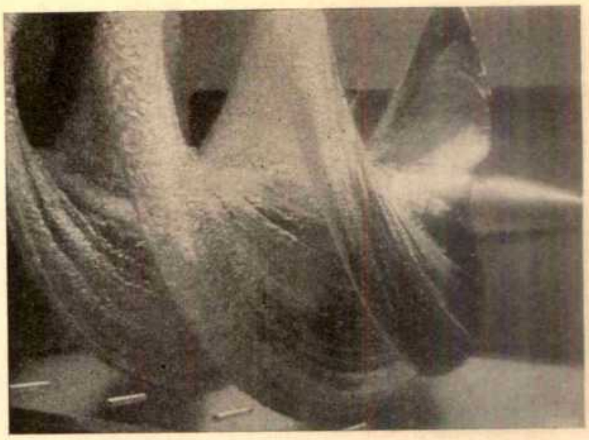

Fig. 3. A fully cavitating propeller in the National Physical Laboratory 44-in. diameter water-tunnel

The signal from a solid-state detector is a quantity of charge produced by ionization and collected by an applied field. To obtain a realistic signal-to-noise ratio combined with rapid collection, the solid must be a semi-conductor. The straightforward 'homogeneous' detector suffers from disadvantages associated with the difficulty of obtaining material of sufficient purity. Junction detectors, which make use of the rectifying junction between *p*- and *n*-type material, are therefore preferred. These are operated with a reverse bias which determines the width of the depletion layer, the sensitive volume of the detector. Junction detectors give good energy resolution, about one order of magnitude better than scintillation counters; very fast response, collection times of the order of 1 nanosecond; and a very thin dead layer.

In the experiment demonstrated a silicon surface-barrier junction detector was being used to measure the α-radiation from a source of americium-241. A similar detector with a wider depletion layer can be used for measurements of β-ray spectra. Future developments that can be foreseen are a fast $4\pi\beta-\gamma$ coincidence system, utilizing solid-state detectors for the β-rays and inorganic scintillators for the γ-rays, and identification of γ-rays from measurements of the photoelectrons ejected from a suitable converter foil.

Exhibits in the Light and Standards Divisions illustrated the Laboratory's responsibilities in the field of standards.

The present primary standard of light is a black-body radiator held at the freezing-point of platinum. While in theory this standard leaves nothing to be desired in accuracy, in practice there has been great difficulty in realizing precisely the theoretical conditions and in achieving good repetition. An alternative approach proposed by the National Physical Laboratory involves the use of a black radiation thermopile, observing all wave-lengths equally, behind a filter which transmits in varying proportions at different wave-lengths in accordance with the spectral sensitivity of the eye. For this measure to be absolute, and hence photometry become a logical branch of radiometry, it is necessary that the filter transmission should be amenable to calibration and that the thermopile be calibrated in terms of known intensities of incident radiation.

As a result of this proposal, the Consultative Committee on Photometry of the Bureau International des Poids et Mesures requested the National Physical Laboratory to organize an international comparison of radiometric scales, as a first step in assessing the possibilities of the proposed method as an internationally agreed substitute for the material primary standard of light. Standards laboratories in seven other countries are participating in this work. Lamps calibrated at the Laboratory for total radiation intensity are being sent to these laboratories for comparison and the results will be reported to the Consultative Committee at its meeting in 1965.

In the Standards Division work is being carried out on the realization of the thermodynamic temperature scale above 175° C, using a method based on the Planck law of radiation. The International Practical Scale of Temperature (IPST) was first defined in 1927 to conform as closely as possible with the thermodynamic Celsius scale as then known. However, recent determinations of thermodynamic temperature above 100° C by gas thermometry have given temperatures higher than those of the IPST, the difference steadily increasing with temperature until it reaches about 1·5° C at the gold point (1,063° C). It is therefore urgently necessary to revise the IPST, or at least to make an authoritative statement of the differences between it and the thermodynamic temperature scale. The Advisory Committee on Thermometry of the International Committee of Weights and Measures is dealing with this problem and the work demonstrated in the Standards Division stems from this Committee's activities.

The apparatus used in measuring temperatures up to the gold point consists essentially of a double monochromator using a prism, followed by a grating, in order to produce a narrow wave-length band at a mean wave-length λ, which can be set accurately. Energy radiated at this wave-length by two furnaces at temperatures T_1 and T_2 is detected by a cooled lead–telluride cell, and the energy ratio R is determined by adjusting the area of the aperture in front of the detector so that equal signals are received from the two furnaces. The energy ratio is then given by:

$$R = \frac{\exp\left(\frac{C_2}{\lambda T_1}\right) - 1}{\exp\left(\frac{C2}{\gamma T_2}\right) - 1}$$

where C_2 is the second constant of radiation in the Planck formula. Since λ is known, T_2 can be determined in terms of T_1. When the most recent assessment of the value of C_2 from atomic constants is used (that is, 0·014 388 4 m deg) the thermodynamic temperatures measured in the experiments are proving to be in agreement with those given by gas thermometry up to 800° C, but somewhat lower above this temperature.

The realization of the temperature scale above 1,063° C is normally based on the use of a visual optical pyrometer. By using a rotating sectored disk to achieve known energy ratios and a photomultiplier cell as a detector the Laboratory is aiming to achieve a ten-fold improvement in accuracy over the visual method. The apparatus on show used at its lower temperature source radiation from a graphite black-body cavity held at 1,063° C and the higher temperatures were produced by special black-body lamps, developed jointly by the Laboratory and the Hirst Research Centre.

Another type of work done by the National Physical Laboratory was also illustrated in the Standards Division, where visitors were able to see new force-measuring columns for 50 tonf and 500 tonf maxima which satisfy the requirements of Grade I proving devices of British Standards 1610 : 1964 for loads ranging from their maximum to less than one-fifth maximum, respectively. These instruments, developed in the Division, have the advantages of being robust, simple in design and accordingly of relatively low manufacturing cost. They may be used for the verification of all types of compression testing machines. The Standards Division also showed an exhibit dealing with the re-definition of the yard and pound as a result of the new Weights and Measures Act which came into force on January 31, 1964. Under this Act the yard is defined as 0·9144 international metre and the pound as 0·453 592 37 international kilogramme.

Open days provide a valuable opportunity for National Physical Laboratory staff to demonstrate the results of their latest work to other scientists and industrial users and at the same time for the staff to widen the already extensive contacts that they have with such people. Visitors are given a cordial welcome and it is hoped that even greater numbers will be able to come to Teddington in 1965 when the open days will take place in the week beginning May 17. Requests for invitations should be addressed to the Director, National Physical Laboratory, Teddington, Middlesex.

A. CHINNECK

NATIONAL LABORATORIES IN INDIA*

By A. RAHMAN, DR. A. GHOSAL, N. SEN, N. R. RAJAGOPAL, MRS. S. DAS GUPTA, S. H. M. HUSSAINI and A. K. ROY

Survey and Planning of Scientific Research Unit, Council of Scientific and Industrial Research, New Delhi

THE post-war period saw a rapid growth of scientific research in India. The Council of Scientific and Industrial Research which was set up in the early 'forties set up a chain of thirty-one laboratories during the period 1946–63. These laboratories were committed to the task of solving industrial and other national problems through research and development, by way of assessing correctly the quality of raw materials available in the country and improvising their proper use. Since industrial research always faces crucial problems in basic science, proper stress was also given to basic research. An investigation has been made by the Survey and Planning of Scientific Research Unit to take a stock of the development of these laboratories by making a statistical appraisal of the research inputs, namely, expenditure, scientific personnel, scientific equipments, personnel and the research outputs in different laboratories.

Expenditure

The total expenditure of the Council of Scientific and Industrial Research increased about sixteen times during the period 1946–62 from Rs. 5·2 millions in 1946–47 to Rs. 81·2 millions in 1961–62. In 1961–62 the recurring expenditure formed 64 per cent of the total expenditure, which again was 17 per cent of the total Government expenditure on scientific research in India (Rs. 469 millions)†.

A detailed statistical investigation has been carried out for the period 1954–62. During this period the expenditure in the laboratories increased rapidly. The increase was very great at the National Metallurgical Laboratory where the expenditure in 1961–62 was eight times that in 1954–55. In other developed laboratories the increase in expenditure was at least 100 per cent. High capital intensive laboratories are the National Aeronautical Laboratory, the National Metallurgical Laboratory, the Central Fuel Research Institute, the Indian Institute of Petroleum, the Central Mining Research Station, the Central Mechanical Engineering Research Institute, the Central Glass and Ceramic Reearch Institute, the Regional Research Laboratory, and the Hyderabad and Public Health Engineering Research Institute, where the capital expenditure formed more than 40 per cent of the total expenditure. High capital expenditure in these laboratories is primarily due to pilot plants and costly equipment. The trend in total expenditure in the developed laboratories with expenditure exceeding Rs. 3 million in 1961–62 is given in Table 1.

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* A comprehensive monograph will be published shortly.

† "A Study of Government Expenditure on Scientific Research", *J. Sci. Indust. Res.*, **22**, 479 (1963).

Table 1. TOTAL EXPENDITURE IN A FEW IMPORTANT LABORATORIES

	Rs. millions	
Laboratory	1954–55	1961–62
National Metallurgical Lab.	1·08	8·78
Central Fuel Research Inst.	2·29	5·61
National Physical Lab.	2·40	4·47
National Chemical Lab.	2·01	4·07
National Aeronautical Lab.	—	3·66
Central Drug Res. Inst.	1·23	3·64
Regional Research Lab. (Hyderabad)	—	3·14
Central Food Tech. Res. Inst.	1·01	3·11

Most of the developed laboratories spent about 50–60 per cent of the total recurring expenditure on salaries and allowances to staff, out of which 9–15 per cent was incurred on salaries of administrative staff. The percentage of total staff expenditure to total recurring expenditure has been increasing in most of the developed laboratories, and it is estimated that if the present trend continues, then by 1970–71 it will be of the order of 80 per cent in many of them.

Most of the specialized equipment and pilot plants have to be imported from abroad. Consequently the work of the laboratories has suffered greatly from the acute shortage of foreign exchange in the country. Foreign exchange spent in a few important laboratories over 1957–62 was as follows: the National Metallurgical Laboratory, Rs. 4·1 million; the Central Fuel Research Institute, Rs. 3·2 million; the Central Glass and Ceramics Research Institute, Rs. 1·1 million; the Regional Research Institute, Rs. 1·8 million; and the National Physical Laboratory, Rs. 1·2 million.

Personnel

A detailed analysis of staff structure in different laboratories has also been made for the period 1954–63. The staff in the laboratories increased from 2,682 in 1954 to 9,014 in 1963, so that the overall rate of growth per annum was roughly 14 per cent. Only two laboratories (the Central Fuel Research Institute and the National Physical Laboratory) had staff strength above 1,000; three (the National Chemical Laboratory, the National Metallurgical Laboratory, and the Central Food Technological Research Institute) had staff strength between 500 and 1,000; five (the Central Mining Research Institute, the Central Glass and Ceramics Research Institute, the Public Health Engineering Research Institute, the Regional Research Laboratory, Hyderabad, and the National Botanical Garden) had staff strength between 300 and 500; and the rest had staff strength below 300 in 1963.

In 1954, out of a total staff of 2,682, 662 (25 per cent) were scientists and technologists directly connected with research, 1,276 (47 per cent) were auxiliary technical persons, assisting the research personnel by performing various technical and skilled work, and 744 (28 per cent) performed administrative work. In 1963, 3,165 out of a total strength of 9,014 were scientists or technologists directly connected with research; 39 per cent were auxiliary technical personnel and 26 per cent were administrative staff. The distribution of research, auxiliary technical and administrative staff varied from laboratory to laboratory depending on its stage of development and nature of work. Technological laboratories employed comparatively more auxiliary technical persons and less research scientists than the laboratories concentrating more on fundamental work.

The number of supporting staff per scientist has been calculated for different laboratories by dividing the sum of auxiliary technical and administrative personnel by the number of research scientists and technologists. The highest figure (5·3) is recorded at the National Physical Laboratory; this figure is high (greater than 3) in technological laboratories like the National Metallurgical Laboratory, the Central Fuel Research Institute, the National Botanical Garden, and the Public Health Engineering Research Institute, while it is low (less than 2·5) in laboratories like the National Chemical Laboratory, the Central Drug Research Institute, the Central Food Technological Laboratory and the Regional Research Laboratory, Hyderabad. There are, however, a few laboratories (for example, the Central Building Research Institute, the Regional Research Laboratory, Jammu, and the Central Leather Research Institute) which do not carry out so much fundamental research, but have a low figure for this ratio.

A three-way classification of scientists by age, qualifications and salary has been examined for the year 1963. Qualifications were broadly grouped under three groups, namely: (1) B.Sc.s and diploma holders in technology; (2) M.Sc.s in general science and graduates in engineering and medicine; (3) doctorates (Ph.D., D.Sc., M.D., etc.). Out of 3,165 scientists about 50 per cent are less than 30 years of age, 35 per cent are in the age-group 30–40, 12 per cent in the age group 40–50 and only 3 per cent are above 50. Out of all scientists 51 per cent are B.Sc.s and diploma holders in technology, 35 per cent are M.Sc.s and 14 per cent hold doctorates. An interesting finding from this survey is that remunerations vary widely among persons having similar qualifications and experience. Thus a Ph.D. in the age group below 50 may get any salary between Rs. 300 and Rs. 2,000 with a mode in the pay-range Rs. 500–1,000; a Ph.D. of more than 50 usually receives a higher salary. The same holds true for M.Sc.s and graduates in engineering and medicine, though for them the modal pay group is Rs. 300–500 for those aged below 40, and Rs. 500–1,000 for those aged above 40. About half the B.Sc. scientists are aged below 30, and most of them have monthly pay below Rs. 300.

So far there is no financial incentive for working scientists to enhance their academic qualifications. Initial qualifications are taken into consideration for determining a scientist's status on entry, and subsequent qualifications earned during service do not play a significant part in his future promotions and pay increases. Since seniority is also taken into consideration for future promotions, it is likely that many B.Sc.s may occupy more responsible positions in the laboratory in future.

Cost per Scientist

A detailed analysis of the cost per scientist has been made for 1961–62 for different laboratories. Highest figures were recorded at the National Aeronautical Laboratory (Rs. 159,000 per annum) and the Indian Institute of Petroleum (Rs. 146,000 per annum)—these figures are comparable to the overall figure for the United States for 1960 (Rs. 173,000)*. At the National Aeronautical Laboratory the high cost is primarily due to the high cost of equipment and high average salaries of trained workers who are scarce in this field in India. At the Indian Institute of Petroleum the high cost is also ascribed to the high remuneration of the foreign scientists. In some of the old-established laboratories like the Central Fuel Research Institute, the National Chemical Laboratory, the Central Leather Research Institute, the Central Building Research Institute, and the National Physical Laboratory, the cost per scientist is comparatively low (less than Rs. 30,000 per annum), while in a few laboratories like the National Metallurgical Laboratory this figure is somewhat high due to heavy expenditure on pilot plants. It appears that in the laboratories engaged in the fields in which there is an abundance of trained workers and low cost chemicals and equipment, the cost per scientist is low. The figures for cost per scientist calculated in respect of the national laboratories will help any organization in the country to gain an idea of the expenditure required for setting up a laboratory in any of the mentioned fields of work.

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* *Reviews of Data on Research and Development* (National Science Foundation, Washington), No. 33, 6 (April, 1962); 1 dollar = Rs. 4·77.

Research Output

In the absence of a suitable measure of research output a study has been made of the research papers produced in different laboratories during the past ten years. In some of the developed laboratories the number of papers has gone down during the past five years or so, due to greater emphasis on the exploitation of the research results in industry. The work in national laboratories is responsible for the framing of many specifications by the Indian Standards Institution in recent years.

Perspective Plan

The Council of Scientific and Industrial Research is drawing up a perspective plan for the expenditure of laboratories in future, in the Fourth (1966–71) and Fifth (1971–76) Five Year Plan periods, on the basis of information gathered for the study referred to here. While arriving at the future estimates of different items, salaries to scientific personnel, salaries to administrative personnel, expenditure on chemicals, equipment, capital equipment, etc., for different laboratories, the rate of growth which was registered during the period 1954–63 will be taken into consideration. In most of the cases it will be assumed that these rates will continue in future, especially due to the under-developed nature of the country, though in a few cases it is necessary to take a view of the future rate of growth on *a priori* consideration. It has been found that most of the developed laboratories have been showing stationary rates of growth during the past ten years or so, particularly for the items of a recurring nature—these rates of growth will be assumed for the future also. For items of a capital nature it is really not possible to have a uniform rate of growth. The investigation also raises the problem of the size of the laboratory, interbalancing of resources and the organizational pattern of research—these are being studied.

THE GULLAND LABORATORY: ARTHUR D. LITTLE, LTD.

IN Britain more than 99 per cent of scientific and technical research is carried out by the universities, industrial laboratories, and the co-operative research associations. Since the War, however, research undertaken by an independent organization on behalf of a client or sponsor on a cost basis has begun to make its mark in Great Britain. This type of research is known as 'sponsored' or 'contract' research, these terms having originated in the United States where the selling of research facilities by independent organizations has reached tremendous proportions since the early 1940's.

The rapid development of sponsored research in the United States since the War was due partly to the realization of the importance of research to industry in the immediate post-war years and partly to the American point of view that industry—and not the Government—should be responsible for its own research and development. This combination of factors created a need for facilities to provide industry with a confidential service offering scientific, technical and economic assistance. This need was met by the setting up of the non-profit-distributing research institutes, some of which are now world famous. In addition to these non-profit institutes there is a host of commercial laboratories in the United States undertaking contract research, but only Arthur D. Little, Inc., in Cambridge, Mass., is comparable in size with the non-profit research institutes.

Established in 1886, Arthur D. Little, Inc., is now an international organization, employing a staff of about 1 400 and offering research, development, engineering and management services to industry, governments and banks throughout the world. There are offices and laboratories in many cities in the United States, and in Europe, Canada and Mexico. It has carried out some 70,000 projects during its seventy-eight years of operation.

In Great Britain the advance of sponsored research has been less spectacular than in the United States but nevertheless significant. One of the newest laboratories of this type is the Arthur D. Little Research Institute, which was formed in 1956 at Inveresk, near Edinburgh, in the laboratories previously operated by the Institute of Seaweed Research. It is a non-profit-distributing organization, which works in association with, but is not a part of, Arthur D. Little, Inc., of Cambridge, Mass. It is unique in undertaking only oriented long-range research, which is aimed at finding new spheres for possible exploitation by industry and is not normally concerned with sponsors' immediate problems. Present fields of investigation include the chemistry of cellulose and other polysaccharides, synthetic carbohydrate chemistry, organic sulphur and phosphorus chemistry, biochemistry and biophysics, synthetic high polymer research, metallurgy and various aspects of physical chemistry.

With the growing realization that research is of vital importance to industrial progress and increased productivity, there is now a demand in Great Britain for the full range of services offered by Arthur D. Little, Inc., in the United States. Consequently, in 1963, Arthur D. Little, Ltd., a wholly owned British subsidiary of Arthur D. Little, Inc., was activated to put the full resources of Arthur D. Little, Inc., at the disposal of firms in Britain. The London office of the new Company is specializing in technical, economic and management services for industrialists who require up-to-date knowledge of technology, markets and industrial trends. Such services include guidance in increasing production, diversification, evaluation of research and development, etc.

At Inveresk, the Gulland Laboratory, originally used by the Institute of Seaweed Research as a semi-technical laboratory, has been rebuilt and equipped by Arthur D. Little, Ltd., to provide facilities for applied research in chemistry and allied sciences, including food technology, pharmacology, and new product development. Unlike the Research Institute, the new building, with a laboratory space of 4,500 sq. ft., is equipped for short-term, confidential work for clients.

The redesigned Gulland Laboratory was officially opened on July 1 by Lord Todd, professor of organic chemistry in the University of Cambridge.

THE INFORMATION EXPLOSION

By Dr. M. P. BARNETT
Institute of Computer Science, University of London

SCIENTIFIC and engineering research throughout the world costs the equivalent of billions of pounds. This expenditure creates information. The volume of books, periodicals, reports, manuals and other publications is exploding. Money and research can be dissipated on experiments which have been done already, perhaps in a neighbouring laboratory, if those who need the answer to a question cannot determine if the question has been answered previously, and if it has been, where the answer is reported. One statistic should be enough to stress the problem's magnitude. Two million scientific and technical articles are published annually throughout the world. To find out what is known about some technical matter can be expensive; but it can obviate experiments of still greater cost and bring financial returns of even greater extent. The time-factor can be vital in medicine, commerce and defence. The value of information is recognized in the United States and in the U.S.S.R. In the United States several hundred million dollars are now spent annually on the information flow, and while the rouble expenditure on corresponding activities may not be publicized so widely, it is certain that the U.S.S.R. has deployed considerable talent and effort on this problem.

Some of the methods that are being used by the engineering community in the United States to deal with the information explosion were described in London during June 29–July 2 by Mr. Bart Holm of the Du Pont Corporation. He spoke to various audiences of research managers, engineering personnel and technical information officers at the request of the Institution of Chemical Engineers, who must be congratulated for organizing the meeting. Mr. Holm gave to these three audiences a virtuoso performance of theme and variations—the polished product of an environment in which things get done, enthusiastically, with ample financial support, in the context of a technology that is advancing on every front.

I returned recently from the United States, and I find the Transatlantic contrasts in this as in other fields rather striking. In the United States, the problem of the information explosion has formed the subject of a report of the President's Science Advisory Committee. Congressional committees are aware of the problem, and even if individual politicians may seem to use particular suggested solutions as cases which it may be expedient to support, their efforts contribute to a forward-going movement, in which the pluralistic structure of American society ensures that all good ideas get ample airing. The Defense Department, the National Institutes of Health and National Library of Medicine and the National Science Foundation maintain extensive information collection, cataloguing and reporting projects, and support many more in universities and industry.

The technical problems which are relevant to this work are very diverse. Library design and operation form ample subjects for study by the methods of operational research, and for mechanization by computers and by special purpose equipment. Improving the standard of technical presentation needs increased attention by schools and colleges. Full-time technical writers are in the ascendancy in many quarters. The literature is swollen not just by the desire to communicate information that its producer thinks is useful. Ego satisfaction, academic promotion, grantsmanship and managerial competition contribute to the volume of both the published literature of books and journals and the sub-literature of reports and memoranda. High quality and elaborated typewriters, multilith machines and, in the offing, phototypesetting (with or without the computer's aid) increase the ease and quality with which in-house documents can be produced. Indexing, abstracting and cataloguing the literature for current awareness and for subsequent literature searches occupies many highly trained personnel, and is itself the subject of extensive research. Computers are being used as powerful aids in some of the routine clerical chores associated with such work, though attempts to use computers to do the actual indexing do not seem to hold out much promise for the near future. Computers are being used to obtain lists of citations to material that has been indexed, and which is sought by specification of index terms. Documents once identified are obtained for use in various ways, including copying of full-size originals, microfilm viewing and preparing full-size copies from microfilm. Still further types of information processing machine are on the horizon.

Educational programmes are being developed to train personnel in the new techniques of information handling. Some of the efforts to secure professional status for this new field of endeavour tend to weaken the case rather than to strengthen it. As in other newly developing fields, a considerable amount of material is produced which is rather weak. There is to some extent a repetition of the social scientist's practice of creating excessive jargon. By and large, however, the picture in the United States is one of general progress in information science that is being helped by, and in turn helps, the general advance of a dynamic technology.

ADVANCEMENT IN COKE TECHNOLOGY

THE twentieth annual report of the British Coke Research Association has been issued recently, shortly after the publication of a special report on work carried out at the Coke Research Centre, Chesterfield, during the five-year period since it was opened by the Duke of Edinburgh. During this time, the Centre has been widely recognized throughout the world as an established authority in its field. This comprises a wide range of research activity from studies of atomic structure to commercial scale operations. Indeed, the Association had its origins very much prior to the Second World War in two research committees, namely, the Midland Coke Research Committee operating in the Department of Fuel Technology of the University of Sheffield, and the Northern Coke Research Committee, still in being, which has always operated from research laboratories associated with the Chemistry Department of King's College, Newcastle (now the University of Newcastle). The latest reports of the Association describe full-scale production work on the 10-ton oven at Chesterfield, as well as investigations at the plants of member organizations. The record over the years is thus truly an achievement in the union of science and practice.

Advances in coke technology must have an important bearing not only on the iron-making industry but also on domestic and other industrial needs, for the technical possibilities of the application to any given requirement of solid, liquid or gaseous fuel are always present. Imported fuel is relatively cheap at the moment and the position of coke in an economic sense is naturally associated with that of coal. Fortunately with regard to the latter, with the foreshadowed increases in the world requirements for fuel, and particularly the increasing demands for electrical power which are not in the foreseeable future likely to be overtaken by the supply of atomic energy, some stabilization of the demand for coal would appear to be probable. Further, coal mines cannot be interrupted in production without serious loss, and if shut down for lack of demand valuable natural resources may be lost for ever. Accordingly, any contribution to the amelioration of the fuel and power position from technical efficiency in the manufacture and utilization of coke must be of prime importance.

Turning to the Director's Report for 1963, the objectives of the research programme relate to the continued furtherance of the investigation of the character of the coke as affected by the composition and preparation of the charge, and the treatment received in the coke oven. The programme also covers certain items of special interest to the outside world such as the abatement of pollution of air and water, and the uses of oven coke in domestic appliances. Of particular interest to the iron and steel industry is the search for new methods of making blast-furnace coke.

Of the high spots that may be selected from the account of the five years' achievements, it is noteworthy that the small and large experimental ovens gave performances that correlated with those of commercial plants, thus justifying the faith of the early protagonists of the scheme, who were practical men. The many factors that determine the satisfactory composition and condition of the coal blend charged to the oven have been meticulously explored, and the preferable practice determined in relation to such factors as bulk density, moisture, particle size and the use of additions such as coke-oven breeze, filter cake, hard coke-oven pitch, char, iron ore and low-volatile non-coking coals, all having been assessed on a practical scale as to their effects on the quality of the resultant coke.

Similarly, in regard to carbonizing conditions, the rate of carbonization had pertinent effects on rate of throughput and on the contents of hydrogen and sulphur in the coke. The rank of the coal would be expected to be of considerable importance, and the degree of soaking applied to the charge was a factor of economic significance. The addition of heavy petroleum oil had promised earlier to be advantageous in coke manufacture, but in point of fact the main advantage resided in some enrichment of the gas made. The outcome was a reduction in the weight of dry coal carbonized per unit volume of coke-oven space per hour.

A useful point of interest to the domestic user of coke for space heating is the effect of size, shape and ash of the coke on the performance of a domestic boiler. The size of coke within the range ¾–1¼ in. mattered little, but the larger sizes were slower in reaching maximum output. The best repeatability occurred with a size range of ¾–1 in. For those who wish to know how best to sustain satisfactory banking conditions at night in their appliances there is an upper limit to the desirable size of coke used. With coke above 1¼ in. combustion over 10-h periods could only be maintained within a relatively small range of thermostat settings. A good reactivity of the coke, that is to say, a tendency to give a bright fire quickly, was preferable, and as it increased combustion could be maintained at progressively lower outputs. Similarly, a reduction in size from 1¾–1 in. to ¾–½ in. brought about a progressive drop in minimum rate of output and hence in the minimum intensity of combustion and degree of aeration practicable.

Conclusions having an important bearing on the suitability of coke both as a metallurgical and domestic fuel were provided from investigations involving the technique of electron-spin resonance. It was shown that a significant fraction of the sulphur reacting with carbon was retained as complexes stable up to 900° C and even higher. In the range of temperature 500°–900° C similar complexes were of the nature of surface sulphides, and their formation was influenced by the available surface of the carbon concerned. Some 50–60 per cent of the total sulphur in the coke was so composed, a pertinent pointer to the difficulties to be overcome in removing sulphur from coke. It is most interesting to note in this context that carbonization at temperatures much higher than those used in conventional practice might offer possibilities of a reduced sulphur content of the coke as well as an increased rate of throughput. It should not be beyond practical possibilities for an economic solution of this problem to be achieved, although it would obviously require time for development and application. The Association has started what promises to be a most interesting investigation in this field.

Of the research work dealt with in greater detail in the Director's report, a subject of considerable interest to the domestic user of coke has been the question of producing reactive coke in coke ovens by inclusion in the charge of 0·75 per cent of sodium carbonate. The operattional conditions required to make this practicable were worked out at a member's plant.

In the Fundamental Studies Laboratory, encouraging success was achieved during the year in the development of experimental methods for use in studies of the fissuring of coke during carbonization; the stress or strain concerned in the fissuring of coke was determined by means of electrical-resistance strain gauges, another successful application of a technique now widely applied in engineering research. For the measurement of stress transmission in coke the determination of the resonant frequency of longitudinal elastic waves in a small cylinder of the coke has been used. The approach by this means is somewhat complicated because the material being examined is both porous and heterogeneous. Preliminary indications were that the mean stress velocity varied with the shatter index of the coke, which is at present regarded as the criterion of a serviceable product. These more basic investigations of fissuring are being followed up in the Applied Studies Laboratory by work on both a laboratory and a pilot scale.

Active development of research on air and water pollution is at present being undertaken. The wastes from coke-oven plants contain a mass of undesirable impurities, but biological treatment of these waste products has been more successful than anticipated. There are, however, inhibiting substances, for example, sulphides, cyanides, phenols and toxic metals, which continue to present problems. Further investigations have been made of the application of the activated sludge and percolating systems. These have become in the investigation of bacteria, genus *Thiobacillus*, complicated enough to require the application of computer analysis to the copious data obtained. A realistic approach to the investigation of the polluting effects of various effluents has been provided by an assessment based on the amount of clean water required to dilute them to a concentration such that fish life might reasonably be expected to be supported. Finally it would appear that coke-oven liquor in limited proportions could be treated with sewage on filters. Where sewage works are being rebuilt or extended it may be possible to make more use of this method of disposal in the future.

The possibilities of developing new or improved processes for the production of a fuel suitable for the blast furnace have been further considered. It appeared to be fairly certain that a coke could be made from coals cheaper than those used in conventional carbonization by a continuous multi-stage process involving mechanical forming of the piece, that is to say, by using a briquetting process. The overall economics of such methods appeared, however, to be generally more attractive in areas of the world where good coking coals were in short supply.

The annual report makes reference to other topics that must be of particular interest to the expert. In addition to the aforementioned special report, there is given a notable list of interesting publications released during the year on a wide variety of subjects, including the following: *Future Trends in the Development of the Coking Industry* by the director, Mr. G. W. Lee (Hodsman Memorial Lecture); a report on *The Russian Coking Industry, 1962*; and the scientific papers emanating from the fundamental studies at the Universities of Newcastle and Hull, carried out respectively under the guidance of Prof. W. F. K. Wynne Jones and Dr. J. J. Kipling.

A final word of comment relates to the benefits emerging from the many years of fruitful effort made by the Association on the scientific control of coke manufacture. This has involved the painstaking development of better methods of analysis and testing, and these have now been demonstrated to the industry with the help of a mobile laboratory and the necessary liaison staff. During recent years both the average level and the consistency of coke quality have improved throughout the industry. This is in no small measure due to the adoption of standard methods of sampling and assessment of the quality of coke, and these methods have also made possible the operation of a price structure based on coke quality.

The mobile laboratory has also served as a base for Association teams carrying out investigations at members' plants. This new service to members has strengthened the link between the staff of the Association and that of the industry served. This is an important feature of the Association's activities and has also been shown to be extremely valuable in other research associations supported by the Department of Scientific and Industrial Research. It goes without saying that success of the sort achieved by Mr. Lee and his staff also implies receptivity on the part of the industrial personnel.

R. J. SARJANT

RADIATION INJURY

UNDER the title *Physical Factors and Modification of Radiation Injury* the New York Academy of Sciences has published a number of papers and discussions given in New York at the end of 1962*. As such the publication is probably one of the most stimulating and rewarding volumes on radiobiology so far available.

The papers in the first section on "Physical Factors Modifying Response to Radiation" are each a delightful balance between a simple review of the subject and presentation of new experimental data with ideas for fundamental analysis and further thought. The subjects of radiation quality and biological effect; the effect of linear energy transfer (LET) as tested in biological systems from bacteria to mammalian cells *in vitro* and *in vivo*, and in whole animals; the relationship of LET to modification of radiation damage by dose fractionation, anoxia and chemical protectors, show how nearly fundamental radiobiological research comes to solving some major problems of radiotherapy. In one group of papers, however, related to the "Diagnosis, Treatment and Prognosis of Human Radiation Injury from Whole-body Exposure", one is struck by the difficulty of applying radiobiological data directly, and the empirical medical approach of considering each case at the bedside must still be the prescribed course.

Somewhat in contrast to the foregoing, the papers in Part 2, resulting from the conference on "Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection", are a mixture of detailed experimental work designed to shed light on one small part of the field, of interest only to the real expert; three or four excellent technical papers grouped together at the end of the volume; and unfortunately, at least one example of an æsthetically unpleasant experimental paper with remote scientific justification on the "Enhanced Survival of Skin Homografts in Mice with a Bacterial Infection".

The volume, with good bibliography, will be essential reading for those concerned with recent developments in radiobiology, and its applications to radiotherapy. It retains some of the overwhelming nature of the meeting itself, but has crystallized the main points of the conferences for easy assimilation.

PATRICIA J. LINDOP

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* *Annals of the New York Academy of Sciences.* Vol. 114, Article 1: *Physical Factors and Modification of Radiation Injury.* Pp. 1–716. By E. P. Cronkite, L. D. Hamilton and 118 other authors. (New York: New York Academy of Sciences, 1964.) 12 dollars.

ELIMINATING DETERGENT FOAM

THE elimination of detergent foam has been a problem of world-wide concern ever since the introduction of synthetic detergents on a large scale after the Second World War. It is still with us, but there are encouraging signs of a solution. The subject is discussed in a short but informative article in *Rohm and Haas Reporter* (Philadelphia, Pa., 22, No. 1, January–February 1964).

Surfactants are commonly classified as either 'soft' or 'hard'; the former are 'biodegradable', readily decomposing in the presence of bacteria in sewage plants, septic tanks and natural waters; the latter are non-biodegradable, resistant to bacterial decomposition. Most synthetic detergents to-day utilize hard surfactants; thus they do not degrade fast enough after fulfilling their cleansing function and their presence is constantly being revealed in rivers, sewage effluents, and so on. It is perhaps commonly assumed that the amount of foam provided by a detergent, for example, in shampoos, washing and dishwashing compounds, is a criterion of the efficiency of its cleaning action; actually the amount of foam has little to do with its cleansing power; it may look good to the housewife but its main function is that of floating out dirt particles, holding them in suspension until they are rinsed away. But it is what happens afterwards to this foam that is the concern of river boards, sewage disposal authorities and public health officials.

The Rohm and Haas Co. claims to be the first firm to offer a non-ionic surfactant for biodegradability by its introduction of 'Surfactant *DN*-65' in the summer of 1963. According to the article: "Now commercially available, the 100 per cent active, nonionic surfactant is supplied as a water-soluble liquid for detergent formulations. Surfactant *DN*-65 is rapidly degradable by the bacteria normally present in waste disposal systems and natural water supplies. The new detergent concentrate is designed to replace the conventional water-soluble octyl and nonyl phenoxy poly-ethoxy ethanol-type surfactants, where biodegradability is required. River

Water DieAway Tests using surface tension measurements as an indicator show Surfactant *DN*-65 to be 95 per cent biodegradable." It is claimed for this product that it is compatible with anionic, cationic, and non-ionic surface-active agents; it is stable, soluble in hydrochloric and phosphoric acids in concentrations up to 5 per cent, and in most common solvents; the foaming, textile-wetting, emulsification properties are said to be excellent and it possesses a hard surface detergency. "It is recommended for use in industrial maintenance formulations, speciality items for home and industrial needs, fabric detergents, metal-cleaning formulations, wax strippers, liquid hand dishwashing detergents, and detergent sanitizers."

Other biodegradable surfactants are being developed in the research laboratories of this firm and they are all geared to the same objective: getting rid of detergent foam.

FURTHER USES OF ELECTRONICALLY SCANNED SONAR IN THE INVESTIGATION OF BEHAVIOUR OF FISH

By Dr. V. G. WELSBY and J. R. DUNN
Department of Electronic and Electrical Engineering, University of Birmingham
AND
C. J. CHAPMAN, D. P. SHARMAN and R. PRIESTLEY
Marine Laboratory, Aberdeen

A PREVIOUS communication[1] described some preliminary trials with a new high-resolution sector-scanning sonar which showed it to have potential usefulness in the investigation of the behaviour of fish, particularly where direct optical observation was impossible. The instrument has recently been used in further examination of the behaviour of fish: (*a*) in large experimental tanks; (*b*) in the vicinity of power station cooling-water intakes.

(*a*) Experiments were carried out on about 100 adult herring (23–29 cm long) in a tank measuring 16 m × 13 m, the depth of water being 1·4 m. The sonar transducer was placed at one end looking down the length of the tank (Fig. 1).

The fish echo-traces on the sonar display screen were photographed with exposures of up to 3 min, thus allowing the moving bright spots to form tracks representing the paths followed by individual fish. A typical result is shown in Figs. 2*a* and 2*b*, taken respectively in light and darkness. The camera film was used, in effect, to carry out a time integration of the movements of the fish. There was a striking difference between the forms of the patterns obtained in daylight and darkness due to changes in the shoaling behaviour of fish. Confirmation of this was obtained when artificial lighting was switched on at night and the 'darkness' pattern immediately reverted to the 'daylight' one.

A further series of experiments was carried out to see how the herring would react to sound stimuli introduced at one end of the tank (as shown in Fig. 1). Figs. 3*a* and 3*b* refer to a typical experiment at night in which a pure tone of 100 c/s was introduced into the tank through an underwater loud-speaker (Type *J*9, Chesapeake Instrument Co.). After an initial quiet period (Fig. 3*a*), the sound was switched on for the duration of the photographic exposure. Fig. 3*b* shows the distribution of fish during this time, and it is clear that the majority of the herring were avoiding the area of the tank in the vicinity of the sound source. The sound was monitored by an underwater hydrophone. Similar reactions to 100 c/s were observed in daylight.

(*b*) The scanner was used to observe fish behaviour in the River Forth in front of the cooling water intakes at Kincardine Power Station. At this site the river is extremely turbid, making photographic observations impossible. The intakes were situated at the end of a pier stretching out 200 yards from the river bank and are positioned at right-angles to the tidal flow. The transducer was mounted 8 ft. above the river bed and directed horizontally down river so that it would scan 30° hori-

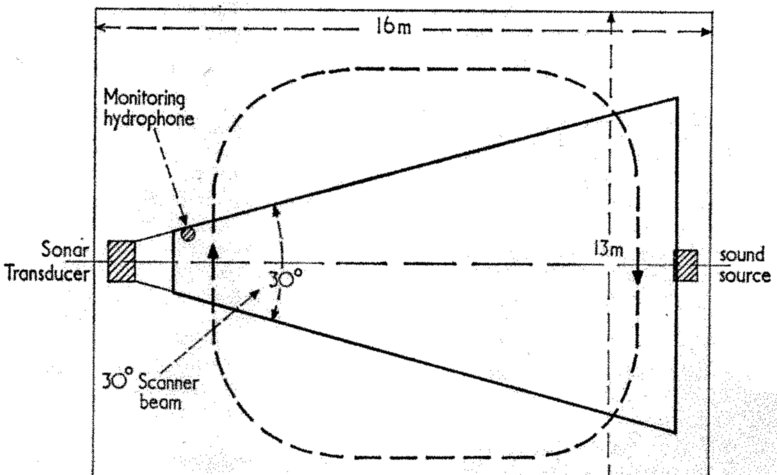

Fig. 1. Diagram of the area of the tank scanned by the sonar set and represented in the photographs of the display screen (heavy line). The circular track of herring shoals in daylight is represented by the broken line

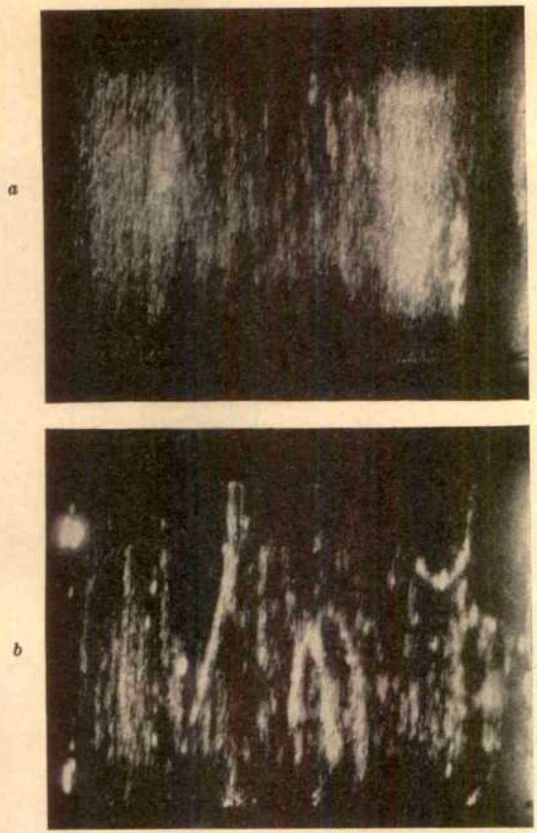

Fig. 2. Time-integrated photographs of display screen showing the distribution of herring: (*a*) in artificial lighting and (*b*) in darkness (3 min exposure)

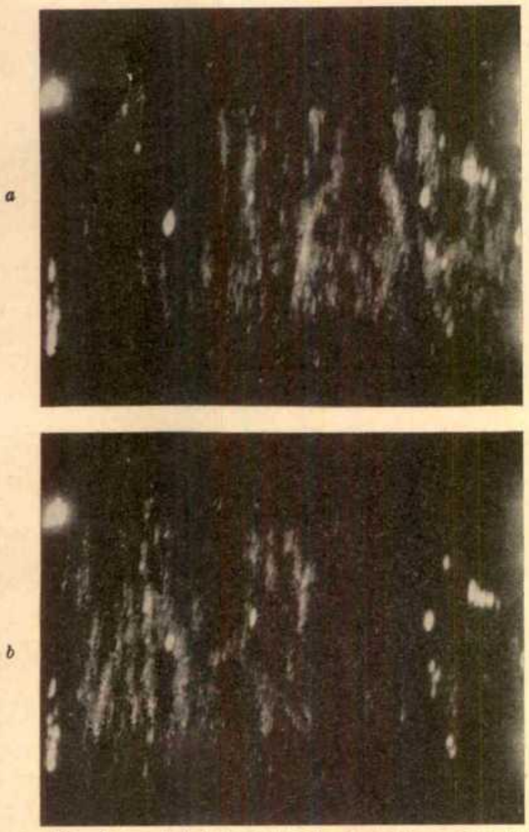

Fig. 3. Time-integrated photographs showing a response to 100-c/s sound: (*a*) before the sound was switched on showing fish near the sound source and (*b*) immediately after switching on the sound showing avoidance of the area in front of the source. (The bright image in the top left-hand corner of the photographs is the monitoring hydrophone; the small bright spots are fixed targets on the bottom of the tank)

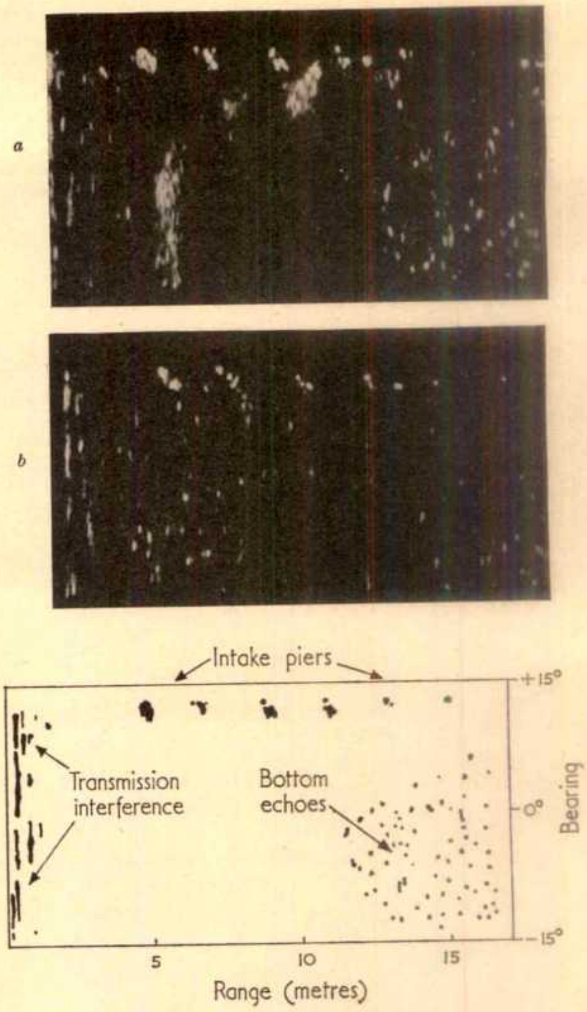

Fig. 4. Two single frames ciné film of the display, taken (*a*) during day, (*b*) at night, show the difference in shoaling behaviour observed off the intakes at Kincardine power station, with an explanatory sketch below

zontally, with a 15° vertical beam, across the intakes. The echo-traces observed on the display screen were recorded on ciné film.

During daylight, tightly packed shoals of fish (probably small clupeids) were observed (Fig. 4*a*). These shoals always moved with the tidal flow, but it was not possible to assess whether the fish were stemming the tide or not. When the ebb-tide had reached its maximum flow (more than 3 knots), air bubbles caused reverberation which completely masked the display.

The scanner was also used in darkness over a similar tidal period to that for the daylight observations. Fish were now observed to be dispersed and were seen as individuals or as small groups (Fig. 4*b*). As with the daylight observations, the fish moved with the tidal flow. The scanner shows convincingly, under these conditions, the complete break-up of the fish shoals at night.

It should be emphasized that in the experimental model described here of the scanning sonar, the scanned sector (Fig. 1) is displayed on a rectangular raster (Figs. 2–4), so that the apparent fish tracks are not geometrically similar to the actual ones. A 'true-motion' display would, however, present no problems and could be provided in future if required. Additional and more detailed experiments are planned, but these observations demonstrate further the usefulness of sector-scanning sonar for fish behaviour research.

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HIGH SALINITY IN SEA WATER

Highest Salinity in the World Ocean?

DURING the International Geophysical Year the R.V. *Atlantis*, passing through the Red Sea (Cruise 242), found an anomalously high temperature in the deep water opposite Jeddah. This was reported by Neumann and Densmore[1] in an unpublished manuscript of the Woods Hole Oceanographic Institution. On its way to join the International Indian Ocean Expedition the new R.V. *Atlantis II* carried out a series of hydrographic stations along the length of the Red Sea. Purposely, the ship

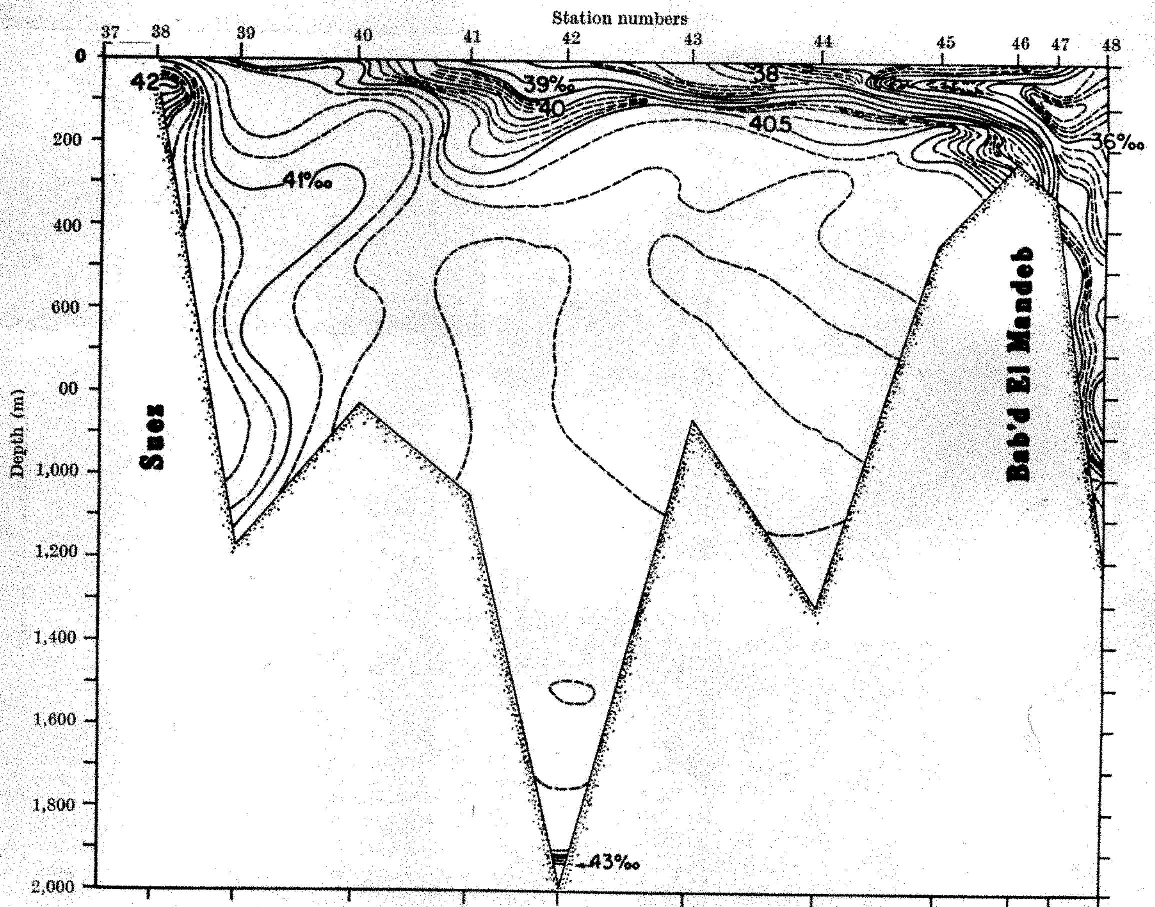

Fig. 1

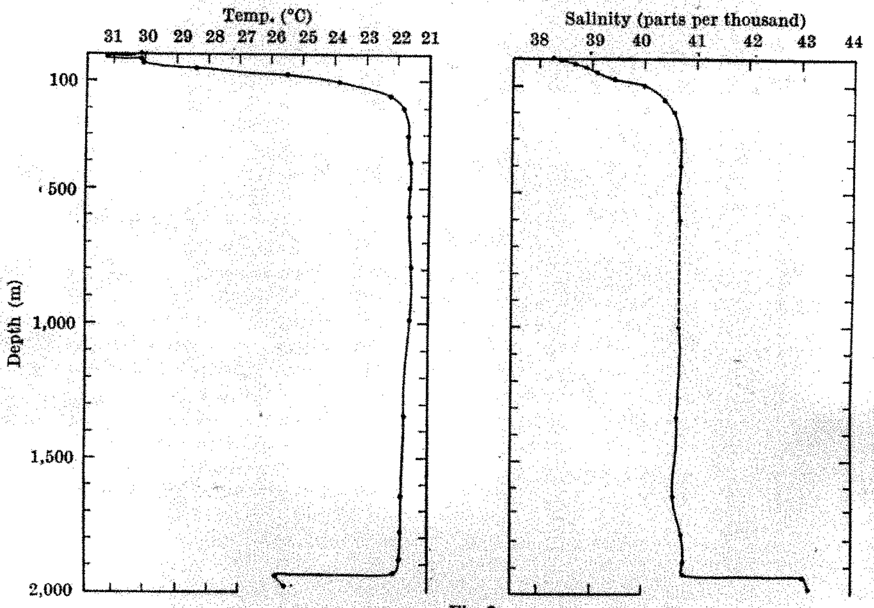

Fig. 2

stopped at the position where Neumann and Densmore[1] found this high temperature. At 21° 21·5′ N., 38° 04·5′ E., Station 42–*Atlantis II*, samples of high-temperature, high-salinity water were obtained close to the bottom.

Fig. 1 gives the profile of salinity distribution from the Gulf of Suez to the Straits of Bab'd El Mandeb as determined from the R.V. *Atlantis II* during July 1963. At Station 42 in the deepest part of the section, 1,931–1,978 m., the water was found to be 25·76°, 3·5° warmer than the rather homothermal water above it. Its salinity was 43·18 parts per thousand, 2·4 parts greater than the surroundings immediately over this band of anomalous water (approximately 50 m thick). Fig. 2 gives the vertical distribution of temperature and salinity for Station 42.

(More recently the British R.R.S. *Discovery* has sampled even deeper depths, 2,400 m, in the neighbouring area and has found clear indications of mixtures of normal Red Sea water and the hot salty water from Station 42.)

Cruise 8 of R.V. *Atlantis II* was sponsored by grant *NSF-GP*821 from the U.S. National Science Foundation.

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Mass.

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Anomalous Bottom Water in the Red Sea

WATER below 1,000 m in the Red Sea usually has a potential temperature between 21·5 and 21·6° C and a salinity close to 40·6 parts per thousand. But in a small basin around 21° 15′ N., 38° 05′ E., three research vessels (refs. 1 and 2 and preceding communication) have reported anomalously warm and salty water in the lowest 50 m. In this small basin, the depth of which is almost 2,000 m, the potential temperature reached 25° C and the salinity almost 44 parts per thousand.

A few miles to the south there is a larger basin, the depth of which approaches 2,400 m. A hydrographic station in this basin (21° 07′ N., 38° 10′ E.) was made from R.R.S. *Discovery* on March 1, 1964. In Fig. 1 the potential temperature is plotted in relation to the salinity so as to show that the water below 1,500 m contained the anomalous water much diluted with typical Red Sea deep water.

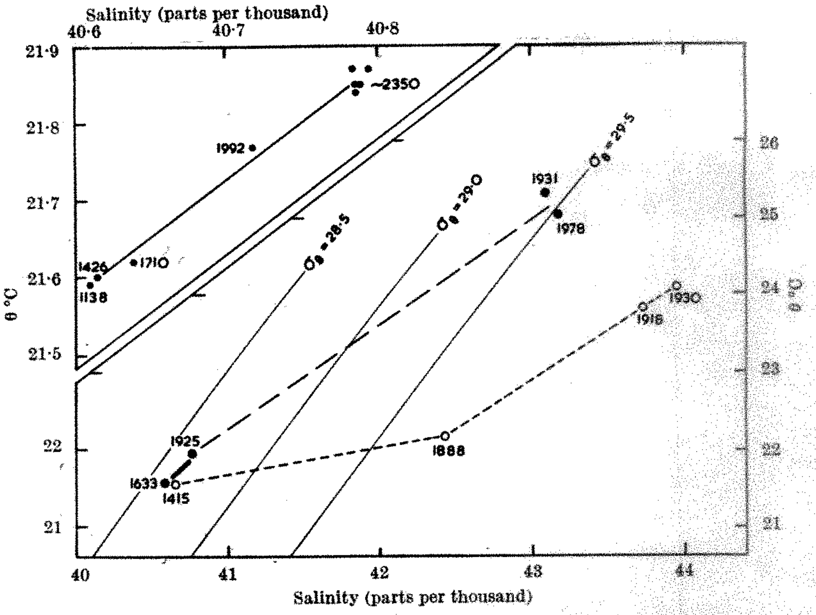

Fig. 1. Potential temperature/salinity diagram for hydrographic stations in the Red Sea. Upper left: ●, *Discovery* 5247; 21° 07′ N., 38° 10′ E.; March 1, 1964; lower right: ○, *Albatross* 254; 21° 10′ N., 38° 09′ E.; May 3, 1948; ●, *Atlantis II* 42; 21° 21′ N., 38° 05′ E.; August 1, 1963; ——, *Discovery* 5247. The figures by the points are depths in metres. The potential density lines are approximate, being extrapolated from Knudsen's formula

The anomalous water is clearly not widely distributed, and its origin is not clear. A plausible explanation is that it forms in a very shallow land-locked basin and is transferred across the sill by wind action, then to descend as a density current on the bottom. This is not inconsistent with Jerlov's[3] report that the anomalous water contains much material in suspension, but it needs to be further examined because of the relatively low oxygen content.

I thank Dr. J. C. Swallow, principal scientist of R.R.S. *Discovery* when this station was done, for permission to quote these observations in advance of publication.

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PATTERN OF CELL MIGRATION DURING CORTICAL HISTOGENESIS

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THE neural elements of the cerebral cortex are derived by mitotic division from a single germinal layer and attain their definitive arrangement through a process of migration from a juxta-ventricular position towards the superficial cortical surface. Since the classical studies of Tilney[1] it has been generally accepted that the cortical laminæ are formed by three successive migratory waves, the first giving rise to the supragranular, the second to the granular and the third to the infragranular layers. Recently, however, Berry and Eayrs[2] were unable to reconcile this concept with the effects of fœtal X-irradiation on cortical structure and postulated that the neuroblasts giving rise to layers V and VI migrated before those destined to form layers IV, III and II. Quantitative comparisons between neurones of normal and irradiated animals, and comparisons between the development of pyramidal cells in the hippocampus and neocortex, supported this hypothesis. It was suggested that more direct evidence might be obtained from an investigation of the behaviour of neuroblasts labelled with tritiated thymidine.

Each of seven groups of fœtal rats, aged 16, 17, 18, 19, 20, 21 and 22 days post-coitus respectively, have accordingly been given a single intra-amniotic injection of tritiated thymidine. Animals were killed 4 h and 2, 4, 6, 8,

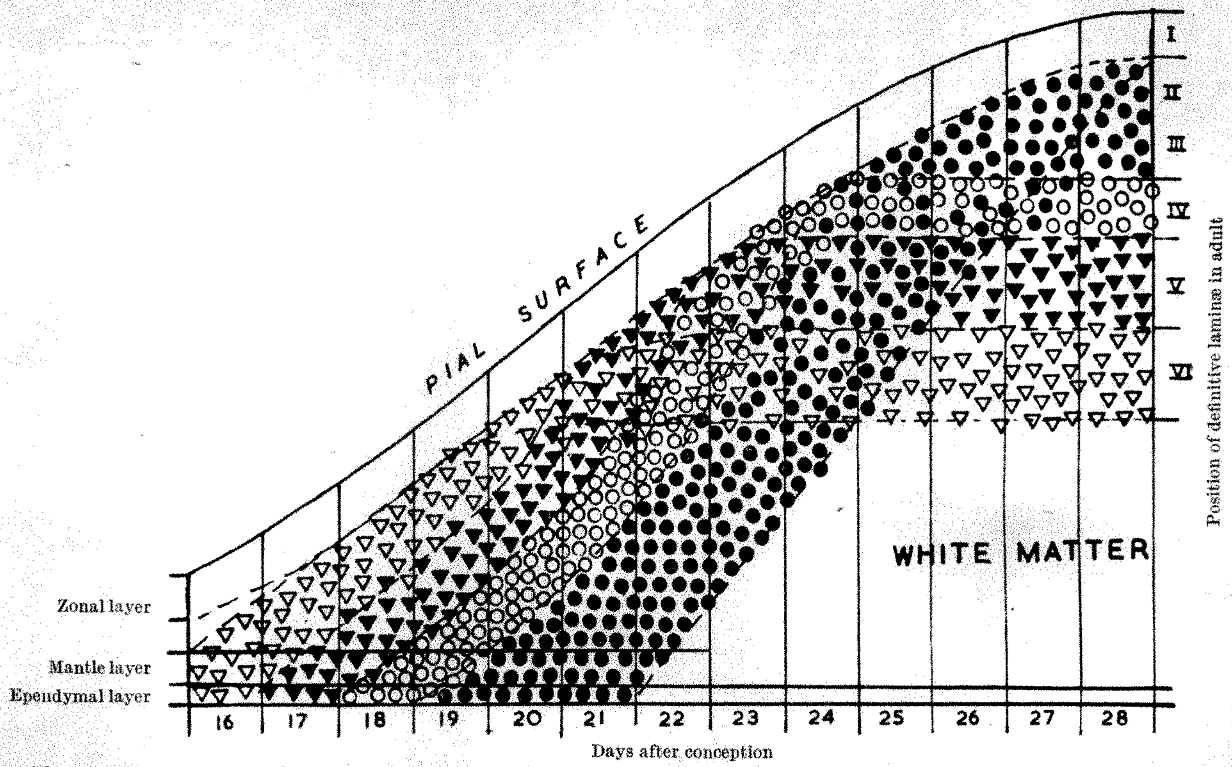

Fig. 1. Schematic representation of the positions occupied by tritium-labelled neuroblasts during successive stages of development. The signals ▽, ▼, ○, ● indicate the ages, shown along the abscissa, at which tritiated thymidine was given. The cells labelled over the period from the 19th to the 21st day of gestation formed the supragranular layers. There was no evidence of lamination into layers II and III by these cells

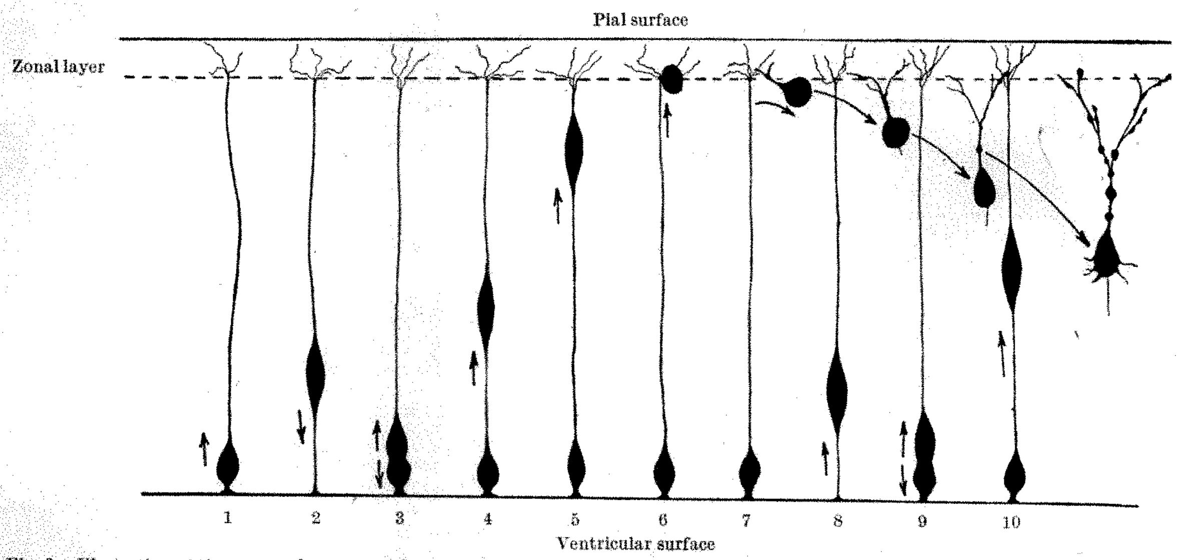

Fig. 2. Illustration of the proposed sequence of events underlying the migration and differentiation of neuroblasts—for explanation see text

10 and 20 days after this injection and autoradiographs of cerebral cortex prepared in a plane passing coronally through the intra-ventricular foramen. Fig. 1, which illustrates diagrammatically the positions occupied by labelled cells at different periods of time after injection, shows that neuroblasts present in the sub-ependymal and mantle layers on the 16th and 17th days of gestation can be traced through successive stages of migration toward the superficial cortex. This position they retain until the 23rd day when cells produced by division on the 18th day near the ventricular surface migrate through them to take up a more superficial position at the pial surface. These cells produced on the 18th day form the granular layer (layer IV) of the adult. The neuroblasts which arise by cell division between the 19th and 21st days migrate through the developing infragranular and granular layers to reach the pial surface about 6 days later, where they differentiate into the supragranular layers II and III. These findings therefore support the hypothesis of Berry and Eayrs[2] and are at the same time consistent with the results of the comparable autoradiographic examinations of Angevine and Sidman[3] using the mouse. In this connexion it is worthy of note that the phylogeny of the cerebral cortex may be closely related to this pattern of histogenesis in so far as the addition of the superficial laminæ, and hence the degree of complexity of the organ, may be attributable to a prolongation of the period during which mitosis may occur in the ependymal layer.

Examination of Nissl, silver and Golgi–Cox preparations in the light of the autoradiographic findings has suggested a possible mechanism by which the migration of neuroblasts takes place. These observations showed that:

(*a*) Ependymal cells, although actively mitotic, remained morphologically unchanged during the developmental period, retaining two processes attached by end feet one to the ventricular wall and the other, much finer, to the pial surface of the cortex. After division, both nucle iappeared to remain within the scanty cytoplasm of the superficially directed process, suggesting that cytoplasmic division did not take place in this region.

(*b*) In regions of the developing cortex which, as shown by autoradiography, contained only migrating cells the perikarya invariably presented a bipolar appearance, the two fine processes coursing radially. The cells contained little cytoplasm except at their two poles and the nucleus was ovoid and darkly staining. The processes, as seen in Golgi–Cox preparations, were indistinguishable from those of the ependymal cells.

(*c*) At later stages of development, two cell types could be identified in Nissl and silver preparations both in the superficial cortex and in those deeper layers in which autoradiographs indicated the presence of some cells that had ceased migration. One had the typical morphology, described above, of a migrating cell; the other possessed a large, pale, spherical nucleus and cytoplasm which was difficult to distinguish from the surrounding matrix. In Golgi–Cox specimens such cells were seen to possess irregularly orientated processes quite distinct from the bipolar processes of the migrating cell.

Although it has not been possible unequivocally to demonstrate a continuous function from a series of static pictures these observations would seem to warrant the hypothesis illustrated in Fig. 2. This proposes that the nuclei of the ependymal cells divide near the ventricular surface of the cortex but that the cytoplasm does not. One of the daughter nuclei remains *in situ*, the other migrating towards the pial surface within the cytoplasm of the superficially attached process of the ependymal cell. In the basal part of the zonal layer cytoplasmic division occurs and the nucleus, with its initially scanty cytoplasm, begins to differentiate independently. During the course of this differentiation the cell is displaced from the pial surface by the subsequent migration and differentiation of succeeding cells.

In this way an anatomical explanation is provided for the orderly way in which cells appear to pass unimpeded through such physical barriers as the dense populations of cells formed by previous migrations and developing fibre tracts.

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MECHANISMS OF SENSITIZATION TO X-RAYS OF MAMMALIAN CELLS BY 5-BROMODEOXYURIDINE

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DJORDJEVIĆ and Szybalski[1] demonstrated that mammalian cells are sensitized to the killing action (defined as loss of ability to divide indefinitely) of X-rays if grown in media containing 5-bromodeoxyuridine (BUDR) under conditions where part of the thymine in DNA is replaced by 5-bromouracil (BU). Szybalski[2] suggested that radiation-induced scission of the main chain of DNA at the phosphate-ester bond was facilitated by the introduction of a bromine atom into the molecule. An alternative mechanism for sensitization that has been envisaged[3] is that BU incorporation renders radiochemical damage sustained by DNA more difficult to repair by post-irradiation metabolic processes. We have attempted to distinguish between these two possibilities: (1) by investigating radiochemical damage in normal and BU-containing DNA; (2) by comparing the degree of sensitization that can be obtained with BUDR in mutants which differ in radiosensitivity because they vary in capacity to undergo post-irradiation repair.

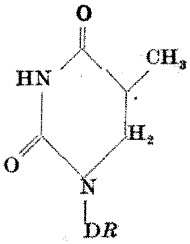
Radiosensitivity of BU-containing DNA

Irradiation of DNA inside cells. E. coli B/r were grown in the sulphonamide-containing medium in which they will incorporate added BU (ref. 4). The extent of incorporation of BU was measured by chromatographic analysis of the nucleotides following enzymatic hydrolysis[5]. Cells were obtained containing DNA in which 49 per cent of the thymine was replaced by BU and which were sensitized by a factor of 3·2 (*Do* reduced from $1 \cdot 3 \times 10^{4}$ to $4 \cdot 0 \times 10^{3}$ rads as a result of BU incorporation) towards aerobic irradiation by 220-kV X-rays in *p*H 7 phosphate buffer. To determine the extent of main-chain scission of DNA these cells were irradiated at 0° C with $1 \cdot 1 \times 10^{5}$ r. of X-rays (at $1 \cdot 1 \times 10^{4}$ r./min), after which they were immediately lysed with dodecylsulphate and their DNA extracted by the Marmur[6] method. After purification the molecular weight of the DNA was determined from measurements of the sedimentation constant by ultraviolet optics and its viscosity in a Couette viscometer extrapolated to zero shear. From these two measurements it is possible to determine the nature of the change in macromolecular structure following irradiation[7]. The only detectable physico-chemical change was a decrease in the average length of the double helices due to the production of 'double breaks' and there was no evidence of cross-linking. The extent of chain scission is measured as a decrease in the average molecular weight, which fell from $10 \cdot 9 \times 10^{6}$ to $6 \cdot 3 \times 10^{6}$ for control cells and from $11 \cdot 3 \times 10^{6}$ to $6 \cdot 5 \times 10^{6}$ for cells with 46 per cent thymine replaced by BU. This experiment indicates that BU does not increase the extent of main-chain scission when the DNA is irradiated *in situ* under conditions where the cells are more radiosensitive.

Irradiation of DNA in isolation. The same conclusion was reached when the DNA was isolated from the cells and then irradiated in dilute solution (0·1 per cent in aerated 0·1 M sodium perchlorate). The extent of main-chain scission measured by the drop in viscosity of the solution was indistinguishable for the control and BU-containing sample (dose to reduce viscosity to one-half being $3 \cdot 5 \times 10^{3}$ r. in both cases). When DNA is irradiated in the dry state, both cross-linking and degradation occur, and the physico-chemical changes are complex and depend very much on environmental factors[8]. Using a number of tests we were unable to detect an effect of the introduction of BU in either cross-linking or degradation following irradiation in the solid state.

Radiation-induced radical formation. While the foregoing experiments appear to eliminate the hypothesis that BU incorporation increases radiation-induced main-chain scission the possibility remains that radiochemical damage at sites other than the phosphate ester bond is enhanced. If BU, when incorporated into DNA, is very much more susceptible to damage than thymine then the resultant overall increase in damage to the DNA might be responsible for sensitization of cells. This view appeared to find support from investigations[9] using electron spin resonance in which it was claimed that the number of radiation-induced radicals which are formed when BU is irradiated at room temperature was nine times that formed in thymine. We were unable to reproduce this result for irradiation either at room temperature or at −196° C (at which temperature the more unstable radical species are trapped) and decided to compare radical formation in normal and 44 per cent bromouracil substituted DNA (BU–DNA) irradiated with cobalt-60 γ-rays (0·5 × 10^6 r./min) under vacuum at −196° C and at room temperature.

The electron spin resonance measurements were made on a conventional X-band spectrometer with a transmission cavity and 105-kc/s modulation. The spectra were plotted as the first derivative of the power absorbed against increase in magnetic field and are shown as such in Fig. 1. Quantitative estimates of free radical concentrations were made by double integration with a second moment balance[10] and comparison with a coal or Mo_2S sub-standard which had been calibrated against DPPH. The number of radicals formed per g DNA as a function of dose are shown in Fig. 2. On irradiation at 77° K the DNA gave an electron spin resonance spectrum which consisted of a single line (Fig. 1). On warming to room temperature, the radicals decayed and the eight line spectrum which has been previously reported[11] became prominent (Fig. 1). This radical is probably formed by the addition of a radiation-produced hydrogen atom to the thymine ring. The changes in the spectrum of DNA on standing at room temperature show that there is more than one radical species present and that this particular thymine-type radical is not the most dominant species formed by radiation, but happens to be the most stable and the most easily observed owing to the spread of the lines. At −196° C before warming to room temperature, it is estimated that the thymine type radical forms less than 10 per cent of the primary radicals formed, but on standing it represents 60 per cent of the total because the other radicals have decayed. There is no foundation for the claim[11] that on irradiation of DNA the predominant radicals formed are those due to addition of hydrogen to the thymine base.



On irradiation at −196° C the BU–DNA gave an electron spin resonance spectrum which was similar to that from DNA (Fig. 1). The radical yield (*G*-value) of BU–DNA was approximately 25 per cent higher than for normal DNA both for irradiation at room temperature and at −196° C (Fig. 2). On warming the sample to room temperature a spectrum slightly different from that of DNA was observed. The major difference was the absence of the eight-line thymine-type spectrum. Since only 44 per cent of the thymine had been replaced by bromouracil it was expected that the thymine-type spectrum would only be reduced by half. Its complete absence suggests that the bromouracil scavenges hydrogen atoms more efficiently than thymine. These results show that the introduction of 44 per cent of BU reduces the average energy required to form a radical in DNA by some 25 per cent. If this increase in radical yield caused a comparable increase in radiochemical damage of the type that results in cell death, the magnitude of such an effect is quite insufficient to explain the increase in cell sensitivities which have been reported. To account for the radiosensitization of cells on a purely radiochemical basis, it would be necessary to postulate that damage to BU had a much greater probability of being lethal than damage to the other bases.

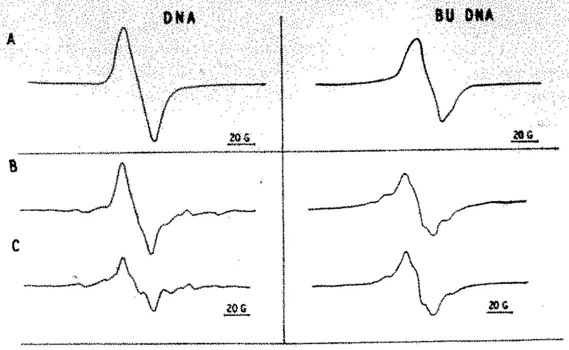


Fig. 1. Electron spin resonance spectra of DNA and DNA in which 44 per cent of the thymine was replaced by 5-bromouracil irradiated at −196° C with 2 × 10^6 rads of cobalt-60 γ-rays *in vacuo*
A, at −196° C; *B*, on warming to room temperature; *C*, after 4 h at room temperature

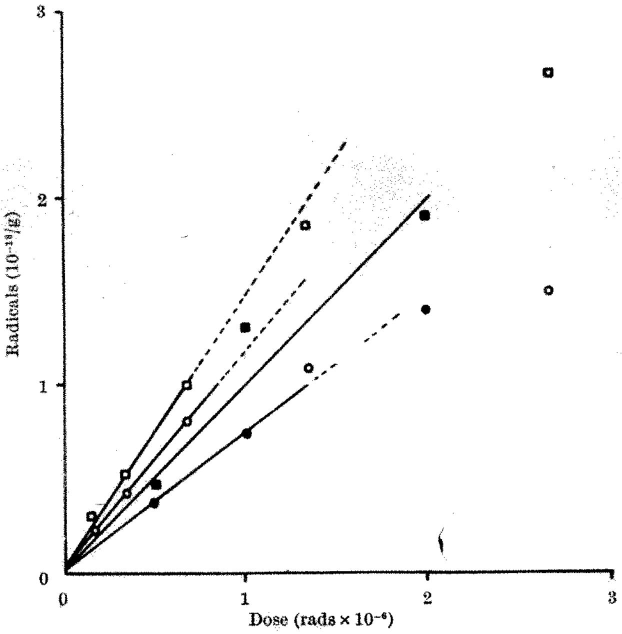
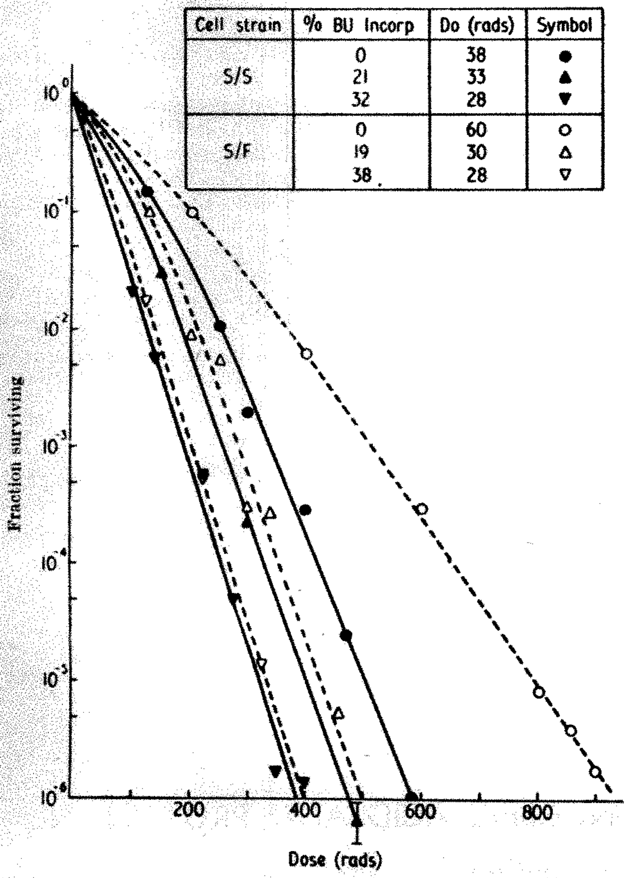


Fig. 2. Number of free radicals produced in DNA and BU-containing DNA by irradiation with cobalt-60 γ-rays *in vacuo*
☐, BU–DNA irradiated at room temperature ($G_{(\text{radicals})} = 2·4$); ■, BU–DNA irradiated at −196° C ($G_{(\text{radicals})} = 1·7$); ○, DNA irradiated at room temperature ($G_{(\text{radicals})} = 2·0$); ●, DNA irradiated at −196° C ($G_{(\text{radicals})} = 1·3$)

Radiosensitization of Murine Lymphoma Cells

Mutant strains of the lymphoma cell, *L* 5178 *Y*, which vary in radiosensitivity have been isolated[12]. For this investigation two strains grown in 'simplified' and full medium[13] and referred to as *S*/*S* and *S*/*F* respectively were used. They were both diploid (85–90 per cent of the cells had 40 chromosomes) and had the same DNA content (0·85 × 10^{-11} g/cell), generation time (10·5–11·5 h at 37° C) and cell diameter (8–10μ). The effect of X-rays on suspensions of these cells in tissue-culture medium is shown in Fig. 3. The surviving fraction was estimated by establishing the maximum dilution at which the cells will grow in medium containing 10^5 irradiated feeder cells per



Cell strain	% BU Incorp	Do (rads)	Symbol
S/S	0	38	●
	21	33	▲
	32	28	▼
S/F	0	60	○
	19	30	△
	38	28	▽

Fig. 3. Survival curves of two strains of lymphoma cells having different BU contents expressed as percentage replacement of thymine (in their DNA) following irradiation with 220-kV X-rays (750 r./min) of an aerated suspension of 10^5 cells/ml. at 37° C

ml. Under these conditions the cloning efficiency from one cell approaches 100 per cent. The survival of the *S/S* cells after irradiation can be enhanced by storage for 12 h at sub-optimal temperature[13]. As had previously been demonstrated for mutants of *E. coli B* (ref. 14), differences in post-irradiation recovery potential are largely responsible for their differences in radiosensitivity. To determine the effect of BUDR on the sensitivity to X-rays of these cells they were grown in media which contained BUDR in the range of 10^{-6} and 10^{-5} M for two and a half divisions. Except at high BUDR concentrations the growth rates of the cells was not affected. Thereafter they were irradiated with 220-kV X-rays, washed and re-suspended in media enriched with thymidine and their survival determined. Depending on the extent of treatment, BUDR rendered up to 70 per cent of the cells non-viable. The average extent of replacement of thymine by BU was determined by chromatographic analysis of the nucleotides of the DNA isolated from the cells[5]. Centrifugation in a cæsium chloride density gradient[15] of native and denatured DNA showed that, except at the higher BUDR concentrations which affected the cellular growth rates, BU substitution occurred in both strands of the DNA in at least 80 per cent of the cells. The survival curves shown in Fig. 3 do not appear to be 'multi-hit' curves in the accepted sense, but for the present we have disregarded this possibility and have expressed radiosensitivity in the conventional way as *Do* (that is, the dose needed to reduce survival by a factor of $1/e$ on the 'linear' portion of the curve). From Fig. 3 it is apparent that the more radio-resistant strain *S/F* is sensitized to a much greater extent than the *S/S* line. When more than 20 per cent of the thymine had been replaced by BU the two strains showed the same radiosensitivity (*Do* ~ 30 r.).

Fig. 4 shows that the two cell lines respond quite differently to the incorporation of BU. The point for *S/S* at 60 per cent incorporation requires qualification, because the survival curves indicate that two cell species are present; 80 per cent of the population show a sensitization of 1·8 while the remainder are only sensitized by a factor of 1·3.

Since the survival of *S/S* can be enhanced by post-irradiation treatments it would appear that the metabolic systems responsible for repair of primary radiochemical damage (probably to DNA) do not operate fully in these cells under normal conditions. The small and progressive increase in radiosensitivity, due to BU incorporation, can be correlated with the greater susceptibility of BU-substituted DNA to undergo radiochemical changes, as shown in the measurements of free radical yield by electron spin resonance. In the *S/F* strain there is a very pronounced sensitization at relatively small BU incorporation. As the BU incorporation is increased beyond 10 per cent the further sensitization occurs much more slowly and resembles the behaviour of the *S/S* line. The sensitization of *S/F* by small amounts of BU can be interpreted as an inhibition of a repair system (probably enzymatic). When this inhibition is complete, further sensitization proceeds in the manner of unprotected DNA, that is, as exhibited by mutant *S/S*. In general, the curve relating cellular radiosensitivity to BU content is made up of two components. One of these may represent the increase in radiosensitivity of DNA and increases slowly and continuously with BU, while the other rapidly reaches a maximum. In the *S/S* line the second of these components is very small and to a first approximation the curve shown for *S/S* in Fig. 4 can be taken to represent the radiochemical component of sensitization. According to this hypothesis the point of inflexion on the composite curve constitutes an approximate measure of the repair capacity of the cell line in question. Erikson and Szybalski's[16] investigation observed this type of relationship for the radiosensitization of human cells with increased incorporation of iodouracil.

BU incorporation could interfere with post-irradiation repair either by inhibiting the complex enzyme system that must be involved or because the radiochemical change which occurs at a BU residue cannot be restituted while that at a thymine residue can. This last mechanism seems unlikely to be the correct one because interference with repair should not reach a limiting value when as

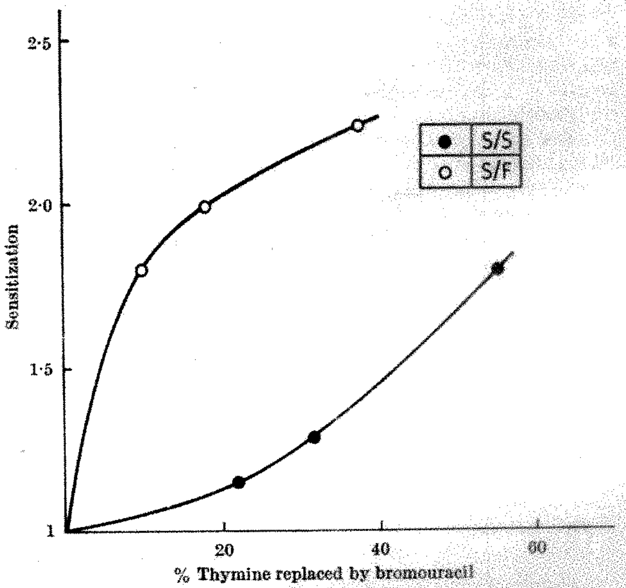


Fig. 4. Relationship between extent of BU incorporation and degree of sensitization to X-rays (expressed as the ratio of the limiting slopes of dose survival curves) for two strains of lymphoma cells

little as 10 per cent of the thymine has been replaced by BU. Blocking of repair enzymes by a process such as substrate inhibition would be expected to show a plateau relationship with BU content. The possibility must be envisaged that other dose-modifying procedures such as the oxygen effect, protection by sulphydryl compounds and sensitization by sulphydryl poisons also work at the level of the repair process. In these cases the protector or sensitizer has to be present during irradiation and it is unlikely that it interferes with the repair reaction directly. However, the repair system may itself be injured by radiation, for example, by damage to membranes or active transport processes. The dose-modifying agents may exert their effect on the radiosensitivity of cells by altering the dose needed to interfere with repair. This would explain why damage to intracellular DNA of cells irradiated under conditions of widely varying radiosensitivity is the same[17], that is, neither protectors nor sensitizers modify the radiochemical damage sustained by the DNA inside the cell. The site of action of the dose-modifying treatments need not therefore be coincident with that of the critical structure. The hypothesis that radiation-induced cell death is determined by two distinct types of primary radiochemical lesions, namely, damage to the critical structure (possibly DNA-nucleoprotein) and the interference with some of the metabolic systems which are responsible for repairing the critical structure, can also explain the great radio-resistance of the micrococcus class of bacteria the DNA of which is very similar in constitution to that of the most radiosensitive Gram-negative cells[17].

We thank Mr. P. Edwards for carrying out the ultracentrifuge measurements. This work was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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TIME OF SYNTHESIS OF DEOXYRIBONUCLEIC ACID AND PROTEIN IN SPORES OF *B. subtilis*

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When trying to understand the factors which govern bacterial spore formation, it is of considerable interest to know the time of synthesis of specific spore macromolecules. The following are the results of experiments designed to obtain information about the time of synthesis of spore DNA and protein in a sporulating strain of *Bacillus subtilis*.

The time of synthesis of spore DNA has been determined by utilizing the technique of 'phosphorus-32 suicide'. This technique is based on the observation that following the disintegration of a critical number of phosphorus-32 atoms in a given molecule of DNA the cell is unable to replicate[1,2]. Thus, measurement of the changes in the number of survivors, in a culture exposed to high doses of $^{32}PO_4^{\equiv}$ during growth, may be taken as evidence for the relative specific activity of phosphorus-32 in the DNA. The time of synthesis of spore DNA was inferred from the relative specific activity of the phosphorus-32 in the DNA of the different spore samples derived from cultures of *B. subtilis* incubated in the presence of a high specific activity of $^{32}PO_4^{\equiv}$ during different phases of their growth-cycle.

Young and Fitz-James[3], using a different technique, have shown that the spore of *B. cereus* receives a part of its parent bacterial DNA during the process of spore formation, and the results reported here are in general agreement with this earlier work.

The time of synthesis of the spore proteins was determined by isotopic labelling with ^{14}C-amino-acids.

DNA synthesis. The general conditions for growth and sporulation of *B. subtilis*, Marburg (wild type), have been described[4,5]. In the experiments reported here a 25-ml. aliquot of an exponentially growing culture was inoculated with carrier-free phosphorus-32 when the $O.D._{650}$ of the culture was approximately 0·3. The Bacto-peptone growth media used contained 22 μg of phosphorus per ml. and the final specific activity was approximately 60 mc./mg phosphorus. Serial aliquots of this high specific activity culture were removed at different time-intervals and transferred to smaller flasks that contained sufficient non-radioactive phosphate buffer to reduce the specific activity of the media 20–40-fold. A separate examination of incorporation of phosphate under these conditions revealed that more than 80 per cent of the phosphate in the medium was incorporated into the bacteria before T_0. (The time of the end of exponential growth has been chosen as an arbitrary time-reference and is designated T_0. The designations T_1, T_2, etc., represent the number of hours after the cessation of exponential growth.) All the cultures were incubated until T_{22}, at which time each flask contained free spores. However, each flask represented a different time-period during which the spore DNA could have been synthesized from the suicide-producing high specific activity pool of $^{32}PO_4^{\equiv}$. At the termination of the experiment, T_{22}, each sample was heated at 80° for 10 min to kill any vegetative cells, and aliquots of the spores were frozen at $-20°$. The number of surviving spores was quantitated at different times thereafter by counting the number of colonies produced when serial dilutions were plated on agar.

When the logarithm of the number of survivors is plotted against $(1 - e^{-\lambda t})$, where λ equals the fractional decay of phosphorus-32 atoms a day, a series of straight lines is produced, and the relative slopes of these lines are, in theory, proportional to the specific activity of phosphorus-

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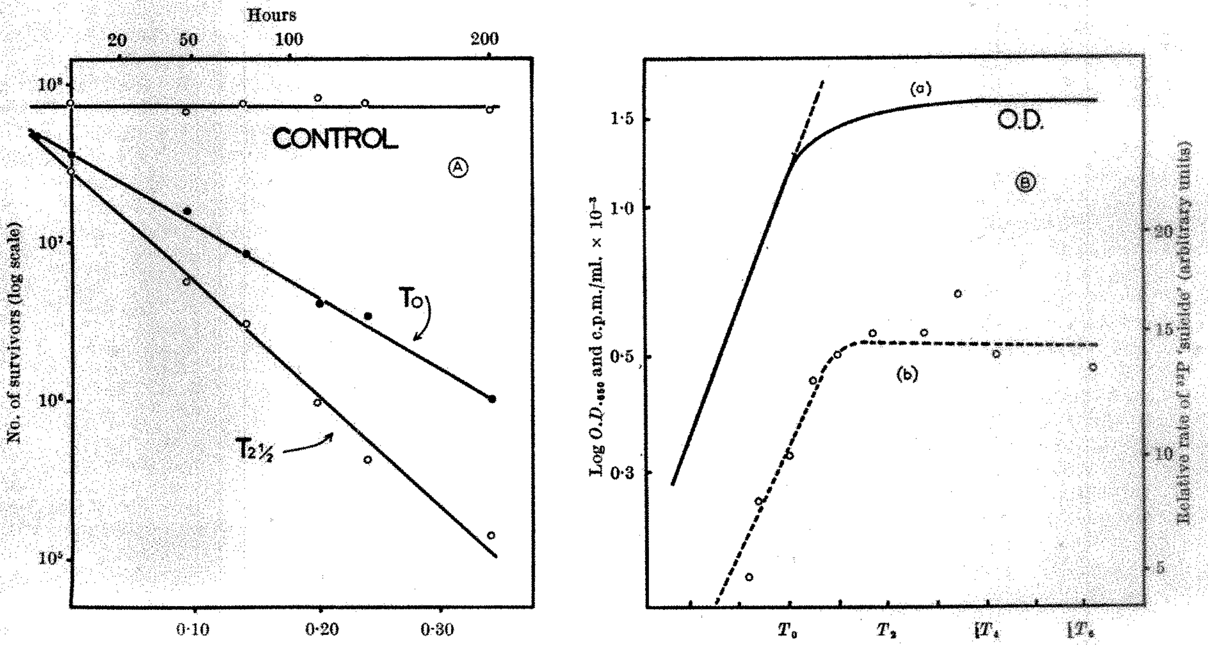

Fig. 1. Inactivation of spores due to disintegration of phosphorus-32. (*A*) Three representative 'suicide' curves depict the number of spores surviving at various times after the completion of the experiment. The samples represented by the two lower curves (T_0 and $T_{2\cdot5}$) were grown in a high specific radioactive medium until T_0 and $T_{2\cdot5}$, respectively, at which times the $^{32}PO_4$ was diluted with non-radioactive phosphate buffer. The control sample contained no radioactivity. (*B*) The relative values of the slopes of all the spore suicide curves (plotted as shown in 1*A*) are represented (curve *b*) versus the time when the high specific activity media were diluted with phosphate buffer. *a*, Growth is indicated by optical density measurements

32 in the DNA of the different samples[1,2]. Some representative spore phosphorus-32 'suicide' curves are shown in Fig. 1*A*.

The relative slopes of all the spore 'suicide' curves are plotted in Fig. 1*B*. Each point on curve *b* relates the rate of spore 'suicide' (ordinate) to the time at which the specific activity of the $^{32}PO_4^{\equiv}$ in the media was diluted (abscissa). It appears that reduction of the specific activity of the phosphate at various times after T_2 does not alter the rate of spore 'suicide', and this is interpreted as evidence that the synthesis of the spore DNA is completed before T_2. The similarity in the shape of the bacterial growth curve (*a*) and the phosphorus-32 'suicide' curve (*b*) suggests that the spore DNA was synthesized as a part of the DNA complement of the last generation of bacteria. As noted earlier, this probability has been suggested by Young and Fitz-James[3] for the spore DNA synthesis of *B. cereus*.

The first spore counts were made 22 h after the termination of exponential growth. Since the results shown in curve *b* of Fig. 1*B* suggest that the spore DNA was synthesized between T_0 and T_2, the 'suicide' curves have been extrapolated to this time, and when this is done, the average yield of the spores is 70–80 per cent of the control value. In a separate control experiment it was found that the phosphate buffer, used to dilute the radioactivity, also slightly inhibited the final yield of spores.

Protein synthesis. The existence of a protein turnover during sporulation of *B. mycoides* and *B. thuringiensis* has been shown by Foster and Perry[6] and Monro[7], respectively. Similar results have been obtained in our earlier investigations on *B. subtilis*[8]. It was suggested that this high turnover in the *Sp*⁺ strains is concerned with the synthesis of the specific spore proteins. The present experiments were designed to determine the time and relative rate of this spore protein synthesis.

Bacteria were grown in Difco-peptone broth in the presence of a mixture of ^{14}C-lysine, ^{14}C-phenylalanine, ^{14}C-proline and ^{35}S-methionine. A parallel culture contained comparable quantities of non-radioactive amino-acids. At various intervals of time, equal aliquots were removed from both cultures and centrifuged at 37°.

The radioactive bacteria were suspended in the non-radioactive supernatant and all the samples were incubated for 48 h. Spores were isolated by the method of Sacks and Alderton utilizing a two-phase system of polyethylene glycol and water[9]. The radioactivity of a measured aliquot of the spore suspension was determined by filtering and washing the spores on 'Millipore' filters which were then mounted on planchets. The number of spores was determined in aliquots heated at 80° for 10 min by plating dilutions on agar.

The results, illustrated in Fig. 2, demonstrate that the longer the bacteria remain in the presence of labelled amino-acids, the higher the ultimate radioactivity of the free spores. However, it is interesting to note that there is an apparent plateau between T_2 and T_5 when transfer of the bacteria does not change the ultimate radioactivity

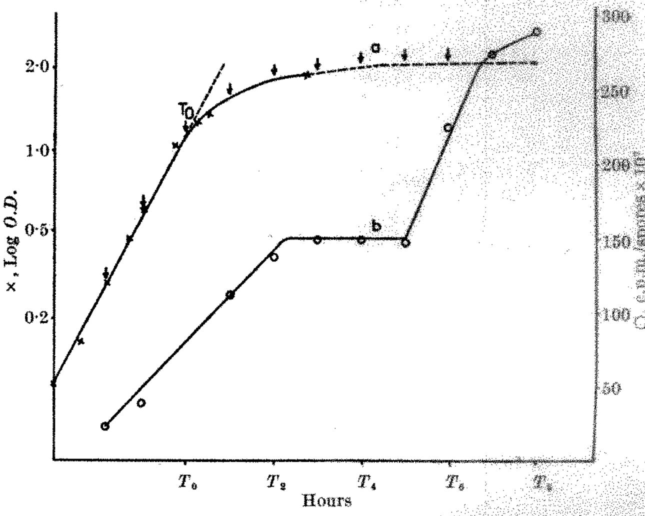

Fig. 2. Synthesis of specific spore protein. Curve *a* is the bacterial growth curve, and the arrows indicate the times at which cultures were transferred to non-radioactive media. Curve *b* (open circles) relates the specific radioactivity of the isolated spores on the ordinate to the time the culture was transferred to non-radioactive media on the abscissa

of the spores. Estimation of the total radioactivity transferred during this time reveals that a relatively constant 5 per cent of the radioactivity in the bacteria at the time of transfer appears later in the free spores. The percentage increases abruptly after T_5 to about 25–30 per cent.

Our interpretation of these results is that the synthesis of most of the specific spore structural protein begins at T_5. Between T_2 and T_5 there is active protein turnover that may involve enzymes necessary for the process of sporulation, but apparently little, if any, specific structural spore protein is made. The 5 per cent of the radioactivity found in the bacteria during this period that does make its way to the free spore may do so by several routes. Probably some is transferred with the DNA as nucleoprotein, while degradation of bacterial protein into the amino-acid pool may provide another route. Very active synthesis of spore protein begins at T_5 and this is consistent with the relatively high rate of amino-acid incorporation that has been noted during this period[8].

When protein synthesis is inhibited at T_5 by the addition of chloramphenicol, the majority of the bacterial population continue to develop heat resistance and to synthesize dipicolinic acid, but the organisms do not achieve the appearance of mature spores[5]. It appears, therefore, that the enzymes involved in the synthesis of dipicolinic acid are formed before T_5 and that the addition of chloramphenicol after this time inhibits the synthesis of protein essential for the morphological and biochemical identity of a complete spore.

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DEOXYRIBONUCLEIC ACID BASE COMPOSITION IN CERTAIN BACTERIA AND THEIR MUTANTS WITH IMPAIRED RESPIRATION

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THE possibility of experimental induction of bacterial mutants with detectable variations in DNA base composition has so far been little investigated. Spirin and Belozersky[1] observed considerable alterations in DNA base composition in some experimentally produced forms of colon bacteria as compared with original strains. Weed[2], examining the action of copper on *Bacillus subtilis*, observed the formation of mutants with the increased guanine–cytosine (GC) content of DNA. Recently, a number of mutants with impaired respiration and altered biochemical characteristics were induced by one of us in various groups of bacteria: *Staphylococcus aureus*[3], *Staphylococcus afermentans*[4], *Bacterium paracoli*[5] and *Bacillus subtilis*[6]. These mutants revealed increased sensitivity to inhibitors selectively suppressing DNA synthesis by cross-linking deoxyguanilic nucleotides. In this connexion the investigation of the GC content in DNA of these mutants was of considerable interest.

Three methods were used in the examination of DNA base composition. First, base composition was determined by the usual chromatographic procedure[1]. Secondly, content of GC was calculated from the melting temperature of isolated and purified samples of native DNA. Thirdly, content of GC was estimated on the basis of extinction ratio at 260 and 280 mμ, *p*H 3 (ref. 7).

It was observed that cells of most mutants were very resistant to different methods of disruption. Therefore, to isolate undegraded DNA they were converted to spheroplasts by the technique of Lederberg[8]. DNA was extracted by phenol from spheroplasts lysed by the addition of sodium dodecyl sulphate and purified according to Marmur's procedure[9]. The melting temperature (T_m) was determined using saline–citrate (0·15 M sodium chloride plus 0·015 M sodium citrate) as a solvent. The T_m for species with high GC content was obtained at a lower ionic strength solvent and corrected to that for saline citrate.

One can see in mutants of *S. aureus UV*-2, *UV*-15 and *UV*-16 a very considerable increase in GC content (69–70 per cent) as compared with that of the parent strain 209 (GC content 32 per cent). Base ratios of mutant 52–1, induced in *B. paracoli*, and of mutants of *B. subtilis*, are also shifted in the same direction (Table 1).

Table 1. DNA Base Composition in some Bacteria and their Mutants as estimated by Chromatographic Procedure

Strains	Molar (%) *G*	*A*	*C*	*T*	$\frac{G+C}{A+T}$	%GC
S. aureus 209	16·5	33·6	15·9	34·0	0·48	32·4
Mutant *UV*-2	35·8	14·4	35·2	14·6	2·45	71·0
Mutant *UV*-15	34·3	15·4	34·9	15·4	2·25	69·2
Mutant *UV*-16	35·4	14·5	35·5	14·6	2·44	70·9
S. afermentans 7503	36·9	12·8	37·4	12·9	2·88	74·3
Mutant 44	36·4	13·6	36·4	13·6	2·68	72·8
Mutant 22	34·9	15·0	35·1	15·0	2·34	70·0
B. paracoli	28·7	21·1	29·1	21·1	1·37	57·8
Mutant 52–1	37·8	12·4	37·6	12·2	3·06	75·4
B. subtilis 168	20·7	29·0	21·3	29·0	0·73	42·0
Mutant *MK*-9	32·4	17·9	31·6	18·8	1·77	64·0
Mutant *MK*-12	31·5	18·2	31·5	18·8	1·70	62·9
Mutant *SC*-22	32·5	17·4	32·6	17·5	1·87	65·1

In the case of *B. subtilis*, mutants *MK*-9 and *MK*-12 were induced by 5-fluorouracil[6], and mutant *SC*-22 was obtained from Dr. Weed, who produced it by copper treatment. All three mutants reveal similar GC content of DNA (63–65 per cent), while the GC content of the parental DNA attains 42 per cent. Mutants of *S. afermentans* 22 and 44 do not show any further increase in GC content. This is probably due to the fact that GC content in DNA of original strain is extremely high (74·3 per cent).

Data on GC content calculated from melting temperature and extinction ratio at *p*H 3 confirm the results obtained by chromatography (Table 2). Considerable discrepancies are observed only with the parental strain of *B. paracoli*.

Table 2 contains additional information concerning mutants of *B. paracoli Bu*-12, induced by bromouracil, and *Cu*-15, induced by copper treatment. These two mutants form small colonies; but they reveal neither any defects in respiration nor increased sensitivity to inhibitors affecting DNA[10]. They also do not reveal any

Table 2. GC CONTENT IN DNA OF SOME BACTERIA AND THEIR MUTANTS AS DETERMINED FROM THE MELTING TEMPERATURE AND EXTINCTION RATIO AT 260 AND 280 mμ, pH 3

Strains	T_m	%GC	$\frac{E_{260}}{E_{280}}$	%GC
S. aureus 209	82·9	33·2	1·579	33
Mutant *UV*-2	98·1	70·2	1·092	71
Mutant *UV*-15	98·1	70·1	1·120	68
Mutant *UV*-16	97·9	69·8	1·122	68
S. afermentans 7503	98·9	72·1	—	—
Mutant 44	98·9	72·1	—	—
Mutant 22	97·1	67·8	1·110	69
B. paracoli	91·5	54·2	1·264	55
Mutant 52–1	100·3	75·5	1·100	70
Mutant *Cu*-15	91·6	54·4	1·266	55
Mutant *Bu*-12	91·4	54·0	1·264	55
Bac. subtilis 168	87·2	43·6	1·412	44
Mutant *MK*-9	95·4	63·3	1·173	63
Mutant *MK*-12	94·6	61·7	1·185	62
Mutant *SC*-22	96·0	65·1	1·161	64

alteration in GC content of DNA as compared with parent strain.

Analysis of DNA melting profiles of all the bacteria examined shows that DNA preparations are fairly homogeneous and contain only one component. It is essential that the width of the melting intervals is the same for the parent culture and for mutants obtained from it. One may therefore conclude that the increase in GC content of DNA as observed in mutants is not accompanied by any significant change in its compositional heterogeneity.

The data obtained clearly indicate that in various groups of micro-organisms, namely, in cocci, Gram-negative bacteria and Gram-positive bacilli, mutants can be induced which reveal large increase in GC content of DNA. These fundamental changes in DNA base composition are accompanied by deficient respiration, increased sensitivity to inhibitors affecting DNA synthesis, as well as by some profound alterations in the variety of enzymatic systems[11].

These unusual mutants were induced by ultra-violet irradiation and by 5-fluorouracil, which can specifically inhibit replication of DNA. After ultra-violet irradiation or thymine starvation, caused by the action of 5-fluorouracil, multiple lesions may form in the DNA molecules as a result of thymine dimer formation[12], or chain breaks[13]. One might suggest that some cells in the bacterial population can 'heal' such lesions during subsequent replication of DNA. During this 'healing process' deoxyguanilic and deoxycytidilic residues might be used preferentially for repair of the lesions and that might cause the observed increase in GC content in DNA of mutants.

This suggestion is supported by the recent results obtained by Bollum and Setlow[14], who reported that replication of ultra-violet-irradiated DNA *in vitro* results in the increased incorporation of deoxyguanilic acid in the newly formed molecules of DNA as well as by experiments of Pettijohn and Hanawalt[15], indicating the possibility of DNA partial replication after ultra-violet irradiation of cells.

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SELECTIVE ALTERATIONS OF MAMMALIAN MESSENGER-RNA SYNTHESIS: EVIDENCE FOR DIFFERENTIAL ACTION OF HORMONES ON GENE TRANSCRIPTION

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CONSIDERABLE experimental evidence indicates the presence in mammalian tissues of rapidly synthesized RNA (refs. 1–5). This RNA has many of the properties of messenger-RNA (*m*RNA), such as rapid turnover and heterogeneity of size, and its synthesis is inhibited by actinomycin; template activity has been demonstrated in the case of this fraction from rat liver in an *E. coli* system which incorporates amino-acids into proteins[6]. While some workers have suggested that rapidly labelled RNA is synthesized and utilized only in the nucleus[4,7], other recent evidence strongly suggests that transfer of this RNA to the cytoplasm does occur[8,9], as would be expected for messenger-RNA which directed cytoplasmic protein synthesis. It is not known, however, to what extent protein synthesis in mammalian cells is dependent on rapidly synthesized RNA as opposed to *m*RNA with slower turnover and perhaps greater stability; it is also not known whether all rapidly synthesized RNA subserves specific messenger functions. Similarly, there is as yet insufficient data to permit extensive direct analogies with bacterial constitutive, inducible and repressible enzyme syntheses, and with present concepts of regulation of microbial gene transcription[10].

Several lines of evidence suggest that the rate of synthesis of various RNA fractions in mammalian cells may be altered under appropriate conditions, notably endocrine ablation and hormone substitution. Thus, Telfer[11] reported increased uterine RNA synthesis following the administration of œstradiol to ovariectomized rats. Butler and Schade[12] observed a decrease in ventral prostate RNA content in male rats following castration or stilbœstrol administration, while Kochakian and Harrison[13] found that androgens restored to normal the low RNA content of kidney, prostate and seminal vesicle in castrated mice. A single dose of cortisone acetate in intact rats results in increased incorporation of ^{32}P and ^{14}C-glycine into nuclear, microsomal and soluble RNA of rat liver[14]. Many of these and other reports relate to some hours or even days after the relevant treatment and indicate a gradual increase in total cellular RNA after the administration of different hormones.

Hormones have been implicated in the regulation of protein synthesis. Insulin[15], growth hormone[16], cortisone[17], œstrogens[18], androgens[19] and thyroid hormones[20] have all been shown to stimulate incorporation of amino-acids into proteins under specified conditions. However, such is not universally true: Manchester, Randle and Young[21] found enhanced incorporation of glycine into protein of diaphragms from adrenalectomized rats, and diminished incorporation of glycine after pre-treatment with hydrocortisone; Korner[22] reported increased incorporation of leucine into protein by rat liver microsomes following adrenalectomy but decreased leucine incorporation some weeks after adrenalectomy.

Induction of specific enzyme synthesis by hormones has been investigated in certain cases; the induction of tryptophan pyrrolase in rat liver by cortisone provides perhaps the best example. Feigelson *et al.*[17] have shown that cortisone acetate causes a net increase in the number of enzyme molecules and that increased synthesis reaches a maximum 4 h after a single dose of the hormone, coincident with the maximal increase in RNA synthesis[14]. Actinomycin[23] inhibits the cortisone-induced synthesis of tryptophan pyrrolase and also of certain hepatic gluconeogenic enzymes[24]. These investigations have led to the inference that the induction of enzyme synthesis by cortisone is regulated at the level of DNA transcription into messenger-RNA.

This conclusion gains some support from a number of recent reports which suggest that insulin[25], growth hormone[16], thyroid hormones[26], androgens[27] and œstrogens[28] as well as cortisone[29] may be involved in the regulation of *m*RNA synthesis, as judged by their effects on the amounts of rapidly labelled RNA in various tissues. Karlson's observations[30] on the effects of ecdysone on RNA synthesis in *Chironomous tentaris* larvæ suggest that this aspect of hormone action is not limited to mammalian systems.

Any attempt to elucidate the molecular site of action of hormones on RNA synthesis must necessarily be directed towards distinguishing between immediate and subsequent events. It is evident already that increased synthesis of nuclear and cytoplasmic RNA accompanies the increase in protein synthesis directed by hormones and that these changes appear to parallel the rate of synthesis of specific induced enzymes. However, these are relatively slow processes and do not necessarily give any indication of the primary site of hormone action. The fact that many hormones increase the synthesis of rapidly labelled RNA indicates that a preliminary step is stimulation of *m*RNA synthesis, but so far the available data suggest that this increase may be a general one, as shown, for example, by the sucrose gradient patterns of rapidly labelled rat liver RNA after the administration of hydrocortisone[29]. These investigations do not distinguish, therefore, among actions on the RNA-precursor pool, activation of RN polymerase and direction of DNA transcription.

We have approached the question of primary action of hormones by analysing their effects on the synthesis of rapidly labelled RNA in rat liver during short time-periods in a system which permits comparative fractionation of this RNA. The term messenger-RNA (*m*RNA) is applied here for convenience to the whole of the rapidly labelled RNA which is extracted with DNA by the 4-aminosalicylate/phenol method[5], although it is not yet certain that all this RNA directs specific polypeptide synthesis. Available fractionation methods such as sucrose gradient centrifugation and chromatography on methylated albumin kieselguhr columns are useful in characterizing *m*RNA but permit only a limited degree of fractionation. We have found that under standardized conditions of *m*RNA preparation countercurrent distribution (CCD) gives the greatest reproducibility of available fractionation techniques. CCD has the added advantage of permitting a high degree of fractionation by the use of a large number of transfers and by varying the solvent system. We have previously applied CCD to the fractionation of *m*RNA from a variety of mammalian tissues[31] and to the demonstration of progressive alteration of *m*RNA synthesis during the growth of a mouse hepatoma[32]. In the present investigations, CCD has been used to demonstrate selective alteration of *m*RNA synthesis in rat liver and the results suggest differential effects of hormones at the level of DNA transcription.

Adult male and female August rats were maintained on a standard high (20 per cent) protein diet, and after removal and extraction of the liver the *m*RNA was isolated and gave reproducible patterns on CCD. Preparations of *m*RNA were made in all cases after a 20-min pulse of 200 μc. of ^{3}H-orotic acid, the pulse-time being assessed from the time of intraperitoneal injection of the label to freezing of the livers in liquid nitrogen. Each preparation was made from the pooled livers of two rats. After extracting ribosomal and soluble RNA with naphthalene-1,5-disulphonate/phenol/8-hydroxyquinoline *m*RNA was extracted together with DNA from the insoluble interfacial material with 4-aminosalicylate/phenol/8-hydroxyquinoline as previously described[5]. By this procedure the rapidly labelled RNA can be quantitatively extracted[31], thus permitting comparative fractionation of preparations made from animals receiving different treatments prior to pulse-labelling.

The *m*RNAs in the DNA/RNA preparations have been characterized by sucrose density gradient centrifugation[5] and fractionated by CCD[31]. Any degradation, which does rarely occur due to nuclease action, can be readily detected by both these methods[31]. Solvent systems for CCD were similar to those developed for ribosomal RNA[33] except that trilithium citrate was substituted for tripotassium citrate in the aqueous phase, as used for CCD of DNA[34]. A standard solvent system was utilized in the present investigations containing the following proportions: organic mixture 28 vols., amine solution 12 vols., 0·033 M trilithium citrate 12·5 vols., and water 39·5 vols. This system was designated as solvent system 150/12·5 referable to the number of volumes of trilithium citrate used. 2 mg of interfacial DNA/RNA were distributed in this solvent system (3 ml. each phase) over 100 transfers. The material was first dissolved in 3 ml. of each phase of a 150/2 system and distributed by transferring through a gradient of increasing lithium concentration (2 tubes) into the 150/12·5 system. After 100 transfers, 1 ml. of ethanol was added to the contents of each tube to give one phase and radioactivity measured in 2 ml. aliquots (10 ml. scintillation mixture) by liquid scintillation spectrometry. Quenching by the solvents was of the order of 5 per cent and was the same for all fractions. A full discussion of CCD of *m*RNA has been recorded elsewhere[31].

Initial experiments examined the effects of starvation on the pattern of rat liver *m*RNA synthesis. Male or female rats were starved for periods up to 24 h following at least 2 weeks on the 20 per cent protein diet; some animals, after a period of starvation, were re-fed on the high protein diet. Starvation produced a marked increase in the amount of *m*RNA per unit DNA, and CCD fractionation showed considerable alteration of *m*RNA pattern (Fig. 1). Some groups of *m*RNA appeared to be affected more than others. The CCD pattern of *m*RNA prepared from rats starved for 24 h, then re-fed for 40 h on the control diet, showed virtual return to that of the control *m*RNA preparation, indicating the reversible nature of the changes induced by starvation.

The effects of hydrocortisone were examined over short time-periods to obtain some indication of the initial actions of this hormone on *m*RNA synthesis. Intact male or female rats were given a single large dose (5–10 mg) of hydrocortisone succinate by intraperitoneal injection (I.P.I), followed after varying time-periods (5–60 min) by an ^{3}H-orotic acid pulse for 20 min. Striking effects were observed on the resulting *m*RNA CCD profiles (Fig. 2). Even in the shortest time of 5 min before injecting orotic acid, hydrocortisone showed a selective action, increasing some and decreasing other *m*RNA fractions. The peak appearing about tube 18 after 5 min was reduced at longer times after hydrocortisone treatment, but otherwise the time-course showed a continued increase in the broad peak

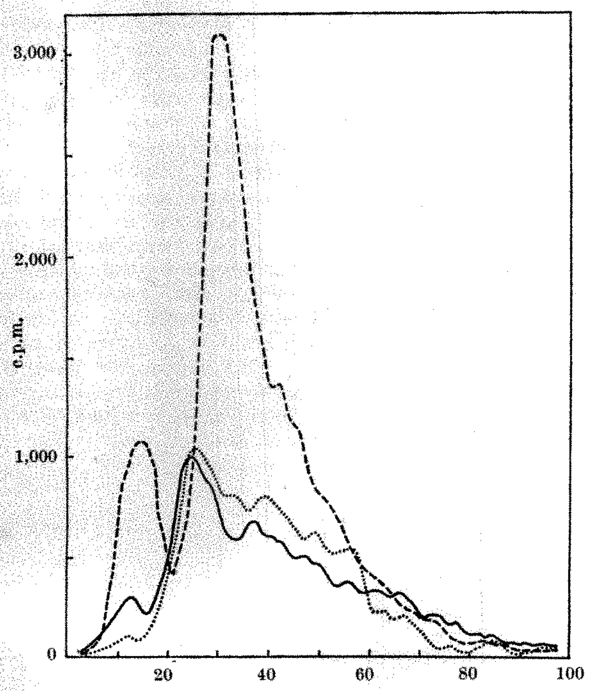

Fig. 1. CCD of rat liver *m*RNA prepared after starvation and re-feeding. 100 transfers in solvent system 150/12·5. ——, control (*m*RNA from rat liver: 20 per cent protein diet); – – –, *m*RNA from liver of rats starved 24 h; . . . . ., *m*RNA from liver of rats starved 24 h, then re-fed on control diet for 40 h

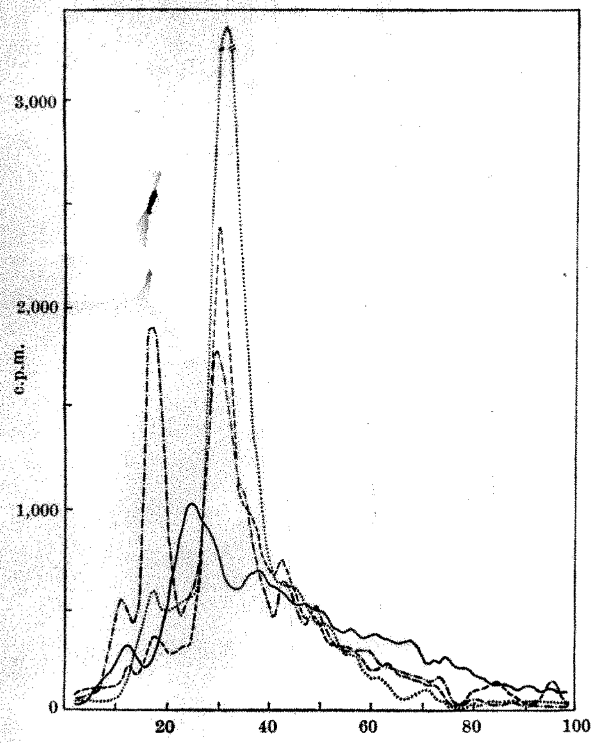

Fig. 2. Effects of hydrocortisone succinate (5 mg/rat) on *m*RNA patterns in rat liver. CCD in solvent system 150/12·5 over 100 transfers. ——, Control (*m*RNA from rat liver: 20 per cent protein diet); —·—·—, *m*RNA from livers of rats receiving I.P.I. hydrocortisone 5 min before pulsing with ^{3}H-orotic acid; – – –, *m*RNA from livers of rats receiving I.P.I. hydrocortisone 15 min before pulsing with ^{3}H-orotic acid; . . . . ., *m*RNA from livers of rats receiving I.P.I. hydrocortisone 30 min before pulsing with ^{3}H-orotic acid

in tubes 25–40. It should be noted that this latter peak is of itself heterogeneous and also that, coincident with the increase in this group of *m*RNAs, other fractions were decreased at all time-periods examined. CCD patterns had returned to normal when examined 24 h after hydrocortisone injection. Whereas these highly selective effects were observed by CCD fractionation, sucrose gradient centrifugation was not sensitive enough as a fractionation technique to show them, and by this latter method we have observed only an apparent general increase in *m*RNA similar to that reported by Kenney and Kull[29].

The effects of insulin on *m*RNA synthesis showed interesting variation with time. Male or female rats were given a single intraperitoneal injection of crystalline bovine insulin over a dose range of 2–20 units per animal, 5 min–2 h before pulse-labelling with ^{3}H-orotic acid. Fig. 3 shows the CCD patterns of *m*RNA after treatment with insulin for 5, 15 and 30 min. In the shortest time-period there appeared to be a general fall in the synthesis of *m*RNA per unit DNA. At 15 min there was a marked stimulation of a fraction in tubes 30–44, while at the same time diminution of other fractions was evident. By 30 min this pattern showed further change but still exhibited differences from the control curve. The patterns illustrated were obtained after treatment with large insulin doses (20 units) but similar selective action, less marked in degree, was observed with much smaller doses. The results of preliminary experiments with alloxan are illustrated in Fig. 4. Alloxan alone over 2 h changed the CCD pattern of *m*RNA, with enhancement of some peaks and diminution of others. Insulin given for 30 min after 1·5 h of alloxan produced a pattern somewhat different to that resulting from either alloxan or insulin alone for the respective time-periods. These results do not, of course, give any indication of the situation which prevails in diabetic animals.

Of the thyroid hormones, only the results of experiments with thyroxine are discussed here. Thyroxine was used over the dose range of 20–100 μg per animal, on male or female rats, over time-periods of 15 min–2 h. Fig. 5

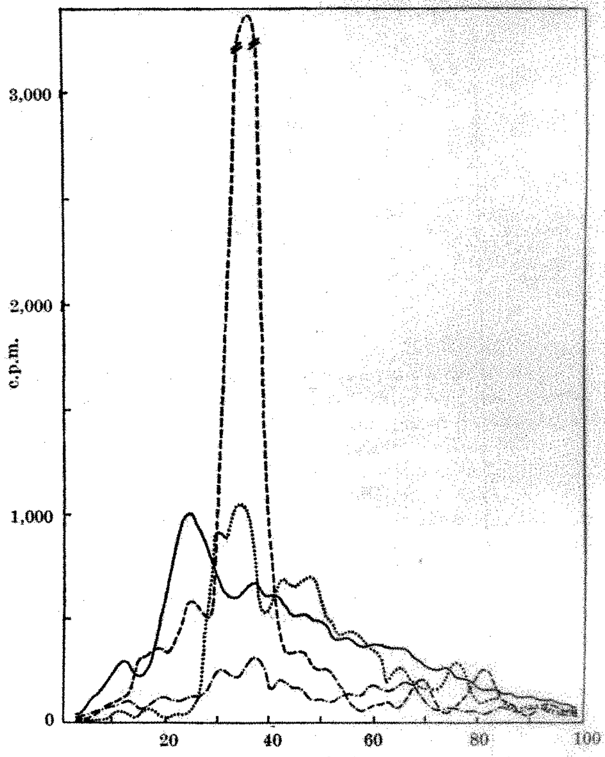

Fig. 3. Effects of insulin (20 units/rat) on *m*RNA patterns in rat liver. CCD in solvent system 150/12·5 over 100 transfers. ——, Control (*m*RNA from rat liver: 20 per cent protein diet); ————, *m*RNA from livers of rats receiving I.P.I. insulin 5 min before pulsing with ^{3}H-orotic acid; – – – –, *m*RNA from livers of rats receiving I.P.I. insulin 15 min before pulsing with ^{3}H-orotic acid; . . . . ., *m*RNA from livers of rats receiving I.P.I. insulin 30 min before pulsing with ^{3}H-orotic acid

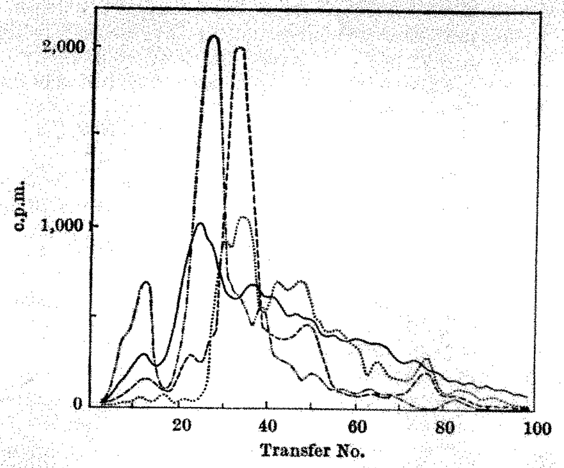

Fig. 4. Short-term effects of alloxan (5 mg/rat) ± insulin (20 units/rat) on *m*RNA patterns in rat liver. CCD in solvent system 150/12·5 over 100 transfers. ——, Control (*m*RNA from rat liver: 20 per cent protein diet); —·—·—, *m*RNA from livers of rats receiving I.P.I. alloxan 2 h before pulsing with ^{3}H-orotic acid; . . . . ., *m*RNA from livers of rats receiving I.P.I. insulin 30 min before pulsing with ^{3}H-orotic acid; – – – –, *m*RNA from livers of rats receiving I.P.I. alloxan 1·5 h, followed by insulin 30 min before pulsing with ^{3}H-orotic acid

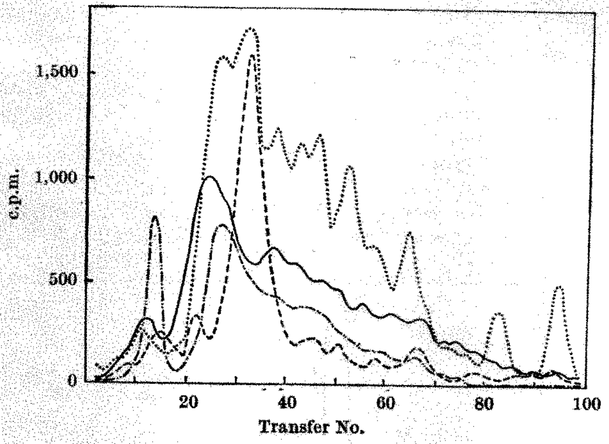

Fig. 5. Effects of thyroxine (30 μg/rat) and thiouracil (100 μg/rat) on *m*RNA patterns in rat liver. CCD in solvent system 150/12·5 over 100 transfers. ——, Control (*m*RNA from rat liver: 20 per cent protein diet); – – – –, *m*RNA from livers of rats receiving I.P.I. thyroxine for 15 min before pulsing with ^{3}H-orotic acid; . . . . ., *m*RNA from livers of rats receiving I.P.I. thyroxine for 1 h before pulsing with ^{3}H-orotic acid; —·—·—, *m*RNA from livers of rats receiving I.P.I. thiouracil for 2 h before pulsing with ^{3}H-orotic acid

shows the effects of thyroxine over two time-periods: from showing a major peak in tubes 28–40 at 15 min, the pattern became more heterogeneous by 1 h with increased synthesis of many peaks. The diminished synthesis of certain fractions evident at 15 min was no longer present at 1 h. Thiouracil appeared to cause some general depression of *m*RNA synthesis per unit DNA; the significance of the enhanced peak about tube 13 (Fig. 5) as of similar peaks in other experiments is uncertain: while it may well represent altered *m*RNA synthesis, residual ribosomal and soluble RNA remain in the first 20 tubes in this solvent system[31] and cannot be distinguished from *m*RNA in this region by CCD alone.

Testosterone was administered to male or female rats by intraperitoneal injection in an oily suspension in single doses of 0·1–1·0 mg per rat. CCD patterns are illustrated (Fig. 6) for *m*RNA pulse-labelled 1 h after testosterone injection. In the livers of male rats there was an increase in *m*RNA per unit DNA; this increase appeared to be a fairly general one involving most fractions as separated by CCD. In female rats somewhat different patterns were observed, the action being more selective.

Stilbœstrol was administered to male and female rats in doses of 0·1–1·0 mg per animal, in oily suspension given by intraperitoneal injection. CCD patterns are shown for a 1-h period in Fig. 7. In female rats there was a rather general increase in liver *m*RNA similar to that produced by testosterone in males. In male rats stilbœstrol appeared to have minimal effect on liver *m*RNA pattern.

The results of these experiments must be considered with certain reservations. The *m*RNA has been examined from a single source, rat liver, which is not necessarily a suitable target organ for examining the action of many hormones. A single radioactive pulse-time has been used for comparative purposes: for more complete information it would be necessary to investigate *m*RNAs synthesized at different rates. The time-periods of hormone action have been designated from the time of administration of hormone to the time of pulsing with radioactive label; since the pulse-time was 20 min the actual times of hormone action were considerably longer than specified, a factor especially important at the shorter times examined.

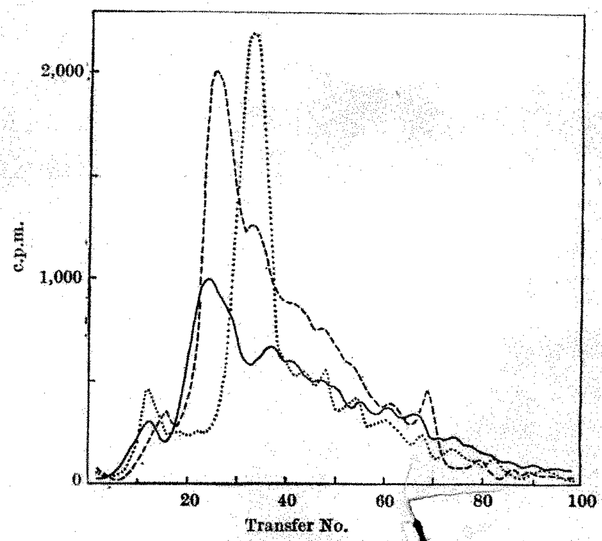

Fig. 6. Effects of testosterone (0·1 mg/rat) on *m*RNA patterns in male and female rats. CCD in solvent system 150/12·5 over 100 transfers. ——, Control (*m*RNA from rat liver: 20 per cent protein diet); – – – –, *m*RNA from livers of male rats receiving I.P.I. of testosterone 1 h before pulsing with ^{3}H-orotic acid; . . . . ., *m*RNA from livers of female rats receiving I.P.I. testosterone 1 h before pulsing with ^{3}H-orotic acid

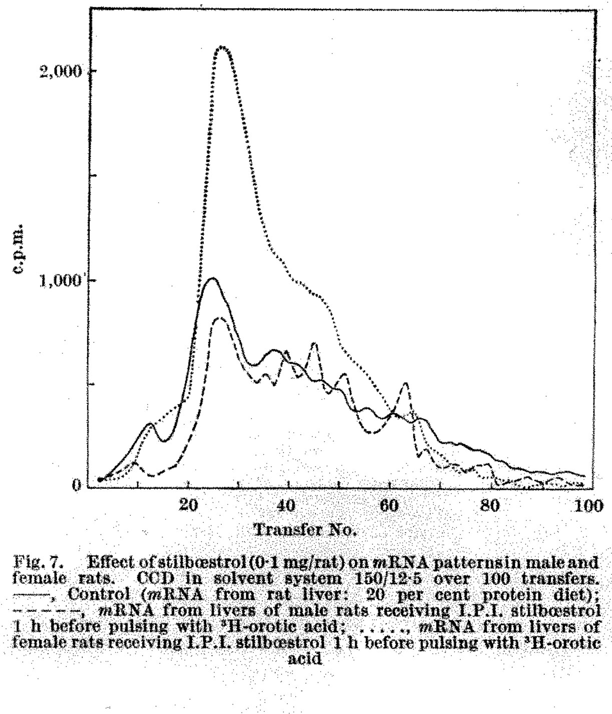

Fig. 7. Effect of stilbœstrol (0·1 mg/rat) on *m*RNA patterns in male and female rats. CCD in solvent system 150/12·5 over 100 transfers. ——, Control (*m*RNA from rat liver: 20 per cent protein diet); – – – – –, *m*RNA from livers of male rats receiving I.P.I. stilbœstrol 1 h before pulsing with ^{3}H-orotic acid; . . . . ., *m*RNA from livers of female rats receiving I.P.I. stilbœstrol 1 h before pulsing with ^{3}H-orotic acid

Where oily suspensions of steroid hormones were used no precise time-course of action can be ascertained owing to delays in absorption. For these reasons the present results cannot be considered to give any clear indication of the initial molecular sites of hormone action but only of early observed effects. Intact animals have been used exclusively and it is clear that detailed investigation of the actions of individual hormones on *m*RNA synthesis will need to examine the effects of endocrine ablation. It is clear, also, that many of the changes noted here may represent multiple effects and not necessarily the direct action of single hormones or their metabolites.

The use of countercurrent distribution to fractionate rapidly labelled RNA has revealed several features of this RNA in rat liver: (1) alteration of the pattern of synthesis of *m*RNA can be brought about by starvation or by hormones; (2) such changes are reversible; (3) both enhanced and diminished synthesis of various *m*RNA fractions occurs; (4) most hormones tested appeared to alter *m*RNA synthesis selectively, increasing some fractions and decreasing others; (5) hormone effects varied with time and in some cases selective alteration of *m*RNA synthesis was recognizable after a very short time.

These findings suggest that regulation of the synthesis of rapidly labelled mammalian RNA may be analogous to the regulation of synthesis of bacterial messenger RNA (refs. 10 and 35). While template activity has been reported for a similar fraction from rat liver[6] we have no indication that all rapidly labelled RNAs direct specific protein synthesis. However, the differential effects of changed environment (starvation) and of the small biologically active molecules (hormones) used would suggest that these RNAs are not precursors of ribosomal RNA (cf. Girard, Penman and Darnell[9]). The results clearly show the importance of fractionating the rapidly labelled RNAs for comparative purposes, rather than assessing gross quantitative change.

The exact manner in which hormones exert selective effects on gene transcription is not clear but the very early changes brought about by some of these hormones could indicate some direct action with the DNA-protein complex where the *m*RNA is synthesized. It could be that hormones interact with repressor proteins in an allosteric manner[36] resulting in altered patterns of synthesis of specific messenger RNAs. It is also possible that some of these rapidly labelled RNAs act directly as repressors at the DNA or ribosomal levels, or that they code for repressor proteins. Further fractionation followed by biological characterization of this RNA should permit a more precise definition of the molecular basis of these aspects of hormone action.

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RELATIONSHIP OF LIVER COMPOSITION TO INTENSITY OF PROTEIN METABOLISM IN DIFFERENT MAMMALS

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AMONG mammals, the intensity of protein metabolism per unit of body-weight becomes progressively smaller with increasing size of species[1]. This is demonstrated by the rate of exchange of nitrogen-15-labelled amino-acids with tissue proteins, by the amount of urinary nitrogen excreted by animals receiving a protein-free diet (endogenous urinary nitrogen), and by the rate of turnover of plasma albumin in various species[1]. Each of these parameters of protein metabolism bears a relationship to body size that follows approximately the 0·7 power of body-weight. Consequently, the intensity of protein metabolism per kg body-weight is about five times greater in an adult rat of 200 g weight than in an adult man weighing 70 kg.

It might be anticipated that these differences in protein metabolic rates of mammals would affect the concentrations of nucleic acids in their tissues, since the nucleic acids are concerned with protein synthesis. Although the published literature[2] contains many estimates of the RNA and DNA concentrations made on mammalian tissues, the considerable errors[3] inherent in most of the assay procedures used in these investigations make strict comparisons between species impossible. We have therefore examined the concentrations of nucleic acids and other cell constituents in the livers of a range of mammals using a uniform procedure, and have related the findings to body size and to intensity of protein metabolism.

The livers examined were those of adult mice, rats, rabbits, dogs and cows receiving their normal diet. Pieces from different lobes of the liver were excised at death. The portions from the liver of 2–6 animals of each species were then pooled for analysis. These were first homogenized in 0·25 M sucrose. Part of the homogenate was retained for whole tissue analysis, and the remainder was spun in a refrigerated centrifuge at 8,500*g* for 10 min to remove nuclei, mitochondria and unbroken cells. The supernatant fraction from this spin was centrifuged for 60 min at 105,000*g* to precipitate the microsomes, which are remnants of the endoplasmic reticulum of the intact cell.

The whole homogenate and the microsome fraction were analysed for nucleic acids, protein and phospholipid content. A modification[4] of the Schmidt–Thannhauser procedure was used for analysis of RNA and DNA. Protein content was estimated after treating samples of the homogenate or microsome fraction with 10 per cent trichloroacetic acid; the nitrogen content[5] of the precipitated material, corrected for nucleic acid and phospholipid nitrogen, provided a measure of the amount of protein. Phospholipid content was obtained by extracting portions of the trichloroacetic acid precipitate with a series of organic lipid solvents[6] and estimating[7] the phosphorus contained in the combined extracts.

Table 1 shows the total amounts of various liver constituents. When expressed per kg body-weight, individual constituents are seen to diminish with increasing size of species. In order to obtain equations describing the effect of body size, the logarithms of the total amount of each liver constituent were plotted against the logarithm of body-weight. In each instance, the relationship approximated closely to a straight line, from which the exponents (Table 2) relating each liver constituent to body-weight were obtained by computing regression equations.

Table 1. LIVER WEIGHT AND COMPOSITION IN RELATION TO BODY-WEIGHT IN A SERIES OF MAMMALS OF DIFFERENT BODY SIZE*

Species	Body-weight (kg)	Total liver content per kg body-weight				
		Wet weight (g)	Protein (g)	Phospholipid (mg)	DNA (mg)	RNA (mg)
Mouse	0·03	53	10·8	1,800	126	560
Rat	0·16	35	7·3	1,190	122	375
Rabbit	2·05	35	6·8	1,000	88	207
Dog	16·0	24	5·6	770	80	138
Cow	400·0†	10	1·9	360	41	53

* Livers from 2–6 animals of each species were analysed.
† Approximate mean weight of group.

Although the various measurements of intensity of body protein metabolism are related to approximately $\mathrm{Wt}^{0\cdot7}$ (Table 2), the total weight of the liver does not diminish so rapidly with increasing body size. From our results, this relationship is expressed by $\mathrm{Wt}^{0\cdot84}$ and from Brody's[8] compilation by $\mathrm{Wt}^{0\cdot87}$. Several liver constituents (protein, phospholipid and DNA content) follow the same relationship to body-weight, but the RNA content of the liver declines as $\mathrm{Wt}^{0\cdot75}$, and is thus more closely related to the effect of body size on body protein metabolism.

The more marked effect of body size on the RNA content of the liver cell is also demonstrated by computing the RNA/DNA and phospholipid/DNA ratios for the livers of different species (Table 3). The extensive decline in the RNA/DNA ratio with increasing body-weight can be contrasted with the relatively slight influence of body size on the phospholipid/DNA ratio (Table 3). The difference is also seen on analysis of the microsome fraction (Table 3), in which a considerable reduction in the percentage of RNA in the microsomes of the larger mammals is accompanied by a small increase in their phospholipid content. Phospholipid is a component of the membranous portion of the microsomes, whereas RNA is contained in the ribosomes attached to the membrane surface. Our observations thus suggest that, in the liver cells of larger animals, there are fewer ribosomes attached to the membranous part of the endoplasmic reticulum, from which the microsomes are formed on homogenization.

Table 2. EFFECT OF BODY SIZE OF MAMMALS ON THE INTENSITY OF PROTEIN METABOLISM AND ON THE TOTAL AMOUNTS OF VARIOUS LIVER CONSTITUENTS

Measurement	Exponent* relating measurements to body-weight
Metabolic measurements‡	
Endogenous urinary N (mg N/day)	0·72
Total body protein synthesis (mg ^{15}N incorporated/day)	0·75
Plasma albumin turnover (days^{-1})	0·66
Total liver constituents†	
Wet weight (g/liver)	0·843
Protein (g/liver)	0·835
Phospholipid (mg/liver)	0·842
DNA (mg/liver)	0·884
RNA (mg/liver)	0·755

* This is the exponent $^{(x)}$ in the equation: observed quantity per animal = a constant X (body-weight)^{x}. It is computed from the slope of the line relating the logarithm of the observations to the logarithm of body-weights.
† Computed from the results given in Table 1. The relationship of liver weight to body size, based on a survey of published data, was found by Brody[8] to follow $\mathrm{Wt}^{0\cdot87}$. From an examination of female mice, rats and guinea pigs, Kosterlitz[9] obtained an exponent of 0·85 for the relationship of the DNA content of the liver to body-weight.
‡ For references, see Munro[1].

Table 3. PLASMA ALBUMIN TURNOVER, LIVER CELL COMPONENTS PER mg DNA, AND COMPOSITION OF LIVER MICROSOMES OF DIFFERENT MAMMALS

Species	Half-life of plasma albumin* (days)	Whole liver composition† (mg/mg DNA)		Microsome fraction (per cent of estimated constituents)		
		RNA	Phospholipid	RNA	Phospholipid	Protein
Mouse	1·2	4·45	14·2	9·6	24·2	66·2
Rat	2·5	3·05	9·8	7·3	22·9	70·0
Rabbit	5·7	2·44	11·6	6·2	29·2	64·5
Dog	8·2	1·69	9·4	—	—	—
Cow	20·7	1·29	8·7	5·9	31·3	62·7

* Results reported by Dixon, Maurer and Deichmiller[10]; the value for the rat is that given by Allison[11].
† Computed from the results given in Table 1.

The endoplasmic reticulum of the liver cell is considered to function in the formation and secretion of plasma proteins. The rate of formation of plasma albumin diminishes with increasing body size (Table 3), and our findings indicate a parallel decrease in the total RNA content of the liver and in the amount of RNA in the microsomes. Absence of a similar correlation with the amount of phospholipid contained in the cell and in the microsomes suggests that, although phospholipid is also a constituent of the endoplasmic reticulum, it is less directly concerned with protein formation.

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MULTIPLE FORMS OF CHOLINESTERASE IN HORSE PLASMA

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THE number of publications dealing with multiple forms of various enzymes has increased rapidly since the introduction by Smithies[1] of starch-gel electrophoresis. Using this technique, Markert and Moller[2] separated lactic dehydrogenase into several molecular forms which they named 'isozymes'. Multiple forms of serum esterases and cholinesterases also have been reported to occur in human and animal sera[3-6]. In extensive human family investigations, Harris, Hopkinson and Robson[7] demonstrated electrophoretically a new genetically controlled variant of serum cholinesterase. This article is concerned with multiple forms of equine plasma cholinesterase, some biochemical characteristics and a genetically controlled variant.

Plasma samples were obtained from 94 horses and the plasma esterases were separated by vertical starch-gel electrophoresis[8] using the discontinuous buffer system[9]. The esterases were characterized using as substrates: α-naphthyl acetate, β-naphthyl acetate, α-naphthyl propionate, α-naphthyl butyrate, α-naphthyl caprylate, naphthol AS acetate, naphthol AS-D acetate and β-carbonaphthoxycholine iodide. Eserine and TEPP were used as inhibitors. Each fraction was eluted from the starch gel using *tris* buffer and its activity was determined spectrophotometrically[10] using benzoylcholine as the substrate. A 'zymogram' is shown (Fig. 1) of the esterase zones detected following electrophoresis and staining with α-naphthyl acetate and naphthanil diazo blue B in phosphate buffer *p*H 6·8

Seven zones, C_1, C_2, C_3, C_4, B, A_1 and A_2, were present in all equine plasma samples examined, C_2 usually being a doublet. Two other zones, C_5 and D, were present in only 4 and 12, respectively, of the samples. Since zones C_1, C_2, C_3, C_4 and C_5 hydrolysed all substrates and benzoylcholine and their activities were inhibited by eserine and TEPP, 10^{-5} M, they were considered to be multiple forms of pseudocholinesterase.

Zone B hydrolysed both the short- and long-chain fatty acid esters but was insensitive towards β-carbonaphthoxycholine iodide and benzoylcholine. It was not inhibited by eserine, 10^{-5} M, but was inhibited by TEPP, 10^{-4} M. Thus it was concluded that zone B contained an aliesterase but not a cholinesterase.

Zone A_2 hydrolysed α- and β-naphthyl acetate, naphthol AS acetate and naphthol AS-D acetate, but not the choline esters, and was not inhibited by eserine or TEPP, 10^{-5} M ~ 10^{-4} M. It appeared that this zone contained an arylesterase but not a cholinesterase.

Zone A_1 had a mobility corresponding with that of albumin. It hydrolysed α- and β-naphthyl acetate, naphthol AS acetate and naphthol AS-D acetate, but did not hydrolyse choline esters. Its activity was not inhibited by eserine or TEPP, 10^{-5} M ~ 10^{-4} M. This zone, containing an aromatic esterase-like activity, probably corresponds to the 'albumin esterase' in human plasma reported by Wilde *et al.*[11].

Zone D hydrolysed only the four short-chain fatty acid esters, α- and β-naphthyl acetate, α-naphthyl propionate and α-naphthyl butyrate, but did not utilize β-carbonaphthoxycholine iodide or benzoylcholine. Its activity was inhibited by TEPP, 10^{-5} M, and eserine, 10^{-4} M, but was partially resistant to eserine 10^{-5} M. It was concluded that this zone was a type of ali-esterase differing from zone B.

Zone LP, a lipoprotein, had the least mobility of the zones observed. It stained slightly with β-naphthyl acetate-dye solution and was identified using sudan black.

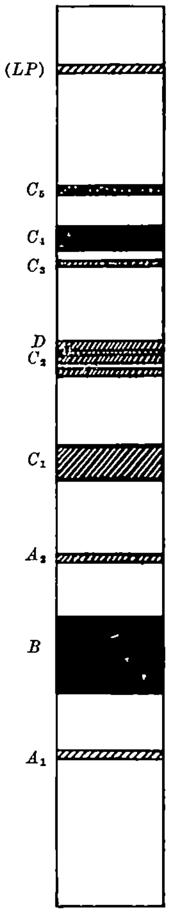

Fig. 1

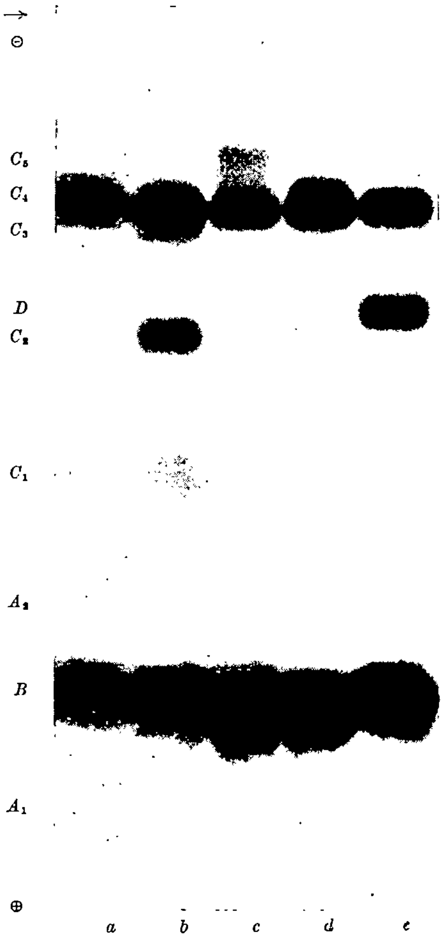

Fig. 2

Fig. 1. Esterase zymogram of horse plasma

Fig. 2. Variations of esterase patterns and activities. (*a*) Normal esterase pattern (light horse); (*b*) highly active zones C_1 and C_2; (*c*) genetically determined variant zone C_5; (*d*) normal esterase pattern (draft horse); (*e*) highly active zone D

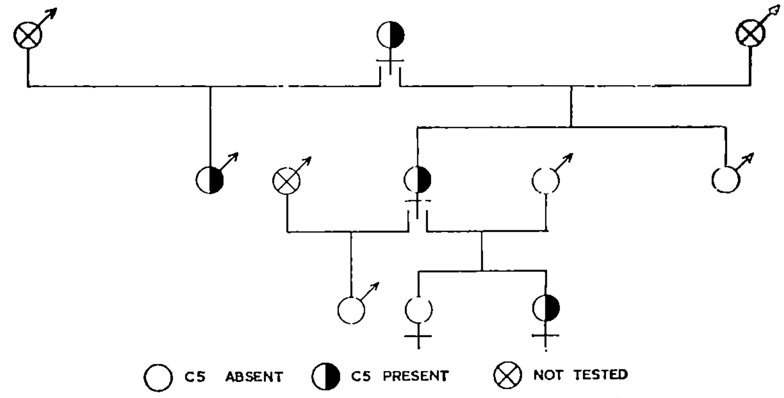

Fig. 3. Pedigree of family in which zone C_5 is segregating

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The hydrolysis of α-naphthyl acetate by zones C_1, C_2 and D was usually weak (Fig. 2*a* and *d*). However, the plasma of certain individuals contained at least one of these zones with considerably greater activity than the average (Fig. 2*b* and *e*). This property appeared to be a constant individual characteristic which was not related to age, sex or breed.

The esterase patterns of the thoroughbred, standardbred, quarter horse, Arab and pony, that is, light horses, were similar, and all lacked zone D, which was common to two of the draft breeds, Belgian and Percheron (Fig. 2*d* and *e*), but not the third, Clydesdale.

Zone C_5 (Fig. 2*c*) was present in only four of the 94 samples examined, these four being members of the same family (Fig. 3). This zone appeared in fresh plasma samples obtained over a six-month period and was not affected by incubation or freezing. The family investigation suggested that this zone, a cholinesterase, may be genetically controlled; each individual may be a heterozygote for an autosomal gene which determines the presence of this component as a phenotype.

From these preliminary investigations, it appeared that: (1) There were four zones of cholinesterase activity in all samples examined; one of these, C_2, was usually a doublet. (2) Zone C_5, a variant form of cholinesterase, may be genetically controlled. (3) Zone B, an ali-esterase, hydrolysing short- and long-chain fatty acid esters, was present in all samples. (4) Zone D, a type of ali-esterase utilizing short-chain fatty acids only, was present in two of three draft breeds examined. (5) Equine plasma contains two arylesterases, one probably corresponding to 'albumin esterase' of human plasma.

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MECHANISMS OF INACTIVATION OF SINGLE-STRANDED VIRUS NUCLEIC ACIDS BY HEAT

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THE two classes of infectious single-stranded virus nucleic acids, exemplified by RNA of tobacco mosaic virus (TMV) and by φ*X*174-DNA, are found to exhibit a marked difference in their response to heat in the physiological *p*H range. This is reflected in the gradual decay of both infectivity and molecular integrity of RNA on storage under conditions in which the single-stranded DNA is stable. Instability and the attendant molecular heterogeneity have in large measure contributed to the difficulty in past attempts to measure the size of the intact and infectious TMV–RNA molecules[1,2]. Heterogeneity could arise from random scission of phosphodiester bonds which hold constituent ribose-nucleotides in a single continuous chain. Kinetic evidence has been presented earlier to suggest that spontaneous decay of TMV–RNA is related to the inherent susceptibility of its phosphodiester bonds to heat-induced hydrolysis[3]. Work of Gordon and Huff[4] and of Eigner, Boedtker and Michaels[5] support this conclusion. However, it is still widely believed that the instability is due mainly to seemingly ubiquitous nucleases carried over into RNA preparations as contaminants.

On the other hand, phosphodiester bonds of single- and double-stranded DNA are stable under thermal treatment at neutral *p*H, but DNA in either form is relatively sensitive to depurination[6-8]. A loss of a single purine is sufficient to destroy the function of a single-stranded cyclic φ*X*174–DNA[8] and presumably that of a genetic marker in transforming DNA[7].

We examine here the kinetics of inactivation of three single-stranded nucleic acids, TMV–RNA, *R*17–RNA and φ*X*174–DNA, under comparable conditions of thermal treatment. The results obtained over a range of *p*H and temperature indicate that the two classes of nucleic acids are inactivated by distinctly different first-order processes. They are consistent with the conclusion that cleavage of a single phosphodiester bond inactivates the RNAs while a single depurination inactivates φ*X*174–DNA.

Preparation of virus nucleic acids. TMV–RNA, *R*17–RNA, and φ*X*174–DNA were prepared essentially by the method of Gierer and Schramm[9], except that the nucleic acid extracts following the phenol treatment were precipitated repeatedly with 95 per cent ethanol to eliminate traces of phenol. Reagent-grade phenol was washed five times with 10^{-4} M versine before use. The parent viruses were purified by differential centrifugation. Heat sensitivity of the nucleic acids obtained from them was found to be independent of the purification steps taken prior to the final centrifugation of the viruses.

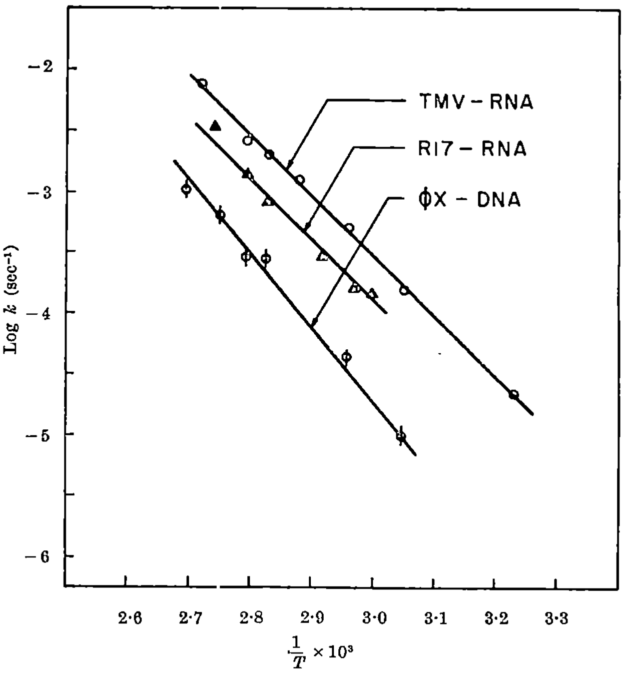

Fig. 1. Temperature-dependence of the rates of inactivation of TMV-RNA, *R*17-RNA and φ*X*174-DNA by heat in 0·1 M sodium phosphate buffer, *p*H 7·15

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Bioassays of these nucleic acids were conducted in manners described in our previous papers[3,11,12].

TMV–RNA, *R*17–RNA and φ*X*174–DNA are all heat-inactivated by a first-order rate law at each temperature and *p*H examined. Their rates show no obvious dependence on concentration at which they are heated. The rate constants of inactivation of the nucleic acids in 0·1 M sodium phosphate buffer at *p*H 7·15 are plotted against temperature and are summarized in Fig. 1. It is immediately apparent that TMV–RNA, though only 1·2 times larger in size than φ*X*174–DNA, is more sensitive to heat than the latter by a factor ranging from 10 at the higher experimental temperature to about 30 at 37°. *R*17–RNA is intermediate in position, its rates of kill being nearly one-half that of TMV–RNA at all temperatures examined. The ratio of the molecular weight of TMV–RNA[11,13–15] to that of *R*17–RNA, $2·0 \times 10^6/0·8 \times 10^6 = 2·5$, corresponds well to the ratio of their rate constants, 2·3. The rates, for example, at 80° for TMV–RNA, *R*17–RNA and φ*X*174–DNA are $1·93 \times 10^{-3}$/sec, $8·3 \times 10^{-4}$/sec and $2·83 \times 10^{-4}$/sec, respectively.

This correlation, together with the closeness of the thermal constants (Table 1) derived from the plot (Fig. 1), suggests that the two RNAs are inactivated by the same mechanism but with rates in direct proportion to their chain-lengths. If such a proportionality is meaningful, it would predict the much smaller, soluble RNA to be a great deal more stable to heat inactivation. Indeed, a very close correspondence has been observed[16]. Gordon *et al.*[17] also showed that TMV–RNA and polio-virus RNA possessing the same molecular weight[1] are heat inactivated at about the same rate.

Table 1

	Medium	*p*H	ΔH^* (kcal/mol.)	ΔS^* (E.U.)	Temp.-range
TMV-RNA	0·1 M phosphate buffer	7·15	+23	−11	37°–95°
*R*17-RNA	,,	7·15	+22	−11	60°–92°
φ*X*174-DNA	,,	7·15	+27	0	55°–98°
*R*17-RNA	Michaelis buffer*	4·2	+35	+20	55°–80°
	,,	5·4	+29	+10	60°–90°
	,,	6·7	+25	−7	60°–90°
	,,	7·5	+29	+5	70°–90°
	,,	8·6	+35	+26	60°–80°
φ*X*174-DNA	,,	4·2	+27	+7	50°–65·5°
	,,	5·4	+29	+17	50°–79°
	,,	6·7	+35	+22	65·5°–95°
	,,	7·5	+35	+21	60°–95°
	,,	8·6	+34	+16	70°–95°
TMV-RNA	0·1 M Na phosphate buffer	5·8	+22	−9	55°–70°
TMV-RNA *S.D.S.* prep.†	,,	5·8	+22	−13	47°–75°
,,	,,	7·15	+19	−19	37°–65°
,,	Michaelis buffer*	8·5	+32	+26	40°–65°

* Michaelis buffer (ref. 18).

† Sodium dodecyl sulphate used for deproteinization (ref. 3).

The second feature of interest is the magnitude of the thermodynamic constants, ΔH^* and ΔS^*, derived from the Arrhenius curves shown in Fig. 1. Our previous values for ΔH^* and ΔS^* were 19 kcal/mol. and −19·5 E.U., respectively, for TMV–RNA prepared by using sodium dodecyl sulphate for deproteinization of the parent virus[3]. The thermal constants for the phenol-prepared *R*17–RNA, however, are in close agreement with the present values for the similarly prepared TMV–RNA. The values for φ*X*174–DNA, particularly the small ΔS^*, suggest a significant difference from those of the two RNAs at the same *p*H, 7·15 (see Table 1 for values at other *p*Hs). The moderate values of ΔH^* obtained for both types of nucleic acids are consistent with inactivation mechanisms involving breakage of a single covalent bond as opposed to co-operative rupture of a large number of hydrogen bonds characteristic of a denaturation reaction. This observation should be taken into consideration when discussing the relevance of secondary structure, reported to exist in various types of RNA[2], to function.

The third notable feature of the Arrhenius plots for the RNAs is their linearity over a wide range of temperature, particularly for TMV–RNA. This suggests that the single-reaction mechanism apparently operating on the RNA over this temperature range is not enzymatic, unless the temperature of maximum enzyme activity of the hypothetical contaminant is 96° or higher.

The experimental results showing the dependence of heat-inactivation rates of *R*17–RNA and φ*X*174–DNA on *p*H are summarized in Figs. 2 and 3. Inactivation rates were measured in Michaelis's 'universal' buffer[18], which circumvents possible specific effects of the buffer ions while keeping the ionic strength reasonably constant over the experimental *p*H range. It is seen from comparing the two plots of rate constants versus *p*H, for example at 70°, that there is a striking contrast between RNA and DNA in their response to *p*H (Fig. 4). For *R*17–RNA, as

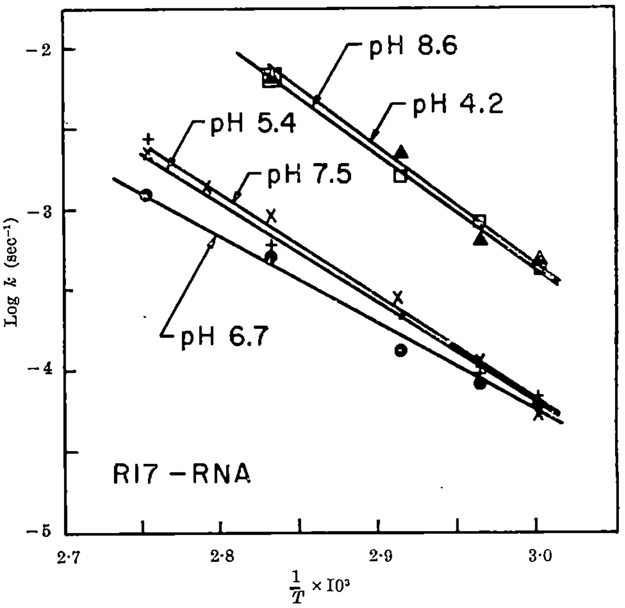

Fig. 2. Temperature-dependence of the rates of heat-inactivation of *R*17-RNA at various *p*H in Michaelis buffer (ref. 18)

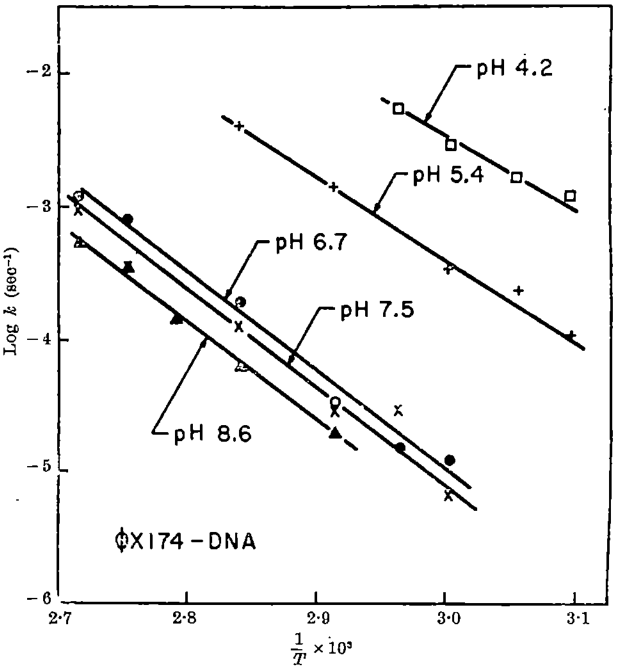

Fig. 3. Temperature-dependence of the rates of heat-inactivation of φ*X*174-DNA at various *p*H in Michaelis buffer

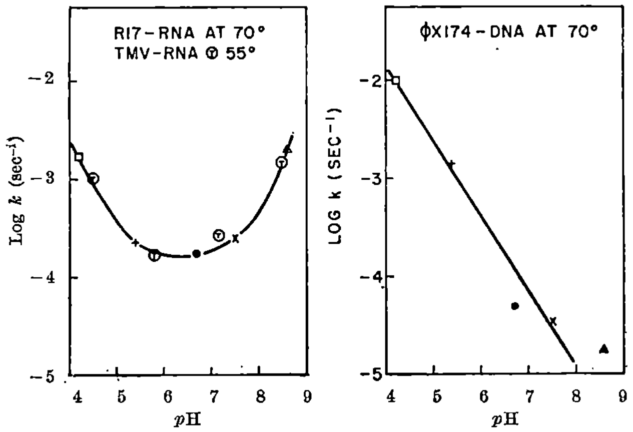

Fig. 4. pH dependence of the rates of inactivation of R17-RNA at 70°, TMV-RNA (T) at 55° and φX174-DNA at 70° in Michaelis buffer. Rate constants for R17-RNA and φX174-DNA are transposed from Figs. 2 and 3

well as for TMV–RNA, the pH of maximum stability is 6–6·5 with progressively increasing sensitivity on either side of this range. This is to be expected if we are to assume that hydrolytic scission of a phosphodiester bond is the cause of the inactivation, for it is known that hydrolytic degradation of RNA is both acid- and alkali-catalysed[19]. The corresponding curve for φX174–DNA shown in Fig. 4, on the contrary, shows that DNA is most stable at the highest pH tested, 8·6, and shows an increasing heat sensitivity with decreasing pH. Vinograd[20] and Fiers and Sinsheimer[8] have shown that φX174–DNA is stable even at pH 12 and, moreover, the latter's data indicate that the inactivation rate of this DNA increases approximately as the first power of the hydrogen ion concentration. Our results in a different buffer system recorded here confirm their findings (Fig. 4). This behaviour is expected from the known chemistry of depurination. The thermal constant, $\Delta H^* = 35$ kcal/mol. at pH 7·5, compares with 34 kcal/mol. over a temperature range of 91°–100° for the single-stranded, transforming DNA in pH 7·15 sodium phosphate buffer[7], and with 35 kcal/mol. for φX174–DNA in glycine buffer, pH 9·0 (ref. 8). In the latter systems depurination was shown to be the most likely cause of inactivation, the reaction course of which follows a 'one-hit' kinetics, and also their evidence shows that chain cleavage is not necessarily a concomitant process, although the phosphodiester bond at the site of depurination may be weakened[21].

It is to be recalled that transforming DNA is heat-inactivated by two principal mechanisms, namely, depurination and denaturation[7,24]. Depurination at temperatures below the melting transition (< 87°) requires very high activation energy as indicated by a rapid decrease in the inactivation rates with decrease in temperature (ref. 7, Fig. 6). This points to a necessity for localized and reversible chain opening of the double helix before a glycosidic bond of a purine can be hydrolysed. Accordingly, the depurination rate for double-stranded DNA is expected to be immeasurably slow compared at 37° to that of the single-stranded φX174–DNA.

It is also worthy of note that the pH of maximum stability for TMV–RNA, as well as for R17–RNA, is close to the pH of maximum activity of tobacco leaf RNase[22,23], a possible contaminant in the former preparation. This fact, taken together with the linearity of the Arrhenius plots shown in Fig. 1 and the reproducibility of the inactivation rates among numerous RNA samples, eliminates nucleases as agents responsible for the inactivation kinetics observed in this series of experiments, at least in the case of TMV–RNA.

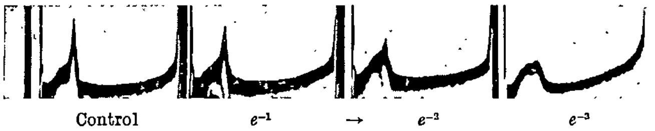

Fig. 5. Ultracentrifuge patterns of TMV-RNA (0·036 per cent in 0·02 M sodium phosphate buffer, pH 7·0) at various levels of survival following heat treatment at 65° in 0·1 M sodium phosphate buffer, pH 7·15. Each picture was taken 64 min after the rotor speed reached 24,630 r.p.m. Bar angle, 15°

In the case of RNA it seems possible that physical methods could provide more direct evidence that chain scission results from one inactivating hit by heat, as single scission should effect a drastic change in its molecular weight. Attempts at correlating the kinetics of biological inactivation of TMV–RNA to physical degradation have been reported[2,3a,15]. Interpretations of these results are, however, beset with serious difficulties, because the usual RNA preparations on which the foregoing kinetic investigations are performed are polydisperse to begin with and the demonstrable biological activity is due to a fraction (~ 0·1 per cent) of the population which is not detectable by conventional physical tools. For example, $S_{20,w}$, usually obtained for the leading main peak seen with the schlieren optics, is 25, from which we calculate a weight average molecular weight of about $1{\cdot}3 \times 10^6$, whereas the molecular weight of the intact biologically functional RNA is $2{\cdot}2 \times 10^6$ (ref. 11). This fact, incidentally, is one of the reasons for the application of radiation target analysis[11]. However, assuming that the physical characteristics of the bulk RNA reflect the properties of the infectious molecules, we have followed the course of molecular degradation by analytical ultracentrifugation and viscometry of TMV–RNA samples heat-inactivated in 0·1 M sodium phosphate buffer, pH 7·15, to e^{-1}, e^{-2} and e^{-3} surviving population. It is found (Fig. 5) that the leading peak decreases in area progressively with each inactivating hit with a concomitant increase in the trailing more diffuse peak, while the intrinsic viscosity falls from 1·92 for the unheated control to 1·59 dl./g for the sample receiving 3 inactivating hits. Allowing the assumption made here, these results support the conclusion that heat inactivation is a direct consequence of a chain scission. Moreover, on very prolonged heating of TMV–RNA in the same buffer but at higher temperatures, poly- and mono-nucleotides and nucleosides but no free bases are found, whereas under comparable treatment calf-thymus DNA yields the expected free purines. This demonstrable stability of glycosyidic bonding of purines to ribose excludes depurination as a significant factor in heat inactivation of RNA.

The striking difference in the behaviour of the phosphodiester bonds of the two types of nucleic acids arises essentially from their chemical difference, namely, that RNA possesses a 2′ OH function in its sugar moiety whereas DNA does not. The effect of acid-base catalysis on the rate of hydrolysis of the phosphodiester bonds of RNA has been long recognized, as has the stability of DNA in alkaline medium. On the basis of their work with model compounds, α- and β-glycerophosphates, Brown and Todd[25] proposed the reaction sequence in Fig. 6 to explain the special lability of the phosphodiester bonds of RNA.

They postulated that the formation of the cyclic phosphate-triester intermediate, B, is essential in order to explain the production of the two isomers, E and F, following alkaline hydrolysis of RNA. The formation of B is possible because of the 2′ OH function, and product C results because of the instability of the triester structure, in which one of three ester linkages becomes sensitive to hydrolysis. Product C, being a diester, is relatively stable at neutral pH and in moderately alkaline medium[4,26].

The thermal constants for both TMV–RNA and R17–RNA (Table 1) are consistent with the Brown and Todd reaction mechanism. The negative entropy of activation, ΔS^*, derived for TMV–RNA and R17–RNA, agrees with the formation of cyclic structure B, the activated state of

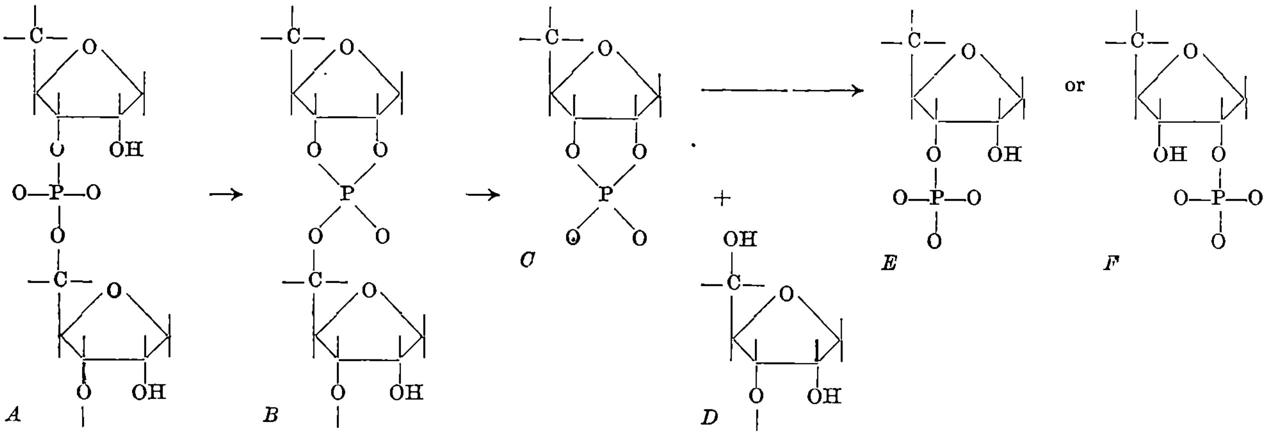

Fig. 6

the RNA molecule, which prepares the phosphodiester backbone for hydrolytic cleavage[3]. Experimental evidence for the involvement of the cyclic intermediate in heat-inactivation of TMV–RNA is shown in the recent work of Gordon and Huff[4].

The entropy of activation for the two RNAs on either side of neutrality (Table 1) becomes increasingly more positive in apparent contradiction to the cyclic mechanism. The reason for the rise may be that acid and base catalysis greatly increases the rate of cyclization so that hydrolysis of the third ester bond, rather than the formation of the cyclic triester, becomes the rate-limiting step in the inactivation (cf. ref. 19).

The demonstrated propensity of the phosphodiester bonds of RNA to disjoint easily without the mediation of enzymes leads us to consider its possible bearing on the short life[27] of messenger-RNA in microbial systems; for it is conceivable that the function of *m*RNA, like the viral RNA *in vitro*, can be disrupted by a single, random scission of these bonds.

As for DNA, in particular at 37°, we conclude that: (1) it is 30 times less susceptible in the single-stranded form to heat-inactivation by depurination than is a comparable-sized RNA to inactivation by phosphodiester backbone scission; (2) because of the large entropy term involved, depurination rate of the double-stranded DNA should be immeasurably slow relative to that of the single-stranded form at this temperature.

We thank Miss Jean E. Kinnear for the ultra-centrifugal analyses and Drs. William Taylor and Joseph Steim for their advice. Part of this work was performed at the Department of Biophysics and Nuclear Medicine, University of California at Los Angeles.

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OPTOKINETIC NYSTAGMUS: AN UNLEARNED RESPONSE ALTERED BY SECTION OF CHIASMA AND CORPUS CALLOSUM IN MONKEYS

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MUCH work has been done in recent years on the role of the corpus callosum in the so-called interhemispheric transfer of learning[1]. Little attention has been directed, however, to the influence of commissural systems in other forms of behaviour, particularly in those which depend scarcely, if at all, on past experience. This article shows that the section of the corpus callosum also disrupts an unlearned response such as optokinetic nystagmus. In this type of behaviour there is a visual input consisting of successively moving stimulus-figures across the visual field, and an oculomotor output manifested by slow conjugate eye deviations in the direction to which the stimuli move, alternating with similar fast movements to the opposite side. Conventionally, the direction of the response is named by the quick phase. In the monkey, output usually outlasts the duration of the stimulus and this is known as optokinetic after-nystagmus.

In primates, as well as in most vertebrates, visual input from homonymous (right or left) half fields of both eyes is conveyed to the opposite half of the brain in a totally crossed fashion[2]. Similarly, pathways for conjugate gaze descend from most of the cerebral cortex towards the brain stem and decussate completely about the level of the third nerve nucleus, as demonstrated in numerous ablation and stimulation experiments[3]. From this latter work it also followed that the efferent pathways responsible for the fast phase of optokinetic nystagmus are the same as those for conjugate gaze. Fig. 1 is a diagram which summarizes some of these functional

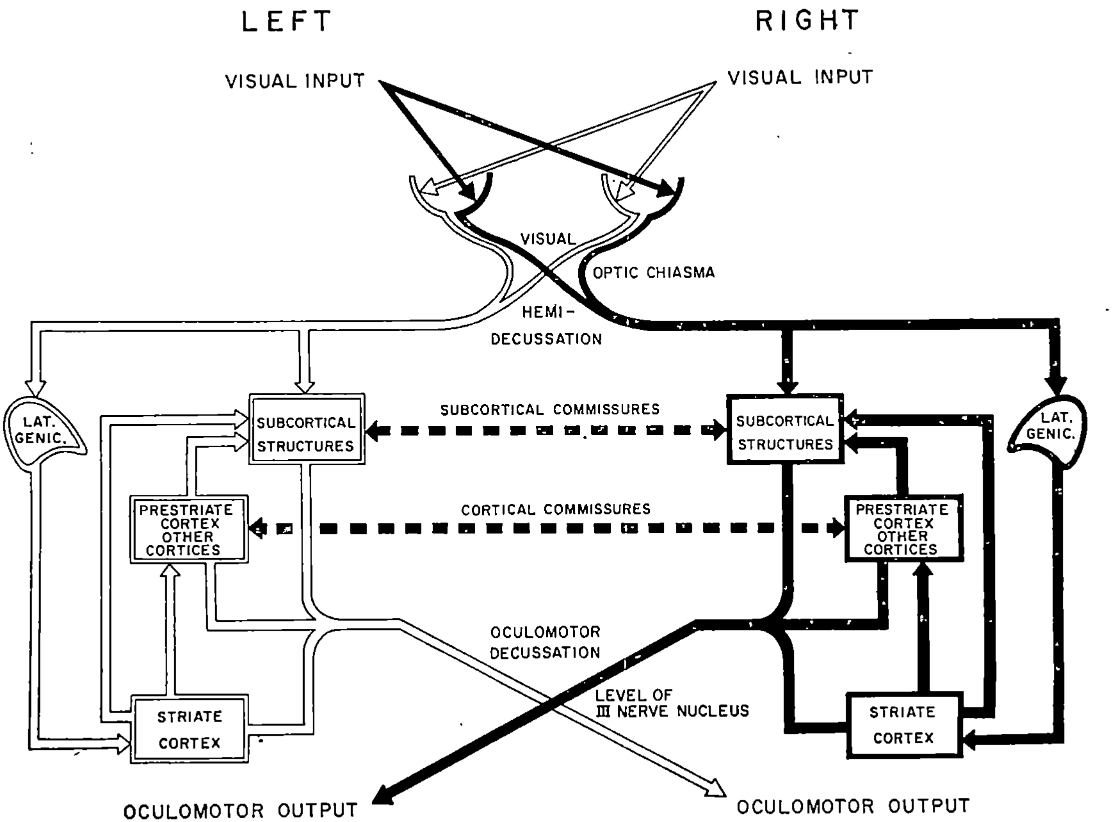

Fig. 1. Functional diagram of visual and oculomotor systems in the monkey. Solid black indicates visual input from the left, reaching the right side of the brain where it initiates oculomotor output to the left (conjugate gaze, fast phase of optokinetic nystagmus). Open lines represent just the opposite functions. Dashed black indicates commissural systems

facts about the visual and oculomotor systems. It should not be taken as representing strict demonstrable anatomic connexions, many of which are still in the process of study and revision. Specifically, visual input from the left field reaches the two right hemi-retinæ and through the hemidecussation at the chiasma conveys the information to the right hemisphere at the cortical level (via the lateral geniculate nucleus to striate cortex) and at the subcortical level (several known and many uncertain structures). The striate cortex projects to the prestriate region and to subcortical areas. Oculomotor outflow may be initiated from striate, prestriate, most of the rest of the cortex as well as from subcortical structures and conveyed through pathways that cross totally about the level of the third nerve nucleus, to drive the eyes to the left. In addition there are commissural systems at cortical and subcortical levels.

While the input and output, that is, visual stimulus and oculomotor response, are rather clear in optokinetic nystagmus, the events in between, the integrating mechanisms inside the central nervous system, are still unknown. Some years ago, we examined the effect of cortical ablations on optokinetic nystagmus in the monkey. Contrary to what might be expected from the previous considerations, unilateral total removal of striate cortex failed to cause any deficit in the response to the side opposite the lesion[4]. After right occipital lobectomy, optokinetic nystagmus was normal and symmetrical to the right and to the left.

The question arose as to how the unilateral visual stimuli are utilized to elicit an optokinetic response to the left in the absence of the right striate cortex. Two basic hypotheses may be formulated to explain this finding. They may be easily followed by referring to Fig. 1. (1) It may be that input from the left visual field is still essential. This input would reach subcortical structures on the right side of the brain which in turn would activate the oculomotor pathways that drive the eyes to the left. (2) It may be, on the other hand, that input now from the right visual field reaches the striate cortex and subcortical areas in the left side of the brain, and from here, by way of commissures, activates areas of the right side of the brain which in turn would stimulate the oculomotor pathways for conjugate gaze to the left.

The work presented here was carried out to test some of these hypotheses. Six monkeys (2·5–3·1 kg) were used. Optokinetic nystagmus was elicited by placing the animal inside a rotating cylinder, 92 cm in diameter, which filled the entire visual field with alternating 2·5-cm black and 33-cm white stripes. This drum could be rotated at constant speed by means of a servo-motor. Details of construction have already been published[3,4]. Stimulation was made binocularly and also with each eye separately with the aid of an opaque sclerocorneal contact occluder. Eye movements were recorded with an electroencephalograph through needle electrodes inserted in both outer canthi and in the bridge of the nose. Polarities were adjusted so that movements to the right produced upward deflexions, and deviations to the left caused downward deflexions. A time-constant of 0·14 sec was used throughout. Recordings were obtained before and after each operative stage. Operations consisted in the sagittal section of the optic chiasma either prior to or following similar interruption of the corpus callosum. Serial sections of all brains, stained with Nissl and myelin sheath methods, confirmed these lesions and also showed that most of the hippocampal commissure had also been severed.

Pre-operative electro-oculograms revealed that optokinetic nystagmus to the right and to the left was symmetrical when elicited by binocular and monocular stimulation. When stimulus-figures occurred at a frequency of 1 per sec (constant angular velocity: 45° per sec) response was quite constant in each animal and varied over the group from 2 to 3 nystagmic beats per sec. Following sagittal section of the optic chiasma, electro-oculograms of optokinetic nystagmus did not differ substantially from those obtained pre-operatively. More

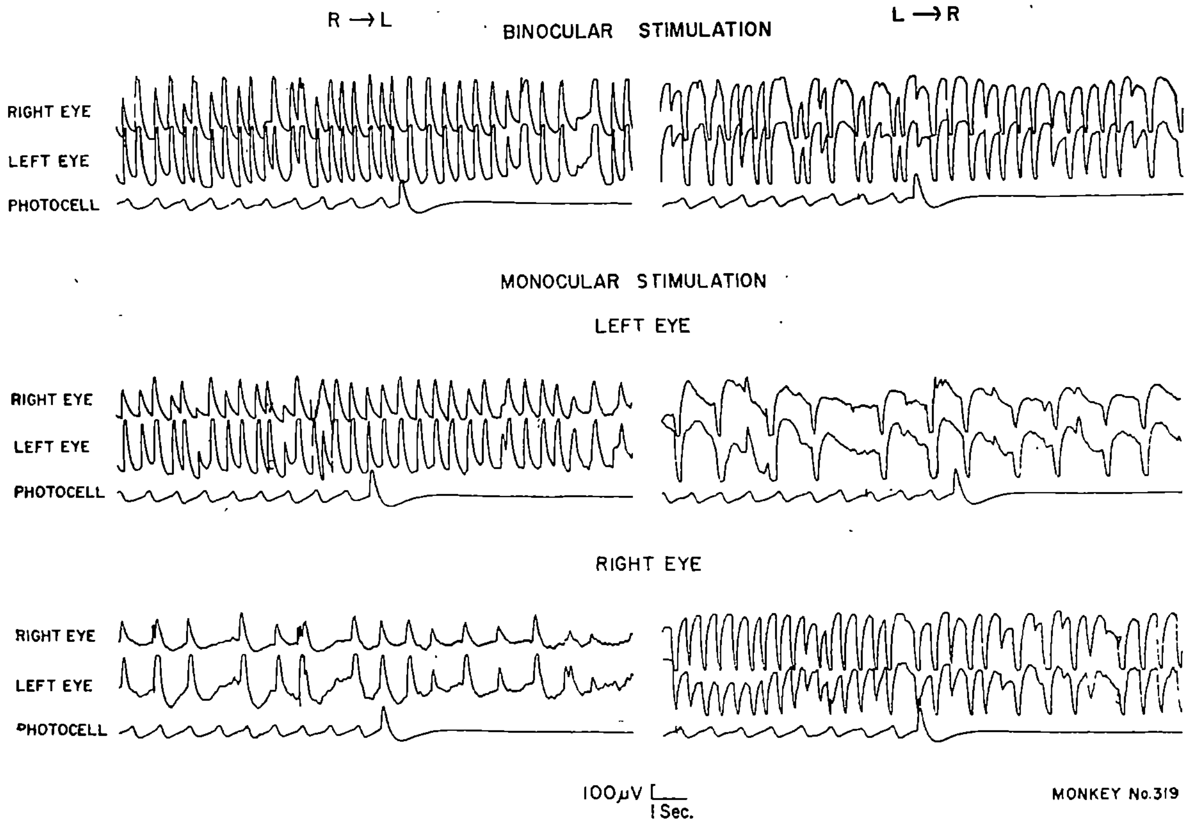

Fig. 2. Electro-oculograms of optokinetic nystagmus after section of chiasma and corpus callosum. Direction of the moving stimuli indicated by *R*→*L*, right to left, and *L*→*R*, left to right. Note the symmetry of the response elicited by binocular stimulation and the marked asymmetry under monocular stimulation. Defective nystagmus and after nystagmus occurred to the side opposite to the visually deprived hemisphere

specifically, when only the left eye was stimulated, no direct primary visual input reached the right hemisphere at a cortical or subcortical level and yet optokinetic nystagmus to the left was normal. Electro-oculograms after section of the corpus callosum in monkeys with the chiasma previously cut are found in Fig. 2. In this illustrative animal, binocular stimulation elicited a normal response with a frequency of 2·7 per sec to the right and 2·3 per sec to the left. Marked asymmetry was revealed on monocular testing. Stimulation of the left eye, that is, visual input restricted to the left hemisphere, caused a normal optokinetic nystagmus to the right, 2·4 per sec in frequency, and a slower, 1·0 per sec, nystagmus to the left. Stimulation of the right eye produced precisely the opposite effect. These recordings have been selected among those showing the least disturbance. In general, nystagmus was not only slower but also irregular, occurring in brief bursts, and at times difficult to obtain. Asymmetries were even more marked when frequency of stimulation was increased. For example, at 3 per sec stimulation (speed: 135° per sec) with both eyes open response frequency to the right and to the left was 3·3 per sec. With only the left eye open, it was 3·1 per sec to the right and 1·0 per sec to the left. These latter figures were reversed when tested with only the right eye open. It should be emphasized that in all instances the response to the same side of the stimulated eye was altered as described, but it was never altogether abolished. Section of the corpus callosum alone produced no such deficits.

These findings suggest that: (1) The first hypothesis (*vide supra*) may not be necessarily true. The response to monocular stimulation in chiasma-sectioned monkeys remained unaltered, and under those conditions, one hemisphere was totally deprived of input. Therefore, visual input to subcortical structures from the opposite field proved not to be essential for optokinetic nystagmus toward that field. (2) The second hypothesis appears to be valid. Oculomotor output for optokinetic nystagmus may be initiated in part by input to the ipsilateral hemisphere, which is conveyed through the corpus callosum to the contralateral side. The fact that some optokinetic nystagmus can still be obtained after sectioning the corpus callosum indicates that other commissural systems might play a part in this phenomenon possibly at a subcortical level.

These results are significant in two general areas of investigation: (1) The elucidation of the mechanisms for optokinetic nystagmus which we have so far discussed; (2) the problem, extensively studied by other workers, of the so-called inter-hemispheric transfer of learning. In the investigation recorded here we have a phenomenon which is not based on previous experience. The response is immediately obtained from naïve animals and man since birth as soon as stimulation starts, and it does not improve with practice. In any event, it is obvious that optokinetic nystagmus does not require any special training of the type involved in discrimination problems, and yet this unlearned response is differentially altered when elicited through one and the other eye in the chiasma-corpus callosum-sectioned monkeys. In view of these considerations, our findings raise the possibility that more elementary dysfunctions are responsible for the deficits in interhemispheric transfer after callosal section, in addition to, or maybe instead of, the postulated alteration in hypothetical mnemonic and gnosic mechanisms.

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REFLEX RESPONSES IN MAN EVOKED BY GAMMA EFFERENT ACTIVATION

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IN the course of observation of the late responses to peripheral nerve stimulation in the small muscles of the hand and foot, a response has been recorded from a small number of normal subjects bearing the characteristics of a reflex arising in the spinal cord from the activation of the muscle spindle fibres, via the high threshold γ-efferent fibres in the mixed peripheral nerve trunk (Fig. 1).

The response is found at stimulus amplitudes, maximal or supramaximal for the α-fibres, appearing at intensities which produce no further increment of the extrafusal fibre electromyogram (Fig. 2). The latency of the response varies from 36 to 45·5 msec, that is 10–15·5 msec longer than the labile '*F*' response[1] normally observed in these muscles.

In one subject where proximal stimulation of the nerve produced the same response (Fig. 1) the response was found to be at a longer latency, indicating the centrifugal nature of the initial component of the response. The difference in latencies over the length of nerve involved gave a figure for the conduction velocity of the γ-motor efferents in man of 45 m/sec. The constancy of the threshold of the response in relation to the maximal motor response suggests a transition of fibre diameter from the highest threshold extrafusal motor fibres to the lower threshold fibres innervating the muscle spindle.

The positive identification of the components of the mixed nerve participating in the response is not possible in man by direct access to the constituent fibres. Techniques have therefore been used to affect selectively first the fine fibres in the mixed nerve making use of the differential blocking effects of local anæsthesia[2,3], and secondly, the largest fibres which are the first to be affected by ischæmia[1]. Selective blocking of the reflex was obtained during the differential blocking phase of 'Xylocaine' anæsthesia, the anæsthetic being introduced into the forearm and hand by the technique described by Bier[4], Adams[5], and more recently by Holmes[6].

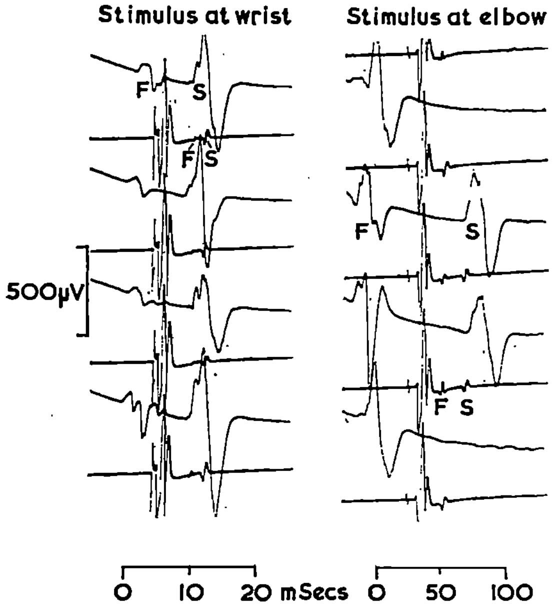

Fig. 1. Records from the first dorsal interosseus muscle showing in the expanded trace the '*F*' response followed at a constant latency by the spindle response '*S*'. With a shift of the stimulus to a more proximal point of the ulnar nerve, the '*F*' response latency is reduced while the latency of the spindle response increases

Note. The polarity of the expanded trace has been reversed.

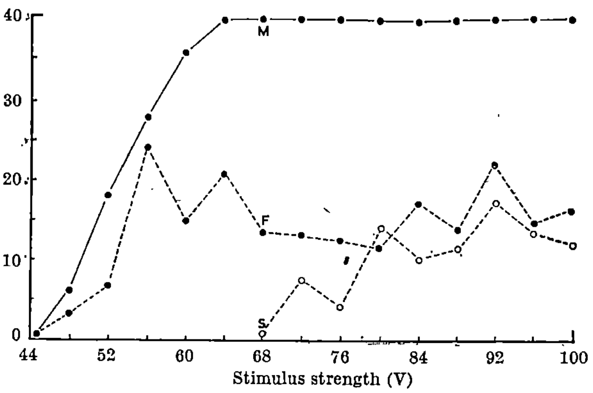

Fig. 2. The relationship between the direct motor response *M*, the '*F*' response and the spindle response '*S*'. The potentials were obtained from two bare needles inserted 2 cm apart into the muscle (first dorsal interosseus). Each point represents the mean of twenty readings. In each of the five subjects exhibiting the responses, its first appearance was made at stimulus intensities maximal or supramaximal for the alpha motor fibres

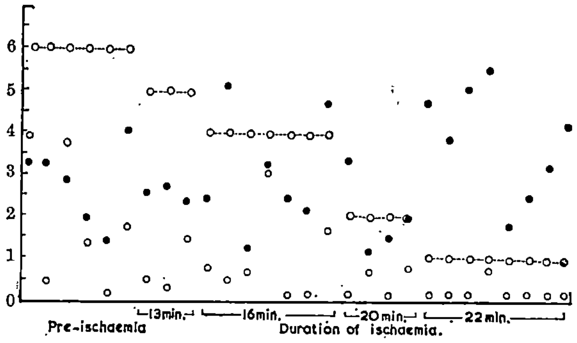

Fig. 3. Marked depression of spindle response produced by ischæmia. Response amplitude depression coincides with the fall in nerve action potential. Normal alpha motor fibres as shown by the '*F*' response. Each point the mean of ten readings

●, Response; o, spindle response; O, nerve action potential

An early depression of the response was obtained while the nerve action potential due to activity in the large 1*a* and 1*b* fibres[7] and the α-motor fibres were relatively unaffected. The depression which occurred after 3 min was not due to pressure of the cuff or ischæmia of the limb, since, as indicated in Fig. 3, which provides a control for the observation, the nerve action potential and spindle response are not attenuated for at least 10–12 min with some activity remaining after 20 min when the limb is exposed to ischæmia alone. This finding provides further evidence, in addition to the high threshold of the reflex for a fine efferent fibre component.

Interruption of the reflex pathway at the other end of the spectrum, that is, by depressing conduction in the group 1*A* spindle afferents, was obtained by occlusion of the blood supply to the forearm. Depression of the response occurs simultaneously with the attenuation of the nerve action potential while integrity of the α-fibres as evidenced by the normal '*F*' responses remains. On release of the tourniquet the return of the response coincides with the resumption of activity in the large afferent fibres, supporting the thesis that the response arises from receptors receiving an afferent supply from the large 1*A* fibres.

Exposure of the muscle to cold (immersion in iced water) produced within 5 min a marked depression of the '*S*' response. The presence of the direct motor response which although prolonged in duration by the cold was unaffected in amplitude and the persistence of the '*F*' wave indicate that the disappearance of the response was not due to failure of nerve or muscle. The sensitivity to cold is in agreement with the findings of Eldred *et al.*[8], who, working with cats, found a reduction of the response of the stretch receptors when the muscle was cooled. The beneficial effects of cryotherapy in spasticity[9] may possibly be related to this effect.

This response arising at the periphery before being attributed to activation of the intrafusal fibres of the muscle spindle must be distinguished from ephaptic activation of the sensory fibres within the terminal portion of the nerve[10,11], mechanical coupling between extrafusal fibres and the spindle[12] and other forms of sensory nerve stimulation such as that shown by Lloyd (1942) to be due to the electrical potentials produced by the depolarized muscle fibres, or antidromically conveyed sensory nerve impulses which provoke stretch receptor response[12].

With stimulation of the ulnar nerve at the wrist the response appears some 10–15 msec later than the '*F*' response. This delay is taken up by the efferent impulse travelling to the muscle and the returning afferent impulses from the muscle. Assuming an efferent delay of 5 msec at a calculated conduction velocity 45 m/sec, and an afferent latency of 3 msec, an additional interval of 2–7 msec must be accounted for. Delays of this order cannot be accounted for on the basis of an artificial synapse.

The responses in all subjects share the property of a long refractory period, varying from 20 to 100 msec. It is proposed that the conditioning volley preceding the test stimulus by an interval shorter than the rising phase of the mechanical response of the muscle unloads the spindle, rendering it insensitive to activation of the intrafusal fibres by the test stimulus. The prolongation of the refractory period in two of the subjects to 90 and 100 msec may be related to the initial muscle tension, a relationship which remains to be demonstrated. Enhancement of the response can be seen when the test response falls immediately after the rising phase of the muscle contraction. This resumption of activity at the peak of muscular contraction with, in some instances, a transient increase in spindle discharge has been observed in mammalian spindles by Hunt and Kuffler[12] and Harvey and Matthews[13]. The depression by cold, too, is unlike the behaviour of the artificial synapse where enhancement of transmission is encountered[14].

The threshold of the '*S*' response relative to the motor response excludes the explanation of this being either α-activation of the intrafusal fibres[15,16], or electrical activation of the sensory fibres arising from currents produced by muscle depolarization, or antidromic sensory impulses provoking a response from the spindle.

It is concluded that the '*S*' wave is a reflex and is obtainable, for reasons as yet obscure, in a small number of normal subjects representing evidence of a muscle spindle response to stimulation of γ-efferent fibres, which via the low-threshold spindle afferents evokes a monosynaptic response from the spinal cord. This can be recorded from the muscle as a small potential occurring at a constant latency.

A knowledge of the conduction velocity and other characteristics of the γ-efferent system in man becomes important in assessing the part which this system plays in the co-ordination or even initiation of voluntary movement in the small muscles of the hand and the extent to which this system can be incriminated in the physiopathology of Parkinson's disease and other disorders of co-ordinated muscular control.

These observations were part of a broader programme of research being carried out while I was in receipt of a research grant from the Polio Research Fund.

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A THERMOELECTRIC DEVICE FOR INDUCING LOCAL TISSUE HYPOTHERMIA AND HYPERTHERMIA

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The device described here was initially designed to cool and heat electronic components in a circuit[1]. The purpose of this article is to discuss its effectiveness in inducing tissue and organ hypothermia and hyperthermia.

A drawing and photograph of the heat pump are shown in Figs. 1 and 2. Two thermoelectric elements provide a positive or negative temperature difference, depending on the polarity of the current. This difference is related to the temperature of the aluminium heat sink which forms the body of the device. The outer surfaces of these elements are connected to the body, which is designed for efficient heat exchange and ease of handling. The inner surfaces make thermal contact with two inserted fittings, shaped to enclose the tissue or organ under study. Several types of fitting are provided, permitting attachment to organs of various shapes and sizes. The fittings are adjustable to allow a narrow opening so that afferent and efferent blood vessels and nerves are not interfered with. A miniature thermistor is mounted in front of the fitting, and protrudes through it, touching the enclosed organ. The temperature of the organ is then read on a meter mounted on the body.

The device is operated from a low-voltage high-current d.c. supply connected to 60-c/s, 110- or 220-V mains. In the application described here the efficiency of heating

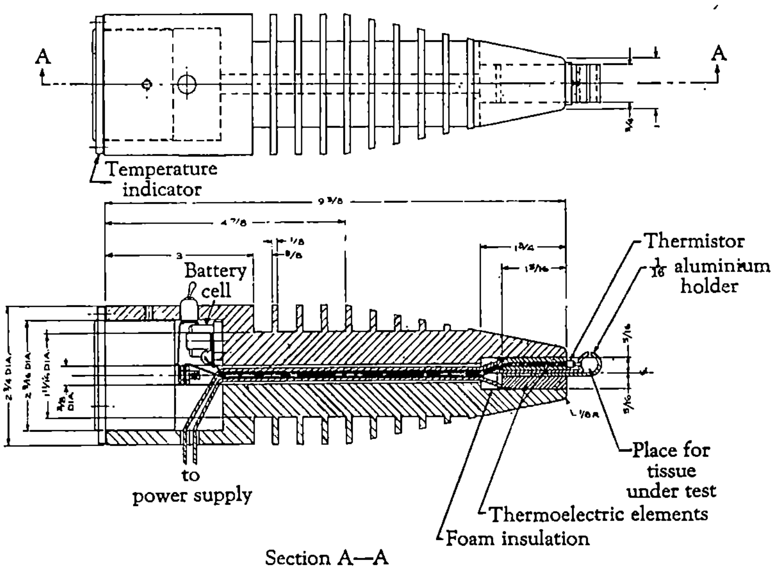

Fig. 1. Thermoelectric device for inducing local tissue hypothermia and hyperthermia: top view and cross-section

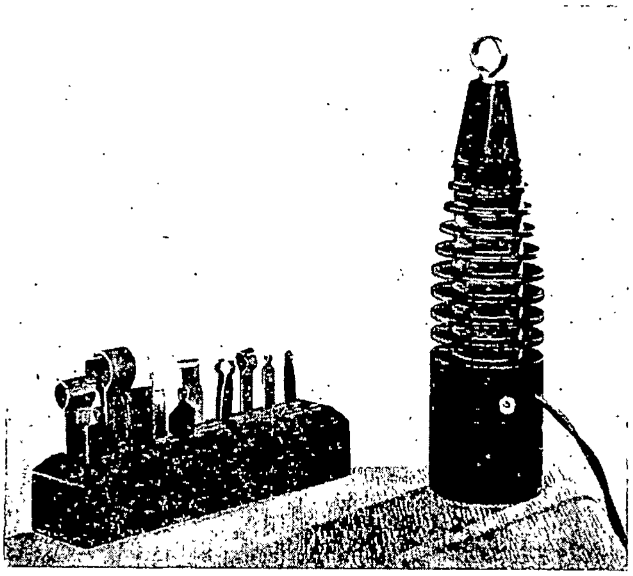

Fig. 2. Photograph of the device. An attachment best suited for the organ under investigation is chosen and inserted into the opening in the upper portion of the unit

or cooling is of no concern, as the cost of the energy consumed is negligible.

In the investigation of tissue and organ hypo- and hyper-thermia, rats were anæsthetized with pentobarbital sodium and various organs were exposed and partially enveloped by suitably shaped attachments that were inserted into the device. The tissues or organs were then subjected to cooling and heating, the degree of which was controlled by the value of the d.c. current, and the tissue temperature at the point of contact with the thermistor was read on the meter.

The lowest temperature that can be achieved is limited by the basic properties of thermoelectric elements[2] (about 65° C below ambient in vacuum with present-day elements). In an actual system the imperfect insulation of the object cooled, from environmental temperatures, reduces the above value. In particular, in the case of living tissue the vascularity of the organ influences the degree of cooling.

The highest temperature which can be achieved is only limited by the melting-point of the solder in the elements, which is about 100° C. In tissue work it must be limited by the damage threshold.

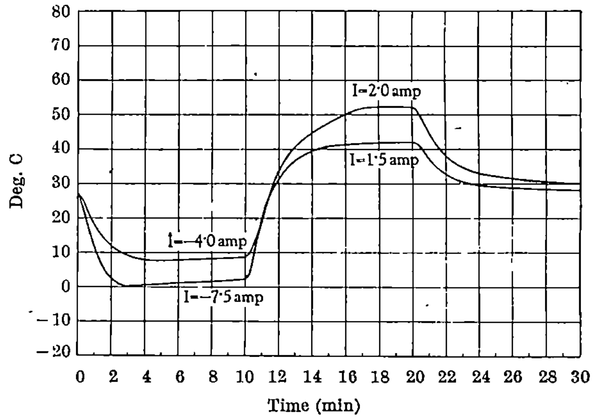

Fig. 3. Temperature of the spleen of a rat for two values of current, as a function of time

Fig. 3 shows the temperature of the spleen of a rat as a function of time, for two values of current in the cooling and heating cycle. The initial negative temperature gradient is steeper at the larger current, and the slight increase of temperature from its lowest value is due to the increase of the heat sink temperature. After 10 min the current was reversed and set to the values shown. The positive temperature gradient is here, also, a function of the current.

Lower temperatures than indicated in Fig. 3 can be achieved by reducing the temperature of the heat sink, for example, with water cooling.

Further tests were carried out on the kidney, uterus, tail, small and large intestine. In peripheral tissues, such as the tail, temperatures to −5° C and steeper gradients were observed, but the general behaviour was found to be similar to that shown in Fig. 3.

This device provides a tool for the investigation of organ function as well as its influence on the metabolism of the whole organism under carefully controlled temperature variations. Applications in therapeutic and surgical techniques are also likely.

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INCORPORATION OF FALL-OUT STRONTIUM-90 IN DECIDUOUS INCISORS AND FŒTAL BONE

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IN a previous article, Rosenthal *et al.*[1] reported that deciduous incisor teeth of children born between 1953 and 1956 who were bottle-fed from birth contained only 25 per cent more strontium-90 than teeth of children who were breast-fed for an average of 25 weeks. On the basis of known ^{90}Sr/g Ca in the mother's diet and known discrimination factors, teeth from bottle-fed children were expected to contain considerably more strontium-90 than that found in the teeth from breast-fed children. Because the strontium-90 content of teeth is thought to represent the strontium-90 body burden during the time the teeth are formed, it was desirable to obtain further data on the differences in tooth strontium-90 between breast-fed and bottle-fed children in order to qualify the discrepancies between theoretical considerations and analytical observations.

Samples of non-carious deciduous incisor teeth of children born between 1950 and 1958 within a radius of 150 miles of St. Louis were pooled and analysed for strontium-90 as previously described[1]. Samples of teeth from children born during 1957 were divided into 3 groups representing teeth of children who were breast-fed for 6 months or more after birth, teeth of children breast-fed for 6 weeks to 6 months, and teeth of children who were bottle-fed from birth. In the latter group, it was deemed advisable to include samples of teeth from children who were breast-fed for less than 6 weeks because these children were probably, and intermittently, bottle-fed. The 1957 samples contained equal amounts (2 g) of tooth crown. Although teeth from the first and last six months of the birth year were analysed separately, the data were averaged for the entire yearly interval. Tooth buds and mandibles were obtained from fœtuses 7–9 months of age between 1961 and 1963. They were defleshed by boiling in distilled water, and the hard tissues from 3–5 fœtuses were pooled for each analysis.

Because analyses of ^{90}Sr content of milk in the St. Louis milk shed did not begin until 1959, the values of the Perry, New York, milk shed obtained by the New York Health and Safety Laboratories of the U.S. Atomic Energy Commission were used between 1954 and 1959. A comparison of the Perry, New York, values with the St. Louis values for 1959–63 demonstrates that St. Louis values average 1·14 times greater than Perry, New York, values. This correction has been made although these small differences do not alter the results. The milk values between 1950 and 1953 are not known and have been estimated by extrapolation of the logarithm of the milk concentration from Perry, New York, versus time for the years 1954–59 using average yearly values.

The observed values obtained for the tooth samples are shown in Table 1, and demonstrate that teeth of bottle-fed children contain 19 per cent more strontium-90 than the teeth of children who were partly breast-fed ($P = < 0.01$) and 33 per cent more than the teeth of completely breast-fed children ($P = < 0.01$). The theoretical ^{90}Sr/g Ca of teeth (C_T) was determined by expansion of the basic equation (1) developed by Reiss[2]:

$$C_T = 0.32 \text{ pre-natal } ^{90}\text{Sr/g Ca} + 0.68 \text{ post-natal } ^{90}\text{Sr/g Ca} \qquad (1)$$

The factors 0·32 and 0·68 represent the fraction of tooth calcium deposited during the pre- and post-natal periods, respectively[2]. For bottle-fed children, equation (1) becomes:

$$C_T = (0.32)\,(1.2)\,C_d^m\,D_m + 0.68\,C_d^I\,D_I \qquad (2)$$

where C_T is tooth ^{90}Sr/g Ca, C_d^m is the mother's dietary intake of ^{90}Sr/g Ca from milk and the total dietary ^{90}Sr/g Ca in the American diet is 1·2 times the amount of ^{90}Sr/g Ca found in milk[3], D_m is the discrimination factor against ^{90}Sr between the mother's dietary intake and her fœtus, C_d^I is the infant's milk intake of ^{90}Sr/g Ca and D_I is the infant's discrimination factor against ^{90}Sr between dietary intake and bone.

Table 1. STRONTIUM-90 CONTENT OF DECIDUOUS INCISORS FROM BOTTLE- AND BREAST-FED ST. LOUIS CHILDREN BORN IN 1957

	No. of samples	pc. ^{90}Sr/g Ca Found ± *S.E.*	Calc.*
Bottle-fed (> 6 weeks)	21	2·79 ± 0·08	2·53
Breast-fed (< 6 months)	12	2·19 ± 0·35	—
Breast-fed (> 6 months)	8	1·73 ± 0·33	0·52

* Calculated from equations (4) and (8). The milk concentration during 1957 averaged 4·28 pc. ^{90}Sr/g Ca.

In order to solve equation (2), values for D_m and D_I must be experimentally determined. A discrimination factor of 0·13 for D_m appears reasonable as determined from fœtal bone investigations in this report and by Reiss[2]. A value for D_I of 0·8 has been selected as an intermediate value on the basis that children under 60 days of age do not discriminate against ^{90}Sr (ref. 4) and ^{90}Sr discrimination in children under 1 year of age is probably less than 0·5 (ref. 5). Using these estimates, equation (2) may be solved to yield:

$$C_T = 0.05\,C_d^m + 0.54\,C_d^I \qquad (3)$$

Because the incisor teeth develop and calcify in less than 1 year, the ^{90}Sr/g Ca in milk (C_d^m and C_d^I) may be averaged for yearly intervals and equation (3) becomes:

$$C_T = 0.59\,C_d \qquad (4)$$

The validity of equation (4) is shown in Fig. 1, where the determined values of ^{90}Sr/g Ca in the incisor teeth of bottle-fed children born during 1950–58 are plotted against the milk nuclide concentration. The determined values compare favourably with the line drawn from equation (4).

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* On leave of absence from Aichi Gakuin University, School of Dentistry, Nagoya.

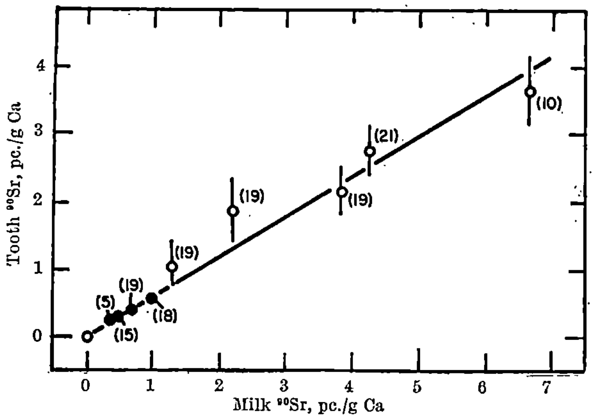

Fig. 1. The ^{90}Sr concentration of deciduous incisor teeth as a function of ^{90}Sr content of commercial milk. The number of samples analysed in parentheses. The vertical bars represent ± 1 *S.D.* The open circles represent values obtained during periods of known milk values between 1954 and 1958. The closed circles represent values obtained for estimated milk values between 1950–53. The line was drawn from equation (4).

The pre-natal contribution from the mother's diet to the fœtal teeth and bone may be defined as:

$$C_T = 1{\cdot}2\ C_d^m\ D_m \tag{5}$$

and with D_m equal to 0·13:

$$C_T = 0{\cdot}16\ C_d^m \tag{6}$$

The values for the tooth buds and bone of fœtuses between 1961 and 1963 (Table 2) demonstrate the equilibrium for ^{90}Sr/g Ca in the hard tissues of the developing fœtus, as has been previously shown[2], and the agreement between equation (6) and the determined values is satisfactory. This agreement also lends validity to the value of 0·13 for D_m, which indicates a discrimination factor of 8 against strontium-90 relative to calcium between the mother's diet and her fœtus. The value for D_m is also in agreement with the value found by Comar[6] in animals but differs from the findings of Kulp[7], who observed that the concentration of strontium-90 in fœtal bone averaged one-twelfth of that in the mother's diet.

Table 2. STRONTIUM-90 CONTENT OF FŒTAL TOOTH BUDS AND MANDIBULAR BONE*

Year	No. of samples	Tooth	Bone	Tooth; bone	Tooth and bone average	Calc.†	Deviation %
1961	6	1·82 ± 0·17	1·62 ± 0·17	1·12	1·72	1·44	−16
1962	7	1·51 ± 0·14	1·66 ± 0·22	0·91	1·58	1·76	+10
1963	17	3·39 ± 0·40	3·68 ± 0·43	0·92	3·54	3·36	−5
			Weighted average	0·96			−4

* Values expressed as pc. ^{90}Sr/g Ca ± *S.E.*

† Calculated from equation (6). Average pc. ^{90}Sr/g Ca for St. Louis milk was 9, 11 and 21 for 1961, 1962 and 1963, respectively.

For children who were completely breast-fed until after the incisor teeth were completely formed, the post-natal portion of equation (2) must be modified to include discrimination of ^{90}Sr between the mother's diet and her milk, which is approximately the same as the discrimination between the mother's diet and her fœtus, and equation (2) becomes:

$$C_T = (0{\cdot}32)\ (1{\cdot}2)\ C_d^m\ D_m + (0{\cdot}68)\ (1{\cdot}2)\ C_d^l\ D_m\ D_l \tag{7}$$

When C_d^m and C_d^l are the same, equation (7) becomes:

$$C_T = 0{\cdot}135\ C_d \tag{8}$$

Solution of equations (4) and (8) for completely bottle-fed and completely breast-fed children born in 1957 in St. Louis, as compared with the determined values, are shown in Table 1. The teeth of children who were breast-fed for less than 6 months are intermediate between completely breast-fed and completely bottle-fed children. Although equation (4) appears to be satisfactory for bottle-fed children, the determined values for breast-fed children are three-fold higher than expected when the values are calculated from equation (8).

The deviation between the theoretical and determined values for the ^{90}Sr/g Ca in the teeth of completely breast-fed children may be explained on the basis of skeletal turnover time in growing infants. Recently, Bryant[5] has reported a turn-over rate for bone of about 100 per cent during the first year after birth and, at birth, the average American infant contains 28 g of calcium in its skeleton[8]. In 1957, the fœtus would have had a skeletal burden of 0·69 pc. ^{90}Sr/g Ca (equation (6)) or a total content of 19 pc. ^{90}Sr in the hard tissues. During the first year of growth, approximately 2 g of tooth calcium are formed in all the deciduous teeth, and if no additional ^{90}Sr is obtained from the diet, a skeletal turnover of only 20 per cent would supply sufficient ^{90}Sr from the infant's own skeletal stores to account for the determined values. In addition, it is possible that the diet of completely breast-fed children was supplemented with exogenous food because the information supplied by the parents, which depends on memory, is probably inaccurate. In all probability, both factors tend to increase the tooth values of breast-fed children over that expected from equation (8). For bottle-fed children, the dietary intake of ^{90}Sr/g Ca during the post-natal period is 8 times greater than that of breast-fed children (equations (2) and (7)), and the contribution of ^{90}Sr from the bottle-fed infant's skeletal stores becomes negligible within experimental error, resulting in little or no effect on the validity of equation (4).

The peak milk concentration of ^{90}Sr occurred in June 1963, when the St. Louis milk values reached 38 pc./g calcium with a yearly average of 21 pc./g calcium. For children born during 1963, application of equation (4) yields an estimated strontium-90 burden of 12·4 pc./g of incisor calcium. The validity of this estimate must wait until 1968–70 when these teeth are shed and become available for examination.

The data presented in this report demonstrate the value of the ^{90}Sr concentration of deciduous incisor teeth as a measure of ^{90}Sr body burden and the relationship of the body burden to the dietary milk concentration for bottle-fed children at the time the teeth are formed. The difference between the values obtained for bottle-fed and breast-fed children, although statistically significant, is within the analytical errors of the estimates and does not seriously interfere with the usefulness of tooth ^{90}Sr as an index of ^{90}Sr body burden. The parameter relating the tooth ^{90}Sr to the infant's skeletal ^{90}Sr burden accumulated during the pre-natal development and the skeletal turnover during post-natal development needs to be resolved and further investigation of these phenomena is in progress.

The fœtus material was kindly supplied by Prof. Mildred Trotter, Washington University School of Medicine and the St. Louis Anatomical Board. We thank Sylvia Raymond, Sophia Goodman and Yvonne Logan for the collection of baby teeth and the Greater St. Louis Citizens' Committee for Nuclear Information for operation of the Baby Tooth Survey. The ^{90}Sr milk values for St. Louis were made available by C. M. Copley, jun., Deputy Health Commissioner of St. Louis, Mo.

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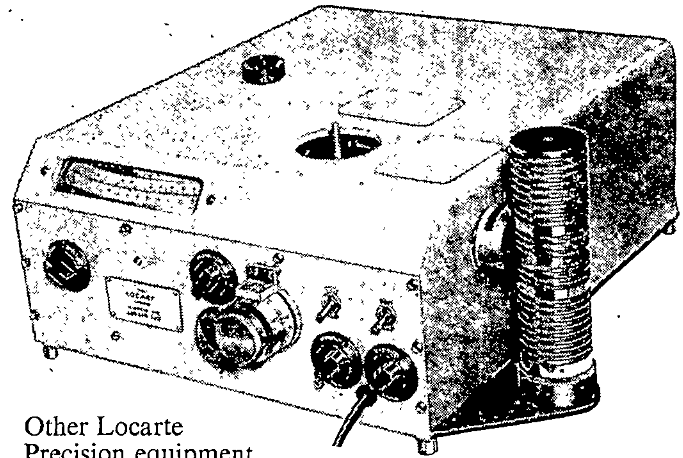

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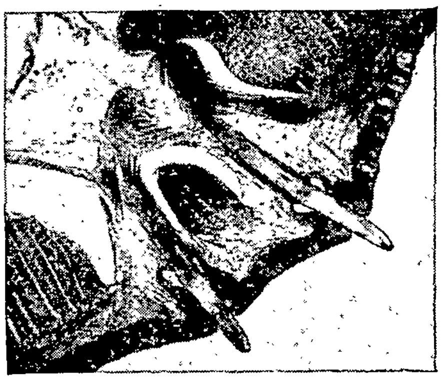

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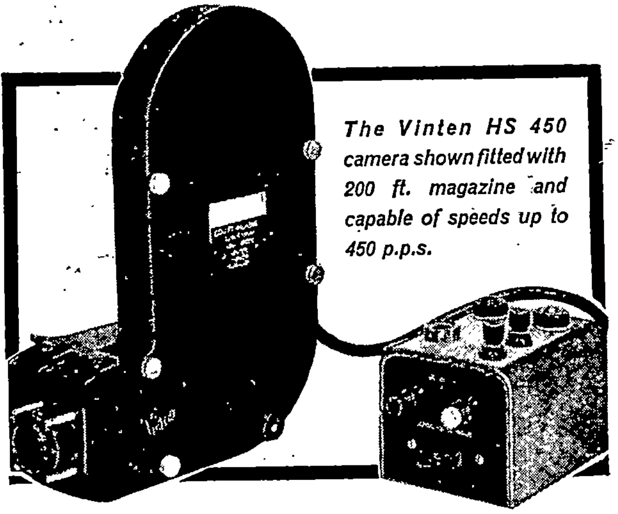

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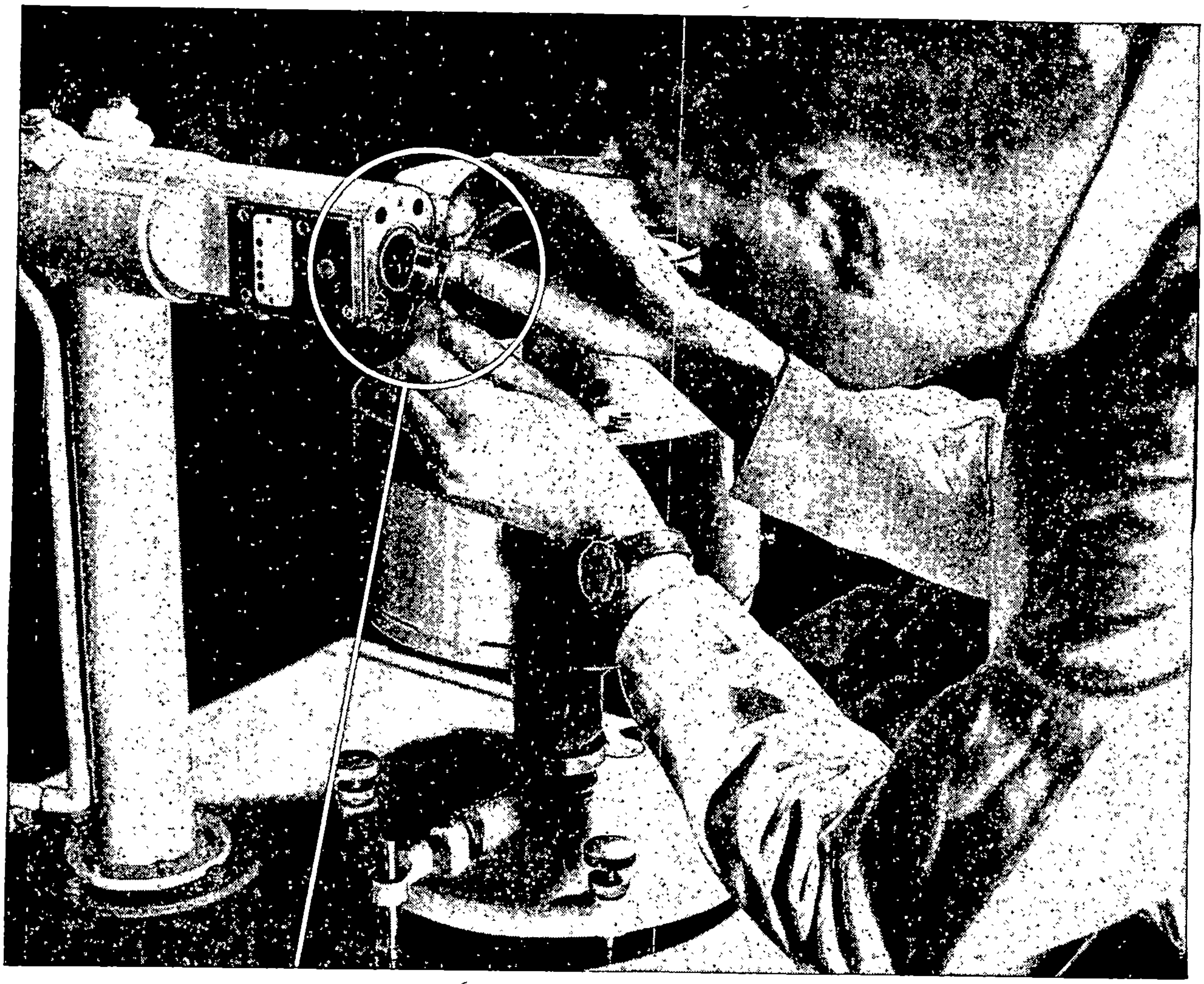

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FALL-OUT IN LONDON RAIN WATER

By Dr. R. WOOD, P. METSON and L. A. RICHARDS

Laboratory of the Government Chemist, Department of Scientific and Industrial Research, London, S.E.1

FOLLOWING the resumption of nuclear testing by the U.S.S.R. in September 1961, regular samples of rain-water were collected on the roof of the Laboratory of the Government Chemist, Clement's Inn Passage, Strand, W.C.2, until the end of 1963, when the Laboratory moved to Cornwall House, Stamford Street, S.E.1. These rain samples were analysed for the presence of various fission products.

The samples were collected using a polyvinyl chloride funnel, having an effective collecting area of 6 ft.². This led to a polythene collection vessel to which had been added an acidified mixed carrier solution containing known amounts of stable nitrates of each of the radionuclides to be determined. Use of this size of funnel enabled analysis to be made on samples collected from rainfalls of 5 mm and upwards. The rainfalls were calculated from the volume of rain collected and the collecting area. Up to and including April 1963, weekly or fortnightly samples were normally taken, and thereafter monthly samples. All samples of rain taken contained variable concentrations of insoluble airborne materials, partly organic and partly inorganic in character. Up to 0·2 g/l. were found, depending to some extent on the volume of sample. The inorganic constituent was mainly siliceous material along with iron and aluminium oxides. Various preliminary treatments were tried. Ultimately, it was found most advantageous to take each sample to dryness and extract the soluble salts with water. The insoluble portion was treated with hydrofluoric and perchloric acids to remove silica and oxidize the organic material. The resulting residue was dissolved in hydrochloric acid. Any residue still remaining was solubilized by means of a carbonate fusion.

Following a suitably modified version of the sequential radiochemical procedure of Osmond *et al.*[1], measurements were made of barium, cæsium, cerium and strontium radionuclides. Later, in this Laboratory, further modifications and additions were made to this sequential scheme which enabled, in addition zirconium, yttrium and rare-earth radionuclides to be determined in the same sample of rain[2]. Initially, part of the sample was examined separately for radioactive iodine nuclides. Later, separate sampling of rain-water for radioiodine assay was done, usually during and immediately after a nuclear test series. The figures for radioiodine nuclides are recorded here only when the sampling coincided with that for other nuclides. Radioiodine was determined by a procedure based on that of Glendenin and Metcalf[3], which involved a series of extractions to separate iodine from other fission products, for example, ruthenium. One of the extraction steps was replaced by a distillation step to eliminate the possibility of active particulate matter being carried between the phases in the extraction.

All the prepared sources were counted in low background anti-coincidence β-counting assemblies, the identity of each nuclide being checked by determination of the half-life when this was practicable and/or by repeating counts through specified absorbers.

Fig. 1 shows the results, plotted on a logarithmic scale, for the various radionuclides measured in London rain over the period September 1961–December 1963, inclusive. All activities have been corrected for decay to the mid-point of the collection period.

During the observation of the decay of the iodide sources it was found that the curve fell steeply for a few hours before steadying off to the characteristic decay of iodine-131 (half-life 8·05 days). This was interpreted as being due to the presence of some short-lived iodine-132 formed from its longer-lived tellurium-132 precursor, as a half-life of 2·2 h for this iodine activity was found.

$$\text{Te-132 } t^{1/2} \xrightarrow[\beta]{77\text{ h}} \text{I-132 } t^{1/2} \xrightarrow[\beta]{2\cdot2\text{ h}} \text{Xe-132 (stable)}$$

The figures for tellurium-132 shown in Fig. 1 were calculated from the activity of iodine-132 obtained by analysis of the total iodine curve.

The presence of cæsium-136 (half-life 12·9 days) in rain collected during the period September to November 1961 at the Atomic Energy Research Establishment, Harwell, has already been reported[4]. This nuclide has also been identified in rain samples collected here over the same period of time and again between August 1962 and January 1963. Its presence was first suspected when lower transmission figures than normal (50 per cent low in some cases) were obtained on counting the cæsium source through a specified absorber as a check on the identity of the 30-year half-life cæsium-137 radiation. This indicated the presence of a cæsium isotope giving a softer β-emission than that of cæsium-137, the main contributor to the activity of the cæsium sources. Counting the cæsium sources at intervals of several days gave a decay curve which was resolved to give the activities of the long-lived cæsium-137 (max.^{m} β-energy 0·5 MeV) and short-lived cæsium-136 (max.^{m} β-energy 0·34 MeV), the identity of

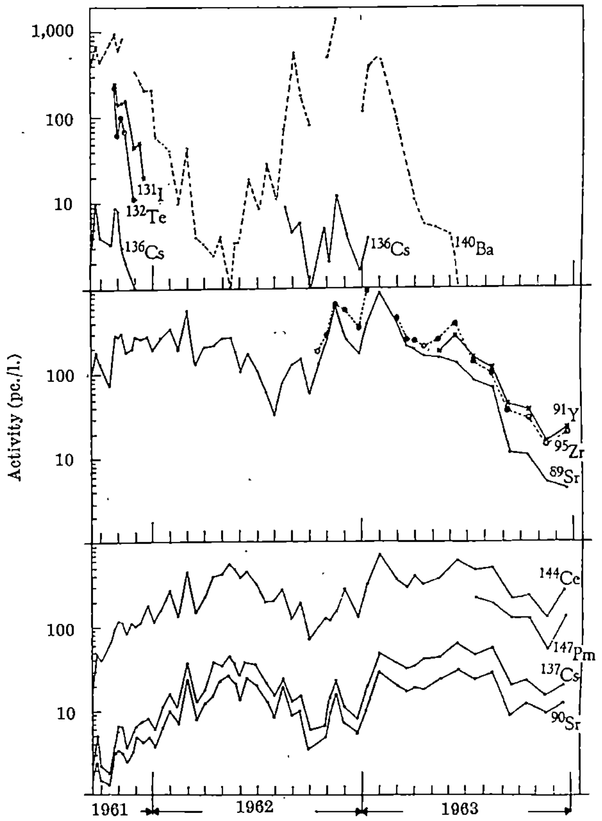

Fig. 1. Radionuclides in London rain: September 1961–December 1963

the latter being confirmed by its 12·9-day half-life. A more accurate estimate of the cæsium-137 activity was made by recounting the cæsium sources three months after the initial count, by which time the cæsium-136 had decayed away. Transmission figures in accord with the presence of cæsium-137 alone were also obtained at this time.

By means of the sequential scheme developed for this work[3] the rare-earth radionuclides were isolated as a group. However, since the determination of this group did not start until July 1963, the only rare-earth radionuclide present was promethium-147 (half-life, 2·6 years). All other fission-produced rare-earth radionuclides would have decayed by this time.

Prior to September 1961 no atmospheric nuclear tests, apart from the three small French tests in the Sahara in 1960, had been conducted since November 1958. Consequently, the only fission products in rain-water before the advent of the 1961 nuclear test series were the long-lived radionuclides such as strontium-90 (half-life, 28 years), cæsium-137 (half-life, 30 years) and cerium-144 (half-life, 285 days). Although no figures are available for the concentration of these three radionuclides in London rain before September 1961, the results obtained by the Atomic Energy Research Establishment, Harwell[5], for its levels in rain collected at Abingdon, Berkshire, in August 1961, serve as a base-line for the results in Fig. 1. These figures were 1·7 pc./l., 2·4 pc./l. and 3·2 pc./l., respectively, for strontium-90, cæsium-137 and cerium-144.

The period covered by the results in Fig. 1 includes the two nuclear test series of the U.S.S.R., that of September–November 1961 and that of August–December 1962, both series occurring almost entirely in the Arctic region. The *Dominic* test series of the United States conducted in the Pacific islands during April–July 1962 and continued in October and November of the same year also come into this period. While the Russian tests were in the main in the megaton yield range and conducted in the northern hemisphere, the American tests were mostly in the intermediate yield range (less than 1 megaton) conducted almost on the equator[6]. Consequently, the high flushes of radioactivity in London rain experienced in the autumn 1961 and again in the autumn 1962 were mainly due to tropospheric fall-out from these Russian tests.

These periods of high flushes of radioactivity were most clearly marked by the rapid increase in the concentration of the short-lived isotopes, principally barium-140 (half-life, 12·8 days) and iodine-131 (half-life, 8·05 days), in successive rain samples. With its short half-life, high-fission yield and consequent high-specific activity barium-140 is a good diagnostic of the presence of fresh fission fall-out material. In the autumn 1961 nuclear test series the level of this nuclide reached a peak of about 900 pc./l. at the end of October. With the cessation of tests the level dropped to approximately 1 pc./l. in May 1962. Thereafter, until the middle of August 1962, the levels began to rise as a result of the American tests started in April 1962, but at a much slower rate than in the autumn of 1961. The higher levels of barium-140 experienced from then on, reaching a peak of about 1,600 pc./l. towards the end of November, were due to the resumption of nuclear testing by the U.S.S.R. Since then, with no further tests taking place, the concentration of this radionuclide diminished until by mid-summer 1963 it was no longer detectable.

It was also during these periods of nuclear testing, and immediately after them, that cæsium-136 (half-life, 12·9 days) was detected. Although of comparable half-life to barium-140, the cæsium-136, a shielded nuclide, is formed in extremely low fission yield[7] and the levels of it in rain soon decayed beyond the limits of detectability.

The determination of trends in fall-out is most easily followed by the measurement of the activity of nuclides having a half-life of several years. Cæsium-137 (half-life, 30 years) and strontium-90 (half-life, 28 years) are particularly useful in this respect. Inspection of Fig. 1 shows that the concentration of these nuclides increased slowly at first in successive samples even during the initial flush of radioactivity in the autumn 1961. Then towards the end of January 1962, approximately three months after the end of the Russian test series, significant increases in the activities due to these nuclides were noted. This was an indication of the start of the annual spring rise in fall-out deposition associated with the transfer of air from the lower stratosphere to the troposphere in northern latitudes at this time of the year[8,9]. The peak of this rise of approximately 26 pc./l. and 46 pc./l. for strontium-90 and cæsium-137, respectively, was reached during May–June 1962, the time agreeing with observations made elsewhere in previous years[10]. Thereafter the levels fell despite the introduction of fresh fission products into the atmosphere from the renewed tests. A similar trend was found in 1963 towards a spring maximum, the peak activities being reached rather later than in 1962 over a period June–August with figures of approximately 33 pc./l. and 64 pc./l. for strontium-90 and cæsium-137, respectively.

It has been established by Peirson *et al.*[11] that settled conditions of fall-out do not occur for several months after the end of a nuclear test series. By this time all material originally injected into the troposphere would have been deposited and all fall-out would now be coming from the lower stratosphere. These settled conditions imply complete mixing of the new and old fission-derived material in the lower stratosphere before its gradual transfer to the troposphere and eventual deposition on the Earth's surface. One indication of this complete mixing is given when the strontium-89 : strontium-90 ratio in the fall-out decays with the half-life of strontium-89 (half-life, 51 days). This condition was first noted in rain samples collected at the end of January 1962, some three months after the cessation of weapon testing (Fig. 2) and continuing until the end of May 1962. After this time the decay of the strontium-89 : strontium-90 ratio proceeded at a slightly but significantly slower rate. This was a sign that very recent fission products, having a high strontium-89 : strontium-90 ratio, from the American nuclear tests in the Pacific (started on April 25, 1962) were now being deposited in London rain. With the recommencement of the new Russian tests on August 5, 1962, the strontium-89 : strontium-90 ratios of successive samples from September 1962 on behaved in an erratic fashion, showing a repeat of the unsettled conditions of fall-out exhibited in the autumn 1961. With cessation of tests at the end of 1962, settled conditions of fall-out appeared to have been established quickly by January 1963. Later, in July 1963, it can be seen from Fig. 2 that the strontium-89 : strontium-90 ratio had moved

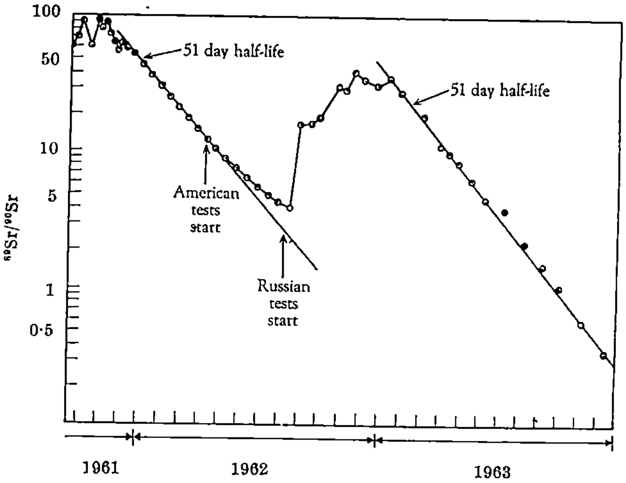

Fig. 2. Ratio strontium-89/strontium-90 in London rain water

Table 1. COMPARISON OF RADIONUCLIDES IN SAMPLES OF RAIN COLLECTED AT CORNWALL HOUSE (C.H.) AND CLEMENT'S INN (C.I.)

Period of collection 1963	Site	Rainfall (cm)	Activity pc./l. ^{89}Sr	^{90}Sr	^{91}Y	^{95}Zr	^{137}Cs	^{144}Ce
November	C.I.	9·20	5·2	9·5	16·0	14·9	16·0	127
	C.H.	9·45	4·2	8·5	18·3	14·5	15·3	131
December	C.I.	1·84	4·1	12·2	23·3	21·5	22·2	275
	C.H.	1·83	5·0	11·8	21·7	20·0	23·0	214

from the strontium-89 decay line and that in subsequent months the ratio tended to move back on to this line. This may be confirmation of the U.S. Atomic Energy Commission announcement[12] on June 30, 1963, of evidence of possible recent nuclear tests of very low yield by the U.S.S.R.

Samples collected between December 1962 and February 1963 consisted partly of rain and partly of snow. The sample collected between January 11 and February 8, 1963, was entirely of melted snow. From the higher activities found in this sample compared with the immediately preceding and subsequent samples it would appear that snow with its greater surface area : volume ratio carries down more fall-out per unit melted volume than rain. The possibility that the high activities found in this sample were due to very recent fission products not found in previous samples can be discounted since the strontium-89 : strontium-90 ratio was in line with that of preceding samples. Neither was any cæsium-136 detected, whereas this radionuclide was found in the preceding samples.

With the impending removal of the Laboratory to new quarters at the end of 1963, duplicate sampling was carried out during November and December of that year at the old and new premises. This was done to see whether any marked change in fall-out conditions existed at the new premises, since fall-out measurements would continue to be made there in the future. Table 1 lists the results obtained. Good agreement was obtained between the levels of each nuclide in the duplicate samples in each month with one exception. In December the concentration of cerium-144 in the sample taken at Clement's Inn (old premises) was 1·3 times that found in the sample at Cornwall House. A chemical re-work of each of the two cerium sources produced no change in their respective activities.

In view of reports[13] of the presence of manganese-54 in fall-out a determination[2] of this radionuclide was made in rain collected at Cornwall House in November and December 1963. Concentrations of 6·9 and 11·3 pc./l., respectively, were obtained for these monthly samples. Manganese-54 (half-life, 290 days) is not a fission product but is formed by radiative activation of material comprising the weapon casing by particles released in the nuclear burst. This radionuclide decays by electron capture followed by emission of a γ-ray, by which means it was detected and measured.

The Atomic Energy Research Establishment, Harwell, publish a detailed yearly report on radioactive fall-out in air and rain. Two such publications[5,14] report on the period mid-1961 to mid-1963 and included are results of radiochemical analyses made on rain samples collected at various sites throughout the United Kingdom. Direct comparison of these results and the London figures for fall-out concentrations in rain collected over the period under review cannot be precise since sampling periods did not coincide and the variability in rainfall and meteorological conditions must contribute to any differences noted. It was noted here that the London figures were generally higher than those of samples collected at Abingdon, near Harwell, 50 miles due west of London. The higher concentrations of fall-out in London rain were especially noticeable from October 1961 to May 1962, and to a lesser extent from November 1962 to March 1963. During a large part of both periods tropospheric conditions of fall-out prevailed. However, a more general comparison between London and the other United Kingdom sites for fall-out concentrations of strontium-89 and strontium-90 over the period under review has revealed reasonable agreement of results, the Abingdon figures being generally lower than those of the other sites, especially during 1962 (ref. 15). The only apparent peculiarity of the Abingdon site is that it is farther inland than the other sites.

At the close of the period under review, of the radionuclides determined here, the main contributions to the radioactivity in London rain, besides the long-lived strontium-90 and cæsium-137, are cerium-144 (half-life, 285 days) and promethium-147 (half-life, 2·6 years). The yttrium-91 (half-life, 57 days), zirconium-95 (half-life, 65 days) and strontium-89 (half-life, 51 days) can be expected to decay beyond the point of detectability during 1964, assuming the nuclear weapon testing moratorium continues.

We thank Dr. D. I. Coomber for his advice.

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ULTRA-VIOLET ABSORPTION SPECTRUM OF THE FREE FULMINATE (C-N-O) RADICAL

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ONE of the first known examples of isomerism was that shown by the cyanates or isocyanates *R*—OCN and *R*—NCO, and the fulminates *R*—CNO. In the cyanate or isocyanate free radical (NCO) the carbon atom occupies the central position, whereas in the fulminate (CNO) this central position is filled by the nitrogen atom. The ultra-violet spectrum of the free NCO radical has recently been obtained in absorption by Holland, Style, Dixon and Ramsay[1] and shown to consist of two distinct systems due to the transitions $A(^2\Sigma^+) - X(^2\Pi_i)$ and $B(^2\Pi_i) - X(^2\Pi_i)$; the system due to the former lying in the region of 4400 Å and the latter occurring between 2650 Å and

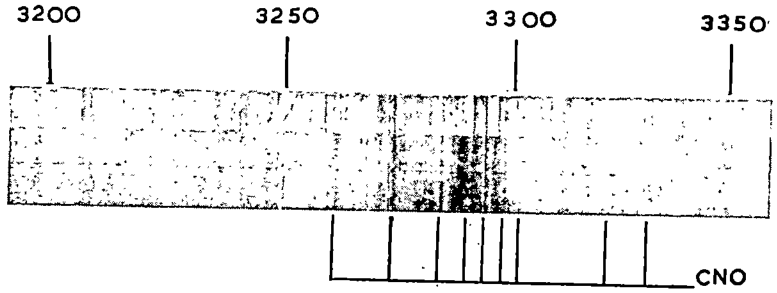

Fig. 1. Absorption spectrum of the fulminate radical taken 15 μsec after flashing a cyanogen/ozone/argon mixture of ratio 5 : 1 : 50. Flash energy, 1,280 J. Pressure of mixture used, 3·5 cm mercury

3200 Å. From a detailed analysis of the $\Sigma - \Pi$ bands Dixon[2] concluded that the NCO radical is linear in both ground and excited states.

We have recently observed these same two systems of NCO in absorption, using the technique of flash photolysis and kinetic spectroscopy, in the investigation of reactions involving oxygen atoms and cyanogen. Together with the NCO bands a new complex system, which has been attributed to the fulminate radical, was observed in the wave-length region 3250–3330 Å. The strongest and best-resolved spectrum of the radical was obtained by flashing a mixture of cyanogen and ozone using excess argon as inert diluent in a conventional flash photolysis apparatus in which the flash tube and reaction vessel were constructed of ordinary transparent quartz and lay side by side in a cylindrical reflector. The type of apparatus used has been fully described by McGarvey and McGrath[3]. Fig. 1 shows the spectrum obtained at a short delay time after flashing such a mixture, and Table 1 lists the wave-lengths of the band heads together with their relative intensities.

When a cyanogen/argon mixture was flashed under similar conditions to those used in the case of cyanogen/ozone/argon mixtures no CN absorption could be detected, and since this radical is a strong absorber the amount of direct photolytic decomposition must be extremely small under these conditions. The effect of the photolytic flash was therefore confined to the dissociation of the ozone in these experiments. From the work of McGrath and Norrish[4] it is known that ozone decomposes under these conditions as follows:

$$O_3 \xrightarrow{h\nu} O_2 + O(^1D) \tag{1}$$

In the absence of any competitive reaction for the removal of oxygen atoms reaction (1) is followed by the fast reaction (2), resulting in the production of vibrationally excited oxygen molecules.

$$O(^1D) + O_3 \rightarrow O_2{}^* + O_2 \tag{2}$$

Table 1

Wave-length in Å (in air)	Relative intensity, (scale 100)	Remarks
3328·1	15	*D.R.*
3318·0	5	*D.R.*
3298·1	10	*D.R.*
3295·1	45	*D.R.*
3292·5	52	*D.R.*
3291·0	58	*L.*
3290·4	100	*L.*
3289·6	38	*L.*
3288·0	55 (at max.)	*D.H.*
3286·4	55	*L.*
3285·0	55 (at max.)	*D.H.*
3283·1	25	*D.H.*
3280·5	38	*L.*
3278·5	25 (at max.)	*D.H.*
3276·5	25 (at max.)	*D.H.*
3270·0	50	*L.*
3267·0	15	*L.*
3258·0	18	*D.R.*
3257·4	13	*L.*

D.R., diffuse band degraded to red; *D.H.*, diffuse headless band; *L.*, line-like in appearance.

When the cyanogen and ozone were present in equimolecular amounts in the cyanogen/ozone/argon mixtures, used in these experiments, vibrationally excited oxygen molecules with v'' up to 17 were produced by reaction (2) and easily detected at short delay times by means of the strong Schumann–Runge absorption system. When the cyanogen/ozone ratio was increased to 5 : 1 this spectrum due to excited oxygen molecules disappeared, indicating the preferential removal of $O(^1D)$ by some reaction involving cyanogen molecules. However, from the absorption spectra obtained at short delay times after photolysing both the foregoing mixtures it was possible to identify the two systems of NCO along with extremely strong CN absorption and the system attributed to the free CNO radical.

All these observations indicate conclusively that the $O(^1D)$ removal reaction involving the cyanogen molecule is as follows:

$$O(^1D) + N \equiv C{-}C \equiv N \rightarrow CNO + CN \tag{3}$$

The $O(^3P)$ ground-state oxygen atom also seems to react according to equation (3), since when nitrogen dioxide was used as the atomic oxygen source absorption spectra taken at short delay times (15 μsec) after flashing again showed the band system 3250–3330 Å attributed to the CNO radical. Attack by an oxygen atom on cyanogen is most likely to produce CNO, but it is possible that NCO could also be produced to a small extent in this way. The principal reactions for the production of NCO must undoubtedly be:

$$CN + O_3 \rightarrow NCO + O_2 \tag{4}$$

$$CN + O_2 \rightarrow NCO + O \tag{5}$$

and it would seem equally likely that reactions (4) and (5) could also result in the formation of the fulminate radical as in equations 6 and 7:

$$CN + O_3 \rightarrow CNO + O_2 \tag{6}$$

$$CN + O_2 \rightarrow CNO + O \tag{7}$$

To provide additional evidence for the assignment of the 3250–3330 Å bands to the free CNO radical reactions (5) and (7) were investigated. In order to carry out these experiments, it was necessary to modify the equipment in such a way as to overcome the weak absorption characteristics of cyanogen and give a reasonable photolytic decomposition into CN radicals on flashing. This was accomplished by combining flash tube and reaction vessel in a condenser-like arrangement in which the flash occurred in the annular space between the inner reaction vessel and the outer quartz envelope. The outer quartz envelope was covered with aluminium foil in order to concentrate the light into the reaction vessel, which was made of spectrosil. With this high-efficiency annular type of flash tube it was possible to dissociate a considerable amount of cyanogen with flash energies of about 1,200 J. Absorption spectra, taken at short time-intervals after flashing the cyanogen/oxygen/argon mixtures of ratio 1 : 1 : 15, showed the presence of NCO and CNO, in accordance with what one would expect from reactions (5), (7) and (8).

$$(CN)_2 + O(^3P \text{ or } ^1D) \rightarrow CNO + CN \tag{8}$$

Another interesting feature of these experiments was that while a cyanogen/argon mixture would show very strong CN radical absorption on flashing, an equivalent mixture in which cyanogen and oxygen were present in equimolar amounts showed no trace of the CN radical even at the shortest delay time used (15 µsec). This complete suppression of the CN absorption by oxygen is indicative of the extreme speed of either one or both of reactions (5) and (7). Of these two reactions (7) must be the more favoured since when oxygen is present even in extremely small concentration the CNO bands at 3250–3330 Å can be detected whereas the NCO absorption does not appear. Indeed, if great care is not taken to remove oxygen from the cyanogen and inert gas these CNO bands appear strongly.

In order to confirm the assignment of the 3250–3330 Å bands to the CNO radical it was decided to investigate the isothermal flash photolysis of gaseous fulminic acid. The gaseous fulminic acid was prepared by reacting formamide oxime (NH_2—CH=N—OH) with an excess of slightly moist hydrogen chloride gas in a sealed tube. The formamide oxime was heated slightly (about 50° C) to initiate reaction and the fulminic acid obtained was diluted with a large excess of argon.

The absorption spectrum obtained at 15 µsec after flashing a 1 : 10 fulminic acid/argon mixture showed the same 3250–3330 Å bands obtained in the previous experiments. The only reasonable primary photolytic process occurring here would be:

$$\text{H—C—N—O} \xrightarrow{h\nu} \text{H} + \text{CNO} \qquad (9)$$

thus providing additional confirmation that these 3250–3330 Å bands are due to the free CNO radical.

A similar series of bands in the same spectral region as those of CNO reported here have been detected in emission from flames supported by active nitrogen by Jennings and Linnett[5]. Unfortunately these authors did not give any figures for the wave-lengths of the band heads and since their published spectrum was a print of a plate taken on a medium quartz spectrograph a direct comparison with the bands reported here could not be made. Jennings and Linnett attributed their bands to an emitter containing only carbon and nitrogen and so concluded that the most likely species were C_2N, CN_2 or some excited state of cyanogen. Of these three possibilities the most likely one to occur during the initial attack of oxygen atoms on cyanogen would be C_2N, produced according to reaction (10).

$$(CN)_2 + O \rightarrow C_2N + NO \qquad (10)$$

If this reaction occurs it would seem equally likely that atomic species other than oxygen, for example, sulphur or chlorine, would also lead to the formation of the C_2N radical. In order to test this possibility an investigation was made of the isothermal flash photolysis of cyanogen/carbonyl sulphide, cyanogen/carbon disulphide and cyanogen/halogen mixtures. The band system in question, that is, the complex system 3250–3330 Å, was not observed during any of these experiments although in the case of the carbonyl sulphide/cyanogen mixtures the sulphur atom seemed to follow a somewhat similar behaviour to the oxygen atom in that the absorption spectrum of the free NCS radical was observed 15 µsec after the photolytic flash.

Since the fulminate radical seems to be very reactive (lifetime not more than 50 µsec) it would seem likely that it plays an active part in the cyanogen/oxygen and cyanogen/ozone flames. The extreme reactivity of this radical especially with oxygen can be deduced from the fact that an increase in the proportion of oxygen in the cyanogen–oxygen mixtures results in the suppression of the CNO spectrum. In order to obtain more relevant information on the participation of this radical in combustion processes its subsequent reactions are at present undergoing a more detailed investigation.

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POSSIBLE STRUCTURES RELATED TO GMELINITE

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IT is well known that closest-packed structures containing spheres of equal radii form hexagonal layered structures. Such structures are often described by means of the symbols *A*, *B* and *C* for the three possible layers of close-packed spheres differing from one another in position[1–3]. Alternatively, the same stacking of layers is sometimes designated by the symbols *h* and *c*, as suggested by Pauling[1], in which the symbol *h* applies to a layer in which the two neighbouring layers are of the same type (that is, both *A*, both *B* or both *C*) and the symbol *c* in which the two neighbouring layers are of different types (for example, layer *B* in contact with the layers *A* and *C*).

There are numerous sequences in which close-packed layers may be stacked. Thus, the smallest possible value of n in a close packing, where n is the number of layers required to complete the sequence, is 2, corresponding to the hexagonal closest packing with an . . . *AB* . . . arrangement. In a like manner, the only possible sequence for $n = 3$ is the cubic closest packing with . . . *ABC* . . . When $n = 4$ the only possible hexagonal closest-packing arrangement is . . . *ABAC* . . . and when $n = 5$ it is . . . *ABCAB* . . . For $n = 6$ it is now possible to arrange the layers in two ways, namely, . . . *ABCACB* . . . and . . . *ABACAB* . . . As pointed out by Smith[2] and Wells[3], beyond $n = 6$ as n increases the number of arrangements of close-packed layers also increases. Only the structures with $n \lesseqgtr 6$ are discussed here. Each structure is hexagonal (except for . . . *ABC* . . . with $n = 3$, which is cubic). The lattice parameter a_0 remains unchanged; only c_0 varies by integral multiples.

It is now shown that such nomenclature may be applied with remarkable success in the classification of the zeolites of the chabazite group. In this class the hexagonal prisms (double six-membered rings) may be considered as the building blocks. Each zeolite differs from the other by the stacking sequence of the various layers, the full symmetry of which depends on the number and arrangement

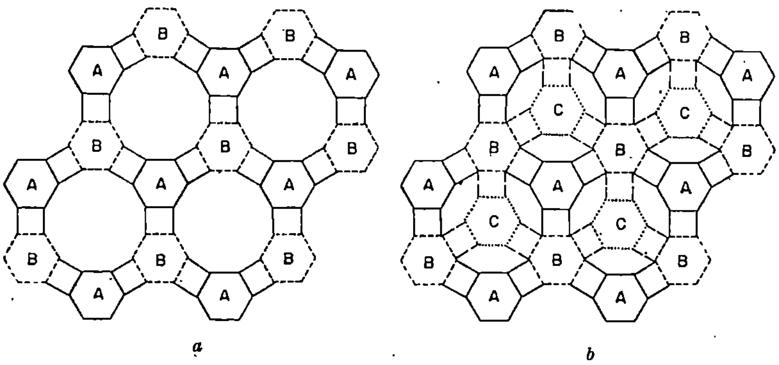

Fig. 1. 001 projections. Each hexagonal ring represents a double six-membered (Si, Al) ring. (*a*) *AB* layer as in gmelinite; (*b*) *ABC* layer arrangement

of layers necessary to complete the stacking sequence. Twenty possible structures in addition to the . . . *AB* . . . (. . . *hh* . . .) and . . . *ABC* . . . (. . . *ccc* . . .) structures for spheres of equal radii are listed by Wells[3]. Theoretically, all twenty could be applied to possible zeolites by replacing each sphere with a double (or single) six-membered ring stacked parallel to the *c*-axis of the hexagonal cells.

Structure of gmelinite, chabazite and erionite. Gmelinite is hexagonal[4] with average lattice parameters $a_0 = 13{\cdot}7_4$ Å and $c_0 = 10{\cdot}0_4$ Å. The (Si, Al)-O framework is formed by joining double six-membered rings through their edges. As is shown in Fig. 1*a*, these prisms form planes stacked parallel to each other and perpendicular to the *c*-axis with an . . . *ABAB* . . . sequence. The gmelinite cavity possesses $3/m$ symmetry and is formed out of the sequence (*ABA*). Since the repeating sequence is . . . *AB* . . . the *c*-dimension of one sequence (layer) is one-half the c_0 parameter, or 5·0 Å.

Chabazite is rhombohedral[4–6]. The (Si, Al)-O framework is formed through an . . . *ABC* . . . sequence with the same type of layers found in gmelinite. Since such an arrangement is cubic for spheres of equal radii, the deviation from cubic symmetry to rhombohedral symmetry is thus expected since, the building blocks are not spherical. Alternatively it is, of course, also possible to describe chabazite with a hexagonal cell with average lattice parameters $a_0 = 13{\cdot}7_5$ Å and $c_0 = 15{\cdot}0_0$ Å (refs. 4–6). Chabazite is thus related to gmelinite by a very simple relationship: in the *c*-direction each set of two layers has been rotated 60° in the same direction about the *c*-axis running through the centre of a double six-membered ring. Thus, . . . *AB*,*AB*,*AB*, . . . of gmelinite becomes . . . *AB*,*CA*,*BC*, . . ., or simply . . . *ABC*,*ABC*, . . . Repetition in the *c*-direction has been increased by one more plane of double six-membered rings; this adds 5·0 Å to the c_0 lattice parameter to yield a theoretical parameter of 15·0 Å, which is 3/2 the c_0 parameter of gmelinite. This is found to be in close agreement with the experimental value.

The . . . *ABC* . . . sequence is shown in Fig. 1*b*. The twelve-membered ring channels of gmelinite are now blocked; strongly suggesting that it is this type of blocking which reduces the sorptive properties of gmelinite. It also strongly suggests that a chabazite sequence would have very little difficulty growing on to a gmelinite sequence, since no change in energy is expected in forming sequence *AB* rather than *AC*.

The structure of erionite has also been reported as being hexagonal[4–6]. It may be described as having an . . . *ABCB* . . . sequence with $n = 4$. Whereas both gmelinite and chabazite contain just double six-membered rings, erionite is considered to have single as well as double six-membered rings periodically linked together. Corresponding, then, to the foregoing nomenclature layers, *A* and *C* consist of the single six-membered rings and *B* the double six-membered rings. The second type of nomenclature suggested by Pauling now becomes very useful and is even more informative. Here the . . . *ABCB* . . . sequence corresponds to . . . *hc* . . . Thus, in erionite we find that the *h* layers correspond to the single and the *c* layers to the double six-membered rings.

Two new proposed structures. In considering the build-up of possible structures containing just double six-membered rings, a new cavity may be formed which is similar to the erionite cavity. Such a cavity is formed when $n = 4$ for the . . . *ABCB* . . . sequence and $n = 6$ for the . . . *ABCACB* . . . sequence. For want of a better name, these two new structures will be called hypothetical structure I and II, respectively, and will be referred to as I and II.

In the structure of I, formed by the . . . *ABCB* . . . sequence with $n = 4$, the large cavities (*ABCBA*) lie along the *c*-direction of the hexagonal unit cell, one above the other and joined through double six-membered rings (rather than through single six-membered rings as in erionite itself). Each cavity has nine non-planar eight-membered ring openings as shown in Fig. 2*c*. The three openings in the top and the three in the bottom half are each shared by other I cavities which are displaced along the *c*-direction by one-half the unit cell-length; the three central eight-membered ring openings are each shared with a gmelinite cavity. Thus, each I cavity is surrounded by six other I cavities and by three gmelinite cavities. The c_0 lattice parameter is theoretically 20·0 Å; a_0, of course, remains unchanged.

Hypothetical structure II, formed by the . . . *ABCACB* . . . sequence with $n = 6$, contains the same large cavity as in I; however, in this structure the chabazite cavity is also present so that now all three cavities—gmelinite, chabazite and I—co-exist, resulting in a hexagonal unit cell the c_0 parameter of which is now 30·0 Å. The framework of these cavities is shown in Fig. 2. This structure may also be described by again considering the cavities which

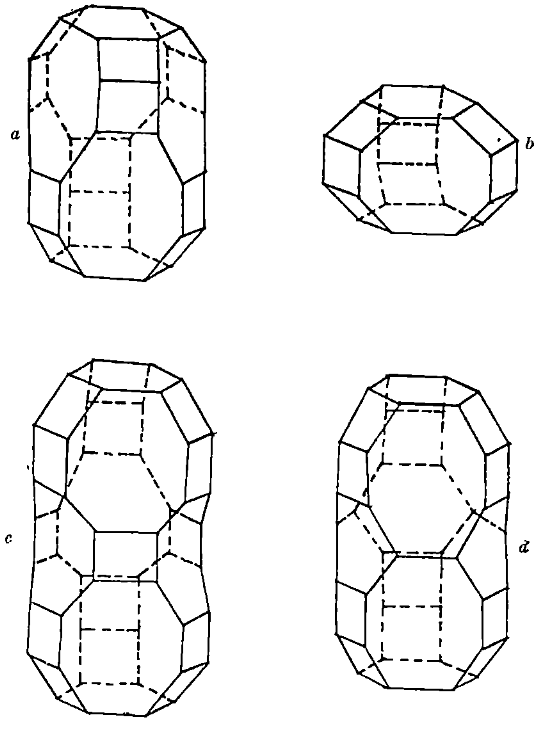

Fig. 2. The cages in (*a*) chabazite, (*b*) gmelinite, (*c*) hypothetical I and II, (*d*) erionite. The aluminium and silicon atoms occupy the corners. The oxygen atoms and cations are not shown

Table 1

Name	No. of layers in repeating unit	Layer sequence	Layer sequence, Pauling notation	*h*	*c*	Length of *c*-axis*
Gmelinite	2	...*AB*...	...*h*...	Double	—	10·0 Å
Chabazite	3	...*ABC*...	...*c*...	—	Double	15·0
Erionite	4	...*ABCB*...	...*hc*...	Single	Double	15·1
Hypothetical structure I	4	...*ABCB*...	...*hc*...	Double	Double	(20·0)
Hypothetical structure II	6	...*ABCACB*...	...*hcc*...	Double	Double	(30·0)

* Experimental values from ref. 4. Theoretical values are indicated in brackets.

are attached to the I cavity: attached to the top three and bottom three eight-membered ring openings are the chabazite cavities, the top three being inverted with respect to the bottom three in accord with the 3/*m* symmetry; the three central eight-membered ring openings are each shared with a gmelinite cavity, which accounts for the additional 10·0 Å in the c_0 lattice parameter relative to that for the I structure.

A summary of the zeolites discussed here is given in Table 1, which includes a summary of those layers consisting of single and double six-membered rings.

In view of the nomenclature discussed here, the similarities in structure of gmelinite, chabazite and erionite are evident. The fact that gmelinite has sluggish sorption and that the sorptive properties are similar to those of the other members of the chabazite group may be qualitatively explained in terms of random or periodic blocking of the twelve-membered ring channels by a layer turned 60° to form an *ABC*, *ABCB* or *ABCACB* sequence. Thus, if the chances of growth of *B* on *A* and *C* on *A* are about equal, it appears that intergrowth of chabazite, gmelinite, I and II would be possible. Finally, in view of the theoretically possible periodic stacking of *A*, *B* and *C* it is evident that many other structures might be possible, consisting of a stacking of planes of single, double or single and double six-membered rings.

We thank Dr. W. M. Meier for his advice.

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CONTAINMENT OF PLASMA IN A MAGNETIC MIRROR, WITH HEXAPOLE STABILIZING FIELDS

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IN the experiments reported here, plasma of density approximately 4 × 10^{10} ions/cm^3 and mean ion energy 1 keV trapped in a magnetic mirror has been found to escape by a rotating flute instability within 60–80 μsec. When hexapole cusp-like fields perpendicular to the mirror field were added, the flute instability disappeared and the density of trapped plasma decayed smoothly with a time constant which could be as large as 400 μsec. Some plasma was observed in the magnetic trap after 1 msec.

A hydrogen plasma was produced by a coaxial gun[1] (peak current of 44 k.amp, rising in 1 μsec) and its properties measured in a uniform guide fields of 600 oersteds at a point 2 m from the gun. The plasma consisted of a fast component of density 3 × 10^{11}/cm^3, mean directed velocity 4·5 × 10^7 cm/sec (corresponding to an ion energy of 1 keV), perpendicular energy 60 eV, electron temperature about 12 eV; followed by a much denser and slower component ($n \sim 3 \times 10^{12}$/cm^2, velocity $\sim 10^6$ cm/sec). The measurements were made with diamagnetic loops, ion collectors, biased Langmuir probes and a special electrostatic ion-energy analyser. The ions in the fast plasma were H$^+$.

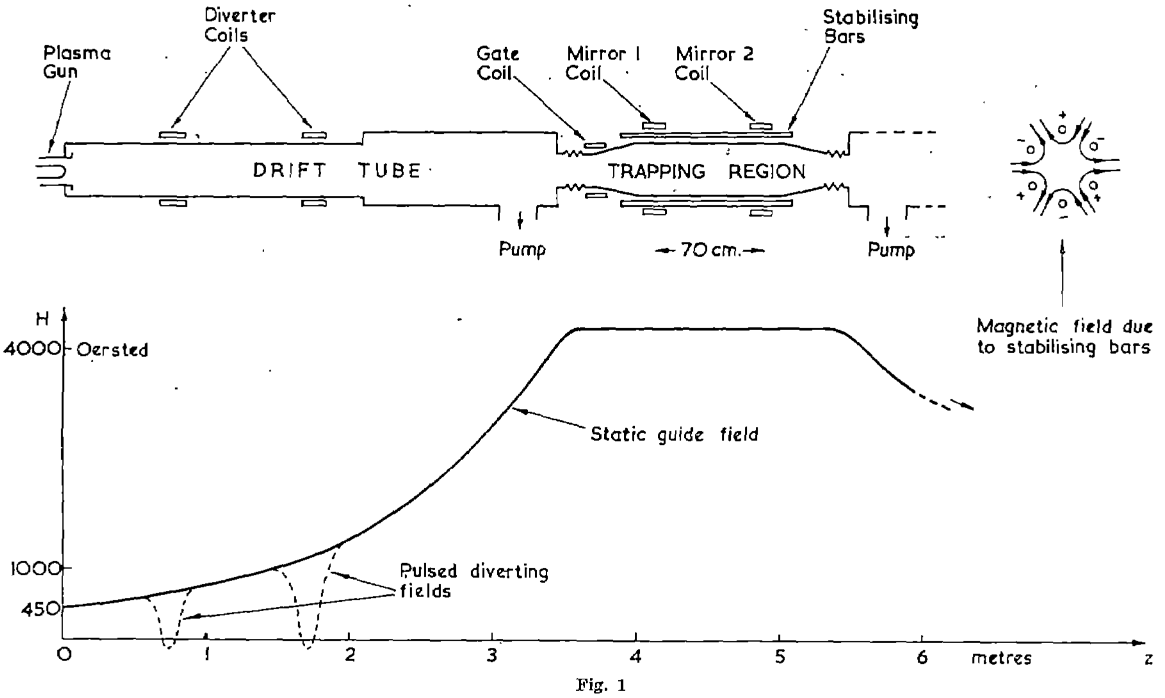
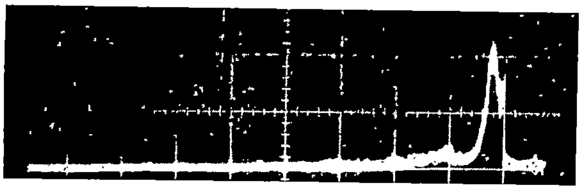


Fig. 1

In the containment experiments the plasma from this gun was injected into a static converging magnetic field which increased smoothly from 450 œ to 4·3 kœ over 3·5 m length, remained uniform over a further distance of 2 m (the central trapping region) and beyond that decreased symmetrically to 450 œ (Fig. 1). The fast plasma moved adiabatically along this field from the gun into the central trapping region. The slow plasma was prevented from following it by the sudden application of pulsed magnetic fields opposite to the guide field, at points 75 cm and 170 cm from the gun. This diverted the slow plasma into the walls of the vessel, which was of 22-cm diameter 'Pyrex' glass in the central section and under the diverter coils, and of 31-cm diameter aluminium elsewhere. The base pressure was 3×10^{-7} torr. Pulsed magnetic fields were produced by the coils marked mirror 1, 2, and 'gate' on the diagram: the mirror coils superimposed on the d.c. guide field an additional 3·6 kœ rising in 50 μsec and decaying with a time-constant of 4·5 msec; the gate coil produced 13 kœ rising in 12 μsec, decaying in 50 μsec. Plasma was trapped by first energizing mirror 2, firing the gun and then applying the gate and mirror 1 fields before the reflected plasma could escape. This produced a simple symmetrical magnetic mirror trap of ratio initially 1·8, changing by about 10 per cent within 1 msec.

The density of the trapped plasma, measured with a 3 cm microwave interferometer, had a mean value of $4 \times 10^{10}/cm^3$ over a diameter of 10 cm. The lifetime of the fast ions was obtained by measuring the flux of fast atoms formed by charge exchange on the background gas: the neutral atom detector incorporated a water-vapour cell in which about 6 per cent of the fast atom flux was converted back into ions by charge exchange, and the flux in a given energy interval was measured. Fig. 2 shows a typical result; the fast ions (of energy 1·6 ± 0·2 keV) escaped from the magnetic mirror in less than 80 μsec. Measurements with double Langmuir probes (1 cm^2 area) arranged around the circumference of the vessel showed that the plasma was rotating at 10^5 radians/sec and also moving radially outwards with an average velocity of 5×10^5 cm/sec (Fig. 3). Signals on probes disposed parallel to the lines of force were highly correlated. High impedance electrostatic probes inserted into the plasma indicated fluctuating electric fields (~ 30 V/cm) consistent with the speed and direction of rotation.



⟵ *t* 100 μsec/cm

Fig. 2. Simple mirror configuration

In subsequent experiments, cusp fields perpendicular to the axis of the vessel were pulsed on at the same time as the mirror 1 and gate fields. The cusp fields were produced by six groups of bars 140 cm long lying parallel to the axis and disposed symmetrically around the central part of the vessel at a radius of about 15 cm. Currents in adjacent groups flow in opposite directions (Fig. 1). The field rose to 11 kœ between bars in 45 μsec and fell by 25 per cent in 1 msec. In this magnetic configuration the flux of neutral atoms decayed smoothly with a time-constant of several hundred microseconds (Fig. 4). The density could not be measured with the microwave interferometer due to the complexity of the magnetic field, but the flux of neutral atoms was the same as in a simple mirror, indicating a comparable density. No rotation or spiralling of the plasma was observed: wall probes along the cusp lines showed a smooth signal for a few hundred microseconds, but other wall probes showed no signal. The electric field in the plasma was less than 1 V/cm.

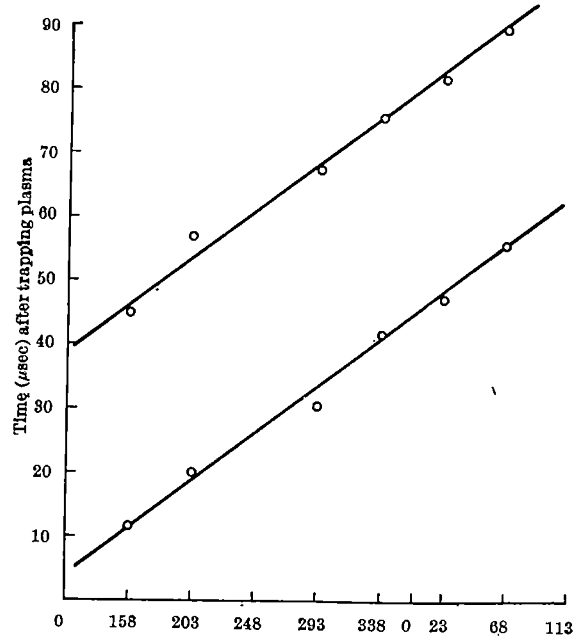
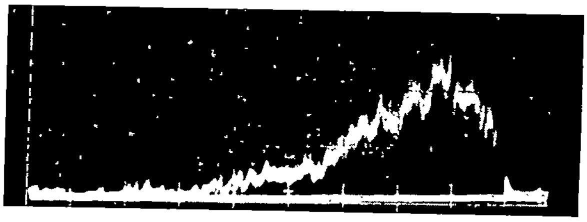


Fig. 3. Time of peak Langmuir probe signal versus azimuthal probe position for circumferential array of eight probes



⟵ *t* 100 μsec/cm

Fig. 4. Mirror-cusp configuration

The decay of the plasma, although smooth, was some four times faster than would be expected from charge exchange on the background gas at the measured base pressure. We believe that the neutral gas density is suddenly increased by gas knocked off the walls due to plasma bombardment observed during the trapping process, a belief supported by mass spectrometer analysis. Moreover, when the amount of injected plasma was reduced by a limiter with a small hole, the decay time of the trapped plasma increased from about 250 to 400 μsec: also, when the diverting fields were not used, the fast plasma disappeared abruptly when the slow plasma arrived. Thus, the residual plasma-loss rate observed with the hexapole fields excited is qualitatively consistent with a charge exchange mechanism.

These results are consistent with those of Ioffe[2] *et al.*, who first used this type of magnetic field configuration but with a different method of plasma generation. They show the advantages of combining the stabilizing properties of cusp fields with the adiabatic confinement due to simple magnetic mirrors, as pointed out by Artsimovich[3] and examined theoretically by Taylor[4].

We thank Mr. K. Plummer and Mr. A. Ellington, who made the apparatus, and Mr. D. Wort, Dr. M. Seidl and Mr. E. Hotston, who made the microwave measurements.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Stability of Hydrogen Compounds on Venus

Recent space probe measurements have confirmed that high-temperature conditions prevail in the lower atmosphere of Venus[1]. Since high temperatures favour the establishment of thermochemical equilibria, these observations require a revaluation of physical and chemical conditions on this planet. I have proposed large-scale interaction of the atmospheric gases and the minerals of the lithosphere to account for the observed atmospheric characteristics[2], and it was demonstrated that the carbon dioxide content of the atmosphere can be accounted for by decarbonation reactions at the inferred temperature of 700° K. In addition, it was proposed that the failure to observe hydrogen compounds is consistent with the expected high rate of escape of hydrogen and the comparatively high oxidation state of the atmosphere.

Unsuccessful attempts to detect CH_4, C_2H_6, C_2H_4 and NH_3 in the atmosphere of Venus were made by Kuiper[3], and recently Spinrad[4] has concluded that the mixing ratio of water is less than 10^{-5} at depths in the atmosphere corresponding to a total pressure of 8 atmospheres. It therefore seems likely that this mixing ratio limit holds to the base of the atmosphere.

The stability of the hydrogen compounds on Venus may be related to the observable parameters $(P_{\mathrm{CO_2}}/P_{\mathrm{CO}})$ and $P_{\mathrm{H_2O}}$ (so far determined as limits only), where the P's refer to the fugacities or partial pressures, depending on the deviation from perfect mixtures. From Kuiper's results[3] we obtain $(P_{\mathrm{CO_2}}/P_{\mathrm{CO}}) \geq 10^3$ if the absorption paths for carbon dioxide and carbon monoxide in the Venus atmosphere are comparable and if the fugacity ratios are roughly proportional to the abundance ratios. Also according to Sagan[5] it seems likely that the total surface pressure is in the range of 10–100 atmospheres. If P(total) $\sim$ 100 atmospheres, then the water mixing ratio limit of 10^{-5} gives $P_{\mathrm{H_2O}} \leq 10^{-3}$ atmospheres at the surface. Thus the atmosphere is very dry at the inferred temperature of 700° K. It is obvious that lower values of P(total) yield even smaller values of $P_{\mathrm{H_2O}}$.

We may now calculate the surface hydrogen pressure limit from the limits of $(P_{\mathrm{CO_2}}/P_{\mathrm{CO}})$ and $P_{\mathrm{H_2O}}$ by the following reaction:

$$CO_2 + H_2 \rightleftharpoons CO + H_2O \qquad (a)$$

This reaction has the equation of equilibrium:

$$\frac{P_{\mathrm{CO_2}}}{P_{\mathrm{CO}}} K_a = \frac{P_{\mathrm{H_2O}}}{P_{\mathrm{H_2}}} \qquad (1)$$

where $K_a = 10^{-0.95}$ at 700° K, from the data of Rossini *et al.*[6]. Accordingly we obtained $P_{\mathrm{H_2}} \leq 10^{-5.05}$ at the surface.

From the foregoing data it is in principle possible to evaluate the stabilities of many hydrogen compounds. For example, it is interesting to examine Hoyle's speculation[7] that large quantities of hydrocarbons may occur on Venus. We may begin by examining the stability of methane, the most stable member of the paraffin series. The pertinent reaction is:

$$2\,H_2 + 2\,CO \rightleftharpoons CO_2 + CH_4 \qquad (b)$$

and the corresponding equation of equilibrium is:

$$\frac{P_{\mathrm{CO}}^2}{P_{\mathrm{CO_2}}} K_b = \frac{P_{\mathrm{CH_4}}}{P_{\mathrm{H_2}}^2} \qquad (2)$$

At 700° K we obtain from the data of Rossini[6] that $K_b = 10^{4.52}$, and if $(P_{\mathrm{CO^2}}/P_{\mathrm{CO}}) \geq 10^3$, $P_{\mathrm{CO_2}} \sim$ atmospheres[2], and $P_{\mathrm{H_2}} \leq 10^{-5.05}$, then $P_{\mathrm{CH_4}} \leq 10^{-10.58}$ atmospheres at the surface. Thus reaction (*b*) is displaced to the left by both the strongly oxidizing conditions and the low hydrogen partial pressure.

We may now also evaluate the stabilities of the higher polymers of the paraffin series by relating them to the hydrogen and methane abundances. For example, for *n*-octane we have the following reaction:

$$8\,CH_4 \rightleftharpoons C_8H_{18} + 7\,H_2 \qquad (c)$$

The equilibrium constant[6] for this reaction is $K_c = 10^{-33.2}$, which shows that this polymer cannot be abundant. Similarly all the higher polymers will be less abundant than methane since the free energies of formation increase by a constant increment of 11,951 calories per CH_2 group at 700° K.

From these tables of free energies of formation we may also compare the relative stabilities of the various hydrocarbon series. It is obvious that such comparisons can be made only when all the gases are in some standard state since the stabilities depend on the gas pressures. We may, for example, compare ethyl*cyclo*hexane with *n*-octane:

$$C_8H_{18} \rightleftharpoons C_8H_{16} + H_2 \qquad (d)$$

ΔG^0 at 700° K for this reaction is 470 calories. The low hydrogen partial pressure that is required to make $(P_{\mathrm{C_8H_{16}}}/P_{\mathrm{C_8H_{18}}})$ large ensures the instability of the hydrocarbons.

The benzene series of hydrocarbons is somewhat more stable than the paraffin series for corresponding polymers under standard conditions. However, the lowest benzene polymer is C_6H_6, and this will be far less abundant than methane. Thus it seems unlikely that any significant quantities of hydrocarbons could occur on the surface or in the lower atmosphere, and this seems also to preclude their occurrence in the higher atmospheric levels.

Most other hydrogen compounds also seem precluded by the Venus environment. For example, we may consider the stability of the ammonia molecule according to the reaction:

$$\tfrac{1}{2}\,N_2 + 3/2H_2 \rightleftharpoons NH_3 \qquad (e)$$

From the data of Kubaschewski and Evans[8] and of Kelley[9] we see that $K_e = 10^{-1.21}$. Thus if $P_{\mathrm{H_2}} \leq 10^{-5.05}$ we obtain the result that:

$$P_{\mathrm{NH_3}} \leq 10^{-1.21}\,(10^{-5.05})^{3/2}\,P_{\mathrm{N_2}}{}^{\frac{1}{2}} \qquad (3)$$

showing that no significant quantity of ammonia can occur if $P_{\mathrm{N_2}}$ has any reasonable value for a terrestrial planet of this size.

No attempt is made here to evaluate the stabilities of other more complex hydrogen-bearing gaseous molecules. However, it is apparent that none of these hydrogen compounds will be favoured in any absolute sense by the high temperatures and low hydrogen partial pressure.

A problem of considerable interest concerns the condensed hydrogen compounds in the lithosphere of Venus and their relation to the atmospheric gases. The stabilities of certain hydrous rock-forming silicates have already been discussed by me[2]. The problem is a difficult one since only a limited number of experimental data exist. However, it seems that the limiting value of $P_{\mathrm{H_2O}} \leq 10^{-3}$ atmospheres at the surface is somewhat below the stability range of at least some hydrous silicates. Thus the thermochemical data obtained by Weeks[10] and by Robie[11] indicate that the $P_{\mathrm{H_2O}}$ required to stabilize the amphibole tremolite with respect to the pyroxenes and free silica is 0·04 atmospheres. However, it seems probable that more stable hydrous silicates than this exist, with complex Mg–Al-bearing amphiboles and micas being the likely species.

The recent suggestion by Walter[12] regarding the possible occurrence of hydrous carbonatite magmas on the Venus surface is certainly in conflict with the cited data of Spinrad on the abundance of water. Such magmas appear to require water partial pressures in the range of ten atmospheres under the most favourable conditions, which as we have seen are four orders of magnitude greater than the deduced limiting value. Also if hydrogen has long been escaping from Venus, as we have reason to believe, then the lithosphere should be depleted of water except for that bound tightly in the silicates. Thus even the existence of hydrous magmas within the crust seems unlikely.

It is apparent that the conditions deduced for the lower atmosphere of Venus militate against the occurrence of life if it consists of the usual hydrogen, carbon and nitrogen compounds. It is remotely possible that some form of life could exist under æolian conditions in the middle atmosphere where the temperature is lower. However, it seems very improbable that such life forms could survive for long under the expected vicissitudes.

Since hydrogen escapes from Venus, we should expect considerable differences in the secular behaviour of the hydrogen compounds as compared with other species. Thus, although the concentrations of oxygen, carbon dioxide and carbon monoxide might be approximately fixed for long periods by the buffering effect of the condensed lithospheric minerals[2], we should expect that the secular depletion of hydrogen would prevent long-term buffer relations with respect to water. The extent to which such differences would show up depends on the rate of the photochemical decomposition of the hydrogen compounds and the escape rate of hydrogen.

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Slowly Varying Component of Solar Radio Emission in Relation to the Solar Cycle

IN a previous communication we showed[1] that the so-called slowly varying component (*S*-component) of solar radio emission, exhibiting the characteristic 27-day variation in the received flux, is limited only within the frequency range 470–29,000 Mc/s and that the amplitude of the characteristic variation would be maximum at a frequency over 4,000 Mc/s. However, the foregoing analysis was performed with the help of solar radio noise flux data for the International Geophysical Year period of 1·5 years only, that is, during the period when the Sun was in the most active form in the present solar cycle. It is quite logical to examine the data over a longer period with a view to seeing whether the above conclusions made for the *S*-component are also valid year to year throughout the solar cycle. We were thus prompted to study the slowly varying component of solar radio emission in relation to solar cycle.

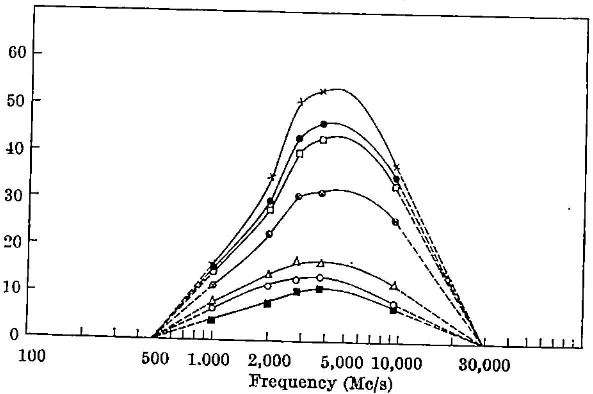

Fig. 1. The curves showing the relation of the variation index with frequency for the years 1957 (×), 1958 (●), 1959 (□), 1960 (⊗), 1961 (△), 1962 (○) and 1963 (■)

The method of analysis adopted in the present case is similar to the earlier one referred to above. In that analysis plots of the daily mean values of solar flux at five different frequencies (9,400, 3,750, 2,800, 2,000 and 1,000 Mc/s) for the International Geophysical Year period were examined. All these plots exhibited pronounced maxima and minima following roughly the 27-day cycle and it was also observed that the amplitude of this variation changed markedly with frequency. A variation index (V.I.) was defined for a particular frequency as the variation of the amplitude from a maximum to the corresponding minimum, showing the characteristic periodicity, averaged over the entire period. Besides the conclusions mentioned here it was also shown that the variation index might conveniently be used as a measure of the correlation between the slowly varying component and the sunspot activity. The present analysis has been carried out for all the above five frequencies over the period 1957–63. For any particular frequency the variation index has been determined individually for every year by the same procedure.

Fig. 1 shows the plot of the variation index against frequency for different years. It is found that all the seven curves are similar and are of the same nature as was previously obtained by us. Moreover, when extrapolated, the continuous curves through the different sets of points would intersect the frequency axis at the same regions—470 Mc/s in the low-frequency side and 29,000 Mc/s in the high-frequency side. It is also observed that the variation index appears to be maximum

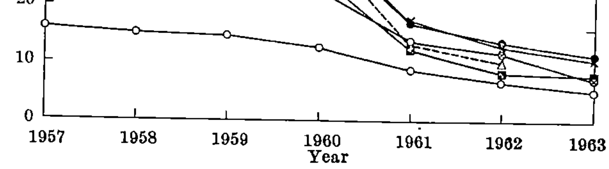

Fig. 2. Plots showing the year to year change in the variation index for the frequencies 9,400 Mc/s (■), 3,750 Mc/s (●), 2,800 Mc/s (×), 2,000 Mc/s (⊗) and 1,000 Mc/s (○). The figure also shows the corresponding variation of the yearly mean Zurich relative sunspot numbers △

in general in the frequency range 4,000–5,000 Mc/s. The results thus obtained for all the seven years confirm, to a large extent, the conclusions of our previous analysis both regarding the cut-off frequencies of the *S*-component of the solar radio emission and also the frequency at which the so-called variation index attains a maximum value. It will be interesting to note here that the curves shown in Fig. 1 are of similar nature to those obtained by Kakinuma and Swarup[2] for the spectra of the *S*-component.

An additional striking feature exhibited by the curves of Fig. 1 is that as we proceed from 1957 onwards the curves gradually come down, indicating a fall in the value of V.I. from year to year. This led us to examine the matter in some detail. Fig. 2 shows the plot of the variation index against year with frequency as the parameter. The yearly mean values of Zurich relative sunspot number are also shown on the same diagram. It will be found that the year to year values of the variation index for all the frequencies show parallelism with those of the sunspot numbers, which clearly indicates the solar cycle dependence of the slowly varying component. The factor by which the index changes from the maximum value in 1957 to the minimum in 1963 are shown in Table 1.

Table 1

Frequency in Mc/s	9,400	3,750	2,800	2,000	1,000
$\frac{\text{V.I. in 1957}}{\text{V.I. in 1963}}$	4·7	4·3	4·8	4·6	3·8

The fact that the variation index depends on the solar cycle led us to examine its variation with sunspot number (Fig. 3). A linear relation is obtained for the two quantities. It is interesting to find that the straight line when extrapolated to zero sunspot number cuts the index axis with a positive intercept which implies that the variation index is not zero, but something positive, though small, even at zero sunspot number condition when the Sun should be completely quiet. The intercepts between the values 5 and 7·5 involve variation of the order of 10–15 flux units.

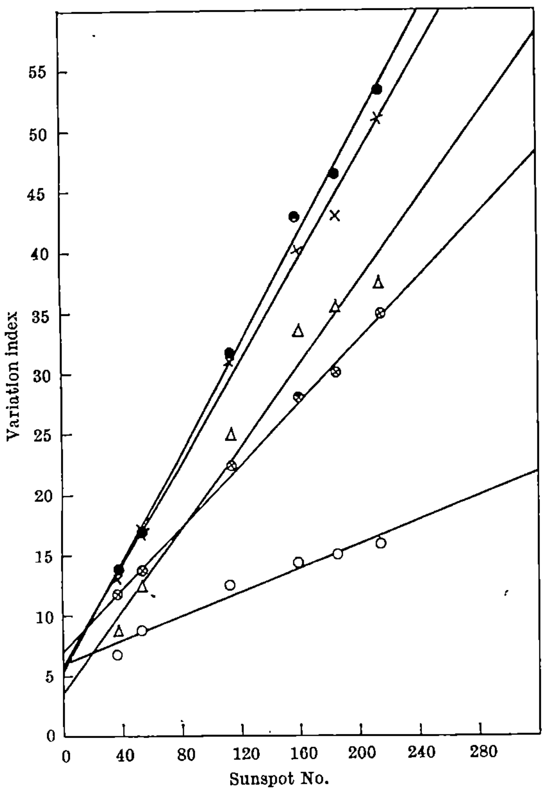

Fig. 3. Plots showing the linear relation between the variation index at frequencies 9,400 Mc/s (△), 3,750 Mc/s (●), 2,800 Mc/s (×), 2,000 Mc/s (⊗) and 1,000 Mc/s (○) and the yearly mean Zurich relative sunspot numbers for the period 1957–62

The foregoing analysis leads us to draw the following conclusions:

(1) The *S*-component of solar radio emission is to be observed only within the frequency range 470–29,000 Mc/s, maximum variation being limited in general in the frequency range 4,000–5,000 Mc/s (Fig. 1). The fact that this is true all throughout the solar cycle is highly significant and needs special attention. It may thus be concluded that the origin of the *S*-component is restricted within two distinct levels of the solar atmosphere and also the maximum variation will occur within a specified zone all throughout the solar cycle.

(2) The *S*-component varies in accordance with the solar cycle (Fig. 2). The factor by which its value changes from the year of maximum activity to that of minimum is slightly over 4. It may be mentioned here that Nicolet[3] also indicated a figure of the same order from a different consideration.

(3) Finally, the slowly varying component is linearly related to the sunspot number. The fact that the component exists even when the sunspot number is zero (Fig. 3) needs further investigation. Possibly this may be due to some active regions on the solar disk with no associated sunspot as have been detected by Covington and Harvey[4].

It should, however, be clearly indicated that the important conclusions drawn here regarding the slowly varying component in relation to solar cycle depend solely on the true nature of the curves shown in Fig. 1. It is thus apparent that in order to evaluate the two cut-off frequencies as well as the frequency at which the variation index becomes maximum more precisely one must consider the individual cases of as many frequencies as possible, particularly in the frequency ranges 500–2,000 Mc/s and 10,000–30,000 Mc/s.

We thank Prof. J. N. Bhar for his advice. One of us (D. B.) thanks the Council for Scientific and Industrial Research, Government of India, for financial assistance. Results used in our analysis were obtained from *CRPL–Solar Geophysical Data Books* and IGY, IGC and post-IGC results published by the Science Council of Japan. We also thank Dr. H. Tanaka of the Research Institute of Atmospherics, Nagoya University, Japan, for sending us some of his solar radio noise data before publication.

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GEOPHYSICS

Instability of Temperature Ice-sheets owing to a Feed-back Mechanism

THE response of a temperate glacier to changes in its specific budget was theoretically examined in 1903 by S. Finsterwalder[1]. He limited himself to the plane problem, and I shall do the same. In 1958 Weertman[2] urged forward the problem by introducing the perturbation method and the incidence of the changes in the superficial slope.

For the calculations, the firn is changed into an equivalent ice-sheet of compact ice. The x axis is taken along the surface in the direction of flow. Let $q_0(x)$, $h_0(x)$ and $\alpha_0(x)$ be the datum-state values of the discharge per unit breadth, the thickness of the ice-sheet and the slope of its surface, respectively. Let $q_1(x, t)$, $h_1(x, t)$ and $\alpha_1(x, t)$ be the per-

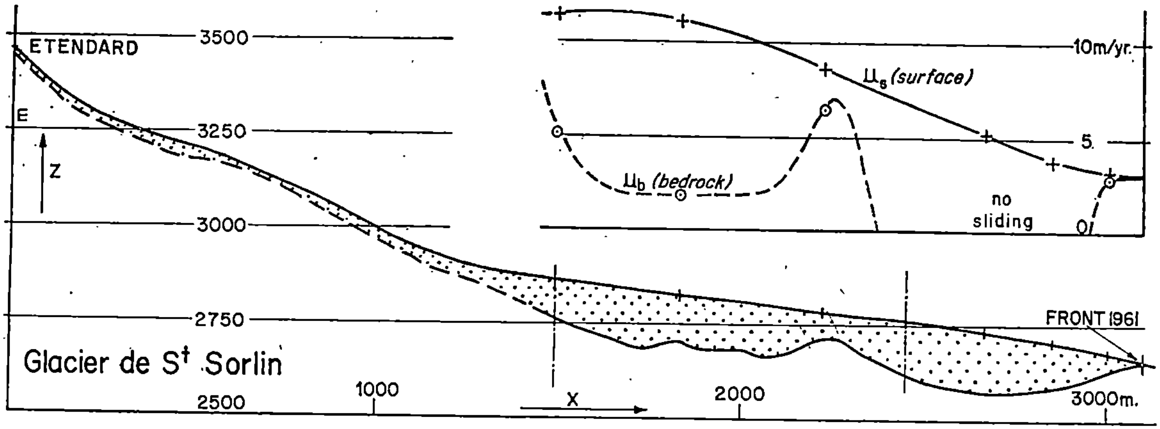

Fig. 1

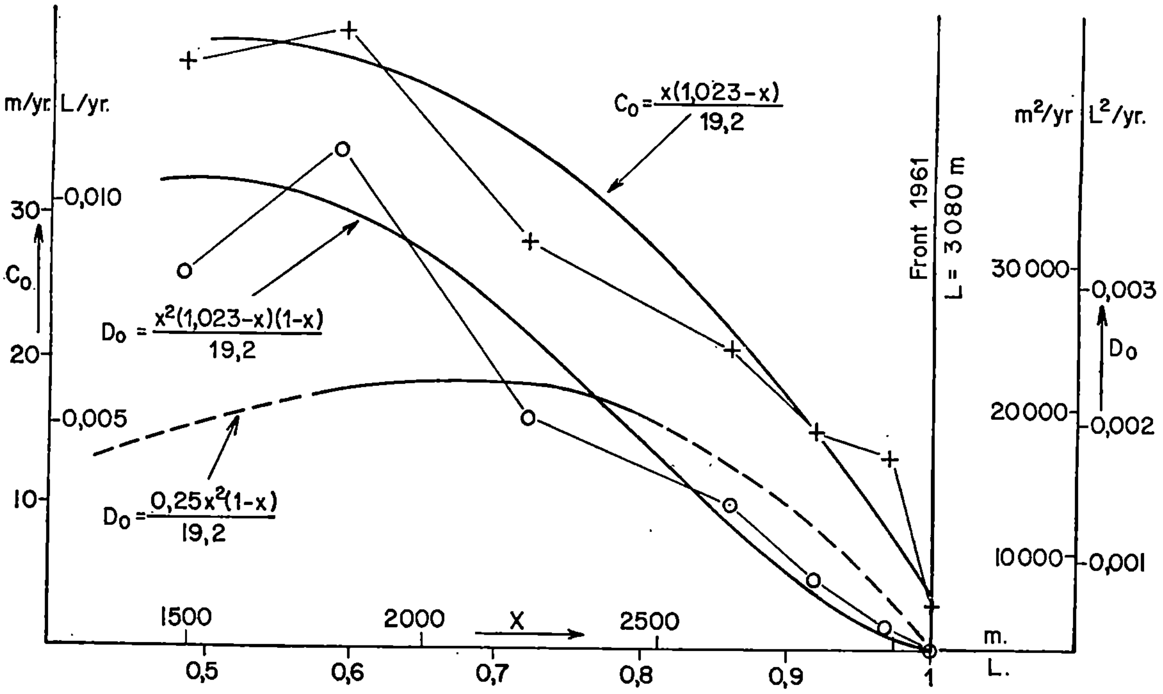

Fig. 2

turbations of these variables. We call b the specific budget (snowfall + superimposed ice-ablation, in height of ice per unit time). b_0 is not the datum-state value, but the value at which the glacier would have been in a steady state at $t = 0$:

$$b_0(x) = \frac{dq_0}{dx}$$

The response of the glacier to a slight change in the specific budget, say, $b_1(x, t)$, is given by the continuity equation. I shall write it as did Nye[3]:

$$\frac{\partial h_1}{\partial t} = b_1 - c_0' h_1 - (C_0 - D_0') \frac{\partial h_1}{\partial t} + D_0 \frac{\partial^2 h_1}{\partial x^2}$$

with:

$$C_0(x) = \left(\frac{\partial q}{\partial h}\right)_0 \qquad D_0(x) = \left(\frac{\partial q}{\partial \alpha}\right)_0$$

Nye has given a very interesting algebraic model. Taking the length of the glacier as the unit of length, let us write it in a slightly different manner:

$$C_0 = \frac{x(1 + \delta - x)}{T} \qquad D_0 = \frac{E x^2(1 - x)}{T}$$

δ, T and E are constants ($\delta \ll 1$). Nye solved the perturbation equation when b_1 is independent of x and $E = 1$. In fact, whenever E is the reciprocal of an integer p, a closed solution can be written. First let b_1 be a step function:

$$b_1 = 0 \qquad t < 0$$
$$b_1 = A \qquad t \geqslant 0$$

Let:

$$h_1 = H_0 + H_1 x + \ldots + H_n x^n + \ldots$$

writing $t/T = \tau$ and:

$$(n + 1)(1 + \delta - nE) = r_n$$
$$[n + 1][1 - (n - 1)E] = s_n$$

one gets the equations:

$$\frac{dH_n}{d\tau} + r_n H_n = s_n H_{n-1}$$

Whence:

$$\left(\frac{d}{d\tau} + r_0\right)\left(\frac{d}{d\tau} + r_1\right)\ldots\left(\frac{d}{d\tau} + r_n\right)H_n = s_1 \ldots s_n AT$$

So H_n cancels when $n > p$. Deriving the solution for a step function we obtain the response of the glacier to an impulse unity at $t = 0$:

$$R(x, t) = \dot{H}_0 + \dot{H}_1 x + \ldots + \dot{H}_p x^p$$

with, for $t \geqslant 0$:

$$\dot{H}_n(t) = s_1 \ldots s_n \left[\frac{e^{-r_0 t/T}}{(r_1 - r_0) \ldots (r_n - r_0)} + \ldots + \frac{e^{-r_n t/T}}{(r_0 - r_n) \ldots (r_{n-1} - r_n)} \right]$$

The damping coefficients r_n are bigger than unity, the last one, $r_p = (p + 1)\delta$, excepted.

The response to any variation $b_1(t)$ is then:

$$h_1(x, t) = \int_{-\infty}^{t} b_1(\theta) \,.\, R(x, t - \theta) \,.\, d\theta = \int_{-\infty}^{+\infty} (\text{the same})$$

As in any filter, the complex amplification (H/A in Nye's paper) is the Fourier transform of $R(x, t)$.

Let us see now if Nye's algebraic model is a realistic one. According to the theory of parallel flow for temperate glaciers and Weertman's theory of sliding, we can approximately write, n being the power in the deformation law of ice, $m = (n + 1)/2$, and C_1 and C_2 two constants:

$$q = C_1 h^{n+2} \alpha^n + C_2 h^{m+1} \alpha^m$$

The measured velocity on the surface is:

$$u_s = \frac{n + 2}{n + 1} C_1 h^{n+1} \alpha^n + C_2 h^m \alpha^m$$

and the less-accurately known sliding velocity on the rock bed:

$$u_b = C_2 h^m \alpha^m$$

Whence, taking the commonly accepted value $n = 3$:

$$C_0 = 4 u_s - u_b$$

$$D_0 = [2 \cdot 4 \, u_s + 0 \cdot 6 \, u_b] \frac{h_0}{\alpha_0}$$

(Nye's statement $u_s \simeq u_b$ cannot be accepted, as Weertman's theory is only valid for very slow sliding velocities[4].)

A longitudinal cross-section of the Glacier de Saint-Sorlin according to seismic soundings[5], the measured values of $u_s(x)$ and the estimated values of $u_b(x)$ are given in Fig. 1. The corresponding values of C_0 and D_0 are given in Fig. 2. By taking $T = 19 \cdot 2$ years and $\delta = 0 \cdot 023$, C_0 fits the algebraic model well. But no value of E can make D_0 fit the model. In fact, it has been proved[6] that E must be equal to 1 near the begschrund and near the front. However, for the ablation zone of the Glacier de Saint-Sorlin, Nye's model can be kept with $E = 0 \cdot 23 \simeq 1/4$ more or less.

Wegmann and Cailleux have noted that the thickening of an ice sheet affords an increase in the specific budget at a given point. This feed-back mechanism can be accounted for by writing:

$$b = b_0(x) + M h_1 + b_1(t)$$

In the ablation zone, for an alpine glacier, $M \simeq 0 \cdot 01$ yr.$^{-1}$. The expression of $R(x, t)$ remains unchanged, but the damping coefficients become:

$$r_n = (n + 1)(1 + \delta - nE) - MT$$

The last one:

$$r_p = (p + 1)\delta - MT \simeq -0 \cdot 1$$

becomes negative. So Nye's algebraic model of a glacier is an unstable one. This conclusion is still effective if we consider b_1 dependent of x, for example, $A = A_0 + A_1 x + A_2 x^2$, as only the first three H_n are changed.

This instability of glacier snouts seems to be real, and may be found with an even more realistic function for D_0. An active ice sheet cannot end on a level bed-rock with a stable edge-shaped snout. It must advance or recede until it reaches some special area. This can be a step of the valley, where the velocity x is increased, and thus δ. It can also be a lake, where the glacier end floats, and then the altitude of its surface becomes about eight times less dependent on glacier thickness, and so also M.

Thus temperate ice sheets fluctuate within areas of stability, and from time to time recede or advance from one area of stability to another. This illuminates the behaviour of glaciers. It explains the formation of recessional moraines during the last stage of the ice-age.

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Seismic Anisotropy of the Uppermost Mantle under Oceans

OLIVINE in rock-forming aggregates has a strong tendency during flow or deformation to develop a preferred orientation of its crystallographic axes. Turner[1] describes a number of olivine fabrics developed under a variety of conditions. The pertinent examples, his figures numbers 7, 10 and 13, show fabrics for banded dunites in which the *b* crystallographic axis is strongly concentrated perpendicular to the banding, and figure 16, in which *b* is perpendicular to a plane of pronounced fissility. The (010) plane of olivine is the plane of the best cleavage and most probable glide plane for this mineral.

Verma[2], working in Francis Birch's laboratory, determined the seismic velocities for olivine as shown in Fig. 1. If the upper mantle has been subjected to flow in the horizontal plane and this has resulted in even a slight statistical orientation of one or more of the olivine crystallographic axes, the differences in seismic velocities are so great that it should be evident in velocities found by seismic refraction (see also Hess[3] and de Roever[4]). The concept was tested against fourteen seismic refraction profiles for an area straddling the Mendocino fracture zone presented in a paper by R. Raitt[5] at the I.U.G.G. meeting at Berkeley in August 1963 (Fig. 2). All profiles which gave both the seismic velocity below the Moho and the azimuth on which they were observed were used with the exception noted here. One would expect the glide planes to tend to line up parallel to the major shear zone so that the low seismic velocities would be perpendicular

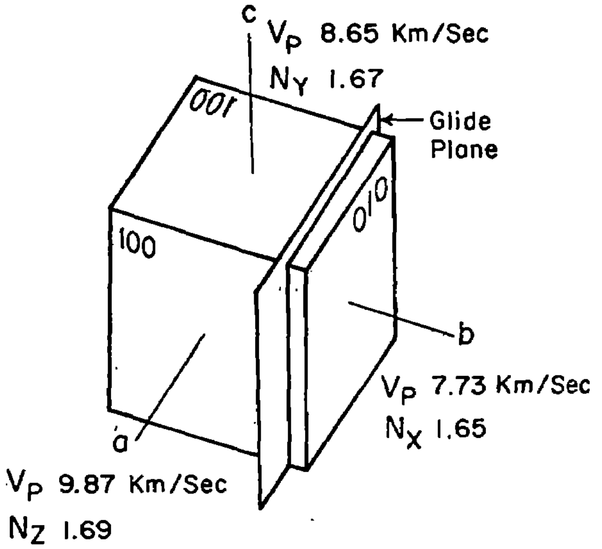

Fig. 1. Orientation of the principal optical directions, seismic velocities (Vp) and glide plane for magnesian olivine

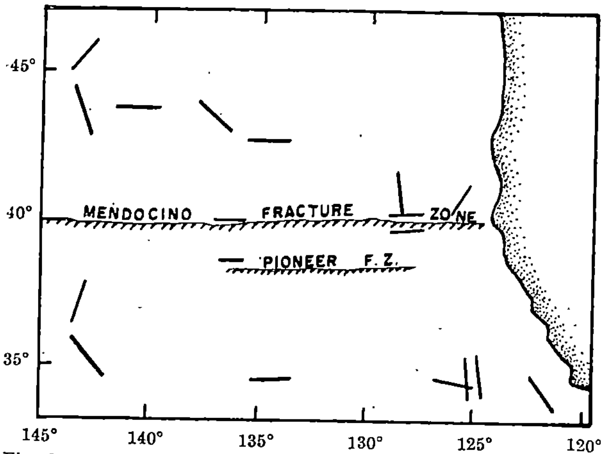

Fig. 2. Location of seismic refraction profiles in Raitt's study of Mendocino fracture zone area off California

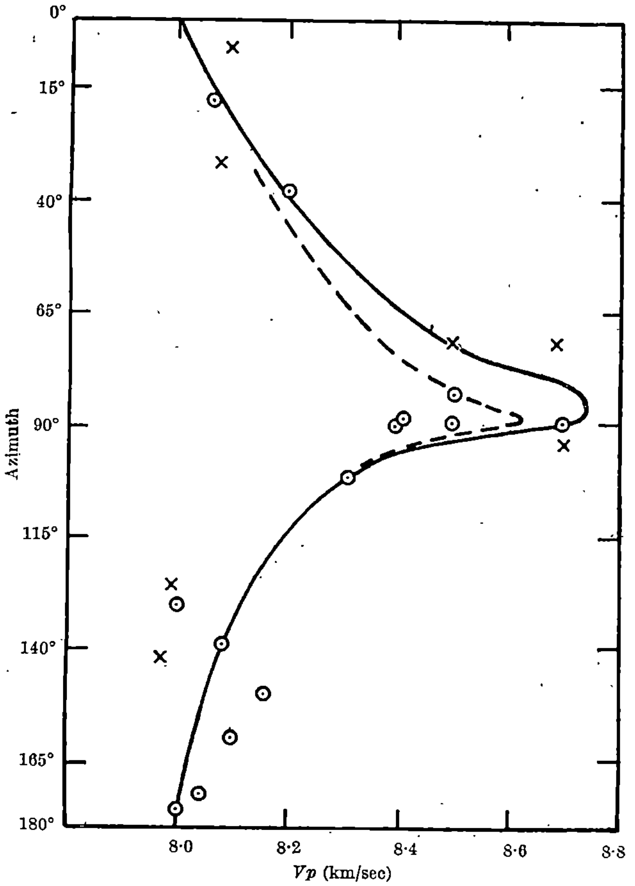

Fig. 3. Seismic velocity, Vp, versus azimuth. Circles, Mendocino area; crosses, Maui area; dashed curve for former, solid curve both areas combined

to it and the higher velocities parallel to it. The Mendocino fracture zone trends almost exactly east–west. Fig. 3 shows the seismic velocities below the Moho versus azimuth and presents a striking agreement between the hypothesis and the observations.

As a further test of the hypothesis, seven recent determinations of velocity versus azimuth by George Shor[6] for the area in the vicinity of the Molokai shear zone (N. 80° E.) were added to Fig. 3, and corroborate the conclusion previously made.

In constructing Fig. 3, all Raitt's measurements available to me were used except those where the sub-Moho velocity was less than 8·0 km/sec. It is evident that in some areas the mantle is altered probably by partial serpentinization so that lower velocities prevail. In an area near the California coast just north of the Mendocino scarp, low velocities for all azimuths were encountered. If this group is taken separately the olivine present still imposes the same velocity differential with respect to azimuth as in the remainder of the area (one near east–west has a seismic velocity of 7·9 km/sec and two which are nearly north–south have velocities of 7·4 and 7·5 km/sec). To determine anisotropy in oceans where no guiding fracture zones are evident it would be well to shoot on three lines at 120° to each other. A single velocity does not mean much because there must normally be other constituents than olivine and pyroxene in variable amounts in the rocks immediately below the M discontinuity. If this were not so the average velocity under the oceans would be near 8·5 km/sec rather than the observed 8·1 km/sec. Thermal expansion in areas of high heat flow, dilation by shearing, fluids such as water in pore space, and in particular hydrated minerals such as serpentine all tend to lower velocity. The amount of such effects is variable from region to region.

As can be seen in Fig. 1, indices of refraction for olivine vary sympathetically with seismic velocities, which is to be expected. Oriented samples from St. Paul's Rock on the mid-Atlantic ridge were examined under the petrographic microscope. These rocks are strongly deformed peridotites–mylonites[7]. Shearing and granulation have been severe so that olivine grains are less than 0·05 mm in diameter. Though it is not possible to orient individual olivine grains and construct a petrofabric diagram, it is evident that most of the grains have a similar optical orientation, extinguishing under crossed Nicols roughly parallel and at right angles to the main shear plane. It was found that $N_{x'}$ (the fast vibration direction for light and the slow seismic velocity direction) is perpendicular to the plane of shearing.

Whereas tests for seismic anisotropy should be made in other parts of the oceans, it seems likely that the oceans everywhere have a fabric such that directions in which the material has flowed in the solid state can be determined. The low velocity is perpendicular to the shear direction and high velocity parallel to it. The low velocity is also parallel to the linear magnetic anomalies off California.

Applying the very fruitful idea introduced recently by Vine and Mathews[8], rates of flow could be added to the structural picture if reversals of the Earth's magnetic field had a regular periodicity. When it becomes possible to drill the hard rocks of the ocean floor an absolute age can be given to a particular linear magnetic anomaly. A series of linear anomalies may then be counted much as are varves on land and another age determination made at perhaps thirty cycles distant so that a relatively good

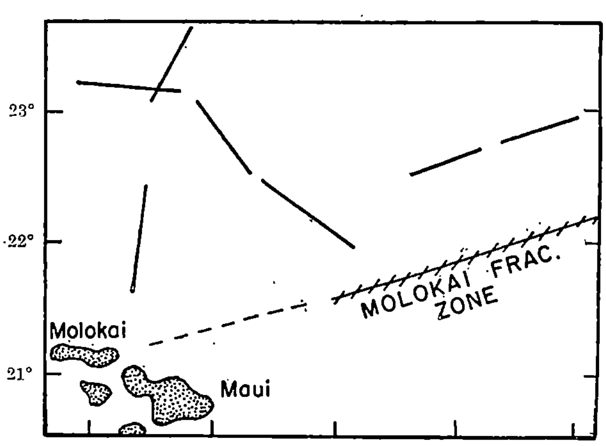

Fig. 4. Location of seismic refraction profiles in Shor's study of area north of Maui, Hawaiian Islands

figure could be obtained for the length of a cycle. Using these tools the structure and history of the whole ocean floor can probably be worked out much more rapidly than the more complicated land surfaces of the Earth. Unfortunately, if my general concept[9] of the history of ocean basins is correct, only something less than the last 10 per cent of geological history can be found in the oceans.

I thank R. Raitt and G. Shor, whose seismic refraction data I have used as a basis for this communication; Ian MacGregor, who collected the oriented samples from St. Paul's Rock; and W. M. Elsasser for helpful discussion of the general problem. This work was supported by a contract with the Office of Naval Research, *Nonr* 1858 (10).

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Electrical Resistivity of the Crust and Upper Mantle at Eskdalemuir, South Scotland

The oscillations in telluric field in magnetic north–south and east–west directions were recorded at Eskdalemuir (55° 19′ N., 3° 14′ W.), South Scotland, over three weeks in December 1963. The electrodes consisted of 2 ft.^{2}, 0·25-in. steel plates buried 2,000 ft. apart. The recording and the La Cour magnetograms made at the observatory yielded the data for this magnetotelluric examination. The period of recorded oscillations ranged from 20 sec to 100 min. The records were visually analysed and only the pulsations with good sinusoidal shapes and having no noticeable vertical component were considered. By the courtesy of G.P.O. Engineering Department, magnetotelluric data were also collected on the cables across the Irish Sea. This was, however, considerably affected at the lower end of the spectrum by near surface features and will only be referred to here. A paper covering the details of this investigation is under preparation, and will be published elsewhere.

The amplitudes of simultaneous oscillations of period T in terrestrial electric (E) and magnetic (H) fields (usually 5–10 mV/km and 5–10γ respectively) have very significant correlation (coefficient 0·75–0·95), but their ratios show considerable scatter. This scatter is independent of period as well as amplitude of oscillations. The mean E/H is used to find apparent resistivity ρ_a according to the formula $\rho_a = 0{\cdot}2T(E/H)^2$ given by Cagniard[1]. The plot of mean ρ_a and its standard error against T is shown in Fig. 1 for profiles in both directions. The two are not identical, thus showing that the surface is anisotropic. Unfortunately, very few oscillations occur simultaneously in both components and it is not possible to evaluate the anisotropy factor. However, the individual values of E/H in the two directions are not very different from normal scatter and both profiles may be combined by taking the geometric mean of ρ_a at each period. The mean profile thus obtained is given in Fig. 2.

The apparent resistivity in the profiles over the Irish Sea is so low at longer periods of the spectrum that the continuous variation of resistivity down to the depths involved is not possible. On the other hand, plausible values are obtained if a layered distribution of resistivity is assumed. The theoretical curves of best fit for the data and corresponding resistivity distributions are also given in Figs. 1 and 2. Mutual consistency of profiles given here as well as five profiles over the Irish Sea helped in choosing the particular standard curves out of many which fitted the data.

The observed spectrum does not extend to sufficiently low periods to establish the depth of the first discontinuity with certainty, but the middle layer must be several km thick. The second discontinuity situated at the

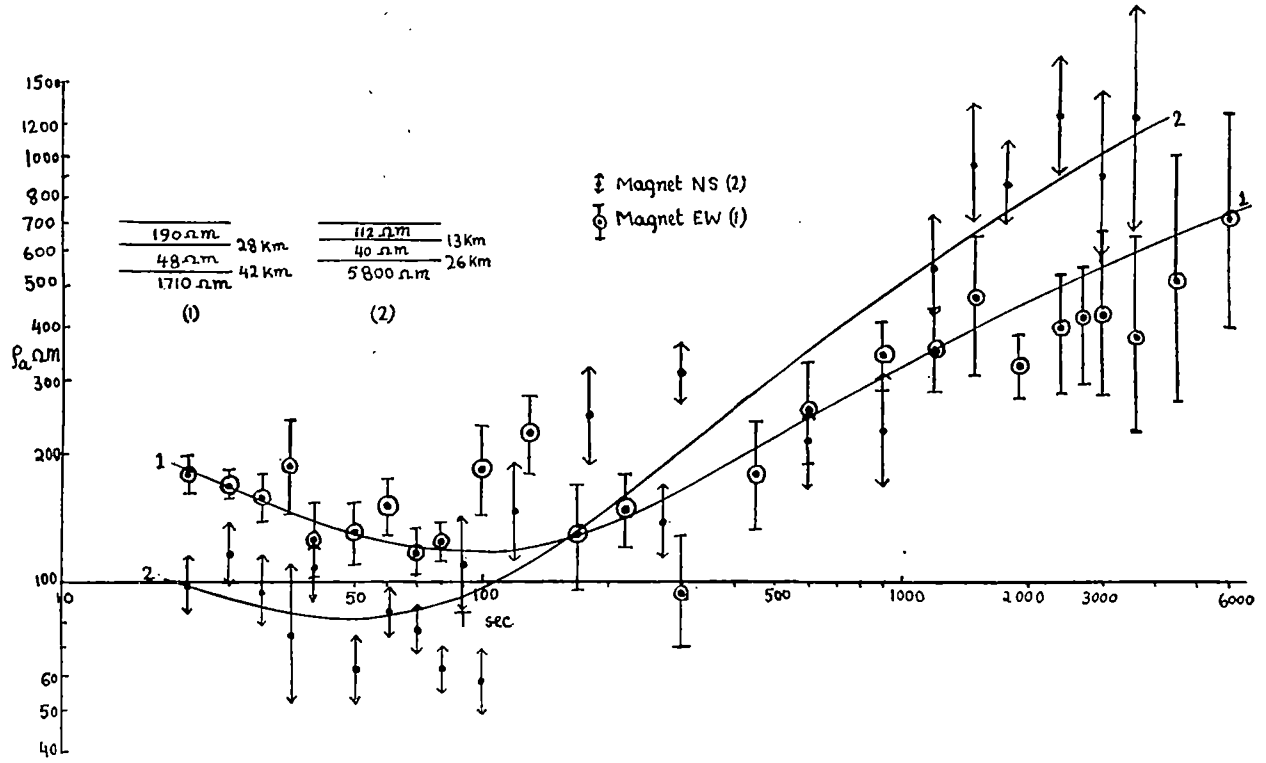

Fig. 1. Observed magnetotelluric profiles at Eskdalemuir

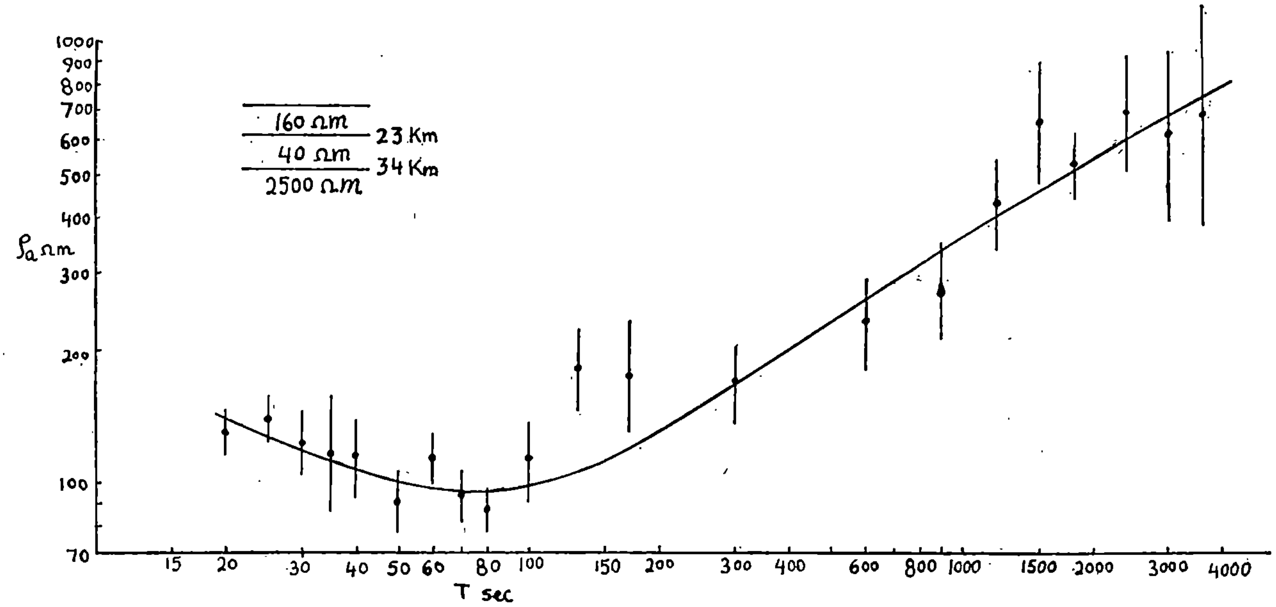

Fig. 2. Mean magnetotelluric profile at Eskdalemuir

depth of 34 km in Fig. 2 and at 29–33 km in the Irish Sea profiles may correspond to Moho. No other measurements of the thickness of the crust in this region are known, but these depths show good agreement with seismic investigations elsewhere in western Europe. The resistivity of the top of the mantle is of the same order as found by other workers, but the profiles indicate the presence of a conductive layer at the bottom of the crust. The resistivity in this depth range varies greatly from place to place; from 1 Ω*m* below the Arctic Sea[2] to 10,000 Ω*m* in Massachusetts[3]. The temperatures necessary to explain these differences by variation in ionic semi-conduction are too high[4] to be unnoticed in the measurements of heat flow in deep wells[5], and it is probable that the impurity semi-conduction plays a more important part in the crust. This explanation is supported by the laboratory investigations of Parkhomenko and Bondorenko[6], who find that the resistivities of different samples of the same rock can be very different at high temperatures and pressures.

I thank Mr. Lamden of the Atomic Weapons Research Establishment, Aldermaston, for permission to use their cables at Eskdalemuir; the staff at the Meteorological Office Observatory, Eskdalemuir; and Dr. C. D. V. Wilson of the Department of Geology, University of Liverpool, who supervised this work.

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PHYSICS

Calorimetric Dose Measurements with 35-MeV Betatron Electron Radiation

THE calorimetric system developed in the International Atomic Energy Agency (IAEA) Laboratory, Vienna, has been used for measuring the electron dose distribution in a 'Lucite' phantom. It may be recalled that the calorimeter[1] is calibrated in rads (1 rad = 100 ergs/g) by accurate determination of the electric energy input.

The 35-MeV electron beam (field size 14 cm × 14 cm at 110 cm focal distance), generated by a Brown Boveri betatron[2], was used for the investigation.

Preliminary measurements with thermistors of the same type and embedded equally in the same material as that used in the calorimeter have shown that they were not affected by doses up to 30 krads produced by 35-MeV electrons.

Further measurements were then undertaken to test the response of the calorimeter in a 'Lucite' phantom (70 mm of material in front, and 30 mm behind the absorber) irradiated with 35-MeV electrons at dose rates of about 250 rads/min. Comparison between the calorimeter and the radiation monitor of the machine (a flat ionization chamber measuring the total electron flux behind the collimators) in the range up to 1,000 rads showed a linear response of the calorimeter with respect to the dose and that the readings of the monitor were proportional to those of the calorimeter.

Simultaneous measurements with a Thimble-Head Ionization Chamber (Baldwin 'Ionex *MK* 3*', volume of the chamber 0·6 c.c.) have shown that this instrument developed serious errors when the applied doses exceeded about 400 r. The errors manifest themselves as a heavy apparent dose-leakage above the critical dose value; for smaller doses, however, the measurements were reproducible to within ± 2 per cent.

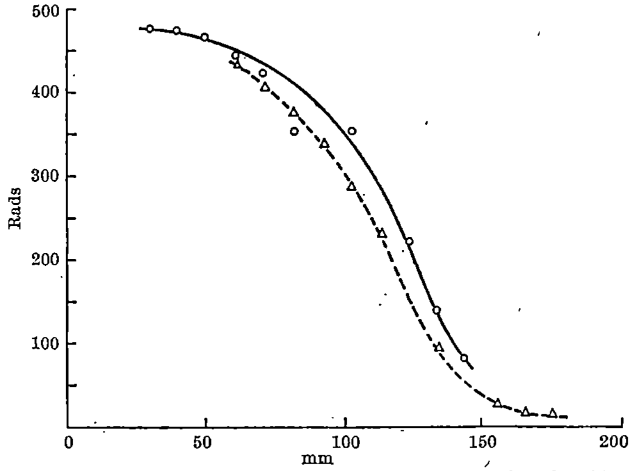

Fig. 1. ○, Calorimeter (rads); △, Thimble-Head Ion Chamber (r)

Dose measurements comparing the calorimeter–dosimeter and the Baldwin instrument, therefore, had to be restricted to doses below 400 rads.

For measurements of 35-MeV electron radiation dose distribution along the axis of the beam, the calorimeter and Baldwin ionization chamber were placed in the 'Lucite' phantom. The 'Lucite' thickness in front of these devices was varied between the limits given in Fig. 1. Here, the full curve represents the calorimetric results, and the dotted curve the ionization values. The results of both methods were reproducible within ± 2 per cent. It is interesting to note that the ionization values are systematically lower than the calorimetric values, with a discrepancy up to 25 per cent.

In a further investigation of this difference the present investigation will be extended to a wider range of radiation energies (also including X-rays) using the calorimetric method and different types of ionization measuring equipment.

We thank Dr. M. Sempert and his staff at the Brown Boveri Laboratory, and Mr. J. Haider (I.A.E.A.), for their kind co-operation.

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Logarithmic Pressure Scale

WITHIN the past year discussions have been published[1,2] which have been critical of the present system of vacuum designation and have advocated logarithmic scales. We have been independently[3] considering this same problem and have arrived at a more specific proposal than that found in refs. 1–3, which encompasses the whole range of pressures from that of the 'highest' vacuum (that is, lowest molecular density) we can attain to the greatest pressures we choose to discuss. It will not ascribe negative values to pressures greater than atmospheric[2].

We propose a logarithmic scale for which there is, as has been pointed out[2], precedence in the chemist's pH system. An additional precedent is that of acoustical, electrical and electronic engineers, who use the 'bel' to denote a ten-fold change in power, and, therefore, a unit change in its logarithm. They find the 'decibel', one-tenth of this step (implying an accuracy of ± 12 per cent), sufficient for expressing these quantities to the precision with which they are ordinarily measured. It is important to note that in neither case is the conversion to an absolute linear system onerous when it is necessary, and such is the adaptability of the human mind that the logarithmic values very soon evoke a direct conceptual response.

We recommend, specifically, that the logarithm to base 10 of the absolute pressure in bars be adopted as the measure of that pressure, and that a unit step on this scale be termed 'one Boyle'. Just as in the acoustical example, it will ordinarily be true that specification to a precision of one 'deciBoyle' (dB), corresponding to a change of 0·1 in the logarithm of pressure, that is, ± 12 per cent. will be sufficient in vacuum work for all but the most precise purposes. It would be convenient for both vacuum and high-pressure work to place the zero point of this scale near one atmosphere, rather than, say, at the present arbitrary reference point at 1 mm mercury. For precise definition, however, we propose to use the 'bar'. Thus, the exact reference point is a pressure of 10^5 joules/metre^3 (10^6 dynes/cm^2), which is greater by the factor of 1·01325 than one standard atmosphere[4], and which, in practice, can often be taken to be 1 atmosphere with negligible error. Thus negative quantities denote vacuum on the proposed scale.

Conversion of a pressure reading of p units to the proposed logarithmic scale is accomplished by the formula:

$$\text{dB} = 10 \log_{10} p + C$$

where the constant C is ten times the common logarithm of the unit prèssure expressed in bars. Values of C for several common systems of units are given in Table 1.

Table 1. CONSTANTS FOR CONVERSION FROM PRESSURE TO THE DECIBOYLE SCALE

Unit	Unit expressed in bars (ref. 4)	C
Bar	1	0
Kilobar	10^3	30
Barye	10^{-6}	−60
Torr	$1·3332 \times 10^{-3}$	−28·7509
Inch mercury	$3·386 \times 10^{-2}$	−14·7026
Millibar	$\times 10^{-3}$	−30
Psia	$6·894 \times 10^{-2}$	−11·6167
lb./ft.^{2}	$4·788 \times 10^{-4}$	−33·1980
g/cm^2	$9·807 \times 10^{-4}$	−30·0847
Cm H_2O at 20° C	$9·789 \times 10^{-4}$	−30·0928
In. H_2O at 20° C	$2·486 \times 10^{-3}$	−26·0444
Atmosphere	1·01325	+0·0572

For example, when p is given in torr, the conversion formula becomes:

$$\text{dB} = 10 \log_{10} (p \text{ torr}) - 28·7509$$

and, for p in atmospheres, it is:

$$\text{dB} = 10 \log_{10} (p \text{ atm}) + 0·0572$$

The two common reference points, 1 torr and 1 atmosphere, are separated by 28·8081, or roughly 30 dB.

Table 2 lists some important points on the pressure scale in the proposed system. Note that the 'torr' is not commonly used in high-pressure work. The advantage of a single logarithmic scale, equally applicable to extreme vacuum and to high pressure, is apparent.

Table 2. COMPARISON OF CONVENTIONAL AND LOGARITHMIC PRESSURE SCALES

	Ref.	p	dB
Normal atmosphere	4	$1·01325 \times 10^5$ joule/m^3	0·0575
Sub-atmospheric			
Vapour pressure of water at 300° K	4	26·7 torr	−14·5
Triple point water—ice I—vapour	4	4·6 ,,	−22·18
'One mm of mercury' = 1 torr	—	1	−28·75
Vapour pressure of mercury at 300° K	4	$2·18 \times 10^{-3}$ torr	−55·4
'One μ of mercury'	—	1×10^{-3} ,,	−58·8
Lower limit of McLeod gauge		1×10^{-6} ,,	−90
Lower limit of ionization gauge		1×10^{-11} ,,	−140
Super-atmospheric			
Critical pressure of air	4	37·2 atmospheres	15·7
Critical pressure of water	4	224·9 ,,	23·5
Triple point water—ice I—ice II	4	2,115 ,,	33·25
Triple point water—ice V—ice VI	4	6,380 ,,	38·05
Phase transition bismuth I—bismuth II	5	25,400 bars	44 05
Phase transition bismuth V—bismuth VI	5	89,400 ,,	49·51
Pressure at the Earth's centre	6	$3·64 \times 10^6$ bars	65 61

Before concluding, there are some points raised in the earlier correspondence which merit attention. It must be agreed that the quantity which the ion gauge samples is more closely related to molecular density than to pressure and it is the molecular density in an experimental enclosure which is most often the quantity of physical interest. It is also true, however: (1) that the ion gauge density as calibrated is really uncertain to the degree that the pertinent temperature within the gauge is uncertain; (2) that the molecular density in the experiment is to be found only by correcting the gauge reading in accordance with the temperature in the experimental enclosure. Surely we should not make the adoption of a rational and useful pressure-designation system contingent on the technical difficulties of measuring molecular density.

By way of recapitulation we enumerate the advantages of the deciBoyle system: (1) It furnishes a unified scale encompassing the whole range from the smallest to the greatest pressures. (2) It centres around a convenient and absoute reference value, approximately one standard atmosphere. (3) The numeric expresses in its units place the accuracy attributable to the majority of pressure designations (that is, ± 12 per cent) which lie outside the range of the simple barometric column or the McLeod gauge.

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METALLURGY

Distribution of Twins around a Microhardness Indenter in Close-packed Hexagonal Metals

Attempts have been made to explain the hardness anisotropy of close-packed hexagonal metals in terms of the slip and deformation twinning modes which are available$^{1-5}$. As a result, simplified models of the stress system around an indenter have been developed in which the shear stresses acting adjacent to the indenter facets are resolved in the slip and twinning planes and in the respective shear directions. The hardness variation was shown to be consistent with the variation in the resolved shear stresses for twinning in the basal planes of zinc, magnesium^3 and beryllium^4 and in the prism planes of zinc and magnesium^3. It was therefore concluded that the hardness anisotropy could be due to twinning.

Following a metallographic examination of the deformation produced in indented zinc and magnesium crystals, it was suggested that slip alone could readily account for the hardness anisotropy found in prism planes and indirectly for the anisotropy in the basal planes^5. However, it was noted that twins were sometimes nucleated around the hardness impressions. It is proposed in this communication to consider in more detail the twinning mode of deformation and to compare the observed distribution of twins around a Vickers diamond pyramid indenter with that predicted by the resolved shear stress models already referred to here.

An impression in the basal plane of magnesium is surrounded by twins at the free surface, whereas in zinc few twins are visible^6. If the surface layer is removed after indenting, twins are found beneath the impression in zinc but not in magnesium. The distribution of twins around an indenter in the $\{10\bar{1}0\}$ prism plane of magnesium is similar to that found in the basal plane of zinc^{5,6}. A similar distribution of twins is also found when a Knoop indenter is used. These observations are summarized diagrammatically in Figs. 1 and 2. It can be seen that although the predicted resolved shear stresses for twinning are almost identical for the equivalent planes in both metals, for the basal planes twins are only found in magnesium around the impression and in zinc below the impression and vice versa for the prism planes.

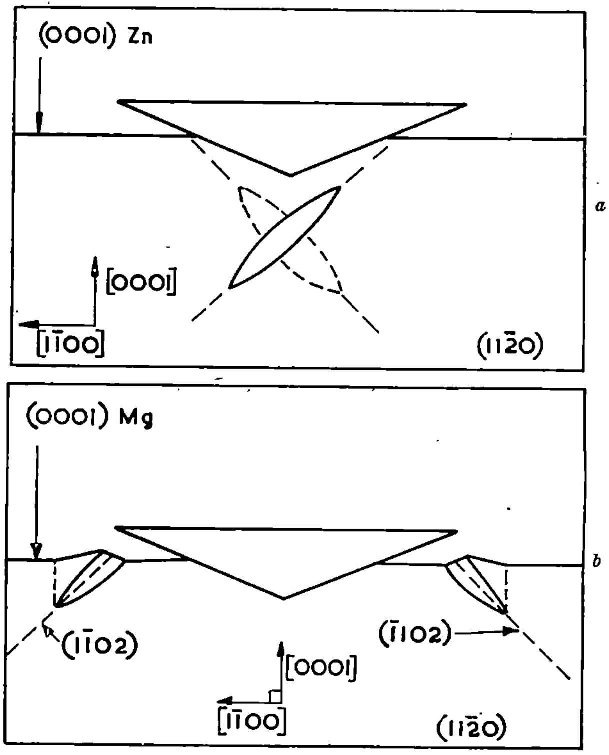

Fig. 1. Distribution of twins around an impression in the basal plane of: (*a*) zinc; (*b*) magnesium

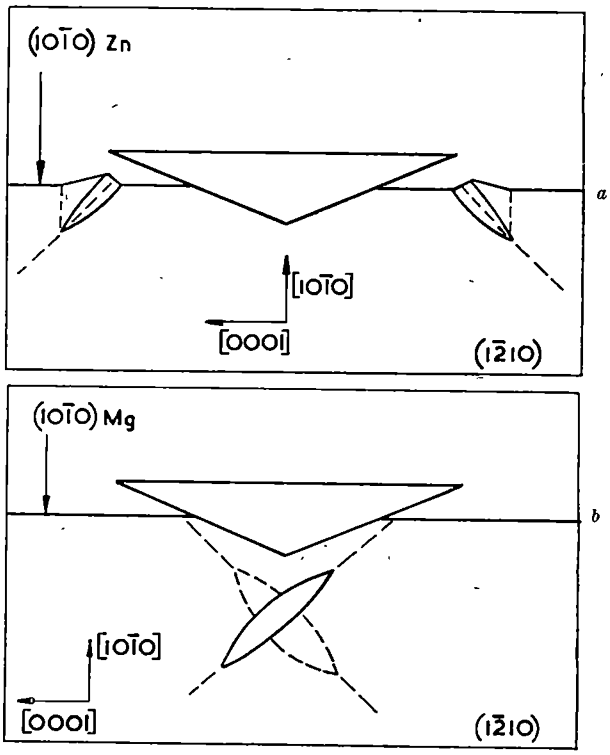

Fig. 2. Distribution of twins around an impression in the prism plane of: (*a*) zinc; (*b*) magnesium

If the changes in shape^{7,8} associated with twinning are considered, the twin distribution can be readily explained. For example, compressive stresses parallel to the *a*-axis favour twinning in magnesium while in zinc compressive stresses parallel to the *c*-axis favour twinning: if the radial compressive stresses around an indenter are resolved parallel and normal to the indented surface, the twins would appear to form in order to relieve the compressive stresses. Clearly a resolved shear stress criterion alone is insufficient to account for the observed distribution of twins and any analysis which attempts to relate deformation twinning with hardness anisotropy must take into account the dimensional changes which occur during twin formation.

It is significant that the hardness anisotropy in the basal planes of zinc and magnesium is similar[5] although the number of twins and their distribution differ in these metals. This suggests that the anisotropy is due to slip rather than twinning, in agreement with the previous results[5]. The effect of indentations on twins already present has also been examined and supports the observations recorded here[6].

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Grain Growth in Cadmium

Much has been written of the growth of the component crystalline grains which occurs when a metal is maintained at a constant high temperature, but hitherto no simple law of growth appears to have been established. Working with cadmium, we have found laws which hold with precision for that metal. Whether they hold for other metals, in particular for metals of face-centred cubic structure, is a matter which we propose to investigate.

In a specimen of which the average grain size is below a certain limit, maintained for a long period at a given temperature, the grains grow to a stable size characteristic of that temperature. If n is the average number of grains intersected by a unit length, taken at random, and n_i, the initial value, is about 1·6, or less, times the stable value n_T ultimately attained, then the law of decrease of n, which is the reciprocal of the average diameter, is:

$$n = n_T\,(1 + pe^{-qt})$$

or:

$$\frac{n - n_T}{n_T} = \frac{\text{number of mortal grains}}{\text{final number of grains}} = pe^{-qt}$$

when p and q are constants. We use the term "number of mortal grains" to indicate the number that vanish in the course of annealing at a given temperature.

This law is obeyed within the limit of experimental error, which is well less than 1 per cent of the value of n. It has been established that no change in the value of n_T occurs in weeks.

If the initial grain size is much smaller than that given by $n_i = 1·6\ n_T$, then there are two preliminary stages of growth, first, a very rapid increase of grain diameter, which at high temperature may lead to a ten-fold increase of the average grain diameter in a matter of seconds, and a second stage in which the exponential law is gradually approached. We have an explanation to offer for this behaviour.

The variation of q with temperature is given by the simple law $q = ae^{-\varepsilon/kT}$, where a and ε are constants. The variation of the stable n_T with temperature is given by $n_T = \dfrac{A}{(T - T_0)^2}$, where A and T_0 are constants.

It is hoped to publish shortly an account of the experiments in question, with an attempted explanation of the results.

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RADIATION CHEMISTRY

Changes in Titanium Dioxide-Silver Nitrate Mixtures after Ultra-violet Irradiation

The photo-darkening of titanium dioxide–silver compound mixtures has been observed by Förland[1] and Goetz and Inn[2] among others. Förland attributed the discoloration to the oxidation of the silver ion to AgO, though Goetz and Inn claimed that it was due to the reduction of the silver ion to metallic silver.

During investigations into this reaction, interesting reflectance changes were observed on specimens after they had been exposed to ultra-violet radiation. The specimens were prepared by mixing 10 gm of a commercial rutile titanium dioxide pigment with 10 ml. of N/10 aqueous solution of silver nitrate. The paste was dried at 80° C for two days in the dark, and formed into compacts to facilitate reflectance measurements. These were evacuated for 1 h at 10^{-2} torr, and then irradiated for two days in a dry nitrogen atmosphere.

The discoloration has a characteristic pink tinge and the reflectance spectrum of a freshly irradiated sample is shown in Fig. 1, curve *A*. The changes after irradiation depend strongly on the presence of moisture, and follow two patterns. If the specimen is kept in a desiccator in the dark, the discoloration slowly dies away, and the reaction appears to be completely reversible. Fig. 2 shows the increase of reflectance at about 5500 Å with time at 20° C. If, on the other hand, the specimen is exposed to moisture, it slowly blackens in the dark. A typical case of a specimen kept in a normal room atmosphere at 20° C is shown in Fig. 1, where curves *B* and *C* show the decreasing reflectance with time. This darkening continues until the specimen appears dark-grey with an overall reflectance of approximately 10 per cent.

This darkening process follows a particular pattern; small black spots form on the surface of the compact, and then grow, bringing about a darkening effect. The formation of these spots depends on the presence of moisture and is irreversible at room temperature. Detailed

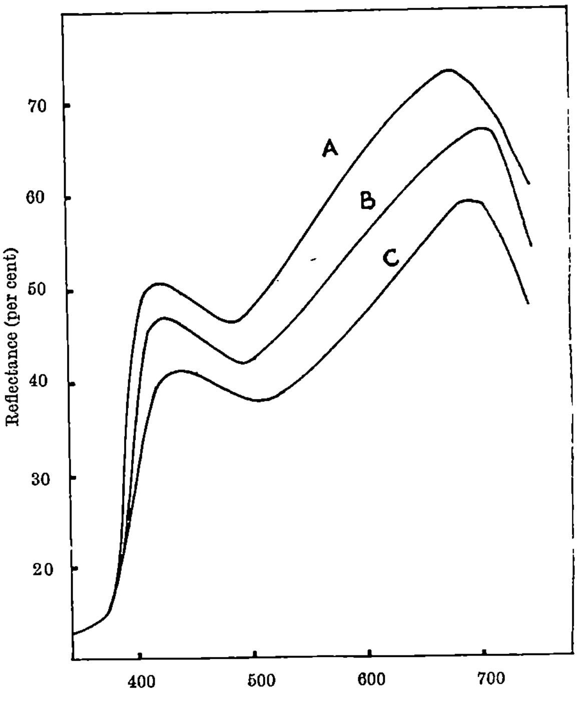

Fig. 1. Reflectance changes measured in a moist atmosphere. *A*, freshly irradiated; *B*, after two days in the dark; *C*, after seven days in the dark

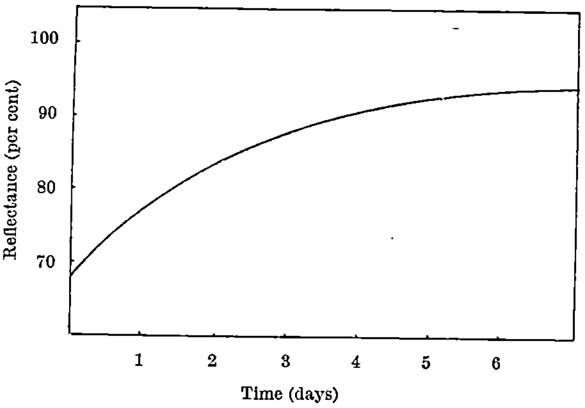

Fig. 2. Reflectance changes measured at about 5500 Å in a dry atmosphere

investigations on the irradiation process to be published elsewhere indicate that these might well be due to colloidal silver, and that the growth is due to silver diffusion over the face of the compact.

The work is part of a general programme dealing with the electrical and surface properties of titanium dioxide. We thank Laporte Titanium, Ltd., for the gift of samples.

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Radiation Chemical Reactions in Deoxygenated Peptide Solutions

Investigations by Garrison *et al.*[1] on the radiation chemistry of acetylglycine contributed to the elucidation of the reactions on C2 of the amino-acids participating in the peptide bond.

Simultaneous and independent experiments were carried out in our Laboratory based on our previous observations[2-9] on the increasing importance of recombination reactions with chain-length of irradiated aliphatic amino-acids in deoxygenated solutions. These investigations were aimed at elucidating the type of recombination products formed from peptides, since these could contribute to clarification of the radiolytic reactions of proteins.

0·05 M oxygen-free solutions of leucyl-glycyl-glycine (leu.gly.gly), leucyl-glycine (leu.gly), glycyl-leucine (gly.-leu), glycine, leucine and a mixture (1 : 1) of 0·025 M glycine and 0·025 M leucine were irradiated by cobalt-60 γ-rays (dose $8 \cdot 2 \times 10^{20}$ eV/ml.). The *G*-values are summarized in Table 1.

Table 1. 100-eV Yield from Peptides

(0·05 M aqueous solutions, cobalt-60 γ-rays, dose 8.2.10^{20} eV/ml.)

	leu.gly.gly	leu.gly	gly.leu	leu	gly	leu + gly*
G (-*M*)	2·2	2·4	2·9	2·1	1·57	*G*(-leu) 3 *G*(-gly) 0·9
G(NH_3 + amines)	1·25					
G(CO_2)	0·15					
G(volatile acids)	0·1					
G(dimers, yielding diaminodicarboxylic acids)	0·55					
G(recomb. yielding monoaminodicarboxylic acid)	0·7					
G(carbonyl compounds)	0·1					

* (1 : 1) mixture of 0·025 M leucine and 0·025 M glycine.

Before hydrolysis several peptides giving a weak ninhydrin reaction were observed in irradiated leu.gly.gly solutions. The amount of carbonyl compounds in the unhydrolysed solution was negligible. Glycine was formed in very small amounts (*G* 0·1); decarboxylation also was very low (*G* 0·15) as well as the amount of acids (*G* 0·1).

After hydrolysis, besides leucine and glycine three diaminodicarboxylic acids (*G* 0·5) were observed corresponding to the three major peptides, that is, in order of importance: (1) dimethyldiaminoadipic acid (recombination product of glycine and leucine); (2) diaminosuccinic acid (recombination product of glycine); (3) diaminodimethylsebacic acid (recombination product of leucine). Since none of these products was present before hydrolysis, the three peptides apparently were formed by recombination of two leu.gly.gly molecules, that is, by formation of new C—C bonds.

This fact can be explained by the formation of a radical on C2 of one of the glycine residues in one leu.gly.-gly molecule and on C4 of the leucine residue in another leu.gly.gly molecule. These radicals then recombine, the new peptide being linked by dimethyldiaminoadipic acid (1), which was found after hydrolysis. Another variety of this reaction is radical formation on one glycine residue in each of two leu.gly.gly molecules, recombination leading to diaminosuccinic acid (2). In the third case only the leucine moieties of two leu.gly.gly molecules participate in the reaction under formation of diaminodimethylsebacic acid (3).

The hydrolysed irradiated leu.gly.gly solution also contained monoaminodicarboxylic acids (*G* 0·7) chromatographically corresponding to α-amino-4-methylpimelic acid and α-amino-3-dimethyladipic acid, indicating that *N*-terminal leucine undergoes reductive deamination under formation of isocaproic acid (without breakage of the peptide bond) which combines with a glycine radical in another leu.gly.gly molecule yielding deaminated peptides from which on hydrolysis the corresponding monoaminodicarboxylic acids are formed. Reductive deamination and recombination appears to be a specific reaction of the *N*-terminal amino-acid. In view of *G* 0·7 of these products, reductive deamination is an important reaction of peptides in irradiated deoxygenated solutions.

These facts and the balance of *G*($-M$) and *G*(products) for leu.gly.gly, which is in comparatively good agreement, point to recombination as the primary reactions in the radiolysis of peptides in deoxygenated solutions.

The low value of *G*(CO_2) instigated an investigation of whether carboxylation[8] occurs and leads to consumption of carbon dioxide. However, carboxylation was found to be negligible.

In the irradiated leu.gly solution the same number of peptides was found as with leu.gly.gly, yielding the same diaminodicarboxylic and monoaminodicarboxylic acids as the peptides formed on irradiation of leu.gly.gly. In the hydrolysate acid (1) is present in the highest amount, the amounts of acids (2) and (3) being equal but lower.

In irradiated gly.leu solutions three peptides were observed yielding diaminodicarboxylic acids only. Acid (3) predominates, followed by (1) and a small amount of (2). The monoaminodicarboxylic acid present in the irradiated solution originates from recombination product of the acetyl moiety of acetyl-leucine (deamination product of gly.leu) with the leu moiety of gly.leu, the product being α-amino-4-methylpimelic acid, whereas recombination between the acetyl moiety and the glycine moiety was not observed.

The results show that recombination is the predominant reaction in irradiated deoxygenated peptide solutions. The recombination reactions observed with peptides are analogous to those observed with the free amino-acids. Rupture of the peptide bond is unimportant. In the presence of an aliphatic amino-acid with longer chain, breakage of the peptide bond is practically completely suppressed in favour of recombination as demonstrated by the small amount of carbonyl compounds and amino-acids in the irradiated solution.

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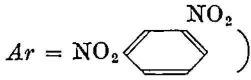
CHEMISTRY

Methanolysis of 2,4-Dinitrophenyl *p*-Toluene Sulphonate

The methanolysis of 2,4-dinitrophenyl *p*-toluene sulphonate has been investigated, in continuance of a series of investigations of the methanolysis of nitrophenyl esters[1,2] both under initially neutral conditions and in the presence of tertiary bases, namely, pyridine and its methyl derivatives. In the cases of picryl acetate[1] and 2,4-dinitrophenyl acetate[2] both the neutral and base-catalysed reactions show acyl–oxygen fission:

$$Ar\text{OCOCH}_3 + \text{CH}_3\text{OH} \rightarrow Ar\text{OH} + \text{CH}_3\text{OCOCH}_3 \quad (1)$$

(where Ar = 

). In the case of picryl benzoate[1] under initially neutral conditions, along with acyl–oxygen fission (1), there also occurred some aryl–oxygen fission (2):

$$Ar\text{OCOPh} + \text{CH}_3\text{OH} \rightarrow Ar\text{OCH}_3 + \text{HOCOPh} \quad (2)$$

Reaction (2) did not, however, occur in the presence of tertiary bases. The order of the catalytic constants (k_2^B) are in each case $k_2^{\text{Py}} > k_2^{\text{Co}} > k_2^{\text{Lu}} > k_2^{\text{Pi}}$, whereas the basic strengths are in the order 2,4,6-collidine > 2,6-lutidine > 2-picoline > pyridine. Thus specific and general base catalysis are ruled out. The mechanism is thus nucleophilic catalysis.

The catalysis of the methanolysis of 2,4-dinitrophenyl *p*-toluene sulphonate by pyridine, 2,6-lutidine and 2,4,6-collidine has been investigated at 45° C. The reaction follows two paths leading to 2,4-dinitrophenol (reaction (3)) and the 2,4-dinitrophenyl pyridinium ions (reaction (4)):

$$Ar\text{OSO}_2\text{C}_7\text{H}_7 + B + \text{MeOH} \nearrow Ar\text{OH} + \text{Me}B^+ + \bar{\text{O}}\text{SO}_2\text{C}_7\text{H}_7 \quad (3)$$

$$Ar\text{OSO}_2\text{C}_7\text{H}_7 + B + \text{MeOH} \searrow ArB^+ + \bar{\text{O}}\text{SO}_2\text{C}_7\text{H}_7 + \text{MeOH} \quad (4)$$

Reactions (3) and (4) were followed chemically by measuring respectively the rate of 2,4-dinitrophenol and complex formation. This is essentially a modification of the method of Olivier and Berger[3]. In the case of pyridine, reaction (4) accounts for some 45 per cent of the total reaction, while for 2,4,6-collidine the amount of reaction going by this path was only 14 per cent. None of the reaction in the presence of 2,6-lutidine appeared to go by this route. The reaction products have all been isolated and identified by comparison of the analysis figures and infra-red spectra with those of the pure compounds. The nature of the products enables the mechanism to be postulated[4]. In the cases of pyridine and 2,4,6-collidine it is concurrent sulphur–oxygen fission (reaction (3)) and acryl-oxygen fission leading to the complex (reaction (4)). In the case of 2,6-lutidine, only the former process is observed.

For a given concentration of base, good first order rate-coefficients were obtained for reaction (3), and these, when divided by the base concentration, yielded the catalytic constants. It is found that $k_2^{\text{Py}} > k_2^{\text{Co}} > k_2^{\text{Lu}}$, which is the same order as was found for the picryl compounds[1] and for 2,4-dinitrophenyl acetate[2]. Once again specific and general base catalysis can be eliminated as possible mechanisms and so nucleophilic catalysis must occur, the catalytic constants being determined in part by the basic strengths and in part by the steric effect of the methyl groups of the base. Reaction (4) is also subject to steric effects as the rate coefficients decrease in the order pyridine 2,4,6-collidine 2,6-lutidine.

Further investigations are being made into the reactions in the presence of 2- and 4-picoline, and in the light of these a fuller discussion of the mechanism will be presented elsewhere.

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Formation of Phase Test Intermediate of Chlorophyll by Electrolytic Reduction

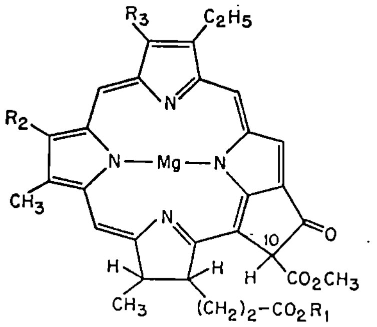
In the course of electrolytic and polarographic studies on chlorophyll and related compounds, we have found that reduction of chlorophyll *a* and *b* in aprotic solvents involves an unusual sequence of events which differ markedly from those observed in protolytic solvents[1,2].

d.c. Polarographic measurements were made in dimethylsulphoxide solvent (distilled from calcium hydride), using 0·1 M tetrapropylammonium perchlorate as supporting electrolyte and an aqueous calomel anode, taking precautions to minimize water diffusion. Controlled potential reductions were carried out at a platinum gauze cathode, using a mercury pool anode and reference aqueous calomel electrode. The electrolysis apparatus permitted also thorough deoxygenation and determination of the absorption spectrum of the solution.

Polarographic results for chlorophyll *a* and some derivatives (Fig. 1) are presented in Table 1. Two waves were observed for all compounds[1,2] and heights for both waves were linear in the square root of the mercury head. The spectral changes following electrolytic reduction of ethyl chlorophyllide *a* at the plateaux of either the first (−1·3 V) or second (−1·6 V) polarographic waves are given in Fig. 2. The resultant spectrum (3, Fig. 2) is unequivocally that of the phase test intermediate (*V*, Fig. 1), as shown by comparison with published spectra[3,4] and by formation of the identical spectrum on addition of aqueous potassium hydroxide. In further agreement with this identification are the following observations on the electrolysed solution: (1) no electron spin resonance was obtained; (2) addition of deoxygenated boric acid solution gave quantitative recovery of *I*; (3) bubbling with air or addition of iodine regenerated a chlorin-type spectrum. Similarly, clear phase-test intermediates were obtained when chlorophyll *a* and *b* were electrolysed.

In two separate electrolyses at the potential of the first wave, coulometric analysis showed that 1·1 and 1·3 electrons were taken up, respectively, per chlorophyllide *a* molecule. Following the reduction, attempts to reduce the solution further at the plateau of the second wave gave no current flow, nor could the solution be oxidized at a potential 0·2 V anodic to the first wave of *I*. Since the product of reduction at the first polarographic wave is reduced at the second wave, in contrast to the behaviour of the phase intermediate, we may conclude that *V* cannot be the primary reduction product.

That the two waves are due to formation of π-electron ions is indicated by similar studies on a series of metallo-tetraphenyl porphyrins, having no other reducible side groups. The polarograms show the same characteristic two waves, with an almost constant separation of 0·44 V, independent of the complexed metal[5]. Previous polarograms of chlorophyll *a* and derivatives in buffered ethanol have also been assigned to chlorin-ring reduction, but show proton involvement in the electrode reaction[1,2]. These results may be reproduced by adding acetic acid to the dimethylsulphoxide solution. Increasing the acid concentration (up to 5 per cent) causes the first polarographic wave to shift anodically and to increase in height at the expense of the second wave. This behaviour is observed also with aromatic hydrocarbons[6], and constitutes further evidence that π-electron ions are initially formed. Finally, the characterization of the porphyrin anions as π-electron ions is substantiated by spectroscopic and chemical evidence[5,7].



Compound (Table 1)	R_1	R_2	R_3	H(C-10)
I	C_2H_5	CH=CH$_2$	CH$_3$	+
II	phytyl	CH=CH$_2$	CH$_3$	+
III	phytyl	CH=CH$_2$	CHO	+
IV	C_2H_5	C_2H_5	CH$_3$	+
V	C_2H_5	CH=CH$_2$	CH$_3$	−

Fig. 1. Chlorophyll derivatives

Evidently, formation of base during the electrolysis would account for the appearance of the phase intermediate. However, addition of chlorophyll to dimethylsulphoxide which had been pre-electrolysed at −1·3 V (very small currents were obtained) gave only the original chlorophyll spectrum. While catalytic base formation cannot formally be ruled out, it is unlikely that the methylsulphenium carbanion[8] would be formed at these low potentials. Moreover, the same polarographic and electrolytic results were obtained in *N*-*N*′-dimethylformamide solvent.

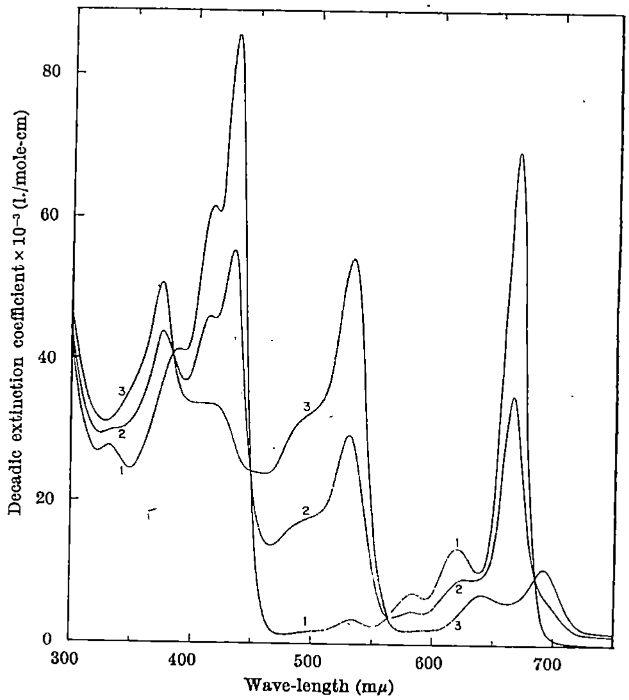


Fig. 2. Spectral changes during electrolysis of ethyl chlorophyllide *a*. (1) Ethyl chlorophyllide *a* in dimethylsulphoxide; (2) intermediate stage of electrolysis; (3) final electrolysis product, phase test intermediate of ethyl chlorophyllide *a*

We therefore suggest the following interpretation of our results. The two polarographic waves are assigned, respectively, to the formation of the π-electron mononegative and dinegative ions of I, II, III and IV. However, under the conditions of bulk electrolysis, the initially formed mononegative radical ion converts to the phase intermediate. This requires the loss of a hydrogen atom from the mononegative radical ion. The nature of the hydrogen acceptor is not clear at this time, and may be the solvent, an impurity, or even another mononegative ion, leading to hydrogen evolution.

If this interpretation is correct, then chlorophyll mononegative ion is unstable with respect to formation of the phase test intermediate and loss of hydrogen to suitable acceptors. Such a transformation may provide a catalytic pathway by which charge-transfer reactions involving chlorophyll may effect hydrogen atom transfer, by virtue of the high stability of the phase intermediate.

This work was supported by grants from the National Institutes of Health (*GM* 108 33-01) and the U.S. Atomic

Table 1. Polarographic Results for Chlorophyll *a* and Some Derivatives in Dimethylsulphoxide* †

Symbol (Fig. 1)	Compound	$-E_{\frac{1}{2}}(1)$	Slope	I_d	n‡	$-E_{\frac{1}{2}}(2)$	Slope	I_d	n‡	$E_{\frac{1}{2}}(1)-E_{\frac{1}{2}}(2)$
I	Ethyl chlorophyllide *a*	1·11	63	0·92§	1·1	1·54	61	0·93§	1·1	0·43
II	Chlorophyll *a*	1·12	61	0·8	0·95	1·54	66	0·8	0·95	0·42
III	Chlorophyll *b*	1·05	59	0·87¶	1·0	1·46	69	0·87¶	1·0	0·41
IV	*Meso*-ethyl chlorophyllide *a*	1·17	57	0·88	1·0	1·64	56	0·84	1·0	0·47

* $E_{\frac{1}{2}}$'s corrected for IR drop, but include junction potentials.

† Units: $E_{\frac{1}{2}}$ in volts versus the saturated calomel electrode (aq.); slope in millivolts of E vs. log $(i/(i_d-i))$; I_d is the diffusion current constant, $i_d/Cm^{2/3}t^{1/6}$; C is concentration in millimoles/l.; i_d is height of diffusion wave (μ amp); m=1·33 mg/sec at column height of 34 cm; t=3·3 sec.

‡ n is number of electrons transferred in reduction; obtained from I_d=0·85n established for zinc tetraphenylporphyrin[5].

§ I_d constant from 0·2 mM to 1·0 mM.

¶ I_d constant from 0·3 mM to 1·0 mM.

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Determination of Electro-oxidation Products using an Auxiliary Mercury Electrode

THE investigation of the mechanism of electro-oxidation is hindered by the lack of information about the reaction products and intermediates. These can be determined in some cases by ordinary analytical methods. In some cases a current-voltage curve obtained by application of a voltage sweep to the electrode during interruptions of the electro-oxidation can be used for determination of products of the electrode reaction, but usually does not give unambiguous results.

If a small mercury electrode is placed in the immediate neighbourhood of the anode the advantages of the polarographic method, that is, the reproducibility and the large cathodic potential range may be used for the determination of reducible products of electro-oxidation which diffuse from the anode. For a rapid and sensitive determination the method must be modified in some way: (1) the potential of the working electrode at which the products are formed must be controlled by means of a potentiostat; (2) the resistance of the solution between the mercury cathode and the reference electrode must be kept low to make possible the application of the linear voltage sweep oscillographic polarography.

Bearing this in mind, the cell and the mercury electrode have been designed as follows. A platinum platinized anode *A* (apparent area 50 mm^2) placed in the centre of the anodic compartment (Fig. 1) is kept at a constant potential by means of a reference electrode (*RE*) joined through a Luggin capillary. The liquid junction potential between the solutions in the compartments of the anode and of the reference electrode may cause uncertainty in the control of the potential of the anode. Therefore a hydrogen electrode according to Giner[1] in the same solution in both compartments has been used. Hydrogen is generated at a platinum black electrode connected to an auxiliary current source. The electrolysis with a current density of 1 m.amp/cm^2 maintains a constant potential of the hydrogen electrode. The cathode is placed in a third compartment (not designed) which is joined to the anodic part by means of a sintered glass plug. The mercury electrode (*ME*) consists of a platinum wire (diam. 0·4 mm) sealed in a glass tube which is ground at the end to a flat tip. The electrode is platinized and coated electrolytically with mercury. It is moved by means of a micrometric screw to a definite distance from the anode. A small piece of glass 0·2 mm thick sealed to the tip prevents the short-circuiting of both electrodes. The solution is deaerated by bubbling with pure nitrogen.

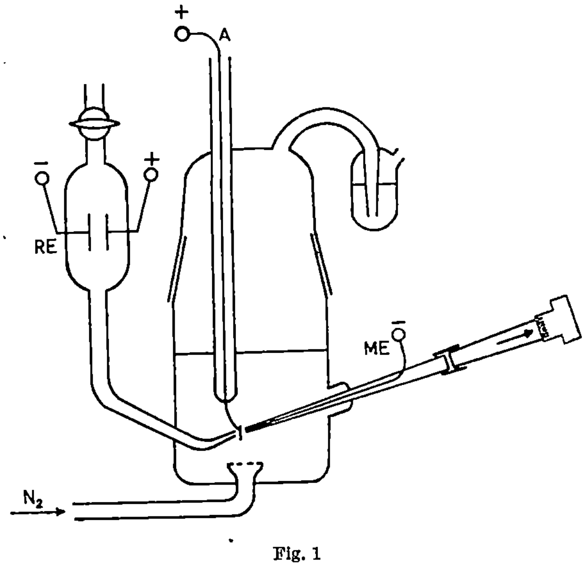

Fig. 1

The cathode (*C*), the anode (*A*), and the reference electrode (*RE*) are connected in a conventional way to an electronic potentiostat (*PO*) (Fig. 2) which is based on operational amplifier units[2]. The mercury electrode is polarized from the differential amplifier (*DA*) through a measuring resistance (*R*). The other imput of the amplifier is switched on a generator (*G*) of a triangular voltage impulse. The anode is earthed and makes the common point of the circuits of the potentiostat and the amplifier, respectively. This connexion is made possible by the fact that the potential of the anode is kept constant by means of the potentiostat. The voltage on the mercury electrode controls the horizontal deflexion of the cathode ray tube of the oscilloscope (*O*), the voltage drop on the resistance (*R*) the vertical deflexion. In this way a current-potential curve is displayed on the screen.

Using this method it was possible to follow quantitatively the rate of formation of formaldehyde during the electro-oxidation of methanol in a buffered solution. The electrolysis at a given potential was switched on and after a delay of 5 min the current-voltage curve of formaldehyde at the auxiliary electrode was recorded. The height of the peak of formaldehyde was compared with the height measured in a solution of a known concentration[3]. The concentration at the anode was then computed using the integrated Fick equation. Similar experiments using a

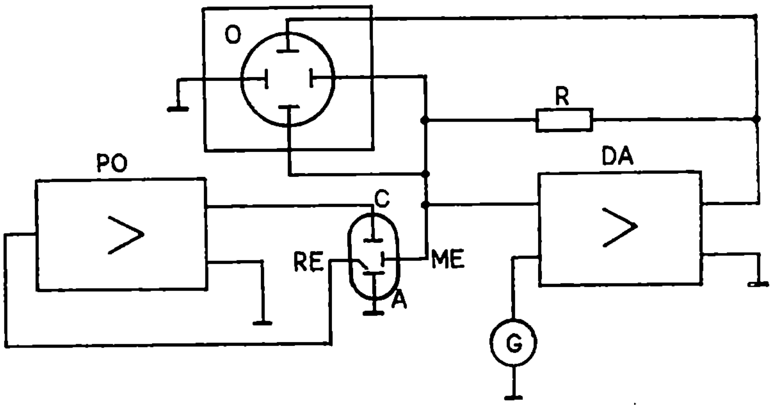

Fig. 2

platinum rotating disk electrode with an auxiliary ring[4] with mercury coating are going on.

We thank Dr. J. Weber for his advice.

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BIOPHYSICS

Electron Spin Resonance in Hæmin, Hæmatin and Hæmoglobin at 4·2° K

The electron spin resonance method is capable of giving fairly detailed information concerning the electron structure of molecules having unpaired electrons. In the past few years D. J. E. Ingram *et al.* have made detailed investigations of the electron spin resonance absorption of single crystals of hæmoglobin and myoglobin compounds. The most accurate measurements on high-spin ferrihæmoglobin and ferrimyoglobin compounds have been made at liquid air or liquid hydrogen temperatures and a well-defined variation of g-value from $g = 2$ to $g = 6$ was obtained[1–4]. Very similar g-values were observed in polycrystalline samples of the prosthetic group hæmin at liquid air temperatures[1,2,5]. No electron spin resonance spectrum was obtained in hæmatin. Measurements in frozen aqueous solutions at 170° K on some hæmoproteins have been reported by A. Ehrenberg[6]. I have recently summarized much of this work up to the first part of 1963[7].

I am undertaking an investigation of hæmin, hæmatin and acid ferrihæmoglobin by electron spin resonance at 4·2° K, to obtain as much detailed information as possible. In these biological complexes the iron atom (Fe^{3+}) is held at the centre of the porphyrin plane, surrounded by a square of nitrogen atoms. The different derivatives can best be classified by the nature of the extra two radicals attached to the central iron atom. In hæmoglobin one of these is a nitrogen atom of a histidine amino-acid of the protein, globin, and the other (sixth) position can be taken by a variety of different radicals. In acid ferrihæmoglobin water is at the sixth position. Hæmatin has water at the fifth position and the OH^- radical at the sixth position. Hæmin has a similar structure to hæmatin but has the Cl^- radical in place of the OH^- radical.

Electron spin resonance spectra were observed using a Strand Labs. spectrometer model 601 with a microwave frequency of 9,400 Mc/s and a field modulation of 6 kc/s. The sample cavity for operation at liquid helium temperature is a rectangular, silver-deposited quartz cavity which operates in the TE_{101} rectangular mode. For measurements of the g-values an Alpha Scientific Laboratories nuclear magnetic resonance gaussmeter *AL* 67 was used. Hæmin and hæmatin were obtained from L. Light and Co. and twice crystallized bovine hæmoglobin from the Nutritional Biochemical Corporation.

The samples of polycrystalline hæmatin show no observable electron spin resonance spectrum at room temperature, possibly due to short spin-lattice relaxation time. At 4·2° K they give a very intense line with $g = 6·3$ and two weak lines with $g = 4·35$ and $g = 2·03$ (see Fig. 1). At 4·2° K the intensity of the line with $g = 4·35$ is smaller than at 90° K. This is in accordance with the theoretical considerations given later because, owing to the Boltzmann factor, the line at $g = 4·35$ should be dependent on temperature in the sense that the intensity should diminish as the temperature goes down. In the powder, a statistical distribution of microcrystals is expected. Previous work on line shapes of powder resonance spectra has shown that $g_{\parallel}$ and $g_{\perp}$ can sometimes be deduced from measurements on polycrystalline samples[8–10]. $g_{\perp}$ is most easily obtained by this method because the expression for the mean g value is strongly peaked in the perpendicular direction. In Fig. 1 we are probably seeing peaks due to three separate environments for the Fe^{3+} ion, of which the g near 6 is presumably the $g_{\perp}$ of the usual high-spin situation observed in hæmoglobin and myoglobin compounds.

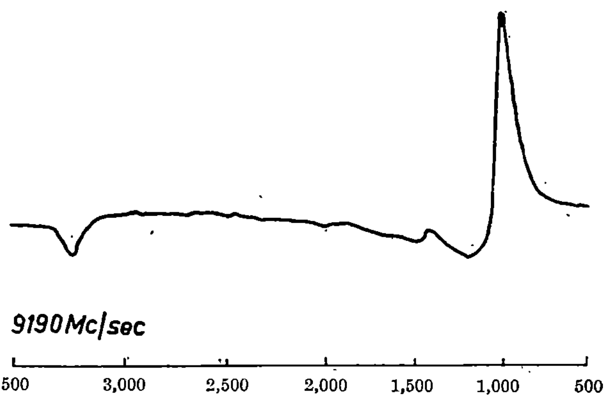

Fig. 1. Electron spin resonance spectrum of hæmatin at 4·2° K

Very similar electron spin resonance absorption was observed for the acidic ferrihæmoglobin, and nearly identical electron spin resonance maxima at $g_1 = 5·82$, $g_2 = 4·25$ and $g_3 = 2·07$ were obtained. For identification of the hæmoglobin used the visible absorption spectrum was measured and typical acidic ferrihæmoglobin maxima at 404·5 mμ, 500 mμ and 631 mμ were found. Similar g-values have been observed for ferric ions occurring substitutionally in glasses[11], in ferric ions of flavin enzymes[12] and also in frozen aqueous solutions of some ferric hæmoproteins[6].

The results for hæmin show that there is a significant difference between the electron spin resonance spectra at 90° K and 4·2° K. At 90° K two electron spin resonance maxima with g-values 2 and 6 were observed. In contrast to these measurements the absorption from hæmin at 4·2° K is a line corresponding approximately to a g-value of 2, and more than a thousand gauss wide. But a very broad line at $g \simeq 7$ was also observed. The coupling of the cavity loaded with hæmin is evidently dependent on the external magnetic field at 4·2° K. It seems most probable that hæmin at 4·2° K is ferromagnetic, but it may be difficult to obtain more precise information with the electron spin resonance method. Measurements of static magnetic susceptibility at 300°–4·2° K are in progress and a systematic survey of this field is planned.

If we assume that the spectrum shown in Fig. 1 is derived from ferric ions in three separate environments we must then ask what are these environments ? The high-spin ferric ion has $S = 5/2$ and it has been argued previously[14] that the spin-Hamiltonian :

$$\mathscr{H}(S) = D\left(S_z^2 - \frac{35}{12}\right) + E(S_x^2 - S_y^2) \qquad (1)$$

is usually adequate for these porphyrin compounds. In equation (1) the x, y and z axes are not necessarily simply related to the porphyrin axes although one might expect them usually to be so. If $E = 0$ we get a g value ranging from approximately $g = 2$ to $g = 6$ with most of the intensity occurring near $g = 6$. Presumably the line near $g = 6$ in Fig. 1 is due to this situation. The line $g = 2$ may well be the typical $g = 2$ line arising from the ferric ion with a fine-structure splitting small compared with the energy of interaction with the external magnetic field, that is, $D \simeq 0$ and $E \simeq 0$. This may be due to some peculiar structure for the hæmatin but is perhaps more likely to be due to a small amount of free ferric ion. Finally the small absorption at $g = 4{\cdot}35$ seems to be genuine. Such a line has been observed for ferric ions elsewhere[11-15] and they have been interpreted as being the case $D = 0$, and E large compared with the magnetic field interaction. If we set $D = 0$ in equation 1 it follows that the ground-term of the ferric ion splits into three Kramers doublets, the middle one of which is predicted theoretically to give an isotropic g value of 4·29 (ref. 15). It has been shown[16] that this behaviour (strictly with E large and D small but not accurately zero) can arise from certain environments. If we suppose that the present g value of 4·35 arises from hæmatin itself then the only one of the three environments considered in that paper which is at all plausible is that of a distorted octahedral arrangement with six identical, or nearly identical, neighbours. Even this environment would seem a little curious in hæmatin and possibly this small line is due to some degradation product.

I thank Prof. J. S. Griffith for giving the theoretical discussion of our experimental data and for kindness in informing us of his theoretical results[16] before publication, also Prof. W. Buckel for the furtherance of this work, and G. Bürk for his assistance in measurements. The programme of which this work is a part was supported by the Deutsche Forschungsgemeinschaft.

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Current Pulses in β-Carotene

WE are at present investigating the absorption spectra and photoconduction behaviour, as well as the electric properties of polyene molecules, in particular, of the molecule β-carotene. This molecule occurs widely in plants and animals. Although β-carotene itself does not occur in the visual pigments it can be considered a precursor of retinene, the C_{20} carotenoid, which occurs in the rods and cones of the retina. An attractive feature of this molecule to us was the possibility of growing single crystals and making our electrical and spectroscopic measurements along the different crystal directions. We have been able to make extremely thin crystals (~ 500 Å thick), as well as much larger crystals, and we shall be reporting a full account of the work elsewhere[1]. Here we wish to point out an interesting phenomenon observed during the electrical measurements.

The crystals used for the electrical measurements were crystallized from a benzene–methanol solution and grew as platelets with the faces in the *ab* plane, with typical dimensions of about 0·2 mm thickness and 10 mm^2 area. 'Aquadag' electrodes were used, including a guard ring electrode to eliminate surface effects. Each crystal was mounted on a quartz plate and enclosed in a 'Pyrex' Dewar flask which could be evacuated and heated to any desired temperature. When a β-carotene crystal is heated in a vacuum for several hours at 120° C and an electric field greater than ~4,000 V/cm is applied, we observe current pulses of the type shown in Fig. 1. This shows the current as a function of time when a steady voltage is applied to the crystal. The pulses are of constant frequency, occur in the dark (frequency ~1/10 c/s) and show no signs of decaying over a period of several hours. The effect of irradiating the crystal with light from a tungsten lamp is to increase the current and also to increase the frequency of the pulses. This effect is also shown in Fig. 1. Increasing the electric field also increases the frequency of the pulses.

A surprisingly marked effect on the bulk conductivity of the crystals, due to oxygen, is observed. When oxygen is introduced into the Dewar flask at room temperature the bulk conductivity increases by several orders of magnitude over a period of several hours. Pumping the Dewar flask causes the conductivity to fall slowly to its original value; raising the temperature of the crystal accelerates this process. A similar effect on bulk conductivity has been observed with at least one other type of crystal[2]. The crystal in a vacuum shows the current pulses at temperatures greater than 90° C. However, when oxygen is present, the current pulses occur at lower temperatures and we have observed them to occur at room temperature.

This is the first time, so far as we are aware, that such current pulses produced by a constant voltage have been reported with an organic molecule. Similar effects have, however, been recently observed with a few inorganic semiconductors[3-5]. Germanium, containing gold impurities, produces current pulses at a field of only 3 V/cm, but these were observed with the crystal at temperatures of 20° K. Gallium-arsenide[5] shows the effect at room temperature at 100–200 V/cm. The pulses occur in a similar way to those observed with β-carotene, both with respect to voltage and to incident light. The current-voltage characteristic has a similar deviation from Ohm's law, showing a flattening in the voltage range in which the pulses occur. A detailed explanation of the effect is not yet certain, but it has been shown that the current pulses are associated with a narrow region of high electric field associated with a charged double layer, which moves across the crystal from one electrode to the other and the transit time of which is related to the period of current oscillations[5]. This region of high field can be in many orders of magnitude more than that which occurs on either side of the region. Qualitative explanations of the way in which the high field region is formed seem to vary. A minority carrier extraction process has been described. It has been suggested that the voltage-threshold for oscillations is reached when the width of the charge depletion layer equals the hole drift length[6]. Another explanation has been given in terms of the trapping of hot electrons, leading to the development of a differential negative resistance which, because of its instability, causes a reorganization of field in the crystal and leads to the mobile domain high field structure[4].

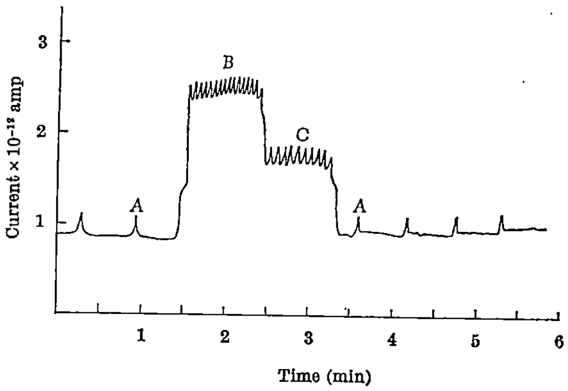

Fig. 1. Current-time relation at constant voltage for a β-carotene crystal in a vacuum showing effect of differing light intensities. *A*, dark; *B*, medium illumination; *C*, weak illumination. Applied voltage, 160 V. Temp., 120° C

It is clearly of interest that organic molecules also can exhibit this effect and we suspect that an explanation of the mechanism will be similar to that provided for the inorganic molecules. The explanations given for the inorganic semiconductors imply that the effect could occur in any heavily compensated high-resistant material, and we expect that other organic molecules, some of which may be of biological importance, will exhibit this effect.

The way in which the frequency of the pulses in β-carotene vary with illumination is very similar to the recording of spike discharges observed with a single optic nerve fibre of *Limulus*[6] when light is directed on to an ommatidium. Here a steady frequency is observed with constant illumination which is approximately linear with the logarithm of the stimulating light intensity. This provokes the question whether a mechanism of the type which we have observed could be an initiating mechanism for the passage of impulses from some receptor site, say the retina, to the optic nerve. It is necessary to be cautious at present about this suggestion. Although it is a precursor, the β-carotene molecule itself does not occur in the visual pigments and these particular pigments will, therefore, require separate investigation. However, even if visual pigments show the effect, then the extrapolation from a single crystal to the biological system may be a difficult one. A satisfactory field strength, sufficient organization and a reasonable minority carrier drift length are some of the requirements for the *in vivo* biological situation. Small voltage differences can give high field strengths if thin enough organized layers are involved and, of course, the visual pigments of the rods are indeed considered to be well organized[7].

We intend to continue investigating β-carotene and also to examine other organic and biologically interesting molecules for this effect. Whether it will prove to have biological significance or whether it is primarily an interesting solid-state electronic effect, which might itself prove to have interesting applications, may be revealed by our further investigations.

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Quantity Production of Synchronized Mammalian Cells in Suspension Culture

Synchronized cultures offer advantages in investigations of the sequence of biochemical changes occurring during the life-cycle of the mammalian cell. Such investigations suffer generally because present methods of producing synchronized cells do not provide immediately sufficient material to permit the use of classical analytical techniques. This communication describes a method by which gram quantities of synchronized cells representing a predetermined segment of the life-cycle can be produced.

Induction of partial synchrony in Chang appendix cell monolayers by reversibly inhibiting DNA synthesis with excess deoxynucleosides was initially reported by Xeros[1]. Recently, Puck[2] (using HeLa cells) and Bootsma *et al.*[3] (using heteroploid human kidney cells) showed independently that synchrony in monolayers can be improved by a double blocking technique using excess thymidine. In the work recorded here, a stable diploid line of Chinese hamster ovary (*CHO*) cells[4] was adapted to suspension culture in Puck's F–10 medium with calcium omitted and supplemented with 10 per cent calf and 5 per cent fœtal calf serum. Cultures seeded at a density of 3×10^4 cells/ml. could be maintained in logarithmic growth to a density of 3×10^5 cells/ml. Life-cycle analysis[5] of the *CHO* cell was performed to determine the optimum times for nucleoside addition and removal. Under conditions of suspension culture the generation time was 12 h, the pre-synthetic period (G_1) was 4·5 h, the DNA synthesis period (S) was 4·0 h, the post-synthetic period (G_2) was 3·0 h, and the mitotic interval (M) was 0·4–0·6 h. Using these values for the major segments of the life-cycle the following general procedure for inducing synchrony in suspension culture was devised. A random culture containing 6×10^4 cells/ml. was blocked for a period equivalent to G_2 plus M plus G_1 (about 9 h) by addition of thymidine. Blockade was released by washing and resuspending the cells in normal pre-warmed medium. After an incubation period slightly in excess of the duration of S (about 5 h), a second block was added and the cells again incubated for a period of 9 h. During the latter incubation period, the entire population presumably accumulated at a point immediately prior to S. Release from the second block, accomplished by again washing and resuspending the cells in pre-warmed normal medium, resulted in all cells entering S as a synchronized wave. Cultures were afterwards sampled at intervals and mitotic indices determined as described previously[5] to assess stability of synchrony for prolonged periods. No significant differences in the quality of the synchrony were observed with blocking concentrations of thymidine ranging from 2 to 25 mM.

Results of a typical experiment using a double thymidine blockage (25 mM) are shown in Fig. 1. Integration of the area under the curve for each generation indicates that in excess of 95 per cent of the population participated in each mitotic wave. The volume of the culture at the time of the first mitotic peak was 900 ml. containing $2 \cdot 2 \times 10^5$ cells/ml., yielding 600 mg of synchronized cells from a single collection.

The utility of this method lies in its adaptability to the production of large quantities of material synchronized within the major segments (for example, G_1, S or G_2) of the growth-cycle. So long as the cells were washed carefully and resuspended in pre-warmed medium, no significant lags or perturbations of the cycle intervals were observed. No constraints on culture volume have been encountered, and 1-g quantities of cells specified as to the life-cycle interval have been provided for investigations involving respiration, isolation of subcellular particles, and histone biosynthesis.

The data presented in Fig. 1 show, however, that the extent of synchrony is subject to rapid decay in subsequent generations. A preliminary investigation was carried out

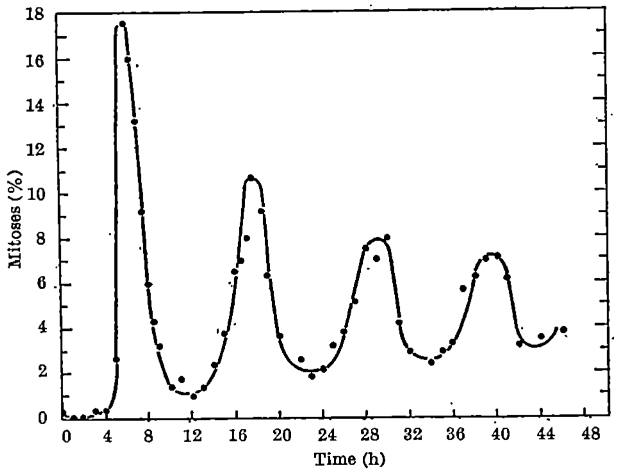

Fig. 1. Mitotic index of synchronized wild-type Chinese hamster ovary cells through four successive generations following release from double thymidine blockade (25 mM)

to test the hypothesis that genetic heterogeneity may account for the rapid reversion of synchronized cultures to random growth[3]. Several clonal lines were derived from the wild-type culture and characterized with respect to generation time and chromosome number. That having a generation time comparable with the wild-type was subjected to the synchronization process during logarithmic growth and followed in two separate experiments through three successive generations. Fig. 2 shows a comparison of decrease in percentage synchronization (as defined by Engelberg[6]) of the parent wild-type cells and cells from the genetically homogeneous clone. These results indicate no great differences in the rate of decay of synchrony between the wild-type line and the line selected for genetic homogeneity. This observation suggests that the capacity of a population of cells to desynchronize may be a fundamental property of the growing cell. Such a mechanism[7] could have significance to survival, since a synchronous population would be subject to annihilation by an insult to which cells in a particular phase of the life-cycle were susceptible but to which cells in other phases were relatively resistant.

These observations suggest also that the degree of synchrony attainable may be an inherent property of the cell. On this basis, synchrony in HeLa cells with approximately 9 per cent variance in generation times[8] may be expected to be superior to that observed in hamster ovary cells for which the variance is about 13 per cent[9]. Preliminary data obtained in our Laboratory by time lapse cinematography of HeLa monolayers are in agreement with results reported by Puck[2], which indicate a three-fold improvement in synchrony over that obtained with *CHO* cells in suspension culture.

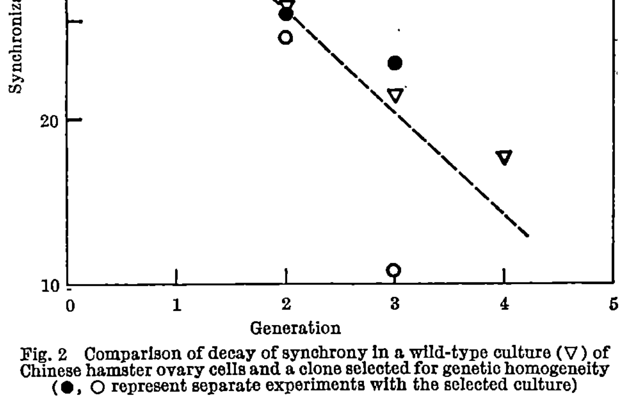

Fig. 2 Comparison of decay of synchrony in a wild-type culture (▽) of Chinese hamster ovary cells and a clone selected for genetic homogeneity (●, ○ represent separate experiments with the selected culture)

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BIOCHEMISTRY

In vivo Effect of L-Lysine on Rat Liver Arginase

PREVIOUS work carried out in this Laboratory was devoted to explaining the effective physiological role of ornithine cycle reactions in urea biosynthesis[1,2]. On the basis of the results obtained, serious doubts arose concerning the role of argininosuccinate synthetase in this process as well as with regard to the position of carbamylphosphate as an intermediate in nitrogen transfer from protein to urea. So far as the problem of urea biosynthesis is concerned, we are now investigating the role of arginase in this process. This communication reports *in vivo* inhibition of rat liver arginase activity by a strong competitive arginase inhibitor, L-lysine[3,4].

Male Wistar rats (average weight, 250 g) were fasted for 12 h with water *ad libitum*. L-lysine (3·0 mmoles/kg) in buffered solution at *p*H = 7·2 was injected intraperitoneally. At the same time control rats were injected with phosphate buffer. At various times after injection (Table 1) the rats were killed by decapitation, and the livers were quickly removed; a 10 per cent (v/v) homogenate with Sorensen phosphate buffer, *p*H = 7·2, was obtained in a Potter–Elvehjem glass apparatus at 0° C. Samples were prepared according to the following scheme: homogenate: 1 ml.; L-arginine: 10 μmoles; final volume: 6 ml. with phosphate buffer, *p*H = 7·2. Samples without substrate, as well as samples inactivated at zero time, were also prepared as blanks. The samples were incubated in a water bath at 37° C for 45 min. The reaction was stopped by adding 1 ml. of trichloroacetic acid (10 per cent). Urea was determined on aliquots of the protein-free filtrate according to Archibald[5].

The results (Table 1) indicate that L-lysine administration is able to inhibit arginase activity in liver. The

Table 1. *In vivo* EFFECT OF L-LYSINE (3·0 MMOLES/KG, INTRAPERITONEAL ADMINISTRATION), ON RAT LIVER ARGINASE

Min after L-lysine administration	μMoles extra-formed urea in the incubation mixture		Inhibition (%)
	Control rats	Rats treated with L-lysine	
30	5·5 ± 0·2	4·6 ± 0·1	16·4
60	5·1 ± 0·3	2·3 ± 0·1	56·0
90	5·6 ± 0·0	2·8 ± 0·2	50·0
120	5·4 ± 0·3	4·1 ± 0·2	24·1
150	5·4 ± 0·2	4·4 ± 0·2	18·6
180	5·7 ± 0·2	4·9 ± 0·0	14·0
210	5·5 ± 0·3	5·0 ± 0·1	9·1
240	5·6 ± 0·3	5·5 ± 0·3	0

inhibition reaches a maximum (56 per cent) 1 h after administration; it gradually decreases with the time, and finally disappears after 4 h.

Dhruba Ken[6] pointed out that intravenously administered L-lysine (1 g/day) strongly depresses uræmia in nephrectomized dogs. However, he did not investigate liver arginase activity. At present we are carrying out experiments in order to investigate the effects on urea formation *in vivo* of the observed arginase inhibition produced by L-lysine.

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Metabolism of Isomeric Synthetic Polypeptides

Previous investigations[1] demonstrated that a synthetic polypeptide containing D-glutamic acid, D-lysine and D-tyrosine is capable of eliciting an antibody response in rabbits: poly D-Glu^{55}D-Lys^{39}D-Tyr^{6}* elicited an average of 26 ± 33 μg Ab N/ml. in 13/17 animals, whereas its L-counterpart, poly Glu^{56}Lys^{38}Tyr^{6}, elicited 91 ± 52 μg Ab N/ml. in 38/38 animals. In view of this finding, we undertook to determine whether the D-amino-acid polypeptide was capable of being degraded *in vivo*. In addition, we planned to explore the metabolism of another L-amino-acid counterpart of the D-polymer, poly Glu^{58}Lys^{36}Tyr^{6} (No. 2), both as a control for the behaviour of the D-polymer and also to examine further some of the anomalous findings of our previous metabolic study on synthetic polypeptides[2].

In order to determine whether the method of iodination and the amount of polypeptide injected had any influence on the metabolic fate of the polymer, separate aliquots of poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) were labelled by the McFarlane iodine monochloride method[3] and by the Talmage method using free iodine in alkaline solution[4]. The experiments were carried out as previously described[2]; the rabbits received 0·05 per cent potassium iodide in their drinking water beginning two weeks prior to starting the experiments. In addition, the serum volume of the rabbit was measured by isotope dilution with iodine-131-labelled poly Glu^{58}Lys^{36}Tyr^{6} (No. 2), since its value greatly affects the calculation of the early rate of elimination; it was found to be 4·4 ± 0·3 per cent body-weight. Four rabbits were injected intravenously with 60 mg of the polymer labelled by the McFarlane method and five rabbits with 60 mg labelled by the Talmage method. There was no significant difference in the serum elimination patterns of the polypeptide labelled by the different methods. The same experiment was repeated using 10 mg of the polymer and again no difference in elimination pattern was found between the aliquots of the polymer iodinated by the two methods.

The serum elimination patterns of the isomeric polypeptides poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) and poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} were compared by injecting intravenously 10 mg of the L-polymer into each of 6 rabbits and 10 mg of the D-polymer into each of 11 rabbits. The elimination patterns were exactly the same in both cases: less than 1 per cent of the radioactivity remained in the serum after 3–4 days. Hence both the D- and the L-polypeptides are removed from the serum at a very rapid rate.

In 4 of the 9 animals receiving 60 mg of the polypeptide intravenously there was considerably more than 1 per cent of the radioactivity remaining in the serum after 3–4 days. In contrast to this, only 2 of the 34 animals in all the experiments in which 10 mg of either poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) or poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} were injected intravenously had more than 1 per cent of the radioactivity in the serum after 3–4 days. When the sera from the animals which retained radioactivity in the serum was passed through 'Sephadex *G*-25', 80–90 per cent of the radioactivity was retained by the column. Hence the persistence of radioactivity in the serum for more than 3–4 days was due to the presence of free iodine-131 or small radioactive peptides rather than the retention of the intact synthetic polypeptide antigen. From these findings we can conclude that the basic metabolic pattern of synthetic polypeptide antigens is that of rapid elimination from the circulation with less than 1 per cent remaining in the serum after 3–4 days. Our previous interpretation[2] involving two fundamentally different types of elimination pattern, depending on the previous immunological history of the animal, was incorrect. Any deviation from rapid elimination, which appears to occur more frequently with large intravenous amounts of antigen, is due to the presence in the serum of free iodine-131 or small radioactive peptides.

In order to demonstrate that the radioactivity in the urine was due to degradation products of the polypeptides, the urine of the animals injected with poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) and of those injected with poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} was dialysed overnight against running water. All the radioactivity was removed, thus indicating that the radioactivity in the urine was due to free iodide or small peptides from the degradation of the synthetic polypeptide antigens.

A series of experiments was performed in order to quantitate the amount of polypeptide metabolized and to assess the influence of previous exposure to one polypeptide isomer on the metabolism of the other. One

Table 1. Percentage of Polypeptide remaining in the Serum and of Radioactivity excreted in the Urine

The same rabbits received 10 mg of poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) intravenously on day 0 and 10 mg of poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} on day 21

	Poly Glu^{58}Lys^{36}Tyr^{6} (No. 2)*			Poly D-Glu^{55}D-Lys^{39}D-Tyr^{6}†		
Day after injection	2	4	15	2	4	15
Serum (%)	3·2	0·4	0·04	2·2	0·5	0·03
Urine (%)	88·0	92·0	95·0	2·8	4·8	12·5‡

* Average of 5 rabbits.
† Average of 3 rabbits (2 of the original 5 died previously).
‡ Subsequent experiments show that up to 20 per cent of the polymer can be degraded and excreted.

Table 2. Percentage of Polypeptide remaining in the Serum and of Radioactivity excreted in the Urine

The same rabbits received 10 mg of poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} intravenously on day 0 and 10 mg of poly Glu^{56}Lys^{36}Tyr^{6} (No. 2) on day 21

	Poly D-Glu^{55}D-Lys^{39}D-Tyr^{6}*			Poly Glu^{58}Lys^{36}Tyr^{6} (No. 2)†		
Day after injection	2	4	15	2	4	15
Serum (%)	4·0	0·7	0·08	2·0	0·3	0
Urine (%)	26·0	27·2	35·0	71·0	78·0	82·0

* Average of 5 rabbits.
† Average of 4 rabbits (1 of the original 5 died previously).

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* The standard abbreviations for the amino-acids are used in the nomenclature of the polypeptides. The L-isomers have no prefix and the D-isomers are preceded by D- (for example, D-Glu). The superscripts denote the molar percentages of the amino-acids in the random copolymers and the number in parentheses following the formula denotes the preparation. (The nomenclature scheme is in *Biopolymers*, in the press.) The molecular weights of the polymers are: poly D-Glu^{55}D-Lys^{39}D-Tyr^{6}, 93,000; poly Glu^{56}Lys^{38}Tyr^{6}, 110,000; and poly Glu^{58}Lys^{36}Tyr^{6} (No. 2), 70,000.

group of 5 animals each received 10 mg of poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) intravenously and 21 days later the same animals received 10 mg of poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} intravenously. The results of this experiment are shown in Table 1. A second group of 5 animals received the same polypeptides in the reverse order and the results are shown in Table 2. The serum elimination patterns were the same in all cases. Only 35 per cent of the D-polymer was degraded and excreted in the urine, whereas 95 per cent of its L-counterpart was metabolized. Previous exposure to one polypeptide isomer did not significantly alter the metabolism of the other isomer when it was injected subsequently. Thus, these experiments show that both the L- and the D-polymers are metabolized and the results strongly suggest the existence of D-proteases and D-peptidases in the rabbit. Previous evidence for the existence of such enzymes was given by the work of Tsuyuki, Tsuyuki and Stahmann on the anti-viral properties of poly D-Lys in the chick embryo[5] and on the metabolism of poly D-Lys by pancreatic extracts[6].

The binding of poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) and poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} by serum from unimmunized animals was studied by starch-gel electrophoresis in borate at pH 8·6 and in acetate at pH 5·5. The D- and L-polymers migrated with the same serum fractions: in borate buffer they were bound to proteins in the α- and β-globulin region and in acetate buffer they were bound in the region of the fastest migrating serum protein.

In order to determine the fate of the synthetic polypeptide antigen remaining after the serum-level had reached a plateau and urinary excretion of radioactivity essentially ceased, the organs of the rabbits receiving 60 mg of poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) and those of the rabbits receiving 10 mg of poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} were homogenized and examined for radioactivity. The results are shown in Table 3. Although the total recoveries in these experiments were not complete, it is clear that in the animals receiving the D-polymer much of the radioactivity retained in the animal, and presumably still in the D-polymer, was located in the kidney and, to a much lesser degree, in the liver. The kidneys were examined histologically to see whether there was any morphological damage that could account for the large amount of poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} retained in these organs. No such damage was found and the kidneys were anatomically normal. To summarize, the difference in the metabolism of the isomeric polypeptides lies in their extravascular fate, since they are eliminated from the serum at the same rate but are not degraded and excreted to the same extent, and the kidney appears to be the major organ involved in the ultimate fate of poly D-Glu^{55}D-Lys^{39}D-Tyr^{6}.

Table 3. PERCENTAGE OF RADIOACTIVITY REMAINING IN THE ORGANS OF RABBITS INJECTED WITH THE ISOMERIC SYNTHETIC POLYPEPTIDES POLY GLU^{58}LYS^{36}TYR^{6} (NO. 2) AND POLY D-GLU^{55}D-LYS^{39}D-TYR^{6}

Organ*	Injected with Poly Glu^{58}Lys^{36}Tyr^{6} (No. 2)† (%)	Poly D-Glu^{55}D-Lys^{39}D-Tyr^{6}‡ (%)
Liver	1·1	6·3
Kidney	0·1	32·7
Spleen	0	0·4
Blood	1·2	0·03
Urine	67·7	12·5

* No significant radioactivity in other organs.

† Nine rabbits; 60 mg per rabbit intravenously and killed on day 12 after injection.

‡ Three rabbits; 10 mg per rabbit intravenously and killed on day 15 after injection. (Received 10 mg poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) previously of which 95 per cent was excreted; see Table 1.)

A preliminary investigation of the cellular metabolism of the isomeric polypeptides was undertaken *in vitro* using peritoneal macrophages prepared from ascitic fluid produced in the unimmunized rabbit by injecting mineral oil intraperitoneally 48 h previously. The results for both the D- and the L-polymer were the same: 15–25 per cent of a 200-µg aliquot of polymer was incorporated in 30 min by a suspension containing 2×10^{6} macrophages. Hence it appears that both polymers are equally susceptible to phagocytosis by macrophages.

Finally, we undertook a series of experiments to investigate the metabolic heterogeneity of the synthetic polypeptide antigen poly Glu^{58}Lys^{36}Tyr^{6} (No. 2). Two rabbits weighing about 2 kg each were injected intravenously with 10 mg of poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) labelled with iodine-131. The antigen disappearance pattern was followed closely for 2 h and then the animals were bled. 30 ml. of plasma containing radioactive antigen (1–2 mg) was taken from each of the two rabbits and injected into two other rabbits; the disappearance rates were again followed for several hours. The elimination rate in the second group of rabbits, that is, in those receiving the antigen after *in vivo* screening, was slower than that following the primary injection of antigen. The difference in excretion rate was greatest during the first 30 min: 35 per cent of the antigen was removed from the serum following its initial injection, whereas only 10 per cent was removed after it had previously been screened *in vivo*. Therefore the synthetic polypeptide antigen is metabolically heterogeneous and there is a fraction that is very rapidly eliminated following intravenous injection.

In conclusion, we have demonstrated the ability of rabbits to degrade a polymer composed entirely of D-amino-acids and hence, by inference, have obtained evidence that suggests the existence of D-proteases and D-peptidases. The rates of elimination from the serum for poly D-Glu^{55}D-Lys^{39}D-Tyr^{6} and poly Glu^{58}Lys^{36}Tyr^{6} (No. 2) are the same: less than 1 per cent of the injected dose remains in the serum after 3–4 days. Only 35 per cent of a 10-mg dose of the D-polymer is degraded and excreted in the urine, whereas 95 per cent of its L-counterpart is metabolized. The majority of the retained D-polymer is in the kidney and a small amount is in the liver.

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A New Protein Factor promoting Contraction of Actomyosin

RECENTLY a tropomyosin-like protein[1,2], or 'native tropomyosin', has been added to a list of proteins participating in muscle contraction. The sensitivity of myosin *B* to the relaxing action of calcium-binding agents including the physiological relaxing factor is ascribed to the presence of 'native tropomyosin' in it together with actin and myosin. Even with these three proteins, however, properties of myosin *B* could not entirely be represented.

On addition of adenosine triphosphate, synthetic actomyosin tends to show a clearing response compared with myosin *B* or myofibrils. This tendency becomes

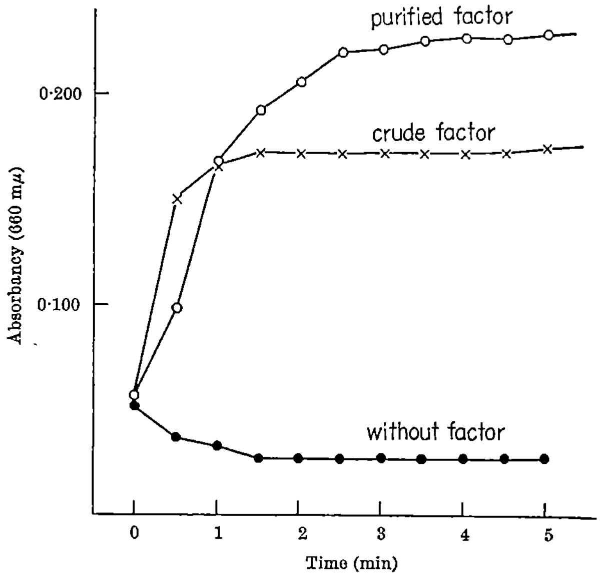

Fig. 1. Effect of contraction-promoting factor on the response of synthetic actomyosin to ATP. Procedures for superprecipitation experiments were essentially the same as already described (ref. 3). Ingredients of the reaction mixtures in final concentrations: 0·8 mg/ml. of synthetic actomyosin, 0·16 mg/ml. of 'crude extract' (ref. 2), 0·24 mg/ml. of purified factor, 0·02 M KCl, 1 mM $MgCl_2$, 1 mM ATP and 0·02 M *tris*-maleate (*p*H 6·8). Actin was obtained from exhaustively washed muscle residue and did not contain a significant amount of the factor

more pronounced when we use specially prepared actin which is free from the new protein mentioned here. As shown in Fig. 1 actomyosin thus prepared shows mainly only clearing response to a moderate concentration of ATP, namely, 1 mM. When the 'crude extract' of muscle residue obtained by the method for the preparation of 'native tropomyosin' is added to the actomyosin preparation, an instantaneous onset of superprecipitation is observed. This actomyosin shows rapid superprecipitation in response to low concentrations of ATP, for example, 10^{-5} M. The function of the factor contained in the 'crude extract', therefore, seems to enlarge the range of ionic strength and ATP concentration in which actomyosin responds to ATP with superprecipitation. Purified 'native tropomyosin' did not show any of this effect. On the contrary, it has rather depressing effect on superprecipitation. Therefore, the 'crude extract' should contain another factor which promotes the contraction of pure actomyosin.

Purification of the factor was performed chiefly by ammonium sulphate fractionation. It was first precipitated by 36 per cent saturation and then, after dissolving in water, by 21 per cent saturation. The protein thus obtained was fairly homogeneous according to the ultracentrifugation diagram. It showed an increasing tendency of aggregating with the elevation of ionic strength; without salt its $S_{20,w}$ was about 10, but more than 40 in 0·1 M potassium chloride. In spite of this remarkable aggregation its intrinsic viscosity remained low, less than 0·2, indicating the formation of a rather random aggregate, and not of end-to-end polymer, as in the case of denaturated actin[4]. Its amino-acid composition was similar to that of actin, and it did not contain any nucleotides. However, any actin preparations, native as well as denaturated, did not function like the new protein. Hence, the protein is now considered to be a new component in contractile elements. The yield of the protein was about 1 g from 100 g of muscle. If we take the loss during preparation into consideration, this amount may fairly correspond to that of actin.

If this contraction-promoting factor was added to *G*-actin, no change in its physical properties took place. However, on addition of salt, the polymerization process of actin was speeded up by the presence of the protein when it was followed by viscosimetry or flow birefringence measurements. On the other hand, no significant interaction could be observed between myosin and the new protein. Thus the new factor appears to act mainly on the *F*-actin moiety of actomyosin. Usual actin preparations contain this component in a varied amount. Therefore, it is possible that some of the properties so far ascribed to *F*-actin might be due to the influence of the new component on actin.

This protein probably plays an important part in contraction of living muscle in which ionic strength and ATP concentration are much higher than those of *in vitro* experiments. In this respect the protein may be regarded as the second protein factor, next to 'native tropomyosin'[1,2], regulating the myosin–actin–ATP interaction. In addition another protein influencing the physical state of *F*-actin has been isolated from myofibrils[5]. To understand the mechanism of muscular contraction in its entirety, we must clarify the mode of action of these natural regulators in detail.

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DNA Polymerase in Thymus Nuclear Fractions isolated with Ca^{++}

Bollum and Potter[1] reported the incorporation of tritiated thymidine into DNA by enzymes present in a soluble high-speed supernatant fraction of aqueous homogenates of regenerating liver and of rat thymus. These enzyme activities were compared in nuclear and cytoplasmic aqueous extracts from Ehrlich ascites tumour cells by Smellie, Keir and Davidson[2] and by Smellie and Eason[3], who found them to be higher in the cytoplasmic fractions. In view of the paradox thus arising from the concept of synthesis of a nuclear component, DNA, by an enzyme apparently present in the cytoplasmic fraction, Keir, Smellie and Siebert[4] re-investigated this problem with revised assay techniques. They found DNA polymerase (DNA nucleotidyltransferase) activity higher in nuclei than in cytoplasm when these were isolated in non-aqueous solvents. Behki and Schneider[5] have reported similar findings in regenerating liver and Novikoff hepatoma nuclei isolated with non-aqueous solvents.

It is the purpose of this communication to present evidence for the presence of DNA polymerase in cellular particulate matter, that is, nuclear fractions of rat thymocytes when extracted with aqueous solvents, as well as the effect of Ca^{++} when included in these aqueous extraction media.

Sprague–Dawley 5½-weeks-old female rats from the animal colony of this laboratory were the source of thymus tissue. The *tris* (*tris*-hydroxymethyl-aminomethane), adenosine triphosphate and the deoxynucleoside triphosphates of adenine, guanine and cytosine were obtained from Sigma Chemical Co. The tritiated thymidine triphosphate, prepared enzymatically from tritiated thymidine (New England Nuclear Corp.), isolated and purified by the method of Lehman, Bessman, Simms and Kornberg[6], was the gift of Dr. E. R. Walwick.

Suitable portions of randomly pooled rat thymus tissue were homogenized with 9 vols. (ratio, 1·00 g tissue : 9·0 ml. buffer) of ice-cold *tris*-Cl (*tris*, 11·0 mM; *p*H 7·7) buffer solutions containing calcium chloride in concentrations shown in Table 1. The homogenates were centrifuged (105,000*g*; 1 h; 1° C) and a part of the clear middle portion of the supernatant fraction was taken for analysis (supernatant fractions *A*, Table 1). The first residue (pellet) fractions obtained were each drained, weighed and homogenized in *tris*-Cl buffer as already indicated, but with no Ca^{++}. Afterwards, all residue fractions were alternately frozen (−196° C) and thawed (2° C) three times, followed by homogenization. The supernatant extracts (centrifugation 105,000*g*; 1 h; 1° C) from the respective first residue fractions were designated 'extracts of first frozen residues' (Table 1, extracts *B*). By repetition of the foregoing process, 'extracts of second frozen residues' (Table 1, extracts *C*) and 'extracts of third frozen residues' (Table 1, extracts *D*), respectively, were obtained.

Each extract was dialysed by the method of Walwick and Main[7] to remove diffusible metabolites and Ca^{++}, assayed for protein concentration by the method of Lowry *et al.*[8], and stored frozen at −196° C until tested for DNA polymerase activity by the modified liquid scintillation counting methods of Main and Walwick[9] and Walwick and Main[7]. In this communication, DNA polymerase activity was determined by measuring the rate of incorporation of tritiated thymidine triphosphate (^{3}H-TTP) into DNA in the presence of the deoxyribonucleoside-5′-triphosphates of adenine, guanine and cytosine under the conditions indicated in Table 1.

When DNA polymerase was extracted from rat thymus cell homogenates by a low ionic strength *tris*-Cl (*tris* hydroxymethyl aminomethane) homogenization medium considerable DNA polymerase remained in the particulate pellet (Table 1, column 6). Thus, additional polymerase could be extracted following rehomogenization, freeze–thawing 3 times, and further homogenization of the pellet. Supplemental polymerase then could be obtained by second and third applications of the above process. These data also indicate that the percentage of total DNA-polymerase extracted from the pellet initially prepared in 2 mM Ca^{++} is more than twice that from the pellet initially prepared in the absence of Ca^{++}. In a second experiment in which the *tris*-Cl homogenization medium was made iso-osmolar (0·25 M) with respect to sucrose, results similar to those above were obtained following triple freeze–thaw extraction of the pellet (Table 2, column 7). Furthermore, in both experiments, as the Ca^{++} concentration of the initial homogenization medium was increased, the amount of polymerase later extracted from the initial pellet increased. One should note the approximately two-fold greater specific activity of DNA-polymerase in the first soluble supernatant extracts prepared in the *tris*-Cl buffer (no sucrose), as compared with corresponding values obtained in the presence of 0·25 M sucrose in the medium.

It is evident from these data that the partition of DNA-polymerase activity between the high-speed supernatant fraction and the centrifugal sediment, particulate fraction of rat thymus homogenates is dependent on the nature of the aqueous homogenization medium used, that is, whether or not it is iso-osmolar, and particularly on the Ca^{++} ion concentration. When Ca^{++} was varied from 0·0 to 6·0 mM a maximal localization of polymerase in the sediment was observed at 2 mM Ca^{++}. This calcium concentration appears to be specially significant, since it is also close to the value (1·8 mM Ca^{++}) found best suited for isolation, in aqueous sucrose medium, of morphologically intact mouse spleen nuclei by Schneider and Petermann[10], and of rat liver nuclei by Hogeboom *et al.*[11]. It is known that calcium ion precipitates the nuclear deoxyribonucleoprotein (DNP) complex[12,13]. The present results indicate that association of DNA-polymerase with the particulate nuclear sediment of thymus homogenates is favoured when the Ca^{++} concentration of the original homogenization medium is 2·0 mM or greater. It may be suggested from these findings that, in intact cells, DNA-polymerase is localized in the nucleus, perhaps in a labile association with DNP. It is of interest that Billen[14] has presented evidence for the association of DNA-polymerase as a complex with DNA in *E. coli* cells. Mazia and Hinegardner[15] found DNA polymerase activity in sea urchin embryos to be associated exclusively with nuclei when the latter were isolated in 1·2 M sucrose in the presence of either 1·0 mM Mg^{++} or 1·0 mM Ca^{++}. These were the only conditions found so far in which the polymerase activity was retained with nuclear material. Our results also suggest that when thymus tissue homogenates are prepared in low ionic strength iso-osmolar aqueous media in the absence of added Ca^{++} the DNP tends to become hydrated and dispersed (column 4, Table 2), and the associated DNA-polymerase is released into the aqueous phase.

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Table 1. DNA-POLYMERASE ACTIVITY IN RAT THYMUS PREPARATIONS AS AFFECTED BY FREEZE–THAWING AND BY Ca^{++}

DNA-polymerase assay: the final incubation medium (0·50 ml.) *p*H 7·4 contained: phosphate buffer (*tris* form) 5·0 μmoles; $MgCl_2$, 3·0 μmoles; KCl, 25 μmoles; ATP (*tris* form) 3 μmoles; each of the deoxyribonucleoside-5′-triphosphates of adenine, guanine and cytosine, 8·33 mμmoles; ^{3}H-TTP (specific activity, $1·2 \times 10^6$ c.p.m./μmole) 8·38 mμmoles; enzyme extract, 0·25 ml. (after dilution to 1·00 mg protein per ml.) of *A* 1–3 or 0·25 ml. (undiluted) of all others; calf thymus DNA primer solution (heated, 100° C, 7 min) 0·150 ml. (100 μg DNA)

No.	Fraction	Ca^{++} conc. in homogenization media (mM)	Protein conc. in extract fractions (mg/ml.)	Specific DNA-polymerase activity (units)*	DNA-polymerase activity (units/g tissue equivalent
A-1	Soluble supernatant fractions	0·0	5·3	8·7	403
A-2		1·0	4·8	8·2	360
A-3		2·0	4·5	5·5	231
B-1	Extracts of 1st frozen residues	0·0	0·8	10·0	87·6
B-2		0·0	0·9	10·2	71·3
B-3		0·0	1·1	29·7	196
C-1	Extracts of 2nd frozen residues	0·0	0·4	2·2	12·2
C-2		0·0	0·2	11·1	19·7
C-3		0·0	0·5	9·6	29·8
D-1	Extracts of 3rd-frozen residues	0·0	0·2	1·7	3·8
D-2		0·0	0·3	1·4	3·1
D-3		0·0	0·2	4·0	5·3

* Expressed as mμmoles ^{3}H-TTP incorporated into DNA/h/mg protein, and based on 30-min rates at 37° C. The time course of incorporation of ^{3}H-TTP into DNA by DNA-polymerase was linear between the limits of 0–60 min.

Table 2. DNA-POLYMERASE IN RAT THYMUS PREPARATIONS AS AFFECTED BY Ca^{++} CONCENTRATION IN THE PRESENCE OF 11 mM *tris*-CL, 0·25 M SUCROSE

No.	Fraction	Ca^{++} conc. in homogenization media (mM)	Pellet weight from 11·8 ml. 1st homogenate (g)	Protein conc. in extract fractions (mg/ml.)	Specific DNA polymerase activity (units)*	DNA polymerase activity (units/g tissue equivalent)
A-1	Soluble supernatant extracts	0·0	—	3·8	6·9	223
A-2		1·0	—	3·6	5·7	190
A-3		2·0	—	3·35	4·6	143
A-4		3·0	—	2·7	5·0	126
A-5		6·0	—	2·6	3·9	95·5
B-1	Extracts of 1st frozen residues	0·0	1·43	1·5	2·8	60
B-2		0·0	1·20	1·7	5·1	75
B-3		0·0	0·91	2·3	8·1	142
B-4		0·0	0·65	3·3	6·0	126
B-5		0·0	0·65	4·4	5·6	103

* Expressed as in footnote to Table 1.

DNA-polymerase assay as in Table 1. Tissue initially homogenized in 11 mM *tris*-Cl, 0·25 M sucrose solution.

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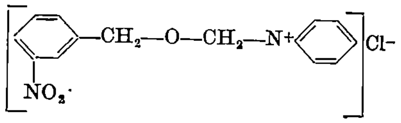
Use of Antibodies on an Insoluble Support for Specific Detection of Radioactive Antigens

When investigating the biosynthesis of different proteins and other compounds it appears to be necessary to isolate them from complex mixtures in order to measure radioactivity. The method of co-precipitation is often used, but it is somewhat limited. We tried to develop a new method for detecting radioactive antigens free of these limitations.

The method proposed is based on the use of immunosorbents (firm complex of soluble antigen with insoluble support) in the form of a suspension able to combine as much as 0·3–1·0 g antibodies to 1 g (dry weight) sorbent[1]. We used such sorbent-antigens for the isolation of pure antibodies and for the quantitative detection of antibodies by measuring the increase in protein or radioactivity on sorbent-antigens[2–4]. The principle of this method rests on the fact that bivalent antibody is combined with the antigen on insoluble support by means of one combining site while the other combining site is free and thus capable of reacting with a soluble homologous antigen.

Such a complex can be used for the isolation of corresponding antigens from the solution. The principal potentiality of this reaction has already been demonstrated[5].

The method of preparation of sorbent-antigens in the form of suspension has already been described[1]. It consists in treating, at 125° C, the cellulose with the following pyridinium salt:



to introduce the organic residue.

After reducing the nitro group to an amino group the product is dissolved in Schweizer's reagent. Cellulose in a form of suspension precipitated after this solution was diluted with large amounts of water was diazotized and coupled to the desired antigen. We used human serum albumin (HSA) and horse γ-globulin (HGG). Complexes capable of combining with antigens (sorbent-antibody) were prepared by adding immune serum containing antibodies corresponding to the sorbent-antigen. The mixture was stirred for 10 min at room temperature and washed three times with 0·85 per cent sodium chloride at *p*H 7 from unbound proteins by centrifugation.

The amount of antibodies needed to obtain active preparation must not be less than the amount able to saturate half the combining capacity of the sorbent-antigen.

HGG fixed with cellulose (C-HGG) treated with corresponding antibodies (C-HGG : anti-HGG) was used in the majority of our experiments. Cellulose-HSA (C-HSA) and C-HGG treated with normal rabbit serum, and C-HSA treated with corresponding antibodies (C-HSA : anti-HSA) served as control preparations.

1 ml. (1·5–3·0 mg dry weight) of one of the control preparations was put into little centrifuge tubes and 2 ml. of the solution containing different amounts of HGG labelled with iodine-131 (ref. 6) were added in different experiments. After stirring for 10 min at room temperature the sediment (sorbent) was separated by centrifugation (5,000 r.p.m.) and supernatant was transferred into the centrifuge tubes which contained the same amounts of sorbent-antibody as in the control. After stirring, the sorbent was separated by centrifugation. The sediments obtained were quantitatively transferred to chromatographic paper (Whatman No. 1) disks (diam. = 20 mm) to make a spot about 10 mm in diameter. To wash out the unbound label these disks were placed in an apparatus for descending chromatography on a sheet of paper (Whatman No. 1). One end of this sheet of paper was placed in the trough containing 0·5 per cent potassium iodine in 0·85 per cent sodium chloride (*p*H 7). This procedure was introduced to detect carbon-14 antibodies by measuring the specific increase in activity of carbon-14 on the sorbent antigen[3,4]. The next day the disks were dried for 15 min at 60° C and then their radioactivity was measured. The results obtained are given in Table 1, which shows that HGG–^{131}I is specifically adsorbed by the sorbent contained anti HGG. Non-specific adsorption does not exceed 3 per cent from specific.

Table 1

Exp. No.	Sorbents		Treated with rabbit serum					Added HGG–^{131}I		All added ^{131}I activity (c.p.m.)	Combined ^{131}I activity c.p.m. sample	Increase in ^{131}I activity on sorbent-antibody (c.p.m.)
	Name	Amount (mg)	Normal	Contained antibodies against HGG		Contained antibodies against HSA		µg	Specific activity (c.p.m.)			
			Volume (ml.)	Volume (ml.)	Antibody protein (µg)	Volume (ml.)	Antibody protein (µg)					
40	C–HGG	1·5	0·63	—	—	—	—	7·5	106	795	23	710
	C–HGG	1·5	—	0·63	540						733	
45	C–HSA	1·5	0·15	—	—	—	—	20	41	810	25	14
	C–HSA	1·5	—	—	—	0·15	300				39	
	C–HGG	1·5	0·15	—	—	—	—	20	41	810	33	577
	C–HGG	1·5	—	0·15	300	—	—				610	

Experiments with the tracer dilution when adding non-radioactive HGG and experiments on measuring absolute increase in protein when being added to a sorbent-antibody showed the combining capacity of the preparations used is 30–50 mg of antigens on 1 g of sorbent-antibody.

Further experiments showed those antigens which are specifically adsorbed by the sorbent antibody keep the capacity to react with corresponding antibodies from the solution added: this was proved by means of ^{14}C-antibodies. Sorbent antibody also appeared to be capable of reacting not only with antigens but also with soluble antigen–antibody complex; it allows presence of radioactive antibodies masked by excess of antigen in the system to be revealed.

The data obtained testify to the possibility of the following reactions between antigens (*Ag*) and antibodies (*Ab*).

$$\begin{array}{ll}
1 & \underset{\text{sorbent-antigen}}{C - Ag} + Ab = \underset{\text{sorbent-antibody}}{C - Ag : Ab} \\
2 & C - Ag : Ab + Ag = C - Ag : Ab : Ag \\
3 & C - Ag : Ab : Ag + Ab = C - Ag : Ab : Ag : Ab \\
4 & C - Ag : Ab + \underset{\text{soluble antigen–antibody complex}}{Ag : Ab} = C - Ag : Ab : Ag : Ab
\end{array}$$

The method described here can be used for the detection of different radioactive antigens. At rather high specific activity one can reveal as much as 0·01–0·001 μg of radioactive antigens. This has certain advantages over that of co-precipitation since it can be applied not only to precipitating but also to non-precipitating systems. Another advantage is low non-specific adsorption and small labour consumption.

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Effect of the Unsaturated Bond on the Degradation of the Tetragalacturonic Acids by a Transeliminase

During the course of investigations of the degradation of the normal and unsaturated-tetragalacturonic acids by a transeliminase[1,2], it was found that unsaturated tetragalacturonic acid was attacked about 1·4 times faster than the normal tetramer. Furthermore, it was observed that the unsaturated tetramer must be almost completely converted to unsaturated digalacturonic acid since there was an approximate doubling (96 per cent increase) in the optical density. All activities were determined by measuring the change in optical density at 232 mμ. Unsaturated galacturonic acid does not absorb at this wave-length, presumably because of isomerization to the keto-deoxy derivative[3]. The observation with unsaturated tetragalacturonic acid was confirmed with the aid of paper chromatography using the ethyl acetate–pyridine–water–acetic acid solvent[1]. Only a spot with an R_F identical to unsaturated digalacturonic acid was detected.

As indicated in a previous publication[1], tetragalacturonic acid is attacked at the two glycosidic linkages farthest from the reducing end (designated bonds 2 and 3; bond 1 being that nearest the reducing group). That bond 1 is not attacked was shown by the complete absence of normal trigalacturonic acid. The relative rates of attack of the two bonds can be determined by comparing the amounts of normal galacturonic acid and normal digalacturonic acid produced since the latter arises only from attack at bond 2 and the former by attack at bond 3. Thus a sample of a 24-h reaction mixture was spotted on paper, developed with the previously mentioned solvent system, the strips cut out and eluted with water. The amount of uronide material was determined in each sample by the carbazole method[4]. In this manner it was determined that bond 3 was attacked about 1·4 times faster than bond 2. All the above results were reproducible.

Since the total cleavage of glycosidic linkages in tetragalacturonic acid is the sum of attack at bonds 2 and 3, it was concluded that the central glycosidic linkage of unsaturated tetragalacturonic acid was attacked about 3·4 times faster than was bond 2 of normal tetragalacturonic acid. Thus if one accepts the previous conclusion that only one enzyme is excreted by *Bacillus polymyxa*[5] one must conclude that the presence of the unsaturated bond somehow inhibits attack at bond 3 but enhances the rate of degradation of bond 2. Whether this is due to the change in configuration of the unsaturated unit (planar) or to other effects has yet to be determined.

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Paper Electrophoresis of Soluble Lens Proteins in Lactose-fed Rats

Proteins of the lens tissue of the eye have been extensively examined because of their relationship with cataract formation. In all forms of cataract a decrease was found in the total amount of lens proteins as compared with normal lens of the same age[1]. Changes in the proportion of the soluble proteins of the lens measured by electrophoresis have been reported in different kinds of cataract; but, according to van Heyningen[2], no consistent patterns have emerged from these investigations. The work recorded here was carried out to determine the behaviour on paper electrophoresis of soluble lens proteins of the rat, as well as the possible changes in the proportion of the different fractions during lactose feeding.

Albino rats, weighing 70–80 g at the beginning of the experiment, were given a 40 per cent lactose diet, and the controls a 40 per cent cane sugar diet, as already described[3]. At appropriate intervals, the animals were killed and the lens homogenate prepared as already described[3]. Paper electrophoresis was performed with a volume of the supernatant of centrifuged lens homogenate, containing 1–1·5 mg of soluble lens proteins. A borate buffer (0·05 M, *p*H 8·6) was used; the potential gradient of electric field was of 5 V/cm for 10 h. The paper strips were stained with bromophenol blue, and the different fractions measured by densitometry.

The soluble lens proteins of normal and lactose-fed animals, separated by means of this method, gave four distinct moieties (Fig. 1), which will be labelled I, II, III and IV, fraction I being the most rapid one. After two months of lactose feeding, it was possible to observe (Table 1) an increase of fraction I (the most rapid one), and

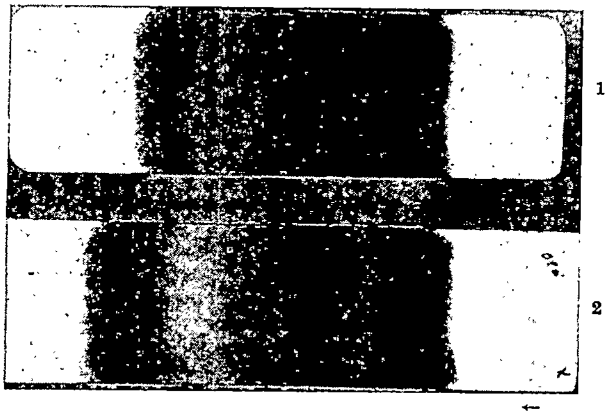

Fig. 1. Paper electrophoresis of soluble lens proteins. 1, Normal rat; 2, lactose-fed rat (2 months)

a decrease of fraction IV (the slowest). These changes occurred in all animals examined. The difference between the mean value of normal and lactose-fed animals, for fractions I and IV, is very significant ($P < 0.001$), which is by no means the case for fractions II and III.

In order to ascertain the progressive changes in the proportions of electrophoretically separated proteins, another group of rats was maintained on the same 40 per cent lactose diet, three rats being killed at different intervals. The first three animals were killed before the lactose diet was started. As shown in Fig. 2, after feeding for 5 days fraction I begins to increase and fraction IV to decrease, but during the first month the values found were within the normal range. After this period, this phenomenon was very conspicuous, and also fractions II and III started to increase, but in a lesser degree than fraction I.

As observable, our results indicate that even using a mild lactose diet, intended to obtain slower changes in the biochemistry of the lens, early changes in the pattern of soluble lens proteins are found in rat lenses. It has been reported that the electrophoretically most rapid fraction corresponds to the α crystallin[4], while all the other fractions may correspond to the β- (and γ-) crystallin[2]. It has been stated that there is a loss of β-crystallin in different types of cataracts[2]. The decrease of fraction IV found in this investigation may correspond to the fall of β-crystallin observed with other methods. The increase of fraction I suggests that there is an increase of α-crystallin.

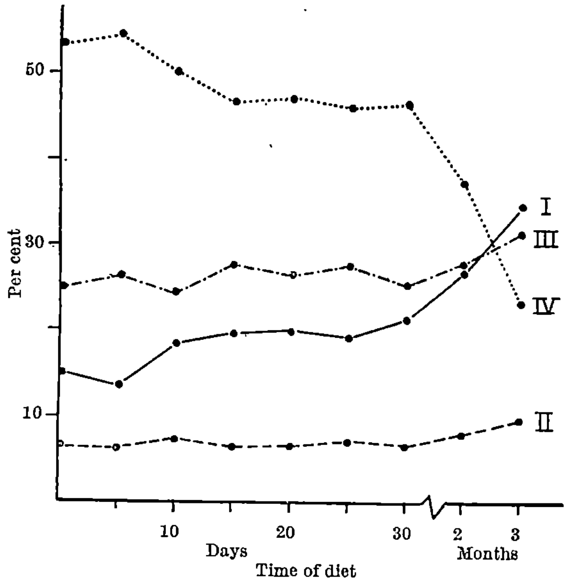

Fig. 2. Paper electrophoresis fractionation of soluble lens proteins in lactose-fed rats at different periods. On each day indicated 3 animals were killed and the stated amounts are the mean values of the three determinations

Table 1. Paper Electrophoresis of Soluble Lens Proteins in Normal and Lactose-fed Rats

	Fraction I	Fraction II	Fraction III	Fraction IV
Normal diet No. of animals, 14	17·7 ± 2·7	7·6 ± 1·3	26·2 ± 2·7	48·5 ± 2·5
Lactose diet No. of animals, 14	27·0 ± 2·2	8·0 ± 1·8	27·8 ± 2·2	37·2 ± 4·0
P	< 0·001	> 0·5	> 0·1	< 0·001

Animals were given lactose diet for 2 months.
All values are given in percentages of the total amount of soluble proteins ± *S.D.*

In cataracts of galactose-fed rats, François *et al.*[5] have found a loss of soluble proteins. With a higher proportion of lactose in the diet as used in this experiment, we found an early fall of the soluble lens proteins[6]; but during this mild lactose diet the amount of total soluble proteins does not decrease until the fortieth day of feeding[7]. Hence, the protein pattern of the lens begins to change before the total amount of soluble proteins starts to decrease.

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Adrenal Glands in Vitamin E Deficiency: *in vitro* Corticoid Synthesis by Quartered Adrenal Glands of Rats deprived of Vitamin E

Recent investigations in this laboratory have indicated an inhibition of corticosterone synthesis in quartered adrenal glands from vitamin E-deficient rats[1]. It was also found that adrenal homogenates of the deficient rats, when properly diluted, undergo *in vitro* lipid peroxidation and formation of malonaldehyde, as determined by the thiobarbituric acid (TBA) test, more readily than any other organ tested[1,2]. However, when incubated adrenal quarters instead of homogenates were assayed for lipid peroxidation by the TBA method, there was very little net production of malonaldehyde, but significant inhibition in corticoid synthesis occurred in the quartered adrenals of vitamin E-deficient rats. This is in contrast to other enzymatic inhibitions which have been demonstrated in vitamin E-deficient rats and shown to be associated with the process of *in vitro* lipid peroxidation[3,4]. The various investigations and aspects of *in vitro* lipid peroxidation in adrenal homogenates of vitamin E-deficient rats will be reported separately. This communication reports preliminary data obtained on corticoid synthesis by the quartered adrenal glands of vitamin E-deficient and sufficient rats.

Diets, the procedures for the handling of rats, and determination of malonaldehyde are modifications of the previous procedure[3]. The animals were fed the diet *ad libitum* for 11–55 days. Corticoid synthesis was examined in two types of experiments: experiment *A* (Table 1) consisted of incubation of adrenal quarters without previous preincubation; experiment *B* (Table 2) consisted of procedures of Saffran and Schally[5] with preincubation of the tissue in isotonic salt medium[6], followed

Table 1. *In vitro* CORTICOID SYNTHESIS BY QUARTERED ADRENAL GLANDS OF VITAMIN E-DEFICIENT RATS AND THEIR CONTROLS

Incubation medium	Prep. of vit. E-def.		Prep. of vit. E-suff.	
	Corticosterone (μg/10 mg/h)	Malonaldehyde *O.D.* 530 mμ	Corticosterone (μg/10 mg/h)	Malonaldehyde *O.D.* 530 mμ
Krebs–Ringer (13)*	0·529 ± 0·142	0·00	0·579 ± 0·114	0·00
Rat serum (22)*	1·360 ± 0·260	0·00	2·380 ± 0·290	0·01

* Numbers in parentheses indicate total number of animals used in each experiment. ± Standard error of mean.

Adrenal quarters were incubated in 1 ml. of serum or 1 ml. of Krebs–Ringer–bicarbonate–glucose for 1 h at 37° C. At the end of incubation time 0·25 ml. aliquots removed and diluted with ethanol to final concentration of 13 per cent and frozen immediately for later assay of corticosterone. Another 0·25 ml. aliquot was removed for malonaldehyde assay.

Table 2. *In vitro* CORTICOID SYNTHESIS AND MALONALDEHYDE FORMATION BY QUARTERED ADRENAL GLANDS OF VITAMIN E-DEFICIENT RATS AND THEIR CONTROLS

Portion of test system assayed	Prep. of vit. E-def.		Prep. of vit. E-suff.	
	Corticosterone (μg/10 mg/h)	Malonaldehyde *O.D.* 530 mμ	Corticosterone (μg/10 mg/h)	Malonaldehyde *O.D.* 530 mμ
(*a*) Medium at end of pre-incubation period	0·224	0·000	0·783	0·000
(*b*) Medium at end of incubation period	0·237	0·043	0·545	0·063
(*c*) Adrenal glands at end of incubation period	0·437	0·000	0·602	0·016

Adrenal quarters equivalent to two adrenal glands from two rats were pooled and preincubated in 2 ml. of isotonic salt medium in Dubnoff shaker for 1 h. At the end of the period the mixture was removed and aliquots brought to final concentration of 13 per cent ethanol and quickly frozen. Fresh medium consisting of 2 ml. serum of rat in each dietary group was added to the adrenals, incubated for 1 h in Dubnoff shaker at 37° C. The values have been corrected for endogenous and blank values of the medium.

by incubation of the tissues in the serum of the same rat. The incubation media in experiment *A* consisted of either Krebs–Ringer–bicarbonate–glucose solution or rat serum, while in experiment *B* the incubation medium consisted of serum only. The gas phase in all the experiments consisted of 95 per cent oxygen and 5 per cent carbon dioxide, and equilibration was achieved in either Warburg vessels (exp. *A*) or in a small beaker placed in a Dubnoff shaker, fitted with a gassing hood (exp. *B*). The incubation time was 1 h at 37° C.

Since the major steroid secreted by the rat adrenal gland preparation is corticosterone[7], its synthesis was assayed by the fluorometric method of Silber *et al.*[8]. Assays were performed on 0·25-ml. aliquots of the incubation medium.

Further evidence demonstrating that the corticoid synthesized in these experiments was corticosterone was obtained by the following procedure: the incubation medium was extracted with methylene chloride and dried under reduced pressure. The residue was taken up in small amounts of ethylacetate and was subjected to thin-layer chromatography[9,10]. The corticoid formed in exp. *A* (Table 1) was chromatographically identical with corticosterone. There also were other polar steroids in the medium which have not yet been identified.

The data in Table 1 indicate that corticoid synthesis *in vitro* by adrenal gland quarters incubated in serum is about 43 per cent less in the deficient group than in the controls. However, the corticoid synthesis was not significantly different in the two dietary groups when the synthetic medium was used instead of serum. Furthermore, the total corticoid synthesis in the synthetic medium was only 1/4 or 1/3 of the serum-incubated glands in both groups of rats. Results shown in Table 2 also indicate that the difference in corticosterone synthesis is also evident when adrenals are pre-incubated before incubation in the serum.

Lipid peroxidation and malonaldehyde formation between the two dietary groups of rats were not significantly different in any of the experiments with quartered adrenal glands. This is in contrast to the results obtained with the adrenal homogenates, in which case large amounts of malonaldehyde are formed in preparations from vitamin E-deficient rats[1,2]. As shown in Table 2, malonaldehyde does not accumulate in the quartered glands (Table 2*c*), nor is it secreted into the media since none of the media tested showed significant net production of this substance (Table 2*a* and *b*). Unlike two other enzyme systems which are inhibited *in vitro* in vitamin E-deficient rats[3,4], the inhibition of corticosterone synthesis under these experimental conditions may not be related to process of lipid peroxidation as measured by malonaldehyde production, and hence, sufficiency of tocopherol in the rat may be directly involved in corticoid synthesis in these experiments.

In vivo determination of corticosterone by the method of Silber *et al.*[8] in the serum and the adrenal tissues of the vitamin E-deficient rats and their controls demonstrated 0·348 and 0·354 μg of corticosterone per ml. of serum of deficient and sufficient rats respectively while the level of corticosterone in the adrenals in 9 pairs of rats was 0·214 and 0·334 μg per 10 mg of tissue in vitamin E-deficient and sufficient rats respectively. The corticosterone level of the adrenals in the deficient rat is significantly 34 per cent less than the control group, but the serum level shows no difference in the two dietary groups.

The *in vitro* addition of tocopherol to the incubation medium for the quartered glands failed to reverse the inhibition of corticoid synthesis in three instances tried.

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PHYSIOLOGY

New Motor End-plates and their Relationship to Muscle Fibre Injury

IT has been shown by Guth and Zalewski[1], Hofmann *et al.*[2], Koenig[3] and Miledi[4] that new motor end-plates will form in denervated skeletal muscle or muscle devoid of its original motor end-plates in mammals and amphibians following implantation of a motor nerve. However, in all these experiments the muscle suffered considerable damage, either during implantation of the nerve or in transecting the muscle to remove the original motor end-plate zone.

When an accessory nerve is implanted into the transected surface of a muscle in which the innervation and original motor end-plate zone are intact, then new motor end-plates will form on the muscle fibres near the cut margin of the muscle[4]. It appears, therefore, that injury to the muscle fibres is a major factor in the causation of these new motor end-plates. However, it has not yet been shown whether nerve fibres can produce new motor end-plates on muscle fibres that have been denervated but not mechanically injured.

The peroneal nerve in rats was cut and laid on the surface of tibialis anterior muscle at its proximal (end-plate free) zone, having first carefully incised the fascia over the superficial surface of the muscle. The nerve was held in place with a small amount of clotted fibrin.

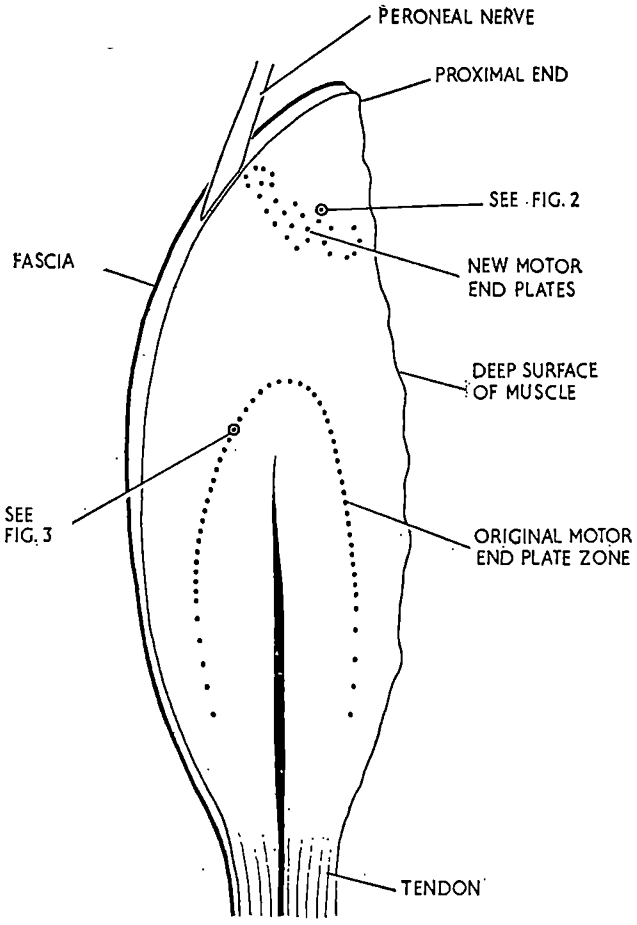

Fig. 1. Diagrammatic radial section of tibialis anterior muscle of rat showing position in which the peroneal nerve is laid on the muscle and also the new and original motor end-plate zones

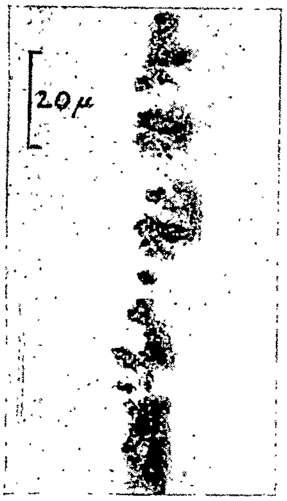

Fig. 2

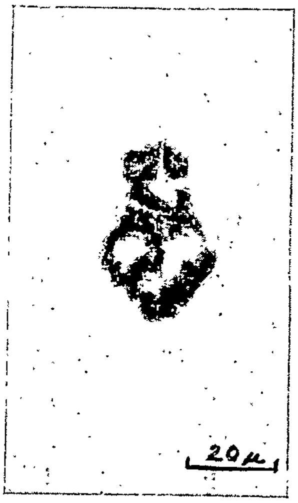

Fig. 3

Fig. 2. New motor end-plate stained by the cholinesterase method 63 days following 're-innervation'

Fig. 3. Original motor end-plate from the same section as Fig. 2 stained by the same method, showing marked degenerative changes

As early as 35 days after 're-innervation' the muscle responded to stimulation of the nerve. Modified Koelle and Friedenwald cholinesterase staining showed deposits of copper sulphide on muscle fibres in the upper region of the muscle, which is normally free of motor end-plates. By counterstaining with silver nitrate, nerves could be followed into these deposits. Silver staining alone showed new motor end-plates in the upper region of the muscle, but the nerves had not re-innervated the end-plates in the original end-plate zone. The new motor end-plates found in the proximal part of the muscle were not restricted to the immediate vicinity of the superficial region of the muscle but had spread to the deeper muscle fibres in the zone (Fig. 1).

Thus while mechanical injury to the muscle fibres may in some cases be a factor in the production of new motor end-plates, it is almost certainly not a necessary factor. Although the most superficial muscle fibres might possibly have been injured in the laying on of the nerve or by some osmotic effect of the fibrin, the more deeply placed ones must have remained intact.

The new motor end-plates are formed on these deeply placed muscle fibres as well as on the more superficial ones.

The main factor in the production of these new motor end-plates in mammalian skeletal muscle would appear to be the availability of denervated muscle fibres and not mechanical injury to the muscle.

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Nicotinic and Muscarinic Receptors of Renshaw Cells

The method of electrophoretic ejection from multi-barrel micropipettes, in conjunction with the recording of electrical responses from single neurones[1], provides a unique opportunity for classifying central acetylcholine receptors according to their pharmacological properties. It has been usual to subdivide these receptors into nicotinic and muscarinic types, a classification based on the original use of these terms by Dale[2] to describe the peripheral actions of certain derivatives of choline. The following evidence has suggested that the receptors of Betz cells are muscarinic[3]: nicotine is a weak excitant; acetyl-β-methylcholine and *dl*-muscarine are almost as active as acetylcholine; the excitation by acetylcholine is slow in onset and is readily blocked by atropine but not by dihydro-β-erythroidine. In contrast the acetylcholine receptors of Renshaw cells exhibit nicotinic properties[4]: nicotine is a more powerful excitant than acetylcholine; acetyl-β-methylcholine and *dl*-muscarine are very weak; the excitation by acetylcholine is rapid in action and is readily blocked by dihydro-β-erythroidine but not by atropine. The acetylcholine sensitivities of ventrobasal thalamic neurones, and of cerebellar neurones, are of intermediate types[5].

Spinal cats anæsthetized with pentobarbital were used in the investigation reported here on the acetylcholine receptors of Renshaw cells in the lumbar spinal cord[4]. Cholinomimetics and blocking agents were administered either electrophoretically from multibarrel glass micropipettes or by intravenous injection. The cells were excited synaptically by stimulation of the central end of the appropriate ventral root. As illustrated in Fig. 1*A*, acetyl-β-methylcholine was much slower in time course as an excitant of these neurones and was less effective than acetylcholine. Concentrations of dihydro-β-erythroidine which very effectively depressed the firing produced by acetylcholine (Fig. 1*B*) did not reduce the action of acetyl-β-methylcholine. With other cells it proved possible to suppress completely the action of nicotine without diminishing the excitant effect of acetyl-β-methylcholine. Simultaneously the characteristic repetitive and high-frequency synaptic response of Renshaw cells, of duration 20–60 msec (ref. 4), was reduced to one or two spikes.

On the other hand, after the intravenous administration of atropine sulphate (0·1 mg/kg) the response evoked by acetyl-β-methylcholine was reduced to a much greater extent than that to acetylcholine (Fig. 1*C*). Doses of atropine sulphate as high as 0·5–2 mg/kg completely blocked the action of acetyl-β-methylcholine, but were found to diminish the effectiveness of acetylcholine only by about 40 per cent. Such doses have little action on the synaptic response, which is dihydro-β-erythroidine sensitive[4], but have been observed to depress a previously described late response which follows this high-frequency firing[6].

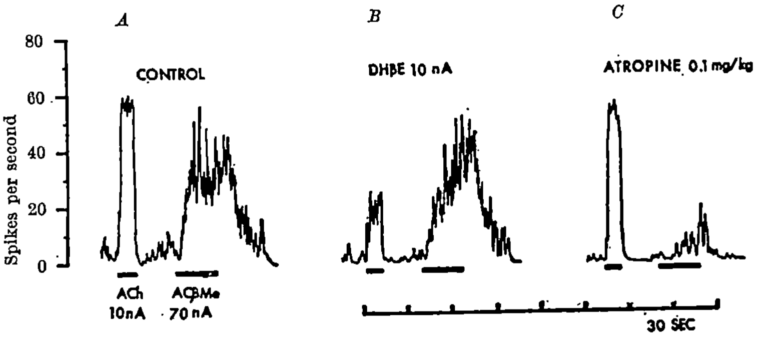

Fig. 1. Frequency of firing of a Renshaw cell, plotted by means of a rate meter and paper recorder (see ref. 5), and produced by electrophoretic ejections of acetylcholine (ACh—10 n.amp) and acetyl-β-methylcholine (ACβMe—70 n.amp), as indicated by the horizontal bars. *A*, control; *B*, during the ejection of dihydro-β-erythroidine (DHβE, 10 n.amp) from a 10 mM solution in 165 mM sodium chloride contained in another barrel of the micropipette. The responses recovered over the next 12 min. *C*, after intravenous injection of atropine sulphate 0·1 mg/kg. Time, 30 sec

The most satisfactory explanation of these results is that each Renshaw cell has two types of acetylcholine-sensitive receptors, both of which are concerned with excitation. The nicotinic receptors are readily blocked by dihydro-β-erythroidine and are presumably located beneath the synaptic terminals of axon collaterals. The muscarinic receptors are atropine sensitive and are apparently associated with a late synaptic response. This duality of acetylcholine receptor sites on a single cell has not been previously demonstrated.

In general the pharmacological properties of Renshaw cells[4] resemble those of neurones within autonomic ganglia, and the present findings lend support to the proposal that certain ganglion cells also have two types of postsynaptic acetylcholine-sensitive receptor[7]. The possibility that the individual cells of other cholinoceptive tissues also have two basic types of receptor may lead to a better understanding of the nature of such receptors, and to a simplification of certain aspects of the mode of action of acetylcholine, cholinomimetics, acetylcholine antagonists and anticholinesterases at and near synapses. Such considerations offer an explanation of the apparently mixed nicotinic-muscarinic properties of the receptors on certain central neurones[5].

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Ionic Changes Responsible for Post-tetanic Hyperpolarization

After repetitive stimulation, the membrane potential of nerve fibres is transiently increased. It has been suggested by Ritchie and Straub[1] that this hyperpolarization could be explained on the basis of two assumptions: that the ionic pump responsible for the coupled efflux of sodium ions and influx of potassium ions[2] becomes hyperactive following stimulation because of the raised intracellular sodium ion concentration[3]; that the extracellular space adjacent to the membrane is confined by a diffusional barrier. Following activity, the hyperactive pump would deplete the potassium in this space and thus raise the membrane potential of the nerve fibre above its resting level. A consequence of this hypothesis is that, in the absence of extracellular potassium, the sodium pump would be unable to lower the potassium concentration further and there would be no post-tetanic hyperpolarization, as Ritchie and Straub[1] indeed found in mammalian *C*-fibres. On the other hand, an increased post-tetanic hyperpolarization in potassium-free solutions has since been reported both in the same fibres[4] and in frog nodes of Ranvier[5].

In the present investigation we have examined post-tetanic hyperpolarization of motor nerve terminals in the isolated rat diaphragm–phrenic nerve preparation under conditions of reduced and increased extracellular potassium concentrations. The results are of particular interest in that we have observed in potassium-free solutions both an increase and decrease in post-tetanic hyperpolarization depending on the exposure time.

The dissection of the preparation, the method of mounting, the composition of the bathing solution and the techniques of location of nerve terminals with a microelectrode have been already described[6]. Neuromuscular transmission was blocked either by raising the magnesium chloride concentration or by raising the magnesium chloride and lowering the calcium chloride concentration of the bathing medium. Individual nerve terminals were located with a microelectrode filled with 4 M sodium chloride with a 1–2μ tip by the recording of extracellular miniature endplate potentials[6]. Short (0·1 msec) cathodal stimulating pulses were then passed through the same electrode from a Grass stimulator. In response to the stimulation, repeated every second, small all-or-nothing antidromic potentials were recorded from the phrenic nerve through a pair of platinum electrodes. The stimulus strength was varied until, over a 60-sec period, 20–80 per cent of the stimuli failed to evoke a response. The phrenic nerve was then stimulated with a tetanus of 1,000 shocks at 100/sec through a proximal pair of platinum electrodes. Following this tetanus, the microelectrode stimulus was continued at the same strength and rate until the number of antidromic responses approximated to the pretetanic level. A rise in nerve threshold was detected by an increase in the number of failures to respond to stimulation and was interpreted as an effect of a raised membrane potential[7], that is, a post-tetanic hyperpolarization.

In Fig. 1*A* a typical control experiment is illustrated. The bathing medium contained 5 mM potassium chloride and before the tetanus 67 per cent of the stimuli failed to evoke an antidromic response (dotted line). After the tetanus (hatched bar) there was no response in the first five successive 10-sec periods (open circles) and the threshold as judged by this technique remained above the control value for some 70 sec. The bathing solution was then changed to one containing no added potassium

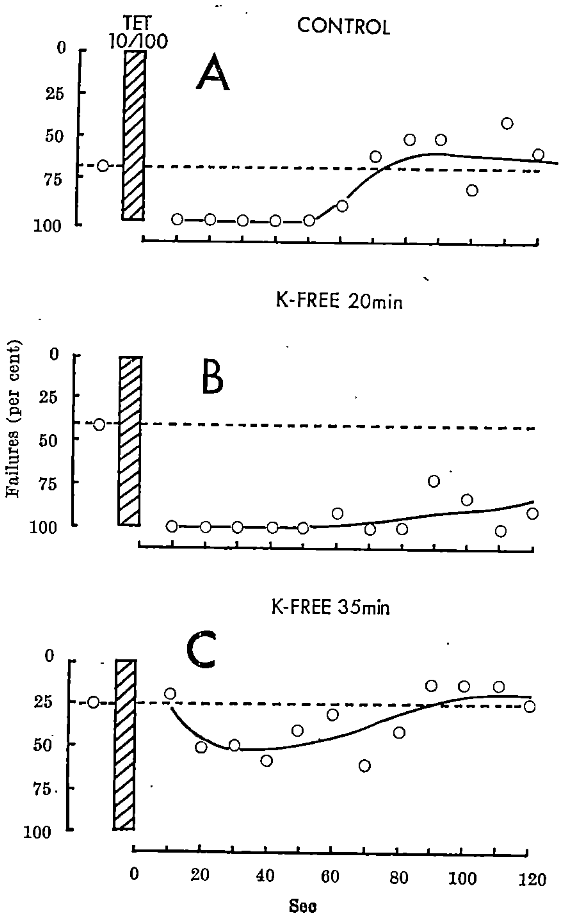

Fig. 1. The threshold of motor nerve terminals in the rat diaphragm *in vitro* after 1,000 orthodromic stimuli at 100/sec. The bathing solution contained 5 mM potassium chloride for the experiment illustrated in *A*, and this fluid was changed to a solution with no added potassium chloride ('K-free') for *B* and *C*. The ordinate indicates the percentage of stimuli at 1/sec which failed to evoke an antidromic response. The percentage was assessed over a 60-sec period before the tetanus, and this control level is shown by the dotted line in *A*, *B* and *C*. After the tetanus (hatched bar) the percentage (open circles) was calculated for successive 10-sec periods. In *A* there was a 70-sec period after the tetanus during which the percentage of failures was greater than the control-level. In *B* after 20 min in the 'K-free' solution this period was more than 120 sec. After 35 min (*C*) there was no change in threshold in the 10-sec period immediately after the tetanus. The bathing solution contained 6·5 mM magnesium chloride and 1 mM calcium chloride. The temperature was 36° ± 1° C

chloride. When the experiment was repeated (Fig. 1*B*) 20 min later, the post-tetanic hyperpolarization inferred from the raised threshold was markedly increased and prolonged, lasting more than 120 sec. After 35 min immersion (Fig. 1*C*) there was a dramatic reduction of the post-tetanic hyperpolarization. Indeed in the 10-sec period after the tetanus the number of responses was above the average for the control period (dotted line). At this stage, by observing extracellular end-plate potentials during an identical nerve tetanus, we established that the nerve impulses had reached the nerve terminals. Full recovery of post-tetanic hyperpolarization was seen when the preparation was again bathed in the control solution containing 5 mM potassium.

In several experiments, the potassium concentration of the bathing solution was raised to 15 mM—three times the control concentration. Invariably the normal hyperpolarization following a tetanus (Fig. 1*A*) was abolished and in fact was often replaced by a phase of depolarization. The abolition was fully reversible on washing with the control solution.

The early increase of post-tetanic hyperpolarization in potassium-free solutions (Fig. 1*B*) agrees with the results of Holmes[4] and Meves[5]. The later decrease in hyperpolarization is similar to the decrease reported by Ritchie and Straub[1]. These varied findings can be explained by the hypothesis put forward by Coombs, Eccles and Fatt[8] to explain after-hyperpolarization in motoneurones; namely, that in the nerve fibres an increased potassium conductance continues after the train of impulses so that the membrane potential approaches the potassium equilibrium potential. Because the contribution to the resting potential of other ions, such as sodium and chloride, cannot be ignored when the extracellular potassium concentration is near or less than the physiological concentration, the resting membrane potential is normally less than the potassium equilibrium potential. Following activity in the nerve we assume a prolonged increase in the permeability of the membrane to potassium ions so that the membrane potential moved closer to the potassium equilibrium potential, so causing a hyperpolarization (Fig. 1*A*). When the extracellular potassium concentration was greatly reduced in our experiments by omitting potassium chloride from the bathing solution, the potassium equilibrium potential would have increased with little increase in the resting membrane potential. Following a tetanus an increase in potassium conductance would then move the membrane potential towards this elevated potassium equilibrium potential, causing more hyperpolarization (Fig. 1*B*). The later reduction in hyperpolarization in the potassium-free solution (Fig. 1*C*) can be explained by the recognized action of a potassium-free solution in decreasing the intracellular potassium concentration[9]. The potassium equilibrium potential would, of course, fall commensurately with this fall in intracellular potassium concentration. Similarly, in solutions containing 15 mM potassium chloride the potassium equilibrium potential and membrane potential would be virtually the same. This would explain the reduction of post-tetanic hyperpolarization in these circumstances.

It is perhaps not unexpected that the same mechanism should be postulated to explain post-activation hyperpolarization in the somas[8], Ranvier nodes[5] and nerve terminals of motoneurones.

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Potentials evoked in the Hypothalamus and Cerebral Cortex by Electrical Stimulation of the Uterus

After the 36th week of pregnancy, labour is readily initiated by such procedures as dilating the cervix, passing uterine bougies and, most effectively, by amniotomy. This ability to initiate labour by mechanical interference suggests the existence of nervous pathways from uterus to hypothalamus. The presence of appropriate receptors in the rabbit uterus has been demonstrated by Ferguson[1] and by Bower[2], but little is known of the central pathways involved. In the experiments recorded here on cats anæsthetized with chloralose we used the evoked potential technique to detect uterine afferent relays to the hypothalamus. Pairs of stainless steel stimulating electrodes were implanted into the myometrium, one pair high in one uterine horn and another pair below the level of fusion of the two horns, but above the vagina. The uterus was then subjected to direct electrical stimulation

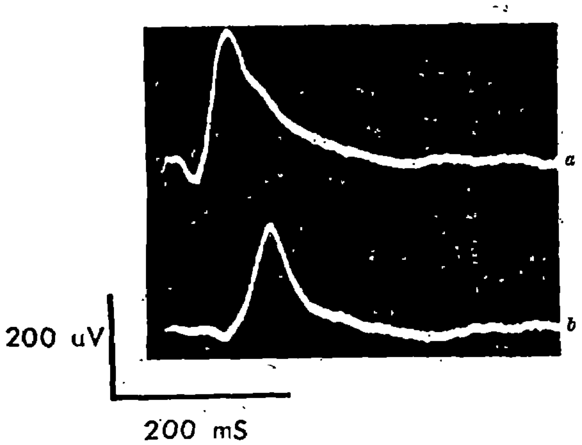

Fig. 1. Responses obtained at the same hypothalamic recording site in response to (*a*) electrical stimulation of the skin of one forepaw and (*b*) electrical stimulation of the myometrium of one uterine horn. Note that uterine stimulation is followed by an evoked potential of small amplitude and long latency

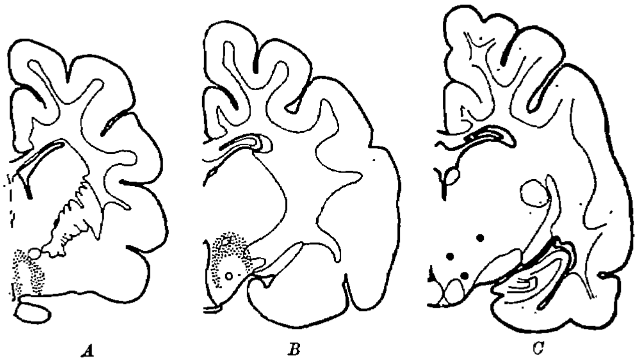

Fig. 2. Diagrammatic coronal sections of the cat brain to show the distribution of potentials evoked in the hypothalamus by electrical stimulation of the uterus. The stippled regions in sections *A* and *B* are those from which responses could consistently be elicited. The three large dots in section *C* indicate where responses were obtained on one occasion

through either pair of electrodes by square wave pulses of 1–3 msec duration.

Evoked potentials recorded from the hypothalamus of cats anæsthetized with chloralose show irregularities[3] due largely to an interaction with background activity[4]. The background activity at the recording site was therefore monitored and stimulation was deferred until a period of 80 msec had elapsed with no such activity in excess of 15 μV negative or positive[5]. The minimum interval between stimuli was 3 sec. The hypothalamus was explored millimetre by millimetre. At each recording site the skin of one forepaw was first stimulated, and stimuli were then applied to each pair of uterine electrodes in turn. Whereas cutaneous stimulation evoked potentials in all hypothalamic regions[3] potentials evoked by uterine stimulation were largely limited to two hypothalamic regions. Fig. 1 compares the potentials evoked by cutaneous and uterine stimulation at the same recording site. In Fig. 2 the distribution of responses to uterine stimulation from 13 experiments is plotted on to three diagrammatic sections of the hypothalamus. It can be seen that the responses are virtually confined to the pre-optic and tuberal regions. The distribution of evoked potentials in any one experiment is more restricted than the composite diagram suggests. In two experiments the electrode passed through the supra-optic nucleus. The results of one of these experiments are shown in Fig. 3 in which evoked potentials resulting from uterine stimulation were confined to the supra-optic nucleus and the region immediately surrounding it. The reason that the area from which evoked potentials are recorded appears larger in the composite diagram than in a single experiment may be attributed to variation in the location and size of the supra-optic nucleus from animal to animal, and the possibility that additional nuclei are activated.

Potentials evoked in the hypothalamus by uterine stimulation appeared less regularly than those elicited by cutaneous stimulation, and with a longer latency. Responses evoked by uterine stimulation had a latency of 30–50 msec, whereas responses to cutaneous stimulation had a latency of 15–17·5 msec. In some experiments responses were obtained from stimulation of the electrodes high in the uterine horn, but not from those in the fused portion of the uterus; in others the opposite was true. In all experiments responses were most consistent when using stimulating pulses of long duration, that is, 3 msec. In a single experiment the cerebral cortex in the region of the sigmoid gyrus was explored with a platinum ball recording electrode, and uterine stimulation was found readily to evoke potentials in the somato-sensory region. Presumably such potentials arise from the activation of receptors or their nerve fibres in the myometrium[2].

Our experiments thus demonstrate that neural pathways exist from the uterus to the hypothalamus and apparently also to the cerebral cortex, but doubt must obtain as to the specificity of these relays. Barraclough and Cross[6] found that units in the hypothalamus of the rat responding to pressure on the os cervicis also responded to chilling or pinching the tail. Their recordings were, however, confined to a region of the lateral hypothalamus from which, in the cat, we did not usually observe a response to uterine stimulation. It is also known[7] that many forms of visceral stimulation can evoke potentials in the cerebral cortex.

It is increasingly recognized[8–11] that the onset of labour in the human being is due to an increase in the sensitivity

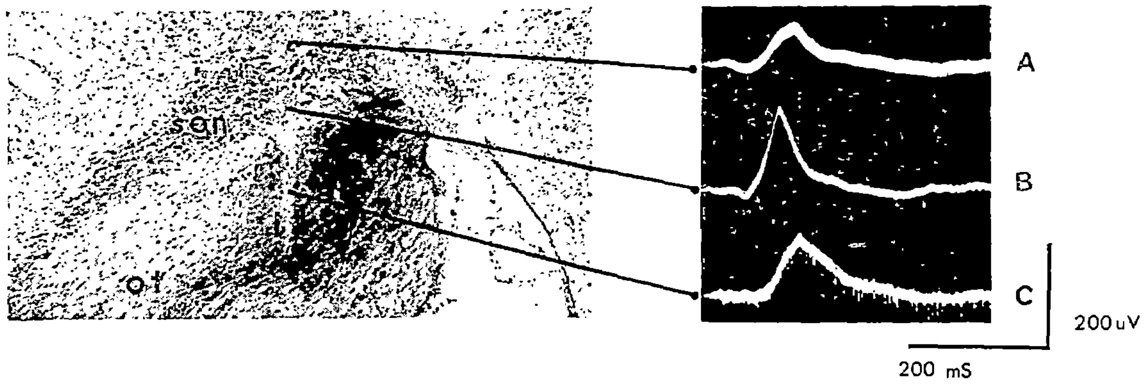

Fig. 3. Localization of potentials evoked by uterine stimulation in the supra-optic nucleus. The photomicrograph shows an electrode track passing through the supra-optic nucleus into the optic tract. The evoked potential illustrated in *A* was recorded just dorsal to the supra-optic nucleus, that in *B* in the ventral portion of the supra-optic nucleus, and that in *C* in the optic tract. *son*, supra-optic nucleus ; *ot*, optic tract

of the myometrium to oxytocin rather than to an increase in the amounts of oxytocin released into the blood. Since the hypothalamus is functionally connected with both neurohypophysis and adenohypophysis it is tempting to speculate that the relay we have demonstrated between uterus and hypothalamus may be concerned not only with the release of oxytocin but also with effecting increased myometrial sensitivity to it, factors which together underly the mechanism of normal parturition.

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PHARMACOLOGY

Isolation and Identification of the 20β-Hydroxy Derivatives of 6β-Hydroxycortisol and 6β-Hydroxycortisone in Liquor Amnii

The presence of C-6 oxygenated steroids in neonatal urine was first reported by Ulstrom *et al.*[1]. It has been demonstrated[2,3] that placental tissue incubated under standard conditions, is able to oxygenate at the C-6 position, while the work of Frantz *et al.*[4] has indicated that during pregnancy the urinary concentrations of the polar steroid 6β-hydroxycortisol is elevated above the normal non-pregnant state. It appeared likely, therefore, that similar polar steroids might be present in liquor amnii.

In a previous communication[5], the isolation and identification of 6β,11β,17α,21-tetrahydroxy-pregn-4-ene-3-20-dione (6β-hydroxycortisol) in liquor amnii were reported. At this time the presence of three additional polar steroids was recognized. Two of these compounds have now been identified as 6β,17α,20β,21-tetrahydroxy-pregn-4-ene-3,11-dione (compound 3) and 6β,11β,17α,20β,21-pentahydroxy-pregn-4-ene-3-one (compound 4).

Liquor amnii, obtained at the time of labour, was extracted with ethyl acetate, using the methods previously described[5]. The purified extract was chromatographed in a modified *BuC* system[6] and the chromatogram treated with a 2 : 1 solution of 10 per cent aqueous sodium hydroxide in 50 per cent methanol and 0·02 per cent blue tetrazolium. It was dried in an oven at 60° C for 10 min. When the chromatogram was viewed under ultra-violet light, using a minus blue 4 gelatine filter (Ilford), three brilliant fluorescent zones were observed. The least polar of these has been previously identified as 6β-hydroxycortisol (compound 1) (ref. 5). Quantities of the other two zones (compounds 3 and 4) were collected from subsequent chromatograms, and were carried through a series of chemical and enzymatic steps. Identification of these 20β-hydroxy derivatives of 6β-hydroxycortisol and 6β-hydroxycortisone was based on the properties as listed in Table 1. The products from each procedure were compared chromatographically with reference steroids. In each case an agreement of R_F values was found.

Table 1. Properties of the 20β-Hydroxy Derivatives of 6β-Hydroxycortisol and 6β-Hydroxycortisone

	Compound 20β-6β-OHF		Compound 20β-6β-OHE	
	Isolated	Ref.	Isolated	Ref.
1 Ultra-violet absorption in ethanol	238 mμ	238 mμ	238 mμ	238 mμ
2 Reaction with blue tetrazolium	Neg.	Neg.	Neg.	Neg.
3 Mobility in chromatographic system Mod. *BuC* (ref. 6)	0·01	0·01	0·03	0·03
4 Mobility after the oxidation of the product from Reaction 3 with sodium bismuthate				
B/50 syst. (ref. 9)	0·24	0·24	0·32	0·32
*LB*21/80 (ref. 9)	0·01	0·01	0·03	0·03
5 Mobility after acetylation of the product from Reaction 4 *LT*/2185 (ref. 9)	0·15	0·15	0·31	0·31
6 Mobility after further oxidation of the product from Reaction 5 using chromic acid *LT*/2185 (ref. 9)	0·31	0·31	0·31	0·31
7 Mobility after reduction of the product from Reaction 4 using *Zn*/*HAc* (ref. 9) *LT*/2185 (ref. 9)	0·24	0·24	0·38	0·38

The configuration of the side-chain was elucidated using the enzyme 20β-hydroxysteroid dehydrogenase[7], and by an examination of the chromatographic properties of the steroids. The reference steroids for this comparison were obtained by treating 6β-hydroxycortisol and 6β-hydroxycortisone with sodium borohydride at 0° C (ref. 8).

This work was carried out during the tenure of a research grant to one of us (M. L.) from the Medical Research Council of Ireland.

20β,6β-hydroxycortisol was kindly supplied by Prof. W. Klyne and Dr. C. B. Thornton from the Steroid Reference Collection, Westfield College, London.

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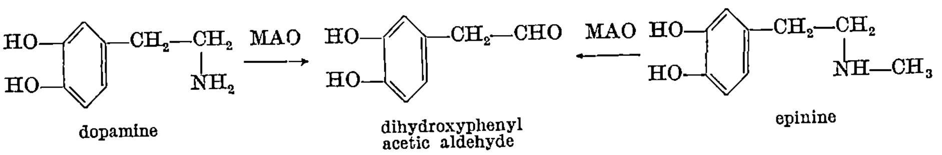
Formation of Tetrahydropapaveroline from Dopamine *in vitro*

In 1938, Holtz *et al.*[1] reported that dopamine, which is pressor in the cat, was converted to a depressor substance after incubation with monoamine oxidase (MAO), for example, extracts of guinea pig kidneys. These authors suggested that the depressor agent might be dihydroxyphenyl acetic aldehyde, the primary deamination product of dopamine, since the addition of semicarbazide prevented the formation of the depressor substance.

To ascertain this assumption, experiments were now performed with epinine, which should also be deaminated by MAO to yield the same aldehyde:



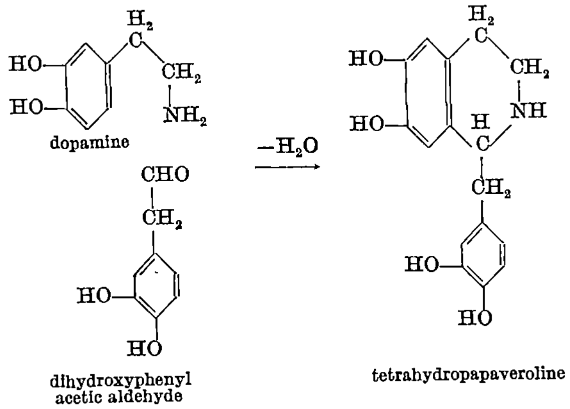
dopamine

dihydroxyphenyl acetic aldehyde

epinine

It was found, however, that epinine was not converted to a depressor agent on incubation with MAO (rat liver mitochondria) but only inactivated[2]. Thus, the blood-pressure-lowering substance formed from dopamine was unlikely to be identical with the dihydroxyphenyl acetic aldehyde.

From previous experiments it was known to us that primary, but not secondary, catecholamines *in vitro* readily condense with aldehydes, for example, pyridoxal 5′-phosphate, to yield Schiff's bases with subsequent formation of tetrahydroisoquinolines[3]. Therefore, we postulated that the dihydroxyphenyl acetic aldehyde formed from dopamine by MAO would similarly react with unchanged dopamine molecules, to form the corresponding tetrahydroisoquinoline derivative: tetrahydropapaveroline (THP), a compound synthesized as early as 1909 by Pyman[4] and reported by Laidlaw[5] to be a blood-pressure-lowering agent:



dopamine

dihydroxyphenyl acetic aldehyde

tetrahydropapaveroline

That THP was indeed formed from dopamine during incubation with MAO is shown in Fig. 1. Incubates deproteinized with perchloric acid were extracted into *n*-butanol at *p*H 4·0, returned to 0·1 N hydrochloric acid and subjected to thin-layer chromatography according to Stahl[6], using *n*-butanol/acetic acid/water (8 : 2 : 2) or *sec.* butanol/formic acid/water (15 : 3 : 2) as solvent systems. This method provided clear separation of dopamine R_F = 0·38 (bottom row) and THP R_F = 0·47 (top row). The chromatograph further demonstrates that in the incubates (*B*, *C* and *D*) THP was formed, and that its amount increased with growing incubation time, 2·5, 5 and 10 min, while that of dopamine decreased. In the incubate *A*, however, the addition of the MAO inhibitor 'Pargyline' (benzyl-benzylmethyl-propynylamine) had prevented the formation of THP.

The deproteinized incubates *A* and *C* were tested on the blood pressure of a cat by intravenous injection. As can be seen from Fig. 2, the incubate *A* with blocked MAO had a pressor effect due to the unchanged dopamine, whereas 5-min incubation of dopamine with the intact enzyme was sufficient to yield a depressor agent, the blood-pressure lowering action of which resembled that of authentic THP (and of isoproterenol) in that it was abandoned after infusion of nethalide (isopropylamino-1-(2-naphthyl)-ethanol hydrochloride), a sympathetic β-receptor blocking agent[7] (Fig. 2*C*). Thus, it was evident that THP and incubate *C*—in contrast with 'musculotropic' papaverine (Fig. 2, *P*)—exerted their depressor actions through the stimulation of sympathetic β-receptors.

It should be pointed out that the—atropine resistant—depressor action of dopamine *per se* in the rabbit[8] was 'papaverine-like', in so far as it remained unaffected by nethalide[2]. Since dopamine in the rabbit was also depressor after the injection of dibenzyline, which abandoned completely the pressor action of noradrenaline[2], it became unlikely that dopamine should lower the rabbit's blood pressure by replacement of vascular noradrenaline, as suggested by Burn and Rand[10].

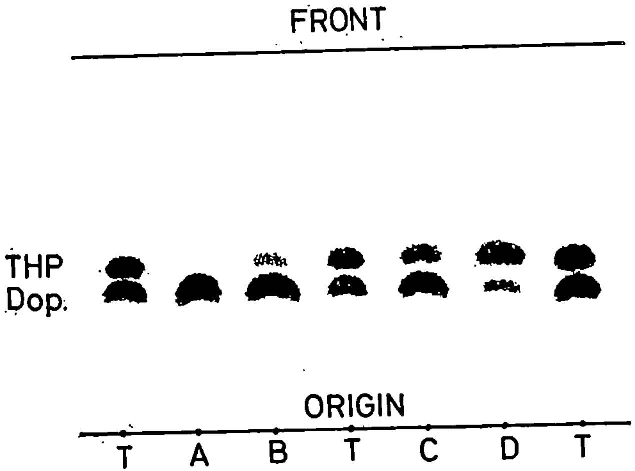


Fig. 1. Formation of tetrahydropapaveroline during incubation of dopamine with monoamine oxidase. Thin-layer chromatography according to the method of Stahl (ref. 6), using silica gel *G* (Merck) suspended in sodium phosphate buffer *p*H 6·8, addition of 1 per cent $NaHSO_3$. Solvent system: *n*-butanol/acetic acid/water (8 : 2 : 2). Spray: Goldenberg's reagent (ref. 9). *T*, mixture of authentic dopamine (Dop) and tetrahydropapaveroline (THP); *A*, *B*, *C*, *D*, incubates of Dop with MAO (rat liver mitochondria). Addition of neutralized ascorbic acid in *A*–*D* to protect the phenolic OH-groups against oxidation, of pargyline in *A* prior to incubation, to inactivate MAO; incubation time 2·5 min (*B*); 5 min (*C*); 10 min (*D* and *A*). 37° C; *p*H 7·4; O_2

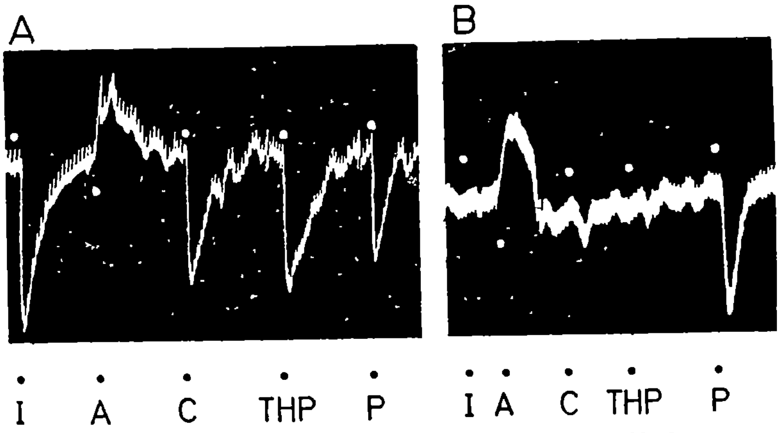


Fig. 2. Action of the incubates *A* and *C* (see Fig. 1) on the cat's blood pressure. Cat (3 kg): chloralose–urethane anæsthesia; intravenous injections of: *I*, 1 μg isoproterenol; *THP*, 10 μg tetrahydropapaveroline; *P*, 1 mg papaverine. *A*, before, *B*, after intravenous infusion of 3 mg/kg nethalide

A paper entitled "Pharmakologie des Tetrahydropapaverolins und seine Entstehung aus Dopamin" will appear in *Arch. exp. Path. Pharmak.*, 248, 387 (1964).

Tetrahydropapaveroline was a gift from the Wellcome Laboratories, London.

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Function of *N*-Acetyl Aspartic Acid in the Brain : Effects of Certain Drugs

N-Acetyl-L-aspartic acid (NAA) was discovered by Tallan *et al.* in 1956 and shown to be unique in its restricted localization to the brain of vertebrate species and in its very high concentration in that organ[1]. Occurring at a level of approximately 1 mg/g of brain, NAA is second only to glutamic acid in the level of free amino-acids in this tissue. Numerous attempts, using a variety of drugs and experimental procedures, to change the concentration of NAA in the brain have met with little success. Thus, electric shock, insulin shock, ammonia intoxication, and the administration to animals of tranquillizers, central nervous system depressants or convulsants, all failed to alter significantly the concentration of NAA in the brain[2-4].

It was reported recently that several drugs, classified as monoamine oxidase (MAO) inhibitors, when administered to rats appeared to produce a rise in the concentration of NAA in the brain[5]. As part of a larger study in this laboratory designed to disclose the function of this unusual amino-acid, it was decided to repeat and extend this observation.

In our investigations, *Ha/ICR* Swiss mice were used. Drugs were administered intraperitoneally as six daily injections, except for hadacidin (*N*-formyl hydroxyamino-acetic acid), which was given as a single dose, and 5-hydroxytryphophan (5HTP) and dihydroxyphenylalanine (DOPA), which were given every 3 h for a total of four injections. The animals were killed by cervical dislocation 4 h after the last injection of drug; the brain tissue was removed and deproteinized. The NAA content of the brain extract was determined essentially by the method of Fleming[6]. This procedure utilizes a kidney deacylase preparation to convert NAA to acetate and aspartate, oxalacetic transaminase to convert aspartate to oxaloacetate, and malic dehydrogenase to convert oxaloacetate to malate. Since the last reaction utilizes NADH, the amount of NAA in the sample can be calculated from the spectrophotometrically determined extent of oxidation of NADH to NAD.

Table 1. Effect of Drugs on the Content of *N*-acetylaspartic Acid (NAA) in the Brain of the Mouse

Drug	Dose (mg/kg)	NAA content of brain μg/g (mean value ± S.D.)	Probability (calculated from *t*-test)
Saline (5)*	—	974 ± 41	—
Iproniazid (9)	100	1,415 ± 245	0·0125
*JB*516 ('Catron') (5)	12	1,120 ± 77	0·025
Tranylcypramine ('Parnate') (10)	15	1,013 ± 82	Not significant
Isoniazid (5)	110	1,339 ± 147	0·005
Imipramine (10)	25	1,165 ± 138	0·005
L-5HTP (10)	50	1,205 ± 79	0·005
L-DOPA (5)	50	980 ± 106	Not significant
Reserpine (10)	0·75	825 ± 114	0·05
LDS-25 (5)	0·25	776 ± 97	0·015
Hadacidin† (5)	100	796 ± 67	0·005

* Number in brackets refers to number of animals.

The following drugs had no effect: pentobarbital, prochlorperazine ('Compazine'), amphetamine, atropine, 4-hydroxybutyric acid, γ-butyrolactone, β-methyl aspartate and NAA.

† The hadacidin was kindly supplied by Dr. H. Shigeura, of Merck, Sharp and Dohme.

The results of these experiments (Table 1) suggest a relationship between NAA and 5-hydroxytryptamine (5HT), since: (*a*) many agents that produce a rise in the level of 5HT in the brain also produce an increased concentration of NAA: (*b*) although some of the agents are monoamine oxidase inhibitors and cause a rise in catecholamines as well as 5HT in the brain, the administration of DOPA, in contrast to 5HTP, had no effect on the level of NAA; (*c*) both LSD-25 (an antagonist to 5HT in peripheral systems) and reserpine (which lowers the brain-level of 5HT) produce a fall in the amount of NAA in the brain. In addition, it should be noted that no correlation exists between brain-levels of NAA and inhibition of monoamine oxidase, since isoniazid caused a rise in the amino-acid concentration but is not an MAO inhibitor, while tranylcypramine ('Parnate') is an inhibitor of MAO and yet does not significantly change the level of NAA in the brain.

The inability of administered NAA to affect the endogenous level of NAA in the brain suggests that the amino-acid cannot pass the blood-brain barrier; this conclusion also is supported by a subsequent experiment in which it was found that the intraperitoneal injection of isotopically labelled NAA did not cause an appearance of labelled NAA in the brain.

Experiments are in progress in an attempt to understand the suggested relationship between 5HT and NAA.

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HÆMATOLOGY

Inherited Variants in Serum Ceruloplasmins of the Pig

By means of chromatography and electrophoresis on starch gel, Morell and Scheinberg[1] showed at least four different molecular components of ceruloplasmin in preparations from the plasma of 9,109 human donors. Then McAlister *et al.*[2] presented evidence for multiple ceruloplasmin components in human sera. Using vertical starch-gel electrophoresis to resolve the ceruloplasmins combined with direct chemical and enzymatic analysis on the gel, they defined five distinct proteins having one or more characteristic properties of ceruloplasmin. The enzymatic analysis used by these workers was based on the work by Holmberg and Laurell[3], who showed that ceruloplasmin could oxidize a number of substances, the best substrate being *p*-phenylenediamine (PPD). Using the PPD oxidase activity as a measure of the ceruloplasmin in sera of different species, it was shown by McCosker[4] that pig sera has more than twice the activity of human sera and four times that of other domestic species.

By combining horizontal starch-gel electrophoresis in a discontinuous system and oxidation of PPD, three inherited ceruloplasmin types have been found in sera from one out of three breeds of pig investigated.

The starch-gel technique of Smithies[5], as modified by Poulik[6] using *tris*-citrate buffer adjusted to *p*H 7·6 for the gel, and sodium hydroxide–boric acid buffer for the tanks as modified by Lush[7] was used to separate the serum proteins. Electrophoresis was carried out in a refrigerator at +5° C, and a voltage gradient of 6 V/cm was applied for 30 min, then adjusted to 10–12 V/cm for the remainder of the run until the brown line was 11 cm from the insert line.

The top half of each gel was stained with 1 per cent nigrosin in a methanol–acetic acid–water solution, and the bottom layer stained according to the method described by Morell and Scheinberg[1] with modifications. In their method, paper strips saturated in a solution of 1·0 M sodium acetate buffer (*p*H 5·1) containing 0·5 per cent *p*-phenylenediamine dihydrochloride were pressed on the cut surface of the gel for 1 min, and then removed. The gel was then placed in a closed, moist chamber and allowed to stand for 1–3 h at room temperature until dark purple bands developed.

In the modified technique for pig sera, a similar solution of acetate buffer (*p*H 5) and PPD was used. Instead of using paper strips, however, it was found, like Uriel[8], that total immersion of the cut gel in the buffer at 37° C gave better results. The optimum time for the ceruloplasmin variants to be clearly distinguishable was approximately 45–60 min after incubation. At this stage the gel was photographed and the different types classified. After incubation for 1 h the oxidase activity of the ceruloplasmin on the PPD resulted in a diffuse purple staining reaction, and began to obliterate any bands which were previously discernible.

The three ceruloplasmin types obtained are illustrated in Fig. 1. The schematic drawing shows the interpretation of these types. It is presumed on the basis of segregation studies shown in Table 1 that the homozygotes appear as three bands with the middle band showing the darker staining reaction. The two homozygous types are recognized and classified as types *a* and *b* with type *a* being the faster-migrating protein. A combination of these two types is shown in the heterozygote type *ab*, which has five bands.

Table 1. SEGREGATION OF CERULOPLASMIN TYPES AMONG OFFSPRING OF DIFFERENT MATINGS

Mating type ♂ × ♀	No. of matings	Segregation of offspring *a*	*ab*	*b*	Total
ab × *ab*	1	2	3	0	5
ab × *b*	2		6	1	7
b × *ab*	1		3	3	6
b × *b*	43			223	223

Table 2. FREQUENCY OF Cp-a AND Cp-b ALLELES IN THREE BREEDS OF PIG

Breed	No. of animals tested	Frequency of Cp alleles *a*	*b*
Large white	113	0	1·0
Landrace	83	0·05	0·95
Wessex	28	0	1·0

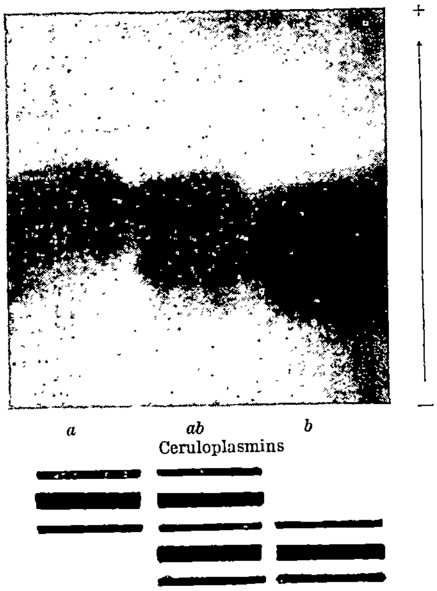

Fig. 1. Starch-gel electrophoresis of pig sera showing the three ceruloplasmin types. A diagrammatic interpretation of these types is also shown

In relation to the other serum proteins of pig blood, the ceruloplasmins are in the same zone as the transferrins at a gel buffer *p*H of 7·6. At this *p*H they appear as three bands in the homozygote form as described above. With a gel buffer *p*H of 8·2, however, they appear as two bands and are in front of the transferrin zone. When staining with benzidine stain[9] for haptoglobins and hæm-binding globulins the oxidizing ability of ceruloplasmins may interfere with the interpretation of the former. At a gel buffer *p*H of 8·2, the ceruloplasmins appear as faint lines between type 3 hæm-binding globulin and the saturated haptoglobin[10].

It is proposed that the three types described above are controlled by two allelic genes at a single locus. The sera from the offspring of 47 different matings of tested parents were examined and the results are shown in Table 1. All mating types were not available, because of the low frequency of type *a* in the parent population. Despite the small numbers for this type, the segregation results appear to be in agreement with the genetic hypothesis of two alleles at a single locus. It is proposed that these alleles be referred to as Cp^a and Cp^b, and the three phenotypes are designated Cp-a, Cp-ab and Cp-b.

The gene frequencies of the Cp^a and Cp^b alleles were calculated from random samples obtained in three pedigree breeds of pigs. These frequencies are shown in Table 2. The type *a* ceruloplasmin was not found in either the Large White or Wessex pigs examined. So far it has only been found in the Landrace breed, and appears to be of relatively low frequency.

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IMMUNOLOGY

Human Pancreas-specific Isoantigens

Pancreas-specific isoantigens have been detected in rabbits[1] and rhesus monkeys[2] following experimental isoimmunization. It has also been reported that isoantibodies and autoantibodies occur in the sera of patients with various types of pancreatic disease[3-5]. Attempts were made in this laboratory to confirm the findings in man using the same techniques of agar-precipitation and indirect hæmagglutination. Difficulty was experienced since normal serum or globulin often gave precipitin lines in agar. Any specific effect from the interaction of serum from patients with pancreatic disease and human pancreas extracts could therefore not be distinguished from non-specific precipitation.

Similar difficulty in the interpretation of agar-precipitation reactions of normal serum or globulin and homologous pancreas extracts has also been reported in pigs[6]. In this instance the serum factor responsible appeared to be a heat stable α-globulin. The substances in porcine pancreas responsible for the reaction were found in all supernates and sediments from differential centrifugation of pancreas homogenates and were inactivated at 56° C for 1 h. Many other workers have reported precipitation reactions in agar which were not due to antigen–antibody systems[7-12]. These usually involved the interaction of normal serum components with various tissue homogenates and in one case a reaction between normal serum and a bovine pancreatic enzyme[12].

Unfortunately, other serological techniques such as complement-fixation and tanned-cell hæmagglutination have given non-reproducible results in the testing of human sera and human pancreas extracts or homogenates. In an attempt to avoid the difficulties of non-specific effects and of non-reproducibility that have plagued the weak reactions of isoimmune sera, the heteroimmunization of rhesus monkeys with individual human pancreas homogenates was considered.

Human pancreas and other organs from individuals of either blood group O or B were obtained at autopsy within 5 h *post mortem* and stored at −50° C until used. Extracts (25 per cent w/v) in isotonic saline were made by homogenization in a high-speed blade-type homogenizer (Servall Omnimixer). The homogenates were then centrifuged at 60,000*g* and the supernates used for testing. The whole homogenate was used as the immunizing antigen.

Young rhesus monkeys 5–10 lb. were injected intradermally and subcutaneously with 1·5 ml. pancreas extract incorporated in 'Difco' Freund complete adjuvant. The animals were trial bled after 2 weeks, and if antibody titres were satisfactory the animals were completely bled. Other monkeys were given a booster immunization with adjuvants and killed 10–14 days later.

The complement-fixation test proved to be the serological test of choice since agar precipitation reactions were also noted with normal monkey serum and human pancreas extract. Antigen titrations were performed by mixing serial dilutions of human pancreas extracts with 1 : 10 diluted monkey serum inactivated at 65° C for 10 min and 1 : 18 diluted guinea pig complement (Hyland Laboratories). Inactivation at 65° C rather than 56° C was used to differentiate immune reactions from the natural complement-fixing antibodies of the Kidd–Friedewald type[13]. The test was incubated overnight at 4° C and then sheep cells sensitized with 2·5 hæmolytic units of amboceptor were added. Antigen titres were expressed as the reciprocal of the highest dilution of antigen giving complete fixation of complement with the 1 : 10 diluted antiserum. Antiserum titres were expressed as the reciprocal of the highest dilution of antiserum giving complete fixation of complement with serial dilutions of antigen.

The sera from monkeys immunized with individual human pancreas homogenates were tested by complement-fixation with an extract of their respective immunizing antigen. Most of the animals immunized had antiserum titres of 20–80 after the primary immunization or after one booster immunization. Antigen titres were usually 40–160. The antisera which reacted with the individual pancreas extract used for their production were then tested with other organ extracts from the same individual in order to establish their serological specificity. Some of the monkeys produced antibodies which reacted with extracts of other tissues from the same individual. These sera were not investigated further. Many of the monkeys produced antibodies that reacted only with pancreas and not with other human tissues such as kidney, liver, spleen, lung, heart, salivary gland, submaxillary gland, parotid gland, gastric mucosa, and intestinal tract mucosa. Absorption with human A, B or O erythrocytes did not inhibit the complement-fixation reaction with pancreas extract. Normal monkey serum and pre-immunization bleedings of the immunized animals, inactivated at 65° C for 10 min, did not react with any of the human tissue extracts tested.

The antisera did not react with any rhesus monkey pancreas extract but some did react with the single extract of chimpanzee pancreas tested. Extracts of other primate pancreases were not tested.

Those immune sera which contained pancreas-specific antibodies were then tested with various individual pancreas extracts by the complement-fixation technique. The reactions of six antisera with ten pancreas extracts are shown in Table 1. The antisera reacted with the particular pancreas extract used for immunization and with some, but not all, of the other individual pancreas extracts. Several different reaction patterns were observed as depicted in Table 1.

Table 1. Complement-fixation Reactions of Rhesus Monkey Anti-human Pancreas Sera with Individual Human Pancreas Extracts

Individual human pancreas extracts	Monkey anti-human pancreas sera						
	M No. 23 (anti-1)	*M* No. 24 (anti-2)	*M* No. 27 (anti-3)	*M* No. 30 (anti-4)	*M* No. 33 (anti-5)	*M* No. 34 (anti-6)	Normal monkey serum
1	+	0	0	0	+	+	0
2	0	+	0	0	0	+	0
3	0	0	+	0	+	+	0
4	0	0	0	+	0	+	0
5	0	0	+	0	+	+	0
6	+	0	+	0	0	+	0
7	+	0	+	0	+	+	0
8	0	+	0	+	0	+	0
9	+	0	+	+	0	+	0
10	+	0	0	0	0	0	0

+, *C.F.* antigen titre >20.

0, *C.F.* antigen titre <10.

Antigen titres were expressed as reciprocal of highest dilution of pancreas extract giving complete fixation of complement with 1 : 10 antiserum.

It was not possible to determine the number of antigenic systems by absorption with individual extracts and re-testing. Sera absorbed with pancreas extracts in a concentration necessary for complete inhibition of antibody activity became strongly anticomplementary. This anticomplementary effect could not be overcome by high-speed centrifugation or addition of enzyme inhibitors to the extracts.

Since the rabbit and monkey pancreas isoantigens had been shown to be present in pancreatic secretions[2], human pancreatic secretions were also examined. Specimens aspirated from duodenal tubes before and after patient stimulation with secretin were tested by complement-fixation for the presence of pancreas isoantigens (Table 2). Antigens which reacted with some, but not all, of the monkey antisera were present in the secretions. Specimens from different individuals reacted with various antisera in a manner similar to individual pancreas extracts. There was no predictable increase or decrease in antigen

Table 2. Complement-fixation Reactions of Rhesus Monkey Anti-human Pancreas Sera with Fluids containing Pancreatic Secretions

Secretions aspirated from duodenal tube	Monkey anti-human pancreas sera *M* No. 23	*M* No. 24	*M* No. 27	*M* No. 30	*M* No. 33	*M* No. 34	Normal monkey serum
Patient No. 1 Pre-secretin stimulation	810	—	—	810	—	810	—
Patient No. 1 10 min post-secretin	270	—	—	270	—	270	—
Patient No. 2 Pre-secretin	—	—	—	10	10	10	—
Patient No. 2 10 min post-secretin	—	—	—	810	90	270	—
Patient No. 2 20 min post-secretin	—	—	—	90	270	90	—
Patient No. 2 40 min post-secretin	—	—	—	270	90	270	—

—, Complement-fixation antigen titre <10; other figures represent reciprocal of highest dilution of secretions giving complete fixation of complement with 1 : 10 antiserum.

titre at various time intervals after stimulation with secretin. Samples taken after stimulation with pancreozymin have not been available for testing.

The nature of the pancreas-specific isoantigens in man, rabbits and monkeys has not been established. A possible function of the antigens could be enzymatic since they are located in the secretory portion of the acinar cells[2] and are present in the secretions of these cells. However, direct demonstration of enzymatic activity of the pancreas isoantigen–antibody complexes or inhibition of enzyme activity by antibody has not as yet been successful. Much further work must be performed before these isoantigens can be associated with pancreatic disease.

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Effect of Hydrocortisone Hemi-succinate on Immune Lysis of Sheep Erythrocytes

The effect of hydrocortisone on immune lysis was examined by titrating guinea pig complement in the presence of various concentrations of the steroid. Sheep erythrocytes, sensitized with rabbit anti-sheep cell hæmolysin, were used as the indicator system for complement activity; a 50 per cent lysis end point was used. Complement activity was not detectable in the presence of 5 mg/ml. hydrocortisone; the titre was reduced significantly by concentrations as low as 0·01 mg/ml.

In order to attempt to determine the mechanism of this antilytic action the following experiments were carried out.

Pre-treatment of complement with hydrocortisone for periods of up to 30 min did not result in any greater reduction in titre than was observed with the same concentration of steroid added at the same time as the sensitized cells. This suggests that the steroid does not have a direct action on free uncombined complement.

Dialysis against isotonic saline of a mixture of sheep red cells, lysin, complement and sufficient hydrocortisone to inhibit lysis was carried out. After dialysis for 2 h complete lysis of the erythrocytes had occurred, indicating that the combination of hydrocortisone with the reactants was unstable, the hydrocortisone passing through the dialysis sac and allowing normal lysis to occur.

A similar unlysed mixture of cells, lysin, complement and hydrocortisone was incubated for 1 h, and the cells were then washed three times in saline. They failed to lyse on further incubation, but could be lysed by the addition of complement, indicating that antibody but not complement was attached to the cells. It is presumed that the complement remained free, and was removed along with the hydrocortisone by the washing procedure. This suggestion was confirmed by the use of the conglutination reaction. Sheep erythrocytes were incubated with either guinea pig or horse complement, both with and without hydrocortisone, and with heated normal bovine serum as a source of conglutinin and naturally occurring anti-sheep cell antibody. Conglutination occurred in the tube containing horse complement but no hydrocortisone; lysis took place as expected in the guinea pig complement control tube. Neither lysis nor conglutination occurred in the two tubes containing hydrocortisone. This experiment and the preceding data would suggest that hydrocortisone hemi-succinate obstructs immune lysis of sensitized erythrocytes by preventing the attachment of one or more complement components. In preliminary experiments hydrocortisone has been found to inhibit the lysis of erythrocytes by hypotonic salt solutions. This observation suggests that the steroid modifies the red cell surface. The modification of cell surface suggested may account for the failure of complement-binding. At the same time the effect of this and other steroids is being investigated using various other lytic systems.

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BIOLOGY

DNA, PNA and Protein Contents in a Rapidly Differentiating System, the Rat Tapeworm (*Hymenolepis diminuta*)

The role of the nucleic acids, deoxypentosenucleic acid (DNA) and pentosenucleic acid (PNA), in the growth and differentiation of organisms has been thoroughly examined and characterized. Rapidly growing tissues usually display rapid synthesis of DNA, PNA and protein. The synthetic rate of these constituents in a given tissue may be estimated by the relative amount of each present. Most of the systems that have been investigated in this regard have required the use of several different individual preparations at different stages of development. Tapeworms, such as *Hymenolepis diminuta*, should be ideal organisms for the examination of certain principles of growth and differentiation, for along the length of the strobila is a gradient of developmental stages, all within the same individual. The experiments reported in this communication were designed to test the usefulness of the tapeworm in a typical examination of growth and development.

The rat tapeworm was maintained in male rats of the Sprague–Dawley strain[1]. Adult worms were flushed from the host's small intestine with Krebs–Ringer solution[2], *p*H 7·4, with 0·05 M 2-amino-2-hydroxymethylpropane-1,3-diol (*tris*) acid maleate[3]. The tapeworms were divided along their length into 3 equal sections, representing the anterior, middle and posterior thirds of the worms. The three portions of worms obtained did not correspond exactly to the immature, mature and pregravid–gravid regions as described by Fairbairn *et al.*[4], but resulted in some overlapping of these regions. Each group was pooled separately and, along with similar batches of whole worms, treated immediately. We used a modification of the procedure outlined by Schneider[5] for the extraction of the nucleic acids. The diphenylamine reaction for deoxypentose[6] and the orcinol reaction for pentose[6], coupled with the Fiske–SubbaRow phosphate determination[7], were used to obtain the amounts of DNA–P and PNA–P present in the tapeworm tissue. Protein was estimated using the method of Lowry *et al.*[8].

The results of this investigation are summarized in Table 1. The values shown represent the average values of at least two separate determinations. Values expressed in Table 1 for DNA–P, PNA–P and protein based on the fresh weight of the tissue corresponded as well to those based on both nitrogen content of the tissue and unit of tissue (for example, μg DNA–P/whole worm). The anterior portion of the worm is known to be a region of rapid growth by cell division, while various stages of differentiation of the tissues and organs occur in the middle and posterior thirds[9]. There is also a metabolic gradient[10], in which the anterior region is the most active. The metabolic activity steadily decreases toward the posterior of the worms.

Table 1. DNA–P, PNA–P and Protein Contents in *Hymenolepis diminuta*

Region of tapeworm	μg of DNA–P/ g tissue	μg of PNA–P/ g tissue	mg of protein/ g tissue	μg of DNA–P/ mg protein	μg of PNA–P/ mg protein	PNA : DNA/ mg protein
Whole worms	36·3	155·3	98·5	0·37	1·58	4·27
Anterior 3rd	52·0	197·5	120·5	0·43	1·64	3·81
Middle 3rd	27·0	149·0	98·0	0·28	1·52	5·43
Posterior 3rd	20·5	137·0	79·0	0·26	1·73	6·65

It was not surprising, then, to find a similar gradient of DNA, PNA and protein in *Hymenolepis diminuta*. The ratios of PNA–P to DNA–P in the different regions of the tapeworms, where the ratio was lower in the more rapidly growing anterior region, and increased in the regions where differentiation of the tissues and organs occurs, agreed with the general pattern found for other growing systems. In addition, our results complement those of Cheng and Jacknick[11], who examined DNA and PNA in the different regions of *Hymenolepis diminuta* using histochemical techniques. The latter authors localized regions with greater concentrations of DNA and PNA at the cellular and tissue level.

We made several unsuccessful attempts to isolate and characterize the DNA from *Hymenolepis* using several different methods[12–14], and modifications thereof. Whether our lack of success was due to the inapplicability of these methods to cestode tissue (simultaneous extraction of mouse liver gave a good preparation of DNA), or due to the tapeworm having a low molecular weight DNA[15], was not established. The diphenylamine-positive material that was obtained by one of these methods[14] possessed no demonstrable thymine derivative.

Campbell[16] has shown that *Hymenolepis diminuta* degrades uracil and thymine to β-alanine and β-aminoisobutyric acid, respectively, by the reductive pathway. Investigations on the regeneration of mammalian liver tissue[17–19] have suggested that the reductive pathway for pyrimidine degradation is involved in the regulation of growth by effectively increasing the pyrimidine pool available for nucleic acid synthesis. *Hymenolepis diminuta*, or similar tapeworms[20], would appear to be an excellent source of material for the further investigation of this problem.

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A Cold Precipitable Protein in the Lens

The biochemical basis for the phenomenon termed 'cold cataract' (that is, opacification of lenses due to cooling) has been elucidated. A soluble protein which comprises approximately 10 per cent of the total soluble lens protein and which precipitates both in lenses and in aqueous extracts of lenses when they are cooled below 10° C has been isolated. This finding validates the suggestion made by van Heyningen[1] that a protein is responsible for clouding of mammalian lenses in the cold. It has been further observed that cold precipitation is a reversible process, since warming the lenses or extracts results in complete restoration of their transparency.

Fig. 1 is a series of photographs of lenses removed from normal seven-week-old Holtzman white rats and cooled by placing them on a watch-glass supported in a beaker of crushed ice. The first photograph (*a*) was taken about 10 sec after the watch-glass was illuminated; the second (*b*) after 20 sec, and the third (*c*) after 30 sec. The change from total opacity to total transparency obviously accompanies the increase in temperature from 4° C up to about 25° C.

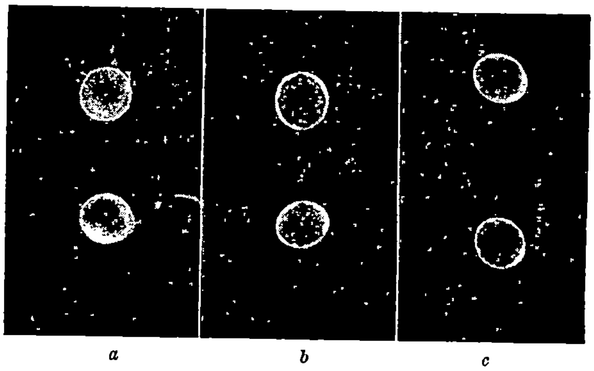

Fig. 1. Solubilization of CPP in cooled rat lenses due to warming (*a*) for 10 sec, (*b*) for 20 sec, (*c*) for 30 sec

Crude preparations of the cold precipitable protein (CPP) are isolated merely by homogenizing lenses in several volumes of distilled water, sedimenting the insoluble matter at 20° C at 20,000 r.p.m. for 1 h and then sedimenting the CPP at 0° C (at which temperature it is insoluble) at 20,000 r.p.m. for 1 h. The cold supernatant contains the total soluble protein minus the CPP. In order to purify the CPP repeated cold recrystallizations are carried out. Fig. 2 is a series of photographs of a test-tube containing an aqueous solution of recrystallized CPP at 12 mg/ml. which was cooled in a beaker of crushed ice. The first photograph (*a*) was taken about 20 sec after removal of the tube from the ice; the second (*b*) after warming the tube by hand for 20 sec; the third (*c*) after warming for about 1 min. Again, the loss of precipitability accompanied the increase in temperature.

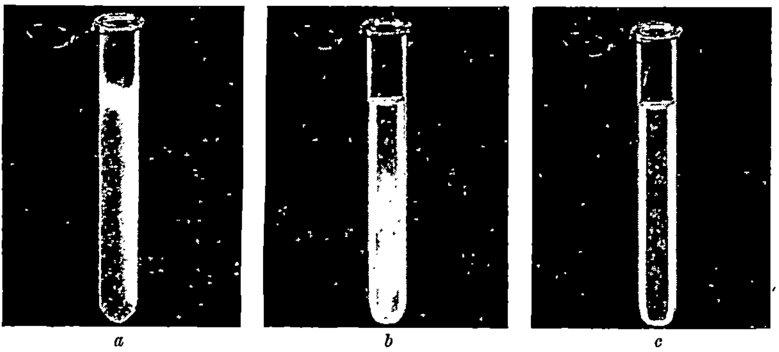

Fig. 2. Solubilization of CPP in a water extract of rat lenses due to hand warming (*a*) for 20 sec, (*b*) for 40 sec, (*c*) for 1 min

The protein nature of this recrystallized, dialysed, cold-precipitated material has been established by ultraviolet absorption, immuno-diffusion, electrophoretic and ultracentrifugal investigations. This work shows further that the CPP contains representative species of each of the three major classes of lens protein. CPP also contains approximately 1–1·5 per cent soluble RNA.

Physico-chemical investigations now in progress indicate that the property of cold precipitability is dependent on the concentration of CPP in solution; a concentration of 3 mg/ml. or more is required for precipitation. Loss of cold precipitability in the lenses of rats 8 weeks of age or older may be due to an increase in lens fluid volume or a decrease in the amount of CPP of a soluble form.

Cold precipitation is also dependent on *p*H; CPP does not precipitate when solution *p*H values are 4·0 or lower.

The solubility or lack of solubility of the protein species making up the CPP appears to depend strongly on the balance between hydrogen bonding and hydrophobic bonding as described by Sheraga[2]. At low temperatures, breakage of some hydrophobic bonds and an increase in hydrogen bonding may lead to precipitation of the CPP sub-units, while at slightly higher temperatures (that is, 20° C or higher) some inhibition of hydrogen bonding accompanied by favourable conditions for hydrophobic bonding may result in dissolution. The dependence of precipitability on *p*H may also be due to changes in hydrophobic and hydrogen bonding.

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Deep Water Corals as Fish Food

DIRECT observations reveal fish to be predators on most species of reef corals. Biting of the tip ends of branches, rasping the surface layers of the polyp, and rasping of the algae or other organisms attached to the non-living surface of the coral, are among the many methods used in obtaining the polyp of the coral and associated animals and plants as food. Direct correlations can be obtained between the occurrence of certain kinds of fish and the type of coral—a relationship which may be largely attributed to the sheltering effect of the coral. Hiatt and Strasburg[1], however, found a direct relationship between the coral and fish occurrences in terms of both habitat and predation.

Demonstration of predation by fish on corals from deeper waters cannot be accomplished so easily, for direct observations cannot be made. The evidence of predation must, perforce, rest on examination of stomach contents of fish, or indirectly on evidence of damage sustained by the coral which is most probably attributed to the biting of fish. To my knowledge there has been no published account of the presence of coral in the gut of fish from deep water. Difficulties are expected in the recognition of coral fragments in fish, for few corals can be taken in their entirety, and the coralla of many forms will be too light and fragile to stand up under the mastication and digestive processes. Mr. Gordon Williams of Auckland, New Zealand, for many years made collections of the stomach contents of fish, and the occurrence of corals in some of these was recently brought to my attention by Dr. A. W. B. Powell, Auckland Institute and Museum. From stomachs of the tarakihi (*Dactylopagrus macropterus*), taken over a period of several years from off Mayor Island, Bay of Plenty, in depths of 35–90 fathoms, Mr. Williams obtained more than 160 species of molluscs (A. W. B. Powell, personal communication) and two species of corals. Although the number of fish stomachs which were examined in order to obtain this collection is not recorded, the relative numbers of the two species of corals are significant. Ninety-eight specimens were recovered, all of which showed some signs of corrosion and some of breakage, although remnants of tissue were present in the internal depths of the coralla of some specimens. Both the species *Kionotrochus suteri* and *Sphenotrochus ralphae* are turbinollid corals which are free living forms, small (up to 1 cm in largest dimension), and have a skeleton which is completely internal to the polyp. Of the 98 specimens of coral, 14 were of *K. suteri* and the remaining 84 of *S. ralphae*. The significance of these proportions is that *K. suteri* is perhaps the most abundant coral in the Bay of Plenty region, being found in great numbers of individuals in a very high percentage of benthonic trawls. *Sphenotrochus ralphae*, on the other hand, although known from a moderately large number of localities, apparently never occurs in abundance. The stomach-content specimens are twice the known number of the species taken alive. Alternative explanations for the disparity are that the tarakihi were feeding in an ecological facies, not yet sampled, in which *S. ralphae* is abundant, or that the larger size of *S. ralphae* makes a more appealing item in the diet of the fish.

Other evidence for the predation of deep-water corals by fish rests with the secondary evidence of the regeneration of coralla severely damaged and broken during life. It is, of course, possible that organisms other than fish may be responsible for some of the observed damage; but the extent and nature of many injuries suggest a large animal, such as a fish. Regeneration of the coral has important implications both in investigations of the growth of coral and in their systematics, for the structures resulting from regeneration may differ significantly in their appearance from those of normal development. Increased recognition of the extent of predation of coral by fish will be of considerable value in both ecological and biological investigations.

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Hypomyces Stage of *Fusarium solani* f. *pisi*

ASCIGEROUS stages have not been established heretofore for either of the *Fusarium* pathogens of pea, *Pisum sativum* L., that is, for *F. oxysporum* f. *pisi* (Linf.) Snyd. and Hans., the cause of a vascular wilt, or *F. solani* f. *pisi* (Jones) Snyd. and Hans., the cause of a cortical foot and root disease. Evidence is presented in this communication that the ascigerous stage of the latter pathogen has been discovered and belongs to the genus *Hypomyces*. Although perfect stages are now known for several formæ in *F. solani*, none has yet been established for any member of the species *F. oxysporum*.

In 1959 one of us (T. M.) reported in Japan[1] the isolation of two formæ of *Fusarium solani* (Mart.) App. and Wr. emend. Snyd. and Hans. from mulberry trees. One of them, called *F. solani* f. *radicola* race 2, was shown later to be pathogenic to pea. This fungus, when properly mated, produced red perithecia in culture and was very similar to *Hypomyces* (*Fusarium*) *solani* f. *cucurbitae* Snyd. and Hans. in appearance and sexual behaviour[2,3], since it was heterothallic, had mating (compatibility) types (+), (−), and through mutation developed heterothallism in respect to sex. Hermaphroditic, male and female clones of the fungus also were found. Random crosses of the Japan fungus attempted with other clones of *Fusarium solani* showed that it would cross successfully with a California isolate of *F. solani* f. *pisi*, obtained from the Berkeley laboratory. Later it was discovered there that the California isolate would cross also with two additional Japan isolates of *F. solani* f. *pisi*[3].

About the same time (1962–63) in the laboratory at Berkeley, the original California isolate sent to Matuo was crossed successfully with a number of clones from a collection of isolates of *F. solani* f. *pisi* obtained from various parts of the United States and from England. All these were typical, tested, highly virulent clones of the pathogen, isolated from pea plants infected with root rot in the field.

All isolates were maintained by single spore culture in PDA tubes at room temperature in diffuse daylight, or in daylight supplemented by 12 h of fluorescent light. Crosses were made 20–30 days after single sporing, by pouring a suspension of conidia in sterile water from one clone into tubes containing another. The time required to obtained fertile perithecia varied from 1–6 months. Single ascospores were isolated from these perithecia, and also single ascospores were recovered from individual asci by the method described by El-Ani[4]. Ascospore progenies were analysed for the inheritance of pathogenicity and mating type.

Pathogenicity tests were made by inoculating emerging pea seedlings of the varieties 'Alaska', 'Little Marvel' and 'Davis Perfection' growing in steamed *U.C.* soil mix, with spore suspensions from PDA slants. The seedlings were not injured during inoculation. Two pots each containing 5–7 plants were used in testing each isolate and the tests were repeated 1–3 times. Controls were uninoculated plants, and plants inoculated with *F. solani* f. *phaseoli* (Burk.) Snyd. and Hans., all of which remained free from disease. Tests were terminated after 4–6 weeks. Plants were removed from the soil, washed, and graded as to the degree of virulence of fungus attack. This varied from severe, with killing of seedlings during the first 3 weeks after inoculation, to a trace or almost no disease, 6 weeks after inoculation.

Some of the crosses resulted in fertile perithecia. Asci were removed from these and the ascospores of several individual asci were recovered and tested separately for mating type and pathogenicity. The perithecia showed a 1 : 1 segregation for mating type when back-crossed to their parents or other tester clones.

Inheritance of virulence was investigated in two ways. (1) Ascospore progeny from the aforementioned asci were tested individually. Evidence was obtained that differences in virulence existed between ascospore pairs within each ascus analysed. (2) Ascospores expelled from perithecia resulting from a cross between two clones were tested at random for pathogenicity. They ranged from very virulent to essentially non-virulent. The colonial characteristics of the progeny showed a wider variation than that of the two parents, some clonal colonies revealing little resemblance to either. The variation between sizes of conidia of different ascospore clones was as great as previously described by Snyder and Hansen[5] for the species *F. solani*. Perithecium and ascospore sizes fell into the range of those given in the same paper for the perfect stage, *Hypomyces solani* (Rke. and Berth.) Snyd. and Hans. The variability obtained from these matings of *F. solani* f. *pisi* was similar to that described for *F. solani* f. *cucurbitae* and showed a pattern usual for a heterothallic fungus.

Crosses of various clones of *F. solani* f. *pisi* show beyond question now that this foot and root rot pathogen of pea has a perfect *Hypomyces* stage, although it has never been reported in the field on its host. It is heterothallic, segregates into two mating types, and while very similar in behaviour to that of *H. solani* f. *cucurbitae*, it is not interpathogenic with its host nor inter-fertile with it. However, perithecia form less rapidly on PDA than on other media such as *V*-8 juice agar[6].

Clones of *F. solani* f. *pisi* derived from ascospores varied widely in their virulence on peas as well as in their cultural appearance. Results from the crosses suggest that a recombination of several genes governing virulence can take place, since random ascospore cultures showed a wide range in virulence.

In 1960 W. L. Gordon[7] pointed out that Buxton's[8] report of a perfect stage in *F. oxysporum* was without fact. *Nectria haematococca* (*Hypomyces solani*), a homothallic fungus associated with the latter species, was shown actually to be the perfect stage of a *Fusarium solani*, not of the wilt fungus *Fusarium oxysporum* f. *pisi*. Gordon suggested, however, that Buxton's *Nectria* (*Hypomyces*) might be a perfect stage of *F. solani* f. *pisi*, but did not test its pathogenicity in order to determine this important point.

In the following year one of us[9] published the results of a series of pathogenicity tests, using Buxton's homothallic fungus. They concurred with Gordon as to the identity of Buxton's *Nectria* (Imp. *F. solani*) and showed it to be a saprophyte, biologically unrelated to *F. solani* f. *pisi*. Repeated tests since that time, using isolates obtained directly from the original source, again have shown no pathogenicity under our conditions. The discovery now of a heterothallic perfect stage of *Fusarium solani* f. *pisi* in two different laboratories shows definitely that it, and homothallic *Nectria haematococca* (*H. solani*), are genetically as well as biologically two different and distinct fungi.

Although both compatibility types of *F. solani* f. *pisi* have been found to occur in the United States, its *Hypomyces* stage has been overlooked up to this time, probably because perithecia do not develop readily on PDA, even when the proper mating types are present, and because perithecia apparently do not occur commonly, if at all, on infected plants in the field.

Perfect stages (in *Hypomyces*) now have been produced in pure culture in the Berkeley laboratory not only for saprophytic *F. solani* (which contains both homothallic and heterothallic groups) but also for the tested, pathogenic formæ, *F. solani* f. *cucurbitae*, r1. and r2., *F. solani* f. *batatis* (Harter) Snyd. and Hans., and *F. solani* f. *pisi*. In addition, the Japan laboratory has reported perfect stages in culture for *F. solani* f. *mori* Sakurai and Matuo, *F. solani* f. *xanthoxyli* Sakurai and Matuo, *F. solani* f. *robinae* Matuo and Sakurai, and *F. solani* f. *pisi*. All these reportedly pathogenic formæ are heterothallic in the usual compatibility sense. Also, Zerova[10] has reported *Hypomyces asclepadis* Zerova as the perfect stage of *F. coeruleum* (Lib.) Sacc. (*F. solani* f. *radicicola* (Wr.) Snyd. and Hans.). Perfect stages so far are not known for *F. solani* f. *phaseoli* and *F. solani* f. *eumartii* (Carp.) Snyd. and Hans.

This ascigerous stage of the pea pathogen is designated *Hypomyces solani* f. sp. *pisi* n. c., pathogenic on the underground parts of pea, *Pisum sativum* L., heterothallic, world-wide, with an imperfect stage in *Fusarium solani* f. *pisi* (Jones) Snyd. and Hans.

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Nature of Growth-promoting Activity associated with the Culture Filtrates of *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. and Hans.

The growth-promoting activity of the filtrates of the genus *Fusarium* has long been recognized[1-5]. Using a bioassay test for auxins, and distilled water as control, Hirata[2] detected indolyl-3-acetic acid (IAA) in concentrated filtrates, whereas Venkata Ram[3] reported its absence. Their media, in addition to the basal medium (g/l. KH_2PO_4, 1·5; $MgSO_4$, 1; $FeCl_3$, 0·01; glucose, 25 and nitrogen source, either ammonium sulphate at 2·0 g, or asparagine at 1·99 g and water 1 l. (ref. 5)), were used to culture the fungus to determine indole production.

Owing to the structural similarity to IAA, tryptophan was substituted as (*a*) nitrogen source and (*b*) both as a combination of nitrogen and carbon source in all media. Two different strains of *Fusarium oxysporum* f. *lycopersici* were used, the strain from the junior author's laboratory and a virulent strain (*R* 5–6).

Media were used either at *p*H 6·5, or unadjusted. Acidity of all media was critically considered, as tryptophan conversion into indole was known to be inhibited in acid condition, while the conversion was favoured in an alkaline reaction[6].

Erlenmeyer flasks (250 ml.) with 50 ml. of sterile medium were inoculated with 1 cm disk of the actively growing fungus and incubated at room temperature (22°–24° C). The filtrates were analysed at 3-week intervals up to a maximum of 12 weeks. Uninoculated sterile media and sterile distilled water were used as checks.

Two flasks of the same medium were pooled and filtered. The filtrates were shaken three times with equal volumes of peroxide-free ether. Solvents were evaporated *in vacuo* and the residue was dissolved in 2 ml. of glass distilled water or in ethanol and was spotted in Whatman No. 1 paper and developed descendingly, using isopropanol–aqueous ammonia–water (10 : 1 : 1 v/v) (ref. 7). For effective separation of indoles from tryptophan, the papers were developed in a solvent system having methyl ethyl ketone–pyridine–water (75 : 5 : 15 v/v) (ref. 8).

Indoles were detected by various spray reagents. Since Salkowski's reagent has questionable specificity[6,9,10], it was used together with that of Ehrlich and a modified Ehrlich reagent, consisting of 2 g of *p*-dimethyl amino benzaldehyde in 6 N hydrochloric acid (100 ml.) and ethanol (100 ml.). Fresh reagents were used for each determination. After spraying, the chromatograms were dried at room temperature (23°–25° C) and the spots were examined in 24 h.

In addition, both oxalic acid paper and Kovac's tests were performed in all filtrates[11]. All tests were repeated at least 10 times and the results are shown in Table 1.

Table 1. Results of Indole Production by *Fusarium oxysporum* f. *lycopersici* in Cultural Media*

Media	Salkowski	Ehrlich	M. Ehrlich	Oxalic acid test	Kovac's test
(1) Richard's	–	–	–	–	–
(2) Tryptophan Richard's (–nitrogen)	–	–	–	–	–
(3) Tryptophan Richard's (–N and C)	–	–	–	–	–
(4) Basal medium	–	–	–	–	–
Ammonium sulphate	–	–	–	–	–
Asparagine	–	–	–	–	–
(5) Czapek's	–	–	–	–	–
(6) Tryptophan Czapek's (–N)	–	–	–	–	–
(7) Tryptophan Czapek's (–N and C)	–	–	–	–	–
(8) IAA control	+	+	+	–	–
(9) Tryptophan control	–	–	–	–	–

* These results were observed regardless of the strains and solvents employed.

Both strains of *Fusarium oxysporum* f. *lycopersici* produced no indole compounds in the filtrates regardless of media. In one instance, a positive spot for Salkowski's reagent in Richard's medium was observed. However, this could not be repeated.

The fungus grew poorly in tryptophan medium. However, greater utilization of tryptophan was noted when it was substituted as nitrogen and carbon sources as revealed by a faint ninhydrin-positive test. Also this material was utilized only slightly as a source of nitrogen in the presence of a sugar. Tryptophan was apparently utilized in a different biochemical pathway leading to the eventual conversion of nicotinic acid as some of the intermediary products were identified in the chromatograms[12], rather than the conventional pathway of tryptophan conversion into IAA.

Using standard bioassay tests, both Hirata[2] and Venkata Ram[3] suggested that *Fusarium* species produced growth-promoting elements. Hirata[2] also showed a positive colour reaction for IAA, especially in the tryptophan–Czapek's filtrates of *F. solani* and *F. oxysporium* f. *vasinfectum*. No mention of a colour reaction was available using *F. oxysporum* f. *lycopersici* filtrates, although the filtrates exhibited a positive *Avena* coleoptile test. The *p*H of the tryptophan–Czapek's medium used by Hirata was not reported. It was noticed in this laboratory that the *p*H of such medium was 4·6, which was not conducive for tryptophan conversion to indole.

Although Venkata Ram[3] reported a positive bioassay test, he detected no indoles in the culture filtrates. He also reported that the filtrates from Richard's medium with carbon sources as glucose and fructose significantly increased the growth-promoting activity of fungal filtrates and also exhibited a positive bioassay test. It is known that fermentable sugars inhibit the production of indole compounds[7].

Under the conditions of the present investigations it is concluded that no isolatable IAA or other derivatives were produced by *F. oxysporum* f. *lycopersici*. It is suggested that the growth-promoting activities associated with *Fusarium oxysporum* f. *lycopersici* is not of auxinic nature. However, these findings do not preclude the possibility that auxins may be produced by the pathogen *in vivo*.

We thank Dr. K. McDonnell of the Department of Botany, University of Cambridge, for supplying the virulent strain, and Mr. Ray Ito, Department of Political Science, University of Rhode Island, for translating the Japanese text of Hirata's paper.

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Influence of the Inoculum and the Medium on the Growth of *Chlorella pyrenoidosa*

I HAVE on more than one occasion considered the practical interest of achieving giant forms of *Chlorella* by treatment with a sugar[1], in order to use them as inoculum in cultures the growth of which it is desired to accelerate. It is known that such giant cells cultivated in a mineral medium devoid of sugar return to their normal form. However, I presumed that the growth of such cultures would be superior to that of cultures inoculated with normal cells. On the other hand, the composition of the culture medium is also an important factor to be considered[4,6].

This communication reveals the decisive influence which the inoculum and the medium, independently, exert on the rate of growth of a culture.

The inoculum is prepared by sowing *Chlorella* cells taken from a mineral medium culture in logarithmic phase of growth, in a medium which induces gigantism with glucose[1]. After four days in this medium, in the dark, at 37° C, the inoculum was centrifuged at 1,000 r.p.m., washed with distilled water, and sown.

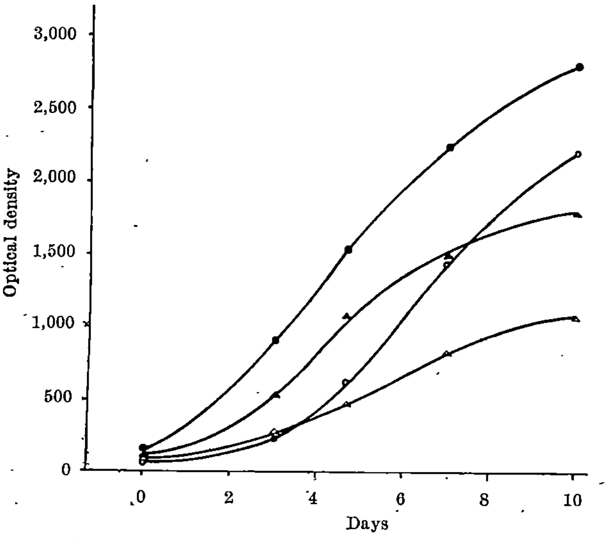

Fig. 1. Growth of *Chlorella* on different media and inocula. Medium *A*: with giant cells (●); with normal cells (○); medium *B*: with giant cells (▲); with normal cells (△)

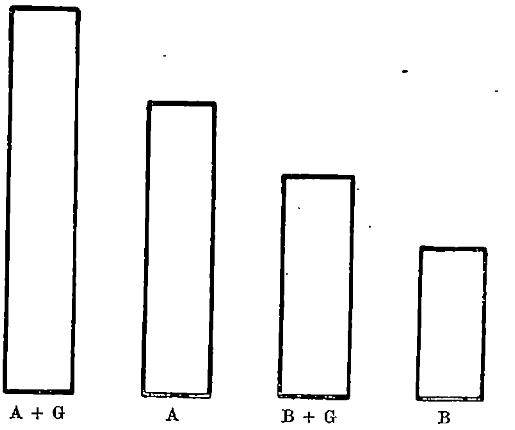

Fig. 2. Relative amount of growth in *Chlorella* cultures of 10-day-old, on different medium, with normal or giant cells (*G*) inoculated

I have examined two mineral media and sown the foregoing inoculum on them with the following compositions and concentrations (g/l.): Medium *A*, KNO_3 1·011; $MgSO_4\cdot 7H_2O$ 0·247; $Na_2HPO_4\cdot H_2O$ 0·621; $NaH_2PO_4\cdot 2H_2O$ 0·089; $CaCl_2\cdot 2H_2O$ 0·015; $FeSO_4\cdot 7H_2O$ 0·007 (in complex form with EDTA); trace elements. Medium *B*. KNO_3 1·250; $MgSO_4\cdot 7H_2O$ 2·500; KH_2PO_4 1·250; $FeSO_4\cdot 7H_2O$ 0·011 (previously dissolved in water acidified with HNO_3); trace elements.

The culture medium is sterilized at 120° C for 20 min. 500-ml. Erlenmeyer flasks were used, in which 200 ml. of medium was placed and sown with approximately equal (in dry weight) inoculations of giant cells. Each experiment was duly checked with an equal dose of normal cells. Six such experiments were made. During the ten days which the experiment lasts the cultures are kept in daylight, at 25° C, and are gassed with a mixture of air and 5 per cent carbon dioxide. The growth of the medium can be measured by optical density using Beckman *DU* with a wave-length of 600 mμ.

Figs. 1 and 2 represent the mean values of the findings in the experiments. The growth is noticeably greater in medium *A* than in medium *B* and within the same medium when giant cells are inoculated. It may be inferred from this that the result of the experiments depends both on the composition of the medium and on the nature of the inoculum. A normal cell culture performed on medium *A* shows, in 10 days, a 100 per cent greater growth than another carried out in the same conditions in medium *B*.

Table 1 gives the quantities of fundamental elements which enter in each medium and their *p*H.

Table 1. PRINCIPAL ELEMENTS IN THE CULTURE MEDIA (g/l.)

Medium	N	P	S	K	Mg	Fe	Ca	*p*H
A	0·140	0·137	0·032	0·392	0·024	0·001	0·004	6·5
B	0·173	0·284	0·324	0·842	0·246	0·004	—	5·5

Medium *B* strikes me by its greater saline concentration; and if it is considered that the absence of chelating agents in it, which would stabilize the solution of trace elements, the abundance of the precipitate which forms during its sterilization and possibly originates unforeseen, ion imbalances may be explained. On the other hand, medium *A* contains the necessary calcium ion, albeit in minimal quantities, for unicellular green algae and specifically for *Chlorella*[2,3]. The *p*H existing in both media are well within the wide margin of tolerance of *Chlorella*.

The inoculum exerts on the growth an action similar to that of the culture medium in Fig. 2. When inoculating giant cells in medium *B*, 85 per cent more growth was obtained than when inoculating normal cells; whereas in medium *A* the growth is only 41 per cent more.

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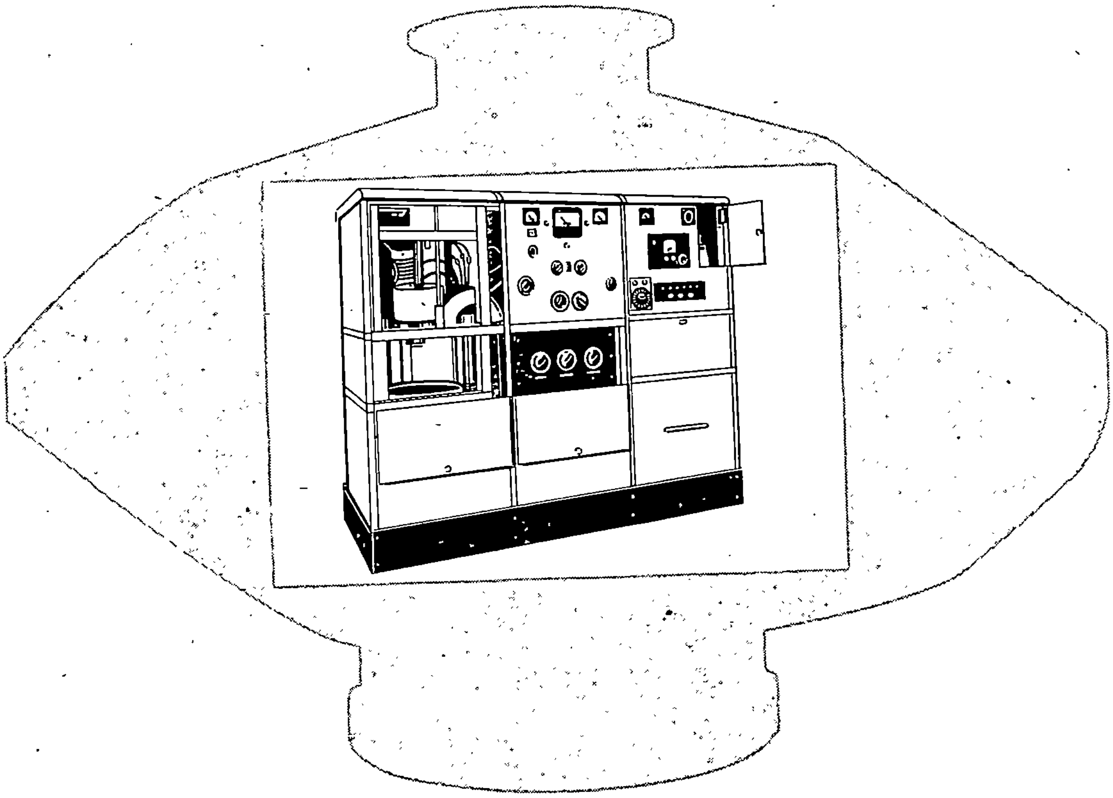

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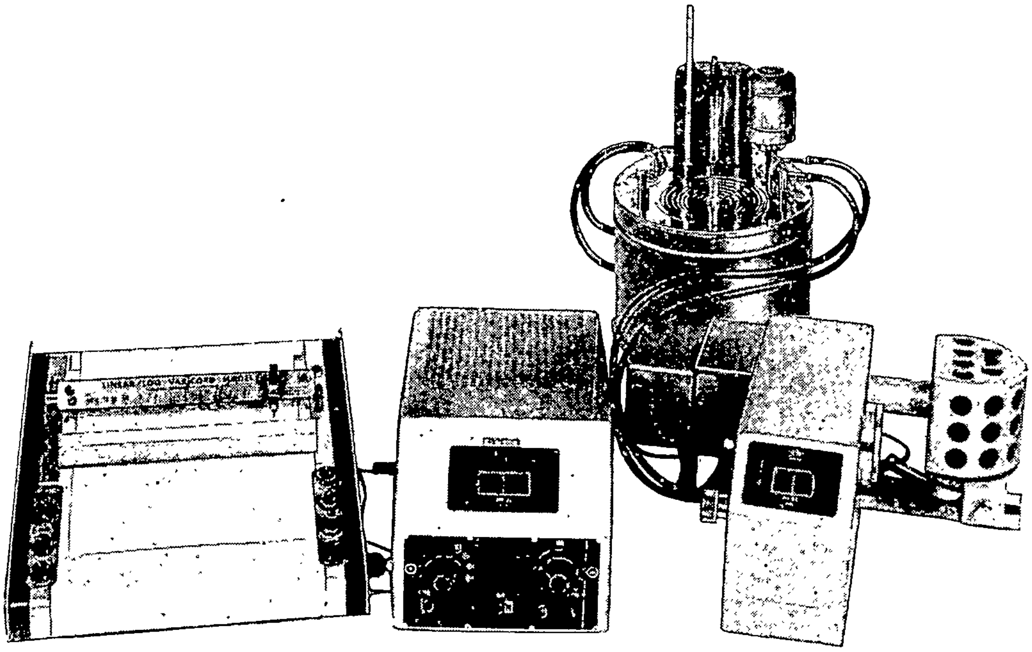

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According to Soeder[4], when the division and sporulation processes are delayed or inhibited generally larger cells are originated and these contain an enormous amount of reserve substances. Griffiths[5] has shown that *Chlorella* cultivated in a medium with glucose has its division inhibited and its volume increased, and that its dry weight is considerably greater than when grown in an inorganic medium. These accumulated reserve substances are those which, as I have shown[1], cause a considerable proliferation of autospores in the giant cells, and these, on being carried to inorganic medium and placed in conditions of autotrophism, break the wall of the mother cell and cause in the culture a growth which is notably superior to normal growth.

It is therefore advisable, whenever it is desired to accelerate the growth of a *Chlorella pyrenoidosa* culture and obtain an abundant mass of this in a short time, to use medium *A* inoculated with giant cells.

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MICROBIOLOGY

Toxic Action of Hæmolytic *Escherichia coli* isolated from Pigs

Certain hæmolytic strains of *E. coli* are associated with œdema disease and gastro-enteritis of young pigs. Timoney reproduced œdema disease in pigs by injecting supernatant material of the centrifuged intestinal contents from pigs which had died of this disease[8]. Since then, Erskine *et al.*, Sojka *et al.* and Sweeney *et al.* have successfully reproduced the disease by injecting bacterial extracts prepared from hæmolytic strains of *E. coli*[2,4,6]. These reports suggest that œdema disease is caused by absorption of a toxic substance produced by specific strains of hæmolytic *E. coli*.

In contrast to the 'toxæmia hypothesis', Lemcke *et al.* observed severe anaphylactic reaction following injections of polyvalent *E. coli* antiserum into pigs that had shown symptoms of œdema disease and concluded that œdema disease was produced due to an antigen–antibody reaction and therefore this disease is a specific anaphylaxis[3]. Further evidence in favour of this 'anaphylactic hypothesis' on œdema disease and gastro-enteritis complex was presented by Buxton and Thomlinson from their observations on passive cutaneous anaphylactic tests in guinea pigs and by Thomlinson and Buxton from reversed anaphylactic experiments in pigs[1,7].

The work recorded here was carried out to test the validity of each of these two hypotheses and to seek a possible reconciliation. Bacterial extracts of a hæmolytic strain of *E. coli*-0141: *K*85*a,c* (*B*), and a non-hæmolytic strain —*Mac* 429 isolated from a pig were prepared by the method of Erskine *et al.* with minor modifications in that the cells were washed twice in 0·85 per cent sodium chloride solution, and disintegrated by sonic oscillation for 30 min[2]. Total protein content was determined by measuring the amount of precipitate produced with trichloroacetic acid[5].

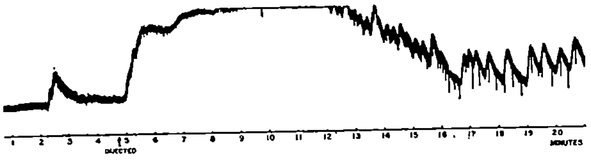

FIG. 1

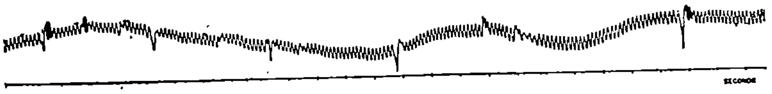

FIG. 2

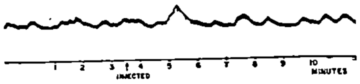

FIG. 3

Fig. 1. Effect of an intravenous injection of 0·5 ml. extract of hæmolytic *E. coli*-0141: *K*85*a,c* (*B*). Speed of the paper 20 mm/min

Fig. 2. Difficulty in expirations, deep and prolonged inspirations accompanied by convulsions are recorded on this graph by running the paper at 20 mm/sec

Fig. 3. Effect of an intravenous injection of 0·6 ml. extract of non-hæmolytic *E. coli*. Speed of the paper 20 mm/min

Each extract was tested on a separate young, healthy, non-sensitized, female guinea pig weighing about 300 g. After anæsthetizing the animal with ether inhalation, its uterus was exposed and the severed right horn was attached to a transducer of an electronic polygraph recorder. About 0·5 ml. of the bacterial extract adjusted to contain 7·0 mg of protein per ml. was inoculated into one of the mesenteric veins.

The reaction of the animal to an injection of hæmolytic *E. coli* extract was observed within 15 sec by a sharp contraction of the uterus and respiratory distress (Fig. 1), the uterus remained contracted for at least 11 min, the rate of respiration increased and the duration of inspirations and expirations became shorter. After this 11-min period of violent reaction, the animal showed signs of respiratory failure with intermittent gasping inspirations and convulsions (Fig. 2) and died within ½ h of the injection.

A lag of 1·5 min followed an injection of the non-hæmolytic *E. coli* extract when the uterus contracted also coincident with respiratory distress (Fig. 3). However, in contrast to the action of the hæmolytic *E. coli*, the reaction lasted for only 1 min and the animal recovered.

The results of these experiments indicate that hæmolytic *E. coli* produce a potent toxin that has a marked effect on non-sensitized guinea pigs. Extracts of non-hæmolytic *E. coli* have a less toxic effect, but whether this difference in potency is due to a different type of toxin produced by the two strains or different amounts of the same toxin is not known. All these data support the 'toxæmia hypothesis'.

We thank Mrs. N. Chopra for protein estimation of the bacterial extracts and Mr. T. W. Chen for his help to run the polygraph.

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ENTOMOLOGY

Diuretic Function of the Labial Glands in Adult Giant Silk Moths, *Hyalophora cecropia*

During experiments on the water balance of pupal and mature *Hyalophora cecropia* (Schneider), it was observed that the facial area of the adult may become moist when the hæmolymph is diluted. A similar, but spontaneous, discharge occurs when the adult is emerging, the watery exudate serving to soften the stiff matted silk of the emergence tunnel by which they escape from the cocoon. The fluid is released from labial glands which open through a medial pore situated on the labium between the rudimentary galeæ. These glands, which secrete silk in the larva, survive metamorphosis to the non-feeding adult as a pair of tubes composed of a thick-walled posterior part lying ventrally in the prothorax and a thin-walled, much folded anterior part which unites with its counterpart and opens at the labial pore. The present experiments show that these glands not only persist, but also continue to secrete in the adult, where they become organs of diuresis. This work records the relationship of secretion to physical restraint, and the high rates of secretion caused by dilution of the hæmolymph.

Experiments were first performed on animals the wings and legs of which had been restrained with cellulose tape to facilitate the collection of samples. The secretion of the labial gland was collected in a calibrated capillary tube with a fine drawn tip fixed over the labial pore. The level of fluid in the tube was marked at intervals and the rate of secretion determined by weighing. In young adults restraint with cellulose tape induced a spontaneous production of fluid within 5–10 min, which reached a rate of about 1 μl./min, 15–20 min later. Thereafter the rate of secretion diminished and finally ceased after about 100 min. The mean rate during the entire period was about 0·5 μl./min.

The rate, volume, and duration of secretion varied greatly and appeared to be related to the water content of the animal at emergence : some unconstrained adults, 1 and 2 days old, secreted copiously after injection with distilled water, while others produced fluid only after constraint and injection. It has not yet proved possible to collect labial gland secretion quantitatively from unconstrained animals.

When water was injected into the abdomen of animals which failed to secrete spontaneously after constraint, or into animals that had completed spontaneous secretion, the glands renewed their secretory activity. Fluid appeared within 5–15 min following an injection of 0·25 ml. distilled water, reached a maximal rate for most animals of about 2 μl./min within 20–30 min and continued at a decreasing rate for 1–2 h, giving an average rate of 0·1–0·2 μl./min. Day-old animals injected with 0·17 M sodium chloride secreted at a rate more than double that of distilled water animals (Fig. 1*A* and *B*). One animal (*A*) which received 0·25 ml. 0·17 M sodium chloride delivered 0·276 ml. at a mean rate of 3·6 μl./min.

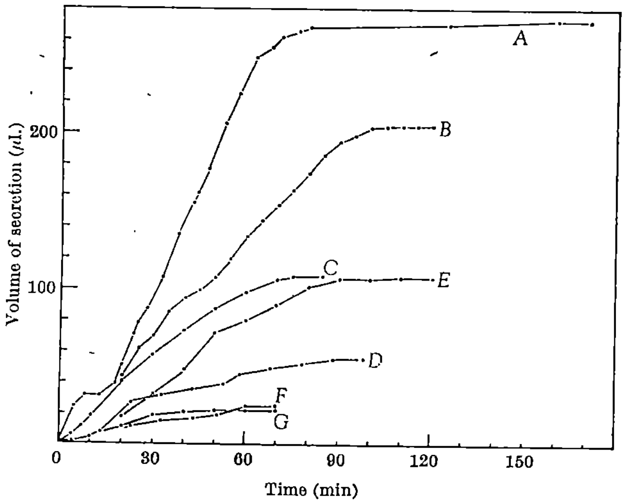

Fig. 1. Volume of labial gland secretion released at labial pore after injection as follows: *A*, 0·25 ml. 0·17 M sodium chloride; *B*, 0·20 ml. 0·17 M. sodium chloride; *C–G*, one animal injected on successive days with 0·25 ml. water

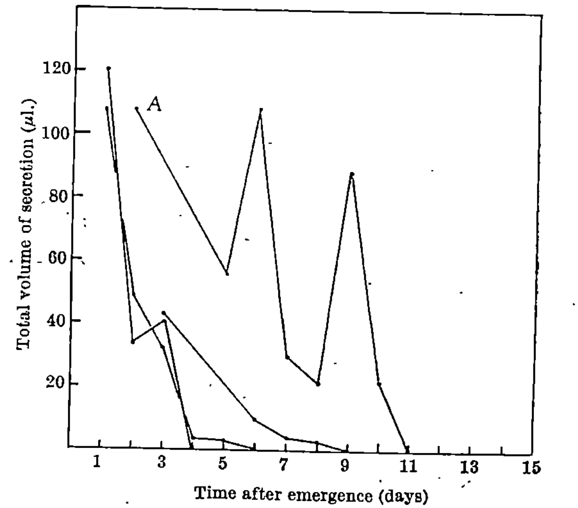

Fig. 2. Change with increasing age in response of labial glands to injections of 0·25 ml. water. Four animals. Animal *A*, which showed a fluctuating change, died on day 15, the others on day 11 or 12

The ability of the labial glands to secrete in response to injection declines during the first few days of the 10–12 day life-span of the adult, but occasional animals have continued to secrete in response to repeated injection of 0·25 ml. of distilled water for as long as ten days (Fig. 2).

Wounding and internal hydrostatic pressure do not appear to be the stimuli for secretion, which could not be induced by injecting peanut oil, though an animal so treated would secrete on receiving an injection of distilled water. Nor does resumption of secretion appear to bear any simple relationship to the osmolarity of the blood, for injections of 1 M glycerol induced secretion as did normal insect saline (0·17 M sodium chloride), and 0·17 M potassium chloride.

Restraint to the legs acting as a stimulus to secretion is probably related to the restraint imposed by the pupal cocoon during emergence for unconstrained animals which failed to respond to injections of water, did so when the legs were restrained, and ceased within seconds when again released. The results so far obtained suggest a reflex mechanism, the threshold for which rises with increasing age. The expenditure of fluid during emergence may influence the extent of subsequent 'spontaneous' discharge.

The striking capacity of the labial glands to respond rapidly to a change in the water content of the hæmocœle suggested the possibility that the labial glands might also play a part in water regulation in the adult. Adult *cecropia*, like many non-feeding adult Lepidoptera, are 'dry' animals : they have a low blood volume, doubtless in the interest of aerodynamic efficiency, and the possibility that the labial glands play a regulatory part in water balance is not yet discounted. A parallel may be found in other labial glands, for example the accessory glands of the heteropteran salivary complex (Edwards, J. S., unpublished results), and possibly the labial glands of the *Thysanura* (Watson, J. A. L., personal comm.). However, the malpighian tubules remain active throughout adult life, as shown by their continued capacity to sequester indigo carmine and to produce urine in response to water load.

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Autogeny and Oviposition in Arctic Mosquitoes

Two species of mosquito, *Aedes* (*Ochlerotatus*) *impiger* (Walk.) and *A.* (*O.*) *nigripes* (Zett.), occur at Lake Hazen, northern Ellesmere Island[1]. This arctic site (71° 18′ W., 81° 49′ N.), where the Canada Department of Agriculture has conducted entomological studies in 1962 and 1963, lies within 100 miles of Alert, the most northerly locality at which mosquitoes are known to exist in Canada[2]. Two findings concerning the mosquitoes at Lake Hazen are described here ; unless otherwise specified all remarks refer to both aforementioned species.

Numerous temporary and permanent pools provide larval habitats in the area. The most widespread flowering plant is *Dryas integrifolia* Vahl. (Rosaceae), which adults of both sexes visit frequently to obtain nectar. During the mosquito flying season (late June–mid-August) about 19 species of bird and 8 species of mammal exist in the area, though at a relatively low density[3].

At emergence, ovaries of female mosquitoes are in stages I–II of Christophers[4] and nullipars usually come to bite in stage II, after having mated. Nulliparous and parous females actively seek vertebrate blood and complete ovarian development (that is, reach stage V) about a week after engorgement. However, nullipars can also complete ovarian development, within about the same time, when maintained from emergence with flowers of *Dryas integrifolia* or with sugar (a 15 per cent solution of sucrose) as their only source of food. Females also oviposited under these conditions, except those *A. nigripes* maintained on sugar. On such blood-free diets, ovarian development was attended by extensive, stepped degeneration of oocytes, and this so greatly reduced fecundity that sometimes only a single egg attained stage V (Table 1). Females maintained solely on demineralized water survived up to 20 days (cf. up to 37 days on *Dryas* and sugar) but their ovaries did not develop beyond stage II, and no oocytes degenerated. Evidently access to nectar or sugar is a prerequisite for the process leading to degeneration of oocytes and translocation of food reserves in this type of autogenous ovarian development.

In another experiment, females (mixed nullipars and pars) attracted in Nature were allowed to penetrate the skin of the human bait (thus unequivocally manifesting their intention to bite) and then treated in one of two ways : (1) allowed to engorge ; or (2) removed within 5 sec of having penetrated, presumably before ingestion of blood could have begun. Both groups were then maintained on *Dryas* and sugar. Ovarian development was then completed in all females (31 *A. impiger* and 9 *A. nigripes*) in group (1), and in 8/25 and 1/14, respectively, in group (2). It therefore appears that in some females (at least) autogeny is facultative. However, until the precise stimulus associated with blood feeding which initiates ovarian development is known, this conclusion must remain provisional.

Autogeny in arctic or subarctic populations of mosquitoes has hitherto been discerned in *Aedes* (*O.*) *communis* (DeGeer) in Canada (although only in populations believed not to take blood[5,6]), and in *A.* (*O.*) *flavescens* (Muller) and *A.* (*O.*) *punctor* (Kirby) in Sweden[7]. The present findings establish the existence of autogeny in blood-sucking mosquitoes in arctic North America ; they show that nectar or sugar is required for its manifestation, as in some but not all other autogenous mosquitoes[8], and that the autogeny is associated with decreased fecundity. They strongly suggest, but do not demonstrate, that autogeny is facultative in individual females. In retrospect, it seems likely that Barlow[9] encountered a similar phenomenon in *Aedes* (*O.*) *hexodontus* Dyar at Churchill, Manitoba, although his use of wild-caught females prevented his reaching certainty on this point.

Table 1. SIZES OF EGG-BATCHES IN MOSQUITOES WHICH COMPLETED OVARIAN DEVELOPMENT

Treatment	Species *Aedes impiger*	 *Aedes nigripes*
(*A*) Emerged in captivity:		
Dryas and/or sugar	1–4 (12)*	1–16 (8)
Dryas and sugar; then blood	8–23 (20)	12–41 (4)
(*B*) Caught biting in Nature:		
denied blood; *Dryas* and sugar	2–21 (8)	7 (1)
allowed to engorge on blood	12–76 (30)	20–47 (10)

* No. of mosquitoes in brackets.

The pattern of autogeny recognized at Lake Hazen may have special adaptive significance for mosquitoes in the arctic and subarctic, where potential vertebrate hosts fluctuate widely in numbers[10]. It provides for reproductive continuity during years when vertebrates are scarce or absent, and for a proportionate, large increase in years when they are plentiful. Thus a rich, temporary supply of protein in one year can be used to increase the number of adult females in the next, when, if blood becomes scarce, their greater abundance can to some extent compensate for their reduced fecundity. Accordingly, populations of such mosquitoes probably show numerical fluctuations broadly resembling those of their principal vertebrate hosts; the mosquitoes, however, unlike the vertebrates, will be ensured (by autogeny) against declining beyond a certain minimum density.

The second finding to be reported here concerns the oviposition of *Aedes nigripes*. At Lake Hazen the Sun remains continuously above the horizon (elevation about 6–32°)[11] throughout the mosquito flying season. Despite this, oviposition is periodic. Females of *A. nigripes* oviposit in clearly visible sites (the characteristics of which will be described elsewhere) frequently rendered conspicuous by the corpses of individuals that die there. Females were often seen ovipositing, but only on surfaces with an aspect currently at right angles (in a horizontal plane) to the rays of the Sun. Laid eggs and dead females *in situ* were found only in sites directly insolated between 0600 and 2100 h suntime (solar noon being 1200 h) ; and the overwhelming majority of eggs and females were at sites directly insolated between 1100 and 1300 h. It may be concluded that oviposition is virtually restricted to the period 0600–2100 h and that it achieves its greatest rate near solar noon. The environmental cues which maintain such periodicity merit study. If, as seems probable, the temperature of the site plays a critical part, the duration of daily oviposition may be expected to decrease as the Sun's maximum daily elevation becomes progressively less during July and August. A consequence of this periodicity is that oviposition sites have a southern to south-westerly aspect and thus are usually among the first parts of the pond margin to become free of snow in June when the overwintered eggs hatch.

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VIROLOGY

Bats and Arboviruses in East Africa

The natural cycles of the majority of the arboviruses are unknown. Even with yellow fever virus—which belongs to Casals's Group *B*—where extensive investigations have shown the natural cycle to involve forest dwelling primates and mosquitoes, many workers think that there still remains at least one other as yet undiscovered cycle of virus maintenance.

In Africa, bats which are abundant and attractive to mosquitoes[1] have as yet been surprisingly little investigated in relation to yellow fever and other arboviruses. Investigations at this Institute during 1951 with the fruit bat *Eidolon helvum* (Kerr) showed that 1/40 serum samples from bats collected in Kampala contained neutralizing antibodies to yellow fever virus, and circulation experiments with this virus detected in one instance a low level of viræmia[2]. We understand that recent work in the United States supports this finding.

In West Africa, Lagos bat virus was isolated from the brain of a fruit bat, *Eidolon helvum* (Kerr)[3], and recently two virus strains belonging to Casals's Group *A* and numerous strains belonging to Casals's Group *B* have been isolated from bats of the genus *Scotophilus* in Senegal[4–6].

In East Africa a Group *B* virus, Entebbe bat salivary gland virus, was isolated from *Tadarida* (*Chaerephon*) *limbata* (Peters) in 1957 (ref. 7). A continuation of this work on bats, which will be reported fully elsewhere, has during the past year led to a number of findings:

(1) Seven strains of two other Group *B* viruses and two further strains as yet unidentified have been isolated from the salivary glands of 479 bats belonging to the genus *Tadarida*.

(2) Hæmagglutination inhibitory antibodies to Group *B* virus antigens have been found in 91 out of 98 (93 per cent) acetone-extracted sera collected from *Tadarida* spp.

(3) Hæmagglutination inhibiting antibodies to chikungunya virus have been found in the serum of one bat and hæmagglutination inhibiting antibodies to Bunyamwera virus have been found in that of another.

(4) In the laboratory it has been shown that two species of the genus *Tadarida* will circulate Bunyamwera virus in titres greater than 3·0 log LD_{50} for at least 6 days. Yellow fever circulation experiments have so far been unsuccessful.

(5) Zika virus circulated in low titres in a bat of the genus *Rousettus*.

The findings suggest that there is an exceptional relationship between bats and Group *B* viruses, for we cannot find records of other apparently healthy vertebrates from which such a high rate of arbovirus isolation can be made and which at the same time show such a high percentage of antibodies. The view that the relationship between Group *B* viruses and bats is not an exceptional local occurrence is supported by the finding of antibodies neutralizing dengue virus in the sera of bats of the genus *Pteropus* in Australia[8] and antibodies neutralizing Tick-borne encephalitis virus in Europe[9]. Furthermore, in America the Group *B* Rio Bravo virus was isolated from bats in Texas[10] and a Group *B* virus has been isolated from bats in Montana[11]. That Group *B* viruses may be found circulating in the blood of bats and thus be available to infect hæmatophagous arthropods is shown by laboratory investigations with Japanese *B*, St. Louis and tick-borne encephalitis viruses[12–14].

The viræmia investigations with Bunyamwera virus indicate that bats may circulate arboviruses of another group. In this respect it appears of great interest that Downs *et al.* have recently reported the isolation from bats of a number of strains of Tacaribe virus, which complement fixation shows to be related to Argentinian hæmorrhagic fever (Junin) virus[15].

It is apparent, therefore, that bats are potentially of great importance in the wild-cycle of arboviruses and warrant further investigation on a wide international basis.

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Recovery of the Viral Antigen in a Cell by means of Double Radioactive Labelling

The use of isotopes has rendered possible the examination of virus development at the cellular level[1,2].

We have investigated the reproduction of the fowl plague virus (FPV) using the method of autoradiography. A double labelling with radioactive isotopes was used for the differentiation of the synthesis of viral RNA and proteins (antigens).

The strain Waybridge of FPV and dermo-muscular cells of human embryos were used. Trypsinized tissue cultures, 2×10^6 cells/ml. suspended in Medium 199 with 10 per cent of bovine serum, were placed on cover slips, 2 cm × 2 cm, and incubated at 37° C for 6 days, and then infected with FPV, 100 ID_{50} per cell. A rat serum with the hæmagglutination-inhibition titre 1 : 1,280 was labelled with iodine-131 according to the techniques of Francis *et al.*[3] and Liebster *et al.*[4], having used sodium salt of iodine, specific activity 6 μc./ml. γ-globulins were obtained by the rivanol method; their specific activity was 0·05 μc./ml.

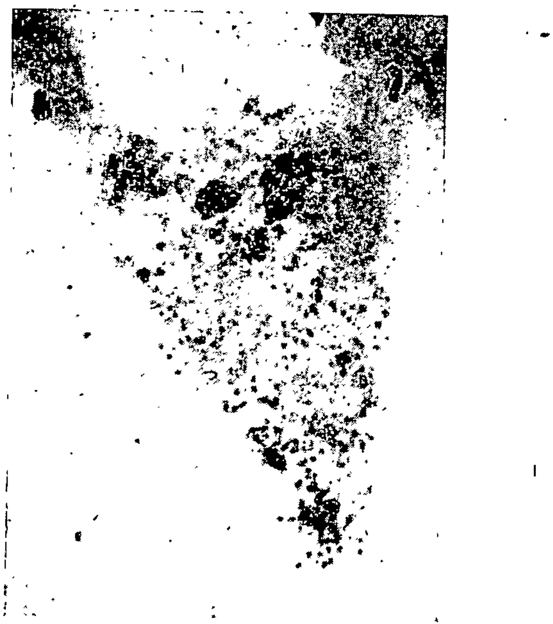

Fig. 1. Autoradiograph of the cell, infected with fowl plague virus

The preparations of the dermo-muscular tissue of human embryos were treated with the virus within 20 min at 4° C; residual virus was removed by triple washing in the cold Hanks's solution; then Medium 199 with 10 per cent serum and ^{14}C-adenine, 0·01 μc./ml., were added and preparations were incubated at 37° C. After exposure for 3 h, cells were washed with Hanks's solution, fixed in Carnoy's solution, covered with γ-globulin labelled with iodine-131, exposed for 20 min and then washed again. Then they were covered with film, type *MR*, and exposed with silica gel in the dark at 4° C for 7 days. Developed preparations were stained with methylene blue.

Fig. 1 demonstrates some results of the method of the double radioactive labelling. ^{14}C-adenine as nucleic acid precursor is presented as small points randomly scattered while γ-globulin labelled with iodine-131, blocking viral antigen, forms large autographs and conglomerates. So far as carbon-14 and iodine-131 have different energies of decay, it is possible to observe simultaneously the reproduction of viral nucleic acid and protein as autographs of different size in the same cell.

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Effect of Actinomycin *D* on Measles Virus Growth and Interferon Production

Actinomycin *D* combines *in vitro* with DNA in mammalian cells and results in impairment of DNA synthesis and of DNA-dependent RNA synthesis[1,2]. This results in uncoupling DNA from cellular protein synthesis.

Certain animal riboviruses (polio, mengo and Newcastle disease viruses (NDV)) have been shown to replicate in actinomycin *D* treated cultures, indicating their independence of cellular nucleic acid function[2,5].

Interferon production in cell cultures occurs in response to treatment of cells with foreign nucleic acids[6]. Because actinomycin *D* inhibits cellular RNA synthesis and hence protein synthesis, it would be expected to inhibit production of interferon.

Bukrinskaya and Zhdanov[7] demonstrated a decrease in the latent period of Sendai virus production in actinomycin *D* treated chick fibroblasts. They attempted to explain their results by suggesting that actinomycin *D* may have inhibited messenger-RNA of the cell, resulting in early direction of cellular enzymatic systems to virus production.

Treatment of chick fibroblasts with actinomycin *D* prior to inoculation with Chikungunya virus resulted in inhibition of interferon production accompanied by enhancement of viral growth[8,9]. Actinomycin *D* has also been shown to cause enhancement of growth of Semliki forest virus (SFV) and NDV (ref. 10).

This communication reports preliminary investigation of the effects of actinomycin *D* on production of interferon and virus titres in measles-infected chick fibroblast cultures.

*H.Ep.*2 cells infected with the 'Edmonston' strain of measles virus were disrupted by three cycles of freeze-thawing. This fluid, which after centrifugation and membrane filtration contained $10^{3 \cdot 7}$ plaque producing doses/ml., was used as inoculum for chick embryo fibroblast monolayers maintained at 37° in medium 199 with 5 per cent fœtal calf serum. After 8 days the whole cultures were disrupted as described and assayed for measles virus by plaque production in *H.Ep.*2 cell monolayers and, after high-speed centrifugation (120,000*g*), for interferon. The end-point of interferon titration was expressed as the reciprocal of the dilution causing 50 per cent inhibition of plaque formation by Sindbis virus in chick fibroblast monolayers.

Both yield of infectious virus and interferon production were compared from normal cultures and from cultures of cells pretreated with actinomycin *D* at a concentration of 0·01 μg/cm^2 of monolayer (0·1 μg/ml.) for 4 h.

Normal cultures yielded fewer than 10 infectious particles/ml. with an interferon titre of 12 while actinomycin-treated cells yielded $10^{2 \cdot 7}$ infectious particles with undetectable interferon production. These results support the view that actinomycin allows increased virus yield by inhibiting interferon production. Thus monolayers of a cell type normally showing a high degree of 'resistance' to measles virus and to which this virus must normally be 'adapted' by repeated passage are rendered more susceptible by suppression of interferon production.

Further experiments are now being carried out to characterize this interferon and examine production of measles interferon in other cell systems.

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Lipid in a Purified Preparation of Tomato Spotted Wilt Virus

Electron micrographs of tomato spotted wilt virus (TSWV), when stained by uranyl acetate or lead hydroxide, revealed the presence of an envelope surrounding an aggregate of several heavily staining spheroidal particles[1]. The envelopes stain only lightly with these reagents, suggesting that the envelopes contained lipid. Accordingly, a purified preparation of the virus was examined to test this hypothesis. By direct extraction with organic solvents the preparation was shown to contain about 20 per cent extractable lipid. Thin-layer chromatography of these extracts revealed a number of spots which gave a blue colour when treated with a molybdenum-based stain[2]. Although lipid has long been known to form part of some animal viruses there is no record of the presence of a lipid in a plant virus.

The virus was a sample of strain *E* of TSWV. The virus was extracted from systemically infected leaves of *Nicotiana glutinosa* by homogenizing them in the buffer solution at *p*H 7, removing non-viral material by alternate cycles of low- and high-speed centrifugation followed by two cycles of sucrose density-gradient centrifugation as described by Best and Palk[1]. The virus was identified in the light-scattering band of the density-gradient tubes by its infectivity and characteristic size and shape under the electron microscope[1]. After removing the sucrose and buffer salts the virus was freeze-dried and then stored over phosphorus pentoxide.

The dry virus was extracted for lipid by the micro-extraction method described by Thomas[3], using the apparatus described by Schaffer *et al.*[4]. Total lipid was determined by extracting 1- to 2-mg portions of the virus preparation in micro centrifuge tubes three times with 100-µl. portions of 1 : 1 chloroform–ethyl ether and two washings with 100-µl. lots of the methanol + ether solvent. The combined extracts and washings from each tube were evaporated under nitrogen to extract all the lipid. This was then extracted ten times with 50-µl. portions of petroleum ether. The solid extracted by petroleum–ether will be called lipid fraction *A*. The residue was also weighed and called lipid fraction *B*. Blanks were run on lipid-free samples of pure tobacco mosaic virus.

The efficiency of the method was tested by extracting known amounts of pure stearic acid. The percentage recovery in four trials using 200-µg samples was 96, 98, 100 and 95 respectively.

Table 1. LIPID CONTENT OF STRAIN *E* OF TOMATO SPOTTED WILT VIRUS

Wt. of virus (µg)	% lipid *A**	% lipid *B**	Total lipid (%)
1,019	8·6	10·3	18·9
1,008	9·1	10·6	19·7
1,066	9·2	n.d.	—
1,495	8·2	9·6	17·8
Mean value	8·8 ± 0·46	10·2 ± 0·55	

* *A*, petroleum-ether-extract. *B*, residue from methanol, chloroform, ether-extract after extraction with petroleum ether.

As shown in Table 1, the total extractable lipid amounted to about 19 per cent of the preparation, and was about equally divided between the petroleum-ether-extract and the residue from the methanol, chloroform, ether-extract.

Although the concentration of virus in the infected leaves is not high it was of interest to compare the lipid content of healthy and virus-infected leaves. Accordingly, on each of five occasions ten *N. glutinosa* plants were selected at random from a uniform batch. Five plants were inoculated with TSWV and the other five were kept as controls. About 3 days after they had developed symptoms the systemically invaded leaves were gathered. The corresponding leaves of the healthy control plants were gathered at the same time. Both lots of leaves were then homogenized and the lipid was extracted from aliquots and assayed by the method of Kates *et al.*[5]. The results in Table 2 show that the infected leaves contain a significantly higher percentage of lipid than the healthy controls. On a fresh-leaf basis the difference is 40 per cent, and on a dry-weight basis of leaves the difference is 10 per cent (the infected leaves having a higher dry-weight content than healthy ones).

Table 2. LIPID CONTENT OF HEALTHY LEAVES OF *Nicotiana glutinosa* AND OF LEAVES INFECTED WITH STRAIN *E* OF TOMATO SPOTTED WILT VIRUS

In all five experiments 5 g of leaves (fresh wt.) were extracted

Expt. No.	Dry wt. as % of fresh wt.		mg lipid per g fresh wt.		mg lipid per g dry wt.	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
1	—	—	8·1	14·2	—	—
2	7·5	8·9	8·1	10·6	108	119
3	7·5	8·8	8·2	11·4	109	118
4	9·0	10·8	9·2	12·8	103	119
5	8·0	8·7	8·5	10·1	107,	116
Mean			8·42	11·82	106·75	118·0
S.E.M.			0·21	0·76	1·315	0·707
Level of significance (probability based on '*t*' test)			1%		0·1%	

It is clear that the differences between healthy and virus-infected leaves is highly significant. The question which now arises is the extent to which the lipid we find in our preparations of TSWV is virus-specific. The electron micrographs of the purified preparations[1] strongly suggest that the lipid is located in the envelopes (as distinct from membranes) which encase several of the 50-mµ particles. The electron micrographs of sections through virus-containing lesions on inoculated leaves suggest that the virus may 'bud-off' from membranous structures of the host-cell cytoplasm[1]. It is, therefore, possible that the envelopes are derived from host material during the 'budding-off' process. In other experiments, the lipid extracted from TSWV preparations was compared qualitatively with extracts of material prepared from control leaves. A preparation was made from these healthy leaves, using differential- and density-gradient centrifugation as for the preparation of virus. A sample was withdrawn from the gradient tubes at the same depth as that from which the virus band was withdrawn. The material was freeze-dried, extracted with organic solvents in the way already described for the virus, and the extracts were run with the extracts from the virus on the same thin-layer chromatograms prepared according to Müldner *et al.*[6]. Using the molybdenum-containing stain of Wagner *et al.* several blue spots were obtained. Some of these were common to both virus and control extracts, but one spot was present only in the extract from the virus. The nature and significance of this are being investigated.

In view of the fact that this virus is transmitted in Nature by an insect in which it has an incubation period[7], it is not so surprising to find that it contains lipid.

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Multiplication of Rauscher Virus in Cultures of Mouse Kidney Cells

RAUSCHER virus induces a characteristic malignant hæmopathy when inoculated into adult or new-born mice of various strains, mainly that of *BALB/c* (ref. 1). In order to handle a system which would give a good production of virus *in vitro*, we cultured cells taken from various organs of animals inoculated with Rauscher virus several days previously. Among the organs tested, we obtained valuable results with cultures of young mice kidneys. The kidney always being spared during the course of Rauscher leukæmia, this production of virus is worth while mentioning.

New-born *BALB/c* mice were inoculated intraperitoneally with 0·1 ml. of an extract prepared according to Moloney's method[2] from spleens of *BALB/c* mice carrying Rauscher leukæmia in the erythroblastic stage. The titre of the inoculated extract varied from 10^4 to 10^6 ID_{50} per 0·1 ml. Animals were killed 6–10 days later. Kidneys were cut up with scissors, washed three times in Hanks's fluid, trypsinized for 2 h in a 0·5 per cent trypsin solution, *p*H 8·0, at 37° C, without stirring, and a strong manual agitation gave a cell suspension which was collected in a medium made of casein hydrolysate containing 15 per cent calf serum. Cultivation was performed in Kahn tubes with an initial inoculum of 5 × 10^5 cells per ml. of nutrient medium, which was changed twice a week. After 5–7 days at 37° C, a continuous cell monolayer was obtained. The following tests were performed: (1) daily observation of an eventual cytopathic effect; (2) weekly hæmagglutination tests with erythrocytes of *BALB/c* mice and of Wistar rats at 37° C, 4° C and 22° C; (3) weekly hæmadsorption tests on the same red blood cells at 4° C and 37° C; (4) weekly assay for an interfering activity using Sindbis virus (100 TCD_{50}/tube) as challenge; (5) every fortnight, search for an interferon activity with the supernatants, using as a test the action on the growth of Sindbis virus (100 TCD_{50}) in cultures of mouse fibroblasts; (6) at varying times, the supernatants or cell-supernatant mixtures, frozen and thawed three times, were inoculated (0·1 ml. intraperitoneally) into new-born mice and these animals were examined each week, in order to detect a splenomegaly.

The kidneys of healthy animals were cultured and tested, following the same procedure.

Moreover, the *in vitro* inactivation of Rauscher virus was examined by inoculating new-born mice with virus incubated alone in the culture medium at 37° C, or in the presence of cells in which we had obtained no multiplication (*KB*).

With kidney cultures originating from mice inoculated with Rauscher virus, no cytopathic effect has been observed, and the examination of hæmatoxylin–eosin stained cover-slips in Leighton tubes confirmed this finding. No hæmagglutinating or hæmadsorbing activity could be detected. The existence of interferon in the supernatants was never demonstrable and no interfering activity on the growth of Sindbis virus was observed. On the other hand, bioassays proved the permanent presence of Rauscher virus in the cultures.

Table 1 gives the details of one among the six experiments performed.

Table 1. INOCULATION OF NEW-BORN *BALB/c* MICE WITH SUPERNATANTS OF INFECTED KIDNEY CULTURES

Time of sampling after initiation of the culture (days)	No. of injected animals surviving weaning	No. of leukæmias	%	Latency (days)	Average latency (days)
14th	21	18	85	41–181	86
32nd	9	9	100	55–127	106
42nd	10	10	100	54–115	98
57th	10	10	100	90–120	103
71st	5	5	100	72–120	98

In nearly all the animals inoculated with material originating from these cultures and sampled between the 7th and 71st day, an erythroblastic proliferation in the spleen and liver, typical of the first stage of the Rauscher disease, was induced.

However, it may be noticed that intrasplenic hæmorrhages were rare, leading to a diminished early death-rate and allowing the apparition of late generalized leukæmias, the cytological type of which was erythroblastic, myeloid or undifferentiated. Two choroleukæmias have been observed, but no lymphoid leukæmia has appeared.

Myeloid leukæmia is not usually described as part of the Rauscher disease but was very frequent in our experiments[3]. We think that one cannot link this fact to the action of any virus other than the Rauscher virus, because antisera prepared either by Dr. Rauscher or in our laboratory cross-neutralize the virus.

Kidney cultures of non-inoculated *BALB/c* mice, examined in the same manner, never produced virus as revealed by the bioassay. Inoculation of young mice with virus incubated in the presence of *KB* cells or in the culture medium alone at 37° C showed that the virus is completely inactivated within 24 h.

These results demonstrate that Rauscher virus actively multiplies in cultures of mice kidney cells. The presence of a residual virus seems very unlikely because of the considerable dilution realized by the renewals of the culture medium (as many as twenty changes before the final inoculation) and by the previously demonstrated conditions of virus inactivation. The type of the disease induced and the relatively long latency period indicate that the amount of virus in the cultures is fairly small, at least 2 log 10 less than in the plasma of leukæmic mice. It is of interest to note that the *in vitro* inoculation with Rauscher virus of cultures of healthy mouse kidneys (still in progress) seems to give much more irregular results.

Rauscher virus, therefore, multiplies in kidney cells as early as 7 days after the inoculation of the animal and can be cultivated in these cells for prolonged periods, although this non-hæmopoietic organ does not show any pathological alteration during the course of Rauscher leukæmia. This phenomenon shows once again that leukæmia viruses grow in many different types of cells and induce a transformation in only some of them.

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GENETICS

Quick Preparation of Corolla Chromosomes from Flower Buds

THE standard practice of obtaining somatic chromosomes from root-tips[1] for counting is often inconvenient, particularly for species in the wild and for perennials in the field. In grafted species, for example, cultivated top-fruit clones, it is impractical to use root-tips and it may be necessary to obtain counts from meioses in pollen mother cells. Several methods are available, however, for examining chromosomes from other meristematic tissues[2] such as young leaves from vegetative tips and stipules, but these do not always prove satisfactory. Burns[3] has recently provided a useful method for making preparations of somatic chromosomes from corollas in *Nicotiana* spp. Her schedule is somewhat lengthy, and the prepared slides are not ready for examination for three days. We have been able to shorten this schedule, and have found the modifications satisfactory with the several genera tested. The abridged technique successfully used for rapidly preparing chromosomes in corolla tissue is given here.

Margins and bases of unexpanded corollas are the most active regions, and buds which are too large must not be taken. Petals from open flowers do not show divisions. Buds 2–3 mm in size are taken in the mornings, and the green corollas are dissected out and placed in a tube containing a few ml. of 8-hydroxyquinoline solution (0·438 g in 1 l. distilled water) to which a drop of saturated maltose is added. This pretreatment should not be allowed to rise above room temperature. The minimum time for pre-treatment is 2 h, but this depends on the material and may be increased to 4 h. The corollas are fixed in modified Carnoy solution (6 parts 95 per cent alcohol : 3 parts chloroform; 1 part glacial acetic acid) which must be freshly prepared for each batch of tissue. A suitable time for satisfactory fixation is 30 min; but the material can be stored in this solution without harm for several weeks at 2° C. Hydrolysis is carried out in a solution made from 2 parts of 1 N hydrochloric acid and 3 parts of the fixative for not more than 5 min at 60° C. Staining is carried out on a slide using lacto-propiono-orcein[4]. Corollas are left on the slide for 2 min with two drops of the stain, heated gently and squashed in the usual manner. Should the chromosomes appear to be clumped together they may be spread by flooding the squash with 45 per cent acetic acid and re-tapping and squashing. If the material is over-stained, de-staining of the cytoplasm can be carried out on the slide using several drops of 45 per cent acetic acid. The chromosomes are very clearly seen under both normal and phase-contrast microscopes[5]. It is possible to count and to photograph selected mitotic plates within 5 h from the commencement of this procedure. Permanent preparations can be made in the usual way[2].

Among the genera and families in which this method has proved successful are *Nicotiana* and cultivated tomato (Solanaceae), *Ribes* (Grossulariaceae), *Rubus*, *Fragaria* and *Malus* (Rosaceae), *Brassica* and radish (Cruciferae) and *Daucus* (Umbelliferae). It was found advisable in *Daucus* to fix portions of the umbel without prior removal of buds and dissection of the tiny corollas. Heterochromatic regions were readily seen in this material following cold treatment of the plants. A particular advantage of making chromosome counts from corollas is seen following the treatment of somatic tissues with colchicine to induce polyploidy. Thus it was possible to confirm the chromosome number $2n = 34$ in the diploid apple variety 'Spartan' (from flower buds collected on a dull, wet day); but a strain of this variety received as a colchicine-induced tetraploid proved also to be diploid, so confirming a previous suspicion deduced from floral morphology and stamen number.

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[1] Darlington, C. D., and LaCour, L. F., *The Handling of Chromosomes* (Allen and Unwin, London, 1960).

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Polymorphism in the Serum Proteins of the Reindeer

Polymorphism in the serum proteins of the reindeer was detected in 1959 (unpublished results) in samples collected by me and investigated by Dr. B. Cohen, then at the University of Edinburgh. Gahne and Rendel[1] reported the findings of six different transferrin types which they explained on a basis of three alleles.

In this communication results from investigations of serum samples from 132 reindeer are reported. Of these, 100 came from the district of Jotunheimen and 32 came from northern Norway. The samples were investigated by two different techniques. One was that used for examining horse transferrins[2]: the other was that developed by Kristjansson[3] for cattle transferrins. The two methods led to the same conclusions; but Kristjansson's technique gave more distinct zones. Accordingly, results obtained by using this last-mentioned technique were photographed for demonstration in the present report.

The reindeer sera investigated in this work showed great variation in the β-globulins. Two, three or four clearly recognizable zones or bands were seen. They appeared in a total of 15 different phenotypes of which four were two-band and eleven were three or four bands. To account for these various patterns a hypothesis of six codominant autosomal alleles was advanced, each allele being responsible for two bands. The products of the individual alleles were named: *C*, *E*, *G*, *I*, *K* and *M*, the fastest migrating being *C* and the slowest *M*. In all these six two-band patterns the slowest band was generally considerably thicker and stained more heavily than the fast band. The following combinations of the two-band patterns were encountered: *CC*, *CE*, *CG*, *CI*, *CK*, *CM*, *EE*, *EG*, *EI*, *EK*, *EM*, *GI*, *II*, *IK* and *KK*. Most common were *C* and *E* types, most rare were the *G* and *M* patterns.

In Fig. 1, which is a photograph of a stained gel, the results from investigation of eight selected serum samples are shown. The positions of the albumins, the α- and the β-globulins are marked on the left of the photograph. Because of the special electrophoretic conditions the albumins are condensed into a thick, heavily stained line going across the gel. The α-globulins are thin, indistinct, curved bands. Three of the samples are from animals being homozygous as to β-globulin types. They are *CC*, *EE* and *II*. The five other samples all show heterozygous animals. Phenotypes representing the remaining two-band patterns *G*, *K* and *M* are seen in all of them. The migration rates for the six two-band patterns are clearly different, the positions of the *G* bands, however, being only slightly slower than the *E* bands under the electrophoretic conditions used in these investigations. There is some indication that the *C* bands actually are of two types, the one, which is rare, being insignificantly slower than the common *C* type.

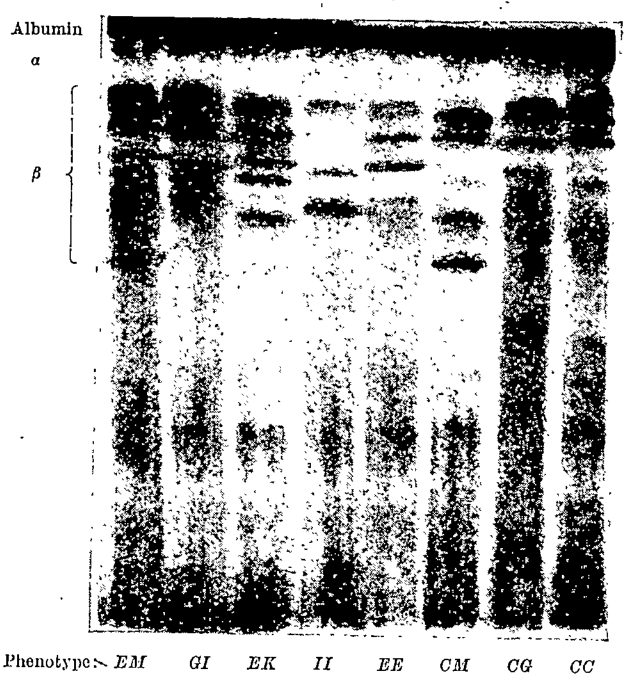

Fig. 1. Photograph of a stained gel showing eight selected reindeer transferrin phenotypes

Besides these different bands in the β-globulin region other but more faint bands or zones may be seen on the photograph, for example, in the samples showing the phenotypes *CC*, *CM* and *EM*. In the first sample there is a faint band in front of the *C* bands. In the two other phenotypes faint bands may be seen between the fastest and slowest two-band patterns of the respective phenotypes. Whether these faint bands belong to the same protein system as those previously mentioned is not known. The occurrence and relative positions of some of these very faint bands in the β-globulin region may, however, indicate that they belong to the same protein system as that already described. This would then suggest that each allele is responsible for three bands or zones.

The serum proteins described by Gahne and Rendel[1] were reported to be transferrins. The proteins dealt with in this work are of a similar type. It is, therefore, concluded that they are transferrins. Accordingly, a genetic theory of six transferrin alleles, Tf^C, Tf^E, Tf^G, Tf^I, Tf^K and Tf^M, is proposed.

I thank Mr. I. Godal, district veterinary officer at Lom, and Mr. S. Skjenneberg, veterinary investigation officer at the State Veterinary Laboratory, Harstad, for their help in securing the samples.

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[2] Brænd, M., and Stormont, C., *Nord. Vet.-Med.*, 16, 31 (1964).

[3] Kristjansson, F. K. (personal communication).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Assistant (inclined towards theoretical physics, with a university degree or diploma in technology with first- or second-class honours) in the Department of Mathematics, to work in the field of absolute rates of electrode processes and associated potential distributions—The Principal, Lanchester College of Technology, Priory Street, Coventry (August 14).

Graduate Chemist or Biochemist to work in the metalloprotein field—The Secretary, Institute of Neurology, The National Hospital, Queen Square, London, W.C.1 (August 15).

Lecturer or Assistant Lecturer (with a good honours degree in civil engineering and good general training and experience, and preferably a knowledge or special experience in surveying and photogrammetry) in the Department of Civil Engineering—The Registrar, The University, Leeds, 2 (August 17).

Research Assistant (veterinary or pure science graduate) in Reproduction for research into problems of male fertility—The Registrar, The University, Liverpool, quoting Ref. CV/25/N (August 17).

Research Assistant (with a degree in one of the social sciences) in the Faculty of Social Sciences—The Secretary and Registrar, The University, Southampton (August 17).

Research Assistant in the Department of Sociology—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (August 20).

Lecturer or Assistant Lecturer in the Department of Statistics, to lecture in probability theory or mathematical statistics and carry out research—The Registrar, The University, Sheffield (August 22).

Lecturer (with experience in one or more of the following fields: numerical analysis, systems programming, engineering aspects of computer design including memory systems and electronic circuits) in the Department of Computer Science—The Registrar, The University, Manchester, 13 (August 22).

Research Assistant (graduate with research experience who may read for a higher degree, or with a Ph.D.) in the Department of Physics to work on the application of electron microscopy to the study of ferromagnetic domains—The Registrar, The University, Sheffield (August 22).

Assistant (graduate in pure science, agriculture, or agricultural economics or economics) to the Principal—The Secretary, The North of Scotland College of Agriculture, Crown Mansions, 41½ Union Street, Aberdeen, Scotland (August 24).

Lecturers (2) in the Department of Physiology, University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (August 24).

Assistant Lecturer (Grade B) in Physics—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (August 27).

Assistant Lecturer in Zoology—The Secretary, The Queen's University, Belfast, Northern Ireland (August 28).

Lecturer in Surgery—The Secretary, The University, Aberdeen (August 28).

Technician (with one of the following qualifications: Ordinary National Certificate in chemistry or equivalent qualifications preferably including chemistry at "A" level) in the Botany Department, to assist with investigations—Prof. D. J. Carr, Department of Botany, The Queen's University, Belfast, Northern Ireland (August 28).

University Librarian—The Registrar, The University, Manchester (August 28).

Assistant Lecturer or Lecturer in Geography—The Assistant Registrar (Arts and Law), The University of Birmingham, Birmingham, 15 (August 31).

Demonstrator in Psychology—The Registrar, The University, Keele, Staffordshire (August 31).

Lecturer (preferably with a veterinary qualification and interested in chemical pathology) in the Department of Pathology—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (August 31).

Lecturer (with an honours degree and experience in the design and use of systems involving a digital computer or in some branch of control engineering) in the Department of Electrical Engineering—The Registrar, The University, Leeds, 2 (August 31).

Lecturer (with at least an honours degree or equivalent qualifications, a good general background and postgraduate research experience in some major field of botany or zoology) in Botany or Zoology in the School of Biological Sciences, University of New South Wales, Sydney—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (August 31).

Research Assistant (with a higher degree or equivalent experience) in the Department of Mechanical Engineering, for an investigation of the use of shock tube techniques in the study of non-equilibrium ionization phenomena—The Secretary, University College, Gower Street, London, W.C.1 (August 31).

Senior Lecturer or Lecturer (preferably with special interests, teaching and research experience and/or research qualifications in one or more of the following fields: protozoology; arthropoda other than insecta; herpetology; mammalia; genetics; cytology or freshwater biology) in the Department of Zoology, Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (August 31).

Lecturer (with appropriate experience in research and teaching) in Physiology at the University of Hong Kong—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, September 5).

Scientific Officer, Zoologist (preferably with an honours degree in zoology) in the Animal Ecology Division Department of Scientific and Industrial Research, New Zealand, to join a small research team working mainly on the basic ecology of ungulates and other introduced mammals in New Zealand—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, quoting Ref. B13/18/26/2651 (September 5).

Staff Tutor in Social Science (with special reference to community tudies) in the Department of Extra-Mural Studies—The Registrar, The University, Liverpool, quoting Ref. CV/249 (September 6).

Assistant Keeper (with an appropriate degree, at least one year's relevant postgraduate experience, and a special knowledge of geology, preferably petrology or mineralogy) in the Department of Geology—The Director, Museum and Art Gallery, New Walk, Leicester (September 7).

Assistant Lecturer (with a degree in mathematics or the physical sciences, and preferably some postgraduate experience) in Computing—The Registrar, University College of North Wales, Bangor, North Wales (September 7).

Lecturer in Chemistry and a Lecturer in Zoology at Njala University College, Njala, Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 10).

Senior Lecturer or Lecturer in the Department of Materia Medica and Therapeutics—The Secretary, The University, Aberdeen (September 12).

Stanley Elmore Senior Research Scholar in the Medical Sciences for research in medicine, preferably in the fields of nutrition or fœtal and neonatal physiology—The Master or Prof. R. A. McCance, Sidney Sussex College, Cambridge (September 14).

Professor of Biochemistry—The Secretary and Bursar, University College, Dublin, Republic of Ireland (September 15).

Chair of Physics as Applied to Medicine at the Institute of Cancer Research—The Academic Registrar, University of London, Senate House, London, W.C.1 (September 23).

Chair of Psychology in the Faculty of Science—The Registrar, The University of Birmingham, Birmingham, 15 (September 25).

Reader in Human Genetics—The Registrar, University of Newcastle upon Tyne, Kensington Terrace, Newcastle upon Tyne, 2 (September 26).

Director of the Closed Circuit Television Service whose responsibilities will include development, organization and administration of the service, interesting and advising academic staff in the uses of television and investigating its place in university education—The Registrar, The University, Leeds, 2 (September 30).

Lecturer (with an appropriate honours degree or equivalent qualifications) in the School of Mechanical Engineering at the University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (September 30).

Professor of Organic Chemistry—The Registrar (Room 22, O.R.B.), The University, Reading (October 24).

Lecturer (with an honours degree in optometry or equivalent qualifications, some clinical experience, and preferably some teaching and/or research experience) in the Department of Optometry, School of Physics, The University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (October 31).

Bacteriological Technicians (preferably with experience in handling laboratory animals) to assist in research in food microbiology, including studies on clostridial spores and the application of new enzymic and serological techniques to the detection of pathogenic bacteria in foods—The Director, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey.

Botanist or Chemist (with a second-class honours degree and interested in plant-soil nutrition)—Reader in Soil Science, Department of Agriculture, The University, Oxford.

Chair of Organic Chemistry—The Registrar, The University, Senate House, Bristol 2.

Chair of Zoology—The Registrar, The University, Senate House, Bristol 2.

Chemist or Biochemist (preferably with experience of analytical techniques) to join a team working on human fertility and the causes of fœtal abnormalities—Prof. J. M. Robson, Pharmacology Department, Guy's Hospital Medical School, London Bridge, London, S.E.1.

Experienced Female Technician for a research unit—The Administrator, Department of Biochemistry, The University, South Parks Road, Oxford.

Graduate (preferably with experience of endocrinology) to join a team doing research on mammalian fertility and anti-fertility drugs—Prof. J. M. Robson, Pharmacology Department, Guy's Hospital Medical School, London Bridge, London, S.E.1.

Junior Scientist (with a good honours degree and a biochemical qualification) for the study of protozoal and helminthic parasites of importance to tropical medicine—The Director, National Institute for Medical Research, Mill Hill, London, N.W.7.

Lecturers or Assistant Lecturers (2) (well qualified academically and preferably some teaching, together with research or industrial experience) in Mathematics—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 12/2/G.

Nematologist (national of the United Kingdom or the Republic of Ireland, with an honours degree in zoology or natural science, plus one year's postgraduate training or experience, preferably in the study of nematodes) in Kenya, to carry out research on nematology in relation to crop husbandry—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/95/03.

Physicist (female graduate) to operate an electron probe microanalyser as a service for various research programmes in the Department—J. A. Charles, M.A., B.Sc., Department of Metallurgy, The University, Pembroke Street, Cambridge.

Physiological or Biochemical Worker for investigating electrical and ionic phenomena in tissues from the brain (micro-electrode and electrophysiological techniques involved)—The Secretary, Institute of Psychiatry, Maudsley Hospital, Denmark Hill, London,-S.E.5.

Plant Breeder (with a degree in botany or genetics, and preferably postgraduate experience in plant breeding) in Northern Rhodesia, to carry through breeding programmes mainly with maize, wheat or ground-nuts, and to produce varieties adapted to Northern Rhodesian conditions—The Appointments Officer, Department of Technical Co-operation, Room 301, Eland Place, Stag Place, London, S.W.1, quoting Ref. RC 213/132/05.

Research Assistant (graduate in microbiology or the biological sciences) in Microbiology, to work on either the production and utilization of algae, or some aspect of food microbiology—Head of the Department of Biological and Health Studies, Battersea College of Technology, London, S.W.11.

Research Assistant (with a Ph.D. or equivalent research experience, and some knowledge of electronics) with a group centred at Silwood Park, near Ascot, working on rocket measurements of magnetic fields in the ionosphere, currently concerned with Centaur and Skylark rockets launched from Woomera and Andöya Is.—Geophysics Department, Imperial College, London, S.W.7.

Research Fellow in the Department of Electrical Engineering for studies in the synthesis of lumped linear electrical networks—The Registrar, The University, Leicester.

Research Fellow (with a good honours degree) in the Physics Department of the United Birmingham Hospitals, to investigate the physical aspects of medical ultrasonics—The Chief Physicist, Queen Elizabeth Hospital, Birmingham 15.

Research Physicist (with a Ph.D. degree or expecting to submit a thesis shortly) in Vacuum Spectroscopy for research which involves the measurement of absorption cross-sections in the vacuum ultra-violet (2000 Å to

400 Å)—The Professor of Physics, J. J. Thomson Physical Laboratory, The University, Whiteknights Park, Shinfield Road, Reading, Berkshire.

SCIENCE GRADUATE BACTERIOLOGIST (Basic or Senior Grade) (with an honours degree) on the staff of the Pathology Department—The Secretary, Birmingham (Selly Oak) H.M.C., Oak Tree Lane, Birmingham, 29.

SENIOR RESEARCH ASSISTANT IN ORGANIC CHEMISTRY, to work on new syntheses of porphyrins with Prof. G. W. Kenner—The Registrar, The University, Liverpool, quoting Ref. CV/243/N.

SENIOR ZOOLOGIST (national of the United Kingdom or the Republic of Ireland, an honours degree in zoology or equivalent, with research or other experience in zoology) IN THE VETERINARY DEPARTMENT, Kenya, for investigations into the treatment of trypanosomiasis, research work in immunity both in the laboratory and in the field, and to give advice on drug regimes in the various areas of the country—The Appointments Officer, Department of Technical Co-operation, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 217/95/04.

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Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 97: Basic Intrusions of the Queen Elizabeth Islands, District of Franklin. By R. G. Blackadar. Pp. 36. 1.30 dollars. Bulletin 99: Surficial Geology of East-Central District of Mackenzie. By B. G. Craig. Pp. viii+41 (13 plates). 1.30 dollars. Paper 63–44: Surficial Geology of Boothia Peninsula, and Somerset, King William, and Prince of Wales Islands, District of Franklin. By B. G. Craig. Pp. 10+map. 75 cents. Paper 63–48: Sedimentology of Hudson Bay, District of Keewatin. By R. J. Leslie. Pp. 31. 35 cents. Paper 64–6: Illustrations of Canadian Fossils—Early Lower Cretaceous (Berriasian and Valanginian) of the Canadian Western Cordillera, British Columbia. By J. A. Jeletzky. Pp. 19 (8 plates). (Ottawa: Queen's Printer, 1964.) [106

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EDUCATION AND MAN-POWER IN EAST AFRICA

THE basic problem of developing countries is to promote economic development to raise their standards of living. In the past decade or so, there has been a remarkable change from a belief that this can best be achieved by the classical method of heavy capital investment to a belief that investment in people through the development of 'human capital' may provide a surer means of achieving this end. The main instrument of such development is seen to be education. The arguments for such a change have been partly economic, but partly also political. For example, post-war American aid to Latin America has taught the United States a sharp lesson that capital investment and aid for economic development do not necessarily mean raised standards of living for the majority of the population of an emergent country, nor do they necessarily stop the growth of Communism—often, indeed, the reverse may be the case. The United Nations Educational, Scientific and Cultural Organization has preached the doctrine (though on what basis it has never been quite clear) that education is a basic human right to which all people are entitled. The people themselves of the developing countries have become utterly persuaded that, more than anything else, education holds the key to their future prosperity and progress. As if to confirm their belief, some of the older countries, more particularly Britain, have in late years also become converted to a similar philosophy on their own domestic scenes.

Education in developing countries, therefore, is now a field of considerable importance and the subject of much thought and planning. Even so, the literature on it is surprisingly sparse, and nowhere is this more so than on the man-power and planning aspects. This makes particularly welcome a recent book giving a detailed case-study of educational opportunity in a developing area, namely, East Africa*. The study represents, in fact, the first stage of a larger project undertaken by Political and Economic Planning with the aid of a grant from the Ford Foundation of America, the main purpose of which was to analyse the conditions which oversea students (particularly Africans) meet during their studies in Britain; the degree to which their courses fulfil their needs and aspirations, and some of the difficulties involved in providing a bigger flow of British teachers to overseas countries. In the words of Mr. Guy Hunter, its author, it is "a detailed case-study of the actual day-to-day problems of educational opportunity in a particular area—the stubborn facts from which wider strategy must be planned". As such, it is most likely to interest the educational planner, but there is also much food for thought in it for all who take an interest in the East African scene.

Several problems stand out starkly in this book, but in no case can they be described as purely East African problems. They are common to the whole of former British Tropical Africa. Undoubtedly, the most important is what is to become of the vast numbers of schoolchildren who will finish their primary education in the next few years and for whom no secondary schooling will be available. After independence, some African countries introduced universal primary education, but all, including the East African countries, had greatly increased the provision of primary school places even before that event. In future years these hordes of frustrated children leaving primary school may well constitute potentially the biggest source in their countries of social and indeed political unrest. However, it is not likely that this problem will be seen by East African Governments as their major educational priority; this will undoubtedly be reserved for secondary education. While the most optimistic forecasts in East Africa over the next few years envisage the possibility of secondary education for 10 per cent of the primary school population, the reality is much more likely to be nearer 6 per cent, a figure which is totally inadequate to meet the higher man-power needs of these newly independent countries. There is no doubt that East African Governments see as their main problem that of replacing expatriates in the public services and obtaining enough local people at least to take a hand in the private sector of the economy and to direct and administer their programmes of economic development. For them therefore above all it is the output of the secondary schools which matters—it is those schools which will produce the teachers which will make the further expansion of secondary education possible and also provide the future agricultural extension workers for the development of agriculture, East Africa's basic industry. So pitifully inadequate will the output of secondary schools be until 1966 that it will not meet even wastage and the lowest economic growth rate over the present. In Kenya in 1961, for example, 182,227 children were enrolled in the first year of the primary school. In the same year only 1,054 were in the fourth form (School Certificate year) of the secondary school. Of these only 540 sat the School Certificate examination and far less passed. The greatest scarcity is undoubtedly that of qualified secondary school teachers, particularly scientists, and the shortfall here will have to be made good by the recruitment on contract of expatriate 'experts', especially to fill the gaps created by the recruitment of African graduates for other spheres of the national life than teaching. Supplying such temporary graduate teachers may well be the greatest contribution that Britain can make at this stage towards helping Kenya, Uganda and Tanganyika in solving its urgent man-power problems.

The greatest problem of all in East Africa, however—and Mr. Hunter's greatest dread—is the possible lowering of educational standards, more particularly at the secondary school and university levels, through indiscriminate and unbalanced expansion. If standards fall at the secondary level, fewer and fewer people will secure the necessary entry qualifications for admission to universities abroad (especially in Britain) or at home, and this could have a disastrous effect on top-level administration and the public service. However, if present standards are maintained the output of the system is patently inadequate to meet present or future needs. The answer, he believes, is to make better use of those post-School Certificate candidates who do not obtain the necessary qualifications to proceed to a university, and at the same time to upgrade after training those people with experience but lacking the paper qualifications who have demonstrated their ability in the various aspects of the national life. A recent educational tour of East Africa showed that Mr. Hunter's worst fears in regard to secondary education are, in fact, already coming true, more particularly in Kenya. There, despite the acute shortage of qualified teachers, and technical facilities such as adequate science laboratories, local government authorities are defying advice from the central government and are setting up —primarily for prestige reasons—their own local, one-stream and inefficient secondary schools. There is every indication that in East Africa this tendency will grow; if it does, educational standards will inevitably fall.

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* *Education for a Developing Region; A Study in East Africa.* By Guy Hunter. Pp. xvi+119. (London: George Allen and Unwin Ltd., for Political and Economic Planning and the Institute of Race Relations. 1963.) 20*s*. net.

Mr. Hunter's solution to the problem of making the greatest use, from a man-power point of view, of post-School Certificate candidates who do not proceed to a university but who enter the economy directly without training is much more debatable. In many developing countries in the Commonwealth novel solutions to the problems of such training and higher education have been devised by the setting up of institutions which owe much to the American idea of the junior college and the liberal arts college. Mr. Hunter sees in such institutions his dreaded bogy of lowered educational standards and he will have none of them. Instead, he would provide diploma and up-grading training in already existing institutions. He is led, equally, to defend the institution of the sixth form, which would inevitably suffer if such new post-School Certificate colleges were set up, since their entry qualification is often a reasonable grade of School Certificate or five Ordinary Level passes in the General Certificate of Education. Senior African opinion in East Africa is strongly in favour of the junior college idea, and African administrators, with hard-headed realism, feel that such a college could do far more to supply diversified training to feed the needs of the economy than the present system. Moreover, the University of East Africa has recently invited the former registrar of the University of the West Indies, a man with much experience of the college idea, to advise it on this matter. The recent report on university education in Malawi has recommended a university which recruits at Ordinary Level or School Certificate, as also has the Lockwood Report on the Development of a University in Northern Rhodesia, a report which has already been accepted by that Government. It is scarcely likely, therefore, that East Africa will depart from such a general pattern, in spite of Mr. Hunter's persuasive arguments. Perhaps the answer to his dilemma is for East Africa to adopt the West Indian model of a post-School Certificate college where diploma-type courses in a variety of subjects will be provided for those who do not have the ability to proceed further to take a degree, while, at the same time, the door is kept open for the later transfer to a university for those who do.

The recent Commonwealth Prime Ministers' Conference in London, with Sir Alexander Douglas-Home's suggestion for further United Kingdom co-operation and assistance in the field of higher education, highlights the importance of many of the themes Mr. Hunter discusses in this book. For this reason it should be read by all who are interested in the development of education, not only in Africa, but also in the younger countries of the Commonwealth.

THE ENERGY OF SCIENCE

Little Science, Big Science

By Derek J. De Solla Price. Pp. 119. (New York and London: Columbia University Press, 1963.) 33*s.* 6*d.*; 4.50 dollars.

THIS is a small but very important book. Based on the George B. Pegram Lectures at Brookhaven National Laboratory (June 19–29, 1962), it contains essays based on one guiding idea—to apply scientific method to the whole structure of modern science itself and thus help to create the new science of science, based not on abstract theory but on actual observations.

Price is attempting to do for science what the pioneers Petty and Graunt tried to do for economics and population in the seventeenth century with a *Political Arithmetic* and *Remarks on the Bills of Mortality*. But, as science deals not so much with heads of population or pounds of goods but with men's intelligence, the science of science also involves a study of that distribution of intelligence which was begun in the nineteenth century by Galton. In modern science numerical analysis can show that, however much individual initiative and genius play in the details of scientific discovery, the whole fabric of science compounds these different initiatives and produces, by a kind of statistical mechanics, ascertainable laws of growth which can be observed in the past and projected into the future. This is, of course, only one aspect of a science of science. It furnishes limiting conditions, size and rate of growth of science as a whole. Price touches also on the other aspects in his latter chapters: the effects of science on social and economic development; the resulting effects on the scientists themselves.

The growth of science in numbers and in cost has been so spectacular in the past twenty years that it appears to be qualitatively different from anything that has gone before. Price is at pains to point out that this is an appearance which any rapidly growing human activity would present at any point in its history. We are informed that 80–90 per cent of all scientists who have ever lived in the world are alive now. This, he points out, would have been just as true in 1900 as it is to-day; but this now presents a qualitative difference—we now feel we are in the new period of "Big Science". The quantitative growth that preceded its appearance has been overlooked in the mass of the monster we have created. The image of the 'little scientist' of the past is romanticized as that of the 'big scientist' of the present is being magnified and coarsened. According to Price:

> ". . . the transition from Little Science to Big Science was less dramatic and more gradual than appears at first. For one thing, it is clear that Little Science contained many elements that were grandiose. And, tucked away in some academic corners, modern Big Science probably contains shoestring operations by unknown pioneers who are starting lines of research that will be of decisive interest by 1975." (P. 3.)

But the essential feature both of 'little science' and 'big science' is the very rapid exponential growth, greater than that of population and that of the economy, doubling every 10–15 years.

This we have long known: where Price breaks new ground is by relating it to other factors particularly the quality, the supply of scientists and the cost of science. Exponential growth measured by the rate of production of scientific publications has scarcely altered even after the great catastrophes of our time. Wars and economic depressions set the curve back by about five to ten years. Then it goes on rising along the same line. Thus, Price has pointed out elsewhere in *Science Since Babylon* (New Haven, Yale University Press, 1961), that exponential rate of growth would produce more scientists than people within two centuries, and this is not going to happen.

Until now the exponential rate has been well maintained. But Price points out that a closer analysis shows that in the advanced countries it is already effectively at an end. The character of any exponential growth in a natural system is to turn over into a so-called logistic curve, an S-shaped curve rising to a certain saturation maximum. He musters evidence to show that the turning point is not somewhere in the near or far future but, for the advanced countries, has already arrived. The symptoms of this are the occurrence of sudden fluctuations; the rising curve is only maintained by bursts and escalations, such as those which occurred in the development of high-energy particle accelerators. Ultimately it is checked by the apparent shortage of trained workers needed for the largest scale of operations.

> "Such an analysis seems to imply that the state called Big Science actually marks the onset of those new conditions that will break the tradition of centuries and give rise to new escalations, violent huntings, redefinitions of our basic terms, and all the other phenomena associated with the upper limit. I will suggest that at some time, undetermined as yet but probably during the 1940's or 1950's, we passed through the mid-period in general logistic growth of science's body politic." (P. 31.)

Here it seems to me that Price is putting forward extremely interesting but not altogether well-founded conclusions.

Going back to Galton, he further considers the distribution of eminence among scientists, the distribution of which, according to a law due to Lotka, is as the square root of the whole number. This law fits very closely the distribution of ability of scientists using the somewhat suspect criterion—but the only one available—of the number of papers published in a working life. He concludes that for a hundred authors publishing only one paper, there will be two who will produce one-quarter of the papers, ten will produce half and the rest will produce the last quarter. This would seem to discount the effect of the single shot genius, while it magnifies the influence of the number of dullards with an itch to write. Nevertheless, it may be quite adequate for statistical purposes. It would not be far wrong to say that the number of average men goes up as the square of the good ones. So that the number of scientists, which doubles every ten years, corresponds to the number of noteworthy scientists who double only every twenty. The number of potential scientists gauged as Ph.D.s shows some relation to the distribution of intelligence quotients of the population. In the United States, where education for the most intelligent is most widespread though by no means ideal, for one Ph.D. in every 300 of the population, intelligence quotients range from 140·3 for physicists down to 123·3 for educationalists. This is essentially prestige selection: education is a much more difficult as well as more essential field than physics.

At that point Price goes over to the consideration of the even or uneven distribution of scientists, particularly good scientists:

> ". . . science displays in several ways a tendency to crystallize out, in the sense that big things grow at the expense of the small ones that constitute a sort of mother liquor. Large fields seem to absorb the manpower and subject matter of small ones. Even though new fields, new departments, new institutions, and even new countries arrive on the scientific scene in increasing number, the few previously existing large ones have a natural growth enabling them in general to maintain their lead." (Pp. 56–7.)

> "About this process there is the same sort of essential, built-in *undemocracy* that gives us a nation of cities rather than a country steadily approximating a state of uniform population density. Scientists tend to congregate in fields, in institutions, in countries, and in the use of certain journals. They do not spread out uniformly, however desirable that may or may not be. In particular, the growth is such as to keep relatively constant the balance between the few giants and the mass of pygmies. The number of giants grow so much more slowly than the entire population that there must be more and more pygmies per giant, deploring their own lack of stature and wondering why it is that neither man nor nature pushes us toward egalitarian uniformity." (P. 59.)

In his third section, Price considers the actual organization or fragmentation of science, particularly in the field of scientific publication. He points out that we are right back in the stage reached by science in the early seventeenth century, but for opposite reasons; previously it was because there were too few scientists, now because there are too many to keep in touch with each other even by publication. A solution has been found in the presence of 'invisible colleges'—the people in the forefront of their own subjects who all know each other and communicate by letter or by direct personal contact, following what he calls the 'high grade and affluent scientific commuter'. This somewhat chaotic development leads to the persistence of multiple discoveries, in fact to its increase. According to Price, premature publication had its *raison d'être* in earlier times as a way of achieving priority but failed to prevent numerous disputes. He thinks that "Scientists have a strong urge to write papers but only a relatively mild one to read them".

One way out of the present plethora of science is the publication of papers by a multiplicity of authors. The latter practice is growing so rapidly that it looks as if the single author paper will disappear in a few years.

> ". . . There is a continuous movement toward an increase in the productivity of the most prolific authors and an increase in the numbers of those minimally prolific. As we approach a limit in both directions, it is clear that something has to give. The most prolific people increase their productivities by being the group leaders of teams that can accomplish more than they could singly. The minimal group are in short supply and we can hardly afford to let them grow until they reach that ripeness of producing significant papers on their own." (Pp. 89–90.)

> "Because of this, one of the great consequences of the transition from Little Science to Big Science has been that after three centuries the role of the scientific paper has drastically changed. In many ways the modern ease of transportation and the affluence of the élite scientist have replaced what used to be effected by the publication of papers. We tend now to communicate person to person instead of paper to paper." (Pp. 90–91.)

Nevertheless, papers can be used to some extent as the measure of the quality of scientific work. The effective value of a paper, by the measure of how much it is read or at least the ascertainable measure of how often it is cited, is a quantity that can now be determined numerically in the new *Science Citation Index*. The degree of citation gives Price an indication of the quality more or less corresponding to the quantity criterion previously made. Price does not claim this as saying anything about the value of the contribution of individual scientists but only the statistical mechanics of scientific man-power and literature which he finds obeying the same general laws.

Surveys of readership show, for example, that the half-life of the scientific paper is in many fields of the order of the doubling time in the subject, which would mean that the probability of being read in any given field remains constant, a rather surprising result which would certainly never hold for physics, where the half-life is only two-and-a-half years. But, as Price points out, in physics the rapidity of publication, with its immense back citation, multiplies the overlapping of discoveries so that much of its apparent growth is fictitious.

> "All this makes for considerable change in the motivation of the scientist; it alters his emotional attitude toward his work and his fellow scientists. It has made the scientific paper, in many ways, an art that is dead or dying. More than this, the invisible colleges have a built-in automatic feedback mechanism that works to increase their strength and power within science and in relation to social and political forces." (P. 91.)

These changes indicate to Price something of the qualitative nature of the change from 'little science' to 'big science'.

In his final and in some ways his most important chapter, Price discusses political strategy for 'big scientists'. Here his outlook becomes international rather than national. He has previously indicated the factors that tend to stagnation, but here he goes further to show that this is due to the fact that in 'big science' the cost of science varies not as the number of scientists but as the square of that number; effectively it tends to bring down the rate of increase of science mitigated only slightly by the importation of scientists from other countries.

> "... the suggestion that this is the cybernetic feedback that is now trying to decelerate science and bring it to a maximum size. This, we maintain, is the prime cause of the present logistic rather than exponential curve. This is the difference between Big Science and Little Science." (P. 94.)

Looking at the world as a whole, it is evident that only a few countries have reached this stage; in fact, strictly, only one—the United States. More than three-quarters of the world science has no large part to play either in education or in utilization. Yet it is in developing countries that it can grow most rapidly because they alone possess large untapped reservoirs of human ability. Price shows that in the past fifty years the balance of scientific effort of the different countries has varied enormously. But while those of the old countries have remained either approximately constant or actually decreased, as has that of France and Germany, that in the countries which have successively entered the scientific world have increased. In the United States, which came in first, the increase which started in the 'eighties has gradually worn itself down to a static condition. The countries with still growing increases are Japan, the Soviet Union and now China.

Once, whether for economic or political reasons, the 'brain drain' of scientists comes to be checked, as is likely in the field of science in the first place, the gap between the rich and poor countries will be filled. When a more or less even level is reached the total productivity of science will be reasonably held at something like five times what it is now. In the event it may even be more; we are, even in the most advanced countries, far from selecting scientists according to their ability out of the general population. An interesting study of the growth of science in Japan shows that it proceeds by a kind of infective mechanism; 15 physicists, for example, sufficed to produce, in a generation from 1868, 60 by 1900 and several thousands now.

At the end of the book Price discusses the effect of these great changes on the scientists themselves and on the image of the scientist in 'big science' and 'little science'. The catching on of the military usefulness of scientists is going to force them willynilly into positions of political responsibility, a kind of feedback between the effects of the scientist on society and the effect of society back again on the scientist.

Power has passed in its different phases to the military, to the financiers and to the lawyers: now it is the scientists' turn.

> "I hope it is not overdramatic to compare the present position of our scientific leadership to that which has existed in other countries, and in this country at other times, among the groups that used to control the means of destiny." (P. 111.)
>
> "In a democracy we are accustomed to finding the leadership taken by men emerging from all these fields that have been crucial to the world's destiny." (P. 112.)
>
> "This is the credit side of the register which balances some of the other, not so good, changes already noticed in the first generation of new-style Big Science. The scientist is accepted by society and must shoulder his responsibility to it in a new way. The rather selfish, free expansion by exponentially increasing private property of scientific discoveries must be moderated when one is in the logistic state. Racing to get there before the next man might well be, in the long run, an impossibly irresponsible action." (P. 113.)

The great merit of Price's book and the reason why it should become compulsory reading for every scientist, 'big' or 'little', is that the statements it contains are based on facts of observation, not on pre-judgments or wishful thinking. His conclusions can be disputed, but it must be disputed henceforth in his terms, that is, in quantitative terms, using science itself as an object for scientific study.

J. D. BERNAL

MEANING IN PHENOMENOLOGY

Edmund Husserl's Theory of Meaning

By J. N. Mohanty. (Phaenomenologica, Nr. 14.) Pp. ix + 148. (The Hague: Martinus Nijhoff, 1964.) 15.30 guilders.

EDMUND Husserl's Theory of Meaning is an able commentary on a particular part of Edmund Husserl's work, namely, his philosophy of thinking and meaning. The author is an Indian professor with wide post-graduate experience in Germany, and thus well fitted for his task. He sets about it by elaborating his theme as follows: 1, analysis of thought; 2, expression and its functions; 3, thinking and meaning; 4, Husserl's philosophy of language; 5, certain associated problems; 6, formal logic; 7, back to experience.

From this scheme, it is evident that the development of logic and semantics since Husserl's day (he died in 1938) has profoundly modified certain aspects of the whole of phenomenology, and that such progress has been critically assessed. The remarkable feature is how closely several recent advances have been anticipated. Husserl was trained under the formidable influence of Weierstrass (though his original thesis was never published), and he retained through his life a large measure of respect for mathematical modes of thought, even if he seemed to follow his own type of subjectivity. Nevertheless, he was hopeful that Hilbert's meta-mathematics would save the day, a view destined to be disappointed by Gödel's classic paper of 1931; a result which only goes to show how fast events were moving in the master's lifetime.

In my opinion, the most valuable part of the volume is to be found in Chapter 7, wherein the discipline of experience is fully manifest. Paragraph 22 is worthy of special comment. Husserl pondered deeply on the technical and symbolic character of the natural sciences, and the tendency of these to increase with time. He thought of such movements as emptying the subject of much of its meaning. Originally, no doubt, there was much truth in this. The crisis (to use Husserl's own word) may have occurred quite unobserved when Thales, fully aware that geometry had proved itself very useful for mensuration in Egypt, started to ask why it worked at all, and what its success implied. It then became obvious that crude need had produced the science of pure geometry.

Following this train of thought to a period nearer our own, there can be little doubt that, as we are reminded on p. 138, Galileo's quantitative physics did more than he knew to divide sense-data from formal explanation, even though he is commonly regarded as its founder of modern experimental philosophy. In its extreme present-day abstraction, physics has wandered a long way from what Husserl would have accepted as experience.

A closely allied topic is that which Husserl liked to call the 'notion of horizon'. For him, this divided into two, outer and inner. The latter appears not only as giving an object the capacity to be more fully determined than at first sight, but also it actually indicates the possibility of achieving such closer analysis. The former, the outer horizon, posits other co-objects of the same field, and they too are at least capable of experience. It is in passages such as these that Husserl displays an instructive sympathy for psychology, including the integrative principle of the Gestalt concept, and problem pictures with their differing degrees of mental awareness.

This is not an easy book to read. The style is difficult in itself, and encumbered with a veritable plethora of exclamation marks. In addition, the author has the tiresome habit of writing 'if' when he means 'whether'.

All in all, this is a welcome new member of the *Phaenomenologica* series, and exhibits Husserl as a precursor of much that is fundamental in modern logic.

F. I. G. RAWLINS

CHURCH AND STATE

Transactions of the American Philosophical Society

New Series—Vol. 54, Part 1: Rome and the City of God: an Essay on the Constitutional Relationships of Empire and Church in the Fourth Century. By Prof. Karl Frederick Morrison. Pp. 1–56. (Philadelphia: The American Philosophical Society, 1964.) 1.50 dollars.

CHRISTIANITY started as a Jewish sect in Palestine. For its members, the Jewish law was obligatory and the outlook on the world in general and the Messiahship of the Founder was Eastern, Syriac and not Greek. Greek thought started with the cosmos and sought to understand human life (and God in relation to life) from the cosmos; Syriac thought started from the idea of the Living God and sought to understand human life in the cosmos from its vision of God.

Palestinian Christianity was largely destroyed during the wars waged by Titus, but previously had taken root in a number of gentile settlements. Not a few missionaries, including Saint Paul, helped in this dispersion of its beliefs. Maybe, too, some gentiles who had been influenced by the new thought, and had been driven out of Jerusalem at the time of the riots which led to the death of Stephen, also played their part in the spread abroad of the new religion. But the gentiles were Greek in thought and certainly were not interested in Jewish laws. After a hard fight, Saint Paul persuaded the Jerusalem Christians to relax most of these laws and practices including, especially, circumcision. However, the problem of making intelligible the essentially Syriac ideas to the Greek mind remained, as well as the organizing of the several little groups of believers into a Church. The latter problem was solved early, indeed before A.D.180, as the Roman Empire could be used as a model by such organizers as Ignatius and others. The Church they built up was flexible enough to allow for expansion and indeed lasted until the time of the Reformation.

The problem of translating Syriac thought into Greek terms was another matter and involved much controversy. Heresies also arose (especially the Arian in the fourth century), and these complicated the problem. The main question was: What was the nature of the Founder and how could it be expressed in words ? It was not until the fifth century that a solution to the problem satisfactory to the majority was attained at the Council of Chalcedon.

When Constantine the Great was converted to Christianity and the Roman Empire became nominally Christian in the middle of the fourth century, still another vital problem arose: What should be the relationship between the State and the Church, which, by definition, already had the Founder as the divine Head? On one hand, the State did not want an *Imperium in imperio*; on the other, the Church obviously should not be controlled by the secular arm. The idea of the 'City of God' emerged and, with safeguards, a duality grew up which lasted throughout medieval times—the spiritual arm, the Pope; and the secular arm, the Emperor. In this connexion the influence and ideas of Saint Athanasius, of Saint Hilary and, a little later, of Saint Ambrose were vital, and Prof. Morrison, in *Rome and the City of God*, considers them in some detail. If the new Church had fallen completely into the hands of the State, humanly speaking it would have died with the Roman Empire, especially in the West. As it was, the opposite occurred and the early Popes inherited much of the power and influence which had previously been wielded by Rome.

Of course, the problem was not settled for all time in the fourth century; the relationship in France between the Church and the State is still uneasy, and the British solution, where the State appoints the chief church officials but then allows them liberty of action, does not appeal to everyone. Prof. Morrison starts with an introduction and then deals in detail first with the legal principles and later with the theological problems involved. The result is a learned work of great interest, but the general reader should have some knowledge of the subject as a whole before tackling it.

M. C. BURKITT

HEAT TRANSFER

Chemical Engineering Practice

Vol. 7: Heat Transfer. General Editor: Herbert W. Cremer. Pp. vi + 460 + xiii. (London: Butterworth and Co. (Publishers), Ltd., 1963.) 100*s*.

VOLUME 7 in the series *Chemical Engineering Practice* covers the ground of heat transfer, a field of major importance in chemical engineering. Four chapters, which form the greater part of the text, are devoted to heat transfer and are followed by two chapters on drying and a final chapter on heating and ventilation.

The first chapter, by Dr. Franklin and Mr. Hartland, on conduction, forms an extremely good introduction to the basic mechanism of heat transfer and provides a general introduction to the text. The authors have succeeded in discussing both steady- and unsteady-state conditions and two-dimensional flow, but there is a somewhat hurried look about parts of the explanations, and the inclusion of a number of worked examples illustrating the techniques would have been valuable to the practising engineer.

The next three chapters, on transfer by convection, by boiling and by radiation, which have been written by Prof. Roberts and Mr. Cass from the Houldsworth School at Leeds, form the most extensive section of the book. The chapter on convection is well presented and the importance of this mode of transfer is well brought out, but there are sections, such as heat flow in annuli, where a more detailed account of the problems would have been appreciated by the reader. The authors have been reluctant to be in any way definite in recommending particular expressions for the calculation of the heat transfer coefficients under varying conditions, and more on the expected accuracy of such correlations would have helped. The use of packed beds for reactors and the ever widening application of the fluidized bed technique are examined, but more information on the performance of large units would have helped the designer, who is at present uneasy about the application of data from small units. There is much research in this field, but the precise mechanism of heat transfer and the problems of geometry have not yet been cleared for accurate design.

Chapter 3, on heat transfer with phase change, deals extensively with evaporation and boiling, but some of the more recent correlations on nucleate and two-phase transfer should have been included. The designers here are perhaps more concerned with heat flux, and the problems of reproducibility and steady values, than with trying to obtain exceptionally high values of transfer coefficients. Some of the practical features of the desirable extent of vaporization, either per pass in a tube or per cycle in a reboiler, have been treated rather thinly. The authors have made a good attempt at condensing our theoretical ideas, but have left the reader undecided regarding application of the theories. The problems of maximum flux and of burn-out have been of vital consideration in the development of nuclear reactors, but neither these nor the question of high fluxes obtained with rockets and sub-cooled boiling are given much coverage.

Chapter 4 covers the less well analysed field of radiation and will undoubtedly prove of real value to all concerned in work at high temperatures and with furnaces. There is plenty of material here for the thinker, and the chapter well deserves study. Our knowledge of the problems of the drying of solids is still sketchy, and Dr. Nissen and Mr. Macmurray have given a useful summary of the position, but the growing importance of spray drying and the use of fluidized bed dryers are not sufficiently outlined. There is a little duplication here of the principles of heat transfer which are discussed in the first chapter, but with a wide range of authors this may be helpful. A good account is given of the drying of paper on a series of cylinders and this provides an excellent indication of the difficulty in applying theoretical expressions to practical problems. Some good drawings of industrial drying plant would have given the reader a clearer picture of the engineering nature of the units, and a discussion of the cost of drying by various methods should have been included to give the practical engineer a guide to the economics of the operation.

The final chapter, on heating and ventilation, is a welcome addition, which has not been included in the chemical engineering field to any great extent in the past.

This book contains a very useful summary of our present knowledge on the basic principles of heat transfer, but it would have been pleasant to report that there were a large number of worked illustrations from practice. These would embody the kind of information which is lacking in literature to-day, and, although it is clearly difficult to be precise, some real attempt to bring home the difficulties of practice might well have been attempted.

The design engineer is eager for illustrations of the right method for use of techniques, and the more abstract the mathematical methods, as with those on unsteady state processes in Chapter 1, the greater the need for confirmation, by example, that those methods are not only reasonably accurate, but well worth while if better plants are to be designed. J. M. COULSON

VACUUM FOR THE TECHNICIAN

Vacuum Technology

By Prof. Andrew Guthrie. Pp. xii+532. (New York and London: John Wiley and Sons, Inc., 1963.) 94*s*.

INTENDED primarily for the technician, this useful book covers most aspects of vacuum technology excluding applications. Very little background knowledge is assumed of the reader; the text is illustrated by good line-diagrams and photographs and is lucidly written, though the author does exhibit occasionally the disconcerting habit of writing very long paragraphs containing unexpected changes in ideas.

Attractive features of this volume are the many worked examples, the carefully selected tabulated data, the repeated emphasis on experimental procedure and correct operation of plant and the useful chapters entitled "Cleaning Techniques" and "Some Fabrication Techniques".

The text is reliable and with a welcome correct use of terminology. However, some controversial points and errors worth mentioning with the view of being helpful and also indicating the treatment adopted are the following. On p. 42 appears the statement, "If it were possible to connect the inlet of a pump directly to the vacuum vessel then pumping speeds at the vessel would be given directly by the speed curve of the pump. In practice, this is not possible, simply due to space considerations". In fact, this direct connexion is practised, as, for example, in connecting an oil diffusion pump to the base-plate of a vacuum coating chamber. Admittedly here an isolation baffle valve is generally desirable between the pump and the chamber and this will reduce the speed, but it is not "space considerations" which are the difficulty. In some cases, for example, the widely used equations (2·8) and (3·2), a formula is given without proof, which is undesirable where the deduction is simple, especially in view of the author's commendable emphasis on an understanding of elementary kinetic theory in the early chapters. Occasionally, the author's clarity of exposition deserts him, as in Fig. 3·2, where the operation of gas-ballast would not be understood in relation to the accompanying text. Errors occur on p. 53, where division by 28·3 becomes equivalent to multiplication by 0·353, in the statement of Example 1 on p. 63 and in the instructions about cutting off the heater on p. 105. The operation of the Becker molecular pump is not clear from the diagrams and description, and the use of a modulator electrode in the Redhead version of the Bayard–Alpert gauge is not explained. It is surprising to see emphasis given to the X-ray limitation of the Bayard–Alpert gauge without it being stressed in describing the conventional hot-cathode ionization gauge. It is difficult, in view of the problems of temperature measurement and inadequate knowledge of accommodation coefficients, to make the Knudsen gauge an absolute instrument as is implied on p. 187.

In many respects this book can be strongly recommended for the technician, and the scientist or qualified technologist will find much useful information in it. The text could have been made shorter by omitting such data as explanations of decimals, simple index notation, logarithms, rather elaborated numerical work in answer to examples and simple physics amply detailed in British text-books for the Ordinary Level of the General Certificate of Education. A shorter text would also have been beneficial in reducing the rather high price.

J. YARWOOD

HOW CLOTTING BEGINS

The Contact Phase of Blood Coagulation

By H. L. Nossel. Pp. xv+160. (Oxford: Blackwell Scientific Publications, 1964.) 35*s*.

UNTIL recently the investigation of the biochemistry of blood coagulation has depended almost entirely on measuring the time between an experimental manipulation and the appearance of the fibrin clot, together with great ingenuity in varying the manipulation. Thus, in *The Contact Phase of Blood Coagulation*, Dr. Nossel writes (p. 2): "The occurrence of the fibrin clot is the only stage of the clotting process which can be directly observed. All knowledge of preceding stages has been inferred from changes in the rate of fibrin formation". Dr. Macfarlane has recently suggested (*Nature*, 202, 498; 1964) that the existence of a long series of reactions leading to blood clotting has a biological purpose, by acting as a biochemical amplifier system. If this is so, it implies that, the earlier a reaction in the sequence, the smaller the amount of chemical change that occurs in it. Progress in coagulation research will, presumably, largely depend on establishing the biochemical nature of the earlier reactions and on making them measurable.

This book describes experiments in which, as usual, the indicator of what is happening is the clotting time. However, Dr. Nossel has used great ingenuity to contribute to our knowledge of the earliest stage of clotting, that is, 'the contact reaction'. This is a demonstrable interaction between 'foreign surfaces', on one hand, and 'contact factors' present in the blood, on the other. The contact reaction ultimately leads to the coagulation of the blood.

So far as normal blood and this research on it are concerned, the only thing that is not a 'foreign surface' is normal vascular endothelium. All other surfaces are foreign, and they may be divided into those that are relatively inert and those that are actively clot-promoting. Examples of inert surfaces are those made of polythene or of glass treated with silicone. Clot-promoting surfaces include those of untreated glass, kaolin, and 'Celite'. The 'contact factors' in normal blood are called Hageman factor and *PTA* factor. When they are absent from blood, the contact reaction does not occur in it; this can be shown and can be used to differentiate blood deficient in these factors from normal blood.

The first chapter summarizes what was known up to the time of the author's own work about the relation between the contact system and the blood-clotting process. The second chapter deals with the relation between different surfaces and the contact system in the blood. The contact system must be 'activated' by contact with an 'active' surface. Activation is a change which can so far be demonstrated only biologically, not chemically. It may involve only minute amounts of material and is, moreover, probably very evanescent.

With the aid of 'Celite', Dr. Nossel has shown that *PTA* factor can be removed from normal plasma leaving Hageman factor behind. Both factors are necessary in the contact reaction to initiate clotting. A 'contact reaction product' is formed which can be eluted from 'Celite'. This new observation suggests an opening into the chemical investigation of this product unless its amounts are just too small. Dr. Nossel also presents evidence that the contact factors can be activated by pure sodium stearate; this is the only substance present in normal human blood known to be able to do this.

With the aid of these observations, Dr. Nossel then investigates the clinical features and establishes the laboratory diagnosis of patients deficient in contact factors. He shows that the contact product activity formed is related directly to the amount of *PTA* factor in the blood. From this and Ratnoff's earlier work it is concluded that the contact product is formed in two stages: in Stage 1, Hageman factor is activated by an active surface; and, in Stage 2, the activated Hageman factor reacts with *PTA* factor to form the contact product. The latter is inactivated by normal plasma. An ingenious extension of the thromboplastin generation test is used to show that the contact product reacts with Factor IX (Christmas factor) but not with any of the other known clotting factors. This demonstration represents the link of the new to what was known before.

In his final chapter, Dr. Nossel considers the relevance of his findings to hæmostasis and to thrombosis, and the crucial and controversial problem whether or not the contact reaction which initiates blood clotting *in vitro* is also involved in initiating thrombosis *in vivo*. The evidence suggests that there are two alternative mechanisms for initiating clotting, one involving tissue fluid and the other the surface contact surface. Deficiency in one system may be compensated for by normality of the other, but sometimes this compensation fails. Dr. Nossel considers the evidence concerning the role of stearate in the blood. Some time ago Esnouf and I observed that, during clotting, some of the lecithin in plasma is rapidly broken down to glyceryl phosphoryl choline and fatty acids including, presumably, stearate. This and Dr. Nossel's results make it conceivable that the contact system may be activated autocatalytically.

The book is written in the language of the blood-clotting community and has a useful glossary of technical terms. One should perhaps criticize the uncritical acceptance of that language which has acquired bad habits with terms such as 'non-contact plasma' or 'non-contacted plasma'; 'celite exhausted plasma'; 'Hageman factor deficient serum', etc. The use of adjectival nouns may be short and accurate, but it is very displeasing. It does not seem impossible to express these concepts more acceptably with the aid of prepositions. A virtue of the book is the detailed description of experimental procedures; this should allow anyone to use the techniques described. The book should be read by all who are interested in understanding blood coagulation and related phenomena and who wish to keep up with the progress now being made.

G. V. R. BORN

THE LEUKÆMIC CELL

The Cytology and Cytochemistry of Acute Leukæmias

A Study of 140 Cases. By G. F. J. Hayhoe, Dennis Quaglino and Richard Doll. (Medical Research Council Special Report Series, No. 304.) Pp. ix+105+74 plates. (London: H.M. Stationery Office, 1964.) 63*s*. net.

FOR nearly seventy years hæmatologists have largely relied on stains of the Romanowsky type (complexes of methylene blue derivatives with eosin) to differentiate between the types of normal and abnormal cells of the blood and blood-forming organs. Their most difficult task has been to distinguish between the cells of different acute leukæmias, and in this field the most experienced observers occasionally differ. To anyone who attempts this feat only occasionally, the marvel is that disagreement is not commoner, and that hæmatological diagnosis so often corresponds with clinical observation.

In this clearly written, well produced and illustrated book, a variety of cytochemical methods of examining the cells of the blood and bone marrow in acute leukæmia have been evaluated, by comparison with each other and with a Romanowsky stain. Some, like the peroxidase test, have been available for many years. Others have been introduced more recently, and their practical value has still to be established. Previous experience with these methods has added a few criteria useful in blood cell diagnosis, but most hæmatologists have not felt that any one of them has made their task more than marginally easier. In *The Cytology and Cytochemistry of Acute Leukæmias* all the most important methods available (new or not-so-new) have been tested on an adequate sample of cases, and the results, though not claimed as revolutionary, will probably convert many former doubters to the view that a battery of tests is likely to give substantial help in cases where one, or perhaps two, have left the nature of the condition uncertain.

Blood and bone marrow smears from 140 consecutive cases of acute leukæmia were investigated by Romanowsky and sudan-black staining, a peroxidase reaction, an azo-dye method for alkaline phosphatase, and the periodic acid–Schiff reaction. Salient characteristics observed by each method were recorded, semiquantitatively whenever possible, and analysed statistically with the aid of a computer. The result directed attention to eleven discriminating characters, on the basis of which it was possible to separate the cases into four groups. Initial consideration of Romanowsky characters alone had left fifty cases unclassified, but analysis of the results of all the tests together reduced this number to fifteen. The relation of the four groups to the conventional classification was next considered, and it became clear that they corresponded to lymphoblastic, myeloblastic, monocytic or myelomonocytic, and erythræmic myelosis types, respectively.

The first group (lymphoblastic) was found to differ, with regard both to cytological and to general biological characters, from the other three, and the authors make the significant deduction that acute lymphoblastic leukæmia is a disease separate from the other types, but that the latter form a related group. They suppose that in the normal person there is one stem cell which can produce lymphocytes only, and another which produces myelocytes, monocytes, or erythrocytes, in response to appropriate stimuli.

Several other observations of interest are discussed. In Romanowsky preparations a few, apparently normal, mature cells are commonly seen in acute leukæmic blood, which are useful in distinguishing the types of the disease. Further, in these preparations, primitive cells often appear similar to corresponding precursor cells in the normal bone marrow. The authors have been able to show that both these appearances are misleading. Cytochemical methods (notably the sudan-black, peroxidase, and periodic acid–Schiff stains) show that both the apparently mature cells and the apparently primitive cells in leukæmic blood differ from their presumptive counterparts in normal blood or bone marrow.

Finally, a hypothesis about the nature of the essential cellular lesion in acute leukæmia is put forward, namely, that there is failure of the proliferative capacity of cells at an early stage of differentiation, with the result that cytoplasmic materials (sudanophilic and periodic acid–Schiff positive), which are present in mature normal cells, accumulate in leukæmic cells without corresponding nuclear development. By analogy with what is known of the megaloblasts of Addisonian anæmia, it is postulated that this failure is due to a prolongation of interphase at the second resting period ("G_2" of Lajtha and Oliver[1]) with consequent asynchrony of nuclear and cytoplasmic developments.

As a student of viral-induced leukæmia in animals (which may or may not be analogous to the human disease) I value this survey of modern hæmatological methods highly. Several murine leukæmogenic viruses induce either lymphoid or myeloid leukæmia under different conditions, while others induce a blood disease in which abnormal cells of all the hæmopoietic series may appear. Clearly, the methods so carefully tested by the authors may help in the analysis of this complex situation, and perhaps give an indication of the point of attack of these viruses. To clinical hæmatologists the book will be stimulating, and valuable as a book of reference for some years to come. They will be grateful to the authors for what must have been a laborious task, and for the factual and restrained manner in which the results have been presented.

M. H. Salaman

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[1] Lajtha, L. G., and Oliver R., in *Ciba Symp. Hæmopoiesis*, edit. by Wolstenholme, G. E. W., and O'Connor, M. (J. and A. Churchill, Ltd., London, 1960).

THE LOWER ORDERS

The Lower Metazoa

Comparative Biology and Phylogeny. Edited by Ellsworth C. Dougherty. Pp. xi + 478. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1963.) 140*s*.

CIRCUMSTANCES prevented the prompt publication by the Kaiser Foundation of the *Proceedings* of their second annual symposium on "Comparative Biology" (1960). Most of the papers, along with some new matter, have now been independently published in *The Lower Metazoa*. The papers collected are inevitably heterogeneous, but the volume is mainly concerned with views about the relationships of the lower animal phyla with each other and with the Protozoa. Only one paper (by D. D. Jensen) looks upward to the vertebrates and derives them, via myxinoids, from the hoplonemertines.

For many lower Metazoa (here taken as extending up to and including the Aschelminthes) there is little or no fossil record. Phylogeny therefore relies heavily on the notions of homology and convergence. There is much room for speculation and speculation readily expands to fill the room available. Remane's criteria of homology are discussed in some detail by Hanson, and Remane himself claims that "morphology can be defined as the search for homologies". The open-minded reader of this book will find that one man's homology is another man's convergence and that there is much dispute over what is primitive and what degenerate. Nevertheless, the book is stimulating, provocative and in places exciting, except perhaps to the purist rigorously analysing his authenticated data. An index would have been useful.

The book opens with 21 papers on comparative morphology and phylogeny, and although it is impracticable to summarize them adequately, some idea of the main positions taken may be given. The earliest metazoan was probably an acoele (Hanson; Steinböck) deriving from some gymnostomatous ciliate (Hanson); or was a planuloid deriving from flagellates (Remane, Hand); or was a sponge deriving from the higher volvocines (Tuzet). As to the cœlom, the enterocœlic origin is best founded (Remane), or the mode of formation cannot yet be decided (Hartman), or it may have been differently evolved in different phyla with much convergence (Clark).

In the cœlenterates are two main branches showing radial symmetry: Hydrozoa and Scyphozoa; the bilateral Anthozoa are an offshoot from the latter (Uchida) and the sub-phylum Ctenophora from the former (Komai). The Turbellaria show trends in their organ systems (Karling) enabling the hypothetical archetype to be both described and illustrated: compared with it, the Acoela are not so primitive! (Ax). Turbellaria arose by progressive neoteny from a parenchymula-like larva of the Cnidaria, and the latter from flagellates (Beklemishev).

Rotifers are closely linked with gastrotrichs (Lang; Ruttner-Kolisko) or are not (Remane): they may have as ancestor a primitive annelid like the Dinophilidae (Ruttner-Kolisko). The ancestral nematode can be described in some detail (Maggenti); free-living Phasmidia living in terrestrial saprobiotic substrates are pre-adapted to parasitism, which is polyphyletic in nematodes (Osche).

There are six papers under comparative physiology. Baldwin postulates that, given the common metabolic ground-plan essential to the survival of animals, possible adaptations are severely restricted and evolution is slow. Dougherty outlines the present position of nutrient media for axenic organisms. G. O. Mackie discusses the nature of individuality in the Siphonophoran bud-colonies, which have reached the organ grade of construction by converting whole individuals into organs. E. J. Martin describes a toxin in the cœlenterate *Rhodactis howesii*. Jennings discusses nutrition in the carnivorous Turbellaria and Rhynchocoela and in the parasitic Trematoda, in which nutritional adaptations are physiological. D. Schneider has investigated phototropism in the bryozoan *Bugula* in which growth is purely apical.

Under other disciplines are seven papers, including C. Lévi on gastrulation in sponges, and Nicholas and Hynes on the embryology and development of *Polymorphus minutus*. Embryology shows a distorted form of spiral cleavage with no clear homologue of the 4*D* cell and no clearly defined entoderm or mesoderm. M. Benazzi describes the chromosome cycle in polyploid biotypes of triclads, and J. H. Phillips presents preliminary evidence for immune mechanisms in cœlenterates. R. W. Pennak considers that the successful invasion of a freshwater habitat from the sea is an evolutionary rarity, more likely to occur via the sandy beach 'psammolittoral' and ground-water 'phreatic' than directly by the estuarine waters route. M. Voge remarks on the profusion of (moist) habitats invaded by flatworms and on their symbiotic relationships. Finally, R. B. Brunson presents some anomalous results in culturing gastrotrichs.

B. G. Peters

Science

Method and Meaning. Edited by Samuel Rapport and Helen Wright. (The New York University Library of Science.) Pp. xii+258. (New York: New York University Press, 1963.) 4.95 dollars.

THIS book, *Science: Method and Meaning*, presents a selection of excerpts from the writings of scientists, dealing first with science and the scientist and, in the second part, with science and its place in the modern world. Those in the first part are chosen to illustrate the nature of scientific activity, the stages of observation, hypothesis and experiment, and the process of discovery. They include a classic statement by T. H. Huxley on the method of scientific investigation and Warren Weaver's discussion of the imperfections or limitations of science. Dr. J. B. Conant's account of the overthrow of the phlogiston theory is also included, as well as passages from the writings of Claud Bernard from J. Livingston Lowe's *The Road to Xanadu*, from W. I. B. Beveridge's *The Art of Scientific Investigation* and from J. R. Baker's *The Scientific Life*. The excerpts in the second part are taken from A. N. Whitehead's *Science in the Modern World* and from the writings of Harlow Shapley, E. W. Sinnott, J. Bronowski, G. T. Seaborg and W. K. Heisenberg.

While the selections have obviously been made with some care, and are fully representative of the varied aspects of scientific activity and its impact on the world, the basis of selection is not clear and the general effect is rather that of patchwork. The book lacks the unity of Sir Richard Gregory's *Discovery*, Prof. W. I. B. Beveridge's *The Art of Scientific Investigation*, or J. R. Baker's *The Scientific Life*, for example, to mention only three which cover essentially the same ground and probably achieve the same objective more effectively. The scope of the book is perhaps slightly wider, however, and it could well serve as an introduction to a somewhat more extensive field of reading. Here it is unfortunate that the references to sources are lacking in precision: readers unfamiliar with the books quoted may have difficulty in locating the originals and there is a slip in the title of Whitehead's book. There is, however, much of merit in the book, but as a whole it does not measure up to the opportunity which the editors have seen but scarcely grasped.

R. BRIGHTMAN

Fundamentals of Palæontology

Reference work for Palæontologists and Geologists of the U.S.S.R. Vol. 15: Gymnosperms and Angiosperms. Edited by A. L. Takhtajan, V. A. Vachrameev and G. P. Radczenko. Pp. 742+80 plates. (U.S.S.R.: Academy of Sciences, Moscow, 1963.) n.p.

THIS impressive book is the final volume of the *Osnovii* series (which covers the whole field of palæontology), and is the second of the two volumes devoted to fossil plants. The earlier palæobotanical volume (*Nature*, 201, 652; 1964) dealt with the lower groups, while the present work covers the seed plants. The general style of treatment, the format and type of illustration are very much the same as in the earlier volume, and most of Harris's comments are equally applicable here. Like the earlier work, there are a considerable number of contributors in addition to the three editors.

To a reader outside the U.S.S.R., the extensive treatment given to the Angiosperms is perhaps the most noteworthy feature of the volume; almost exactly half the work is devoted to them. This is an unusually large fraction compared with other palæobotanical texts, and reflects the interest and considerable contribution made in this field of palæobotany by Prof. Takhtajan. Seward, in his four-volume work on fossil plants (now in many respects out of date, but still probably the nearest equivalent in English to the palæobotanical volumes of the *Osnovii*), significantly never included the Angiosperms, although he dealt with all other groups.

The introductory section of that half of the volume dealing with Angiosperms is particularly useful to the palæobotanist in treating those aspects of the living members of the group which are important to him, but which can only be found rather widely scattered in purely botanical literature; these are, particularly, wood anatomy, leaf form, pollen morphology and fruit structure. The consideration given in the whole volume to fossil pollen is especially salutary, as many Western palæobotanists working on pre-Quaternary material are apt to regard palynology as a branch of stratigraphy rather than an integral part of the general study of fossil plants. The acceptance of the identity of living Gymnosperm genera in the Mesozoic on the basis of their pollen alone (for example, Russian Jurassic *Podocarpus*) may seem an over-optimistic reliance on the systemic value of pollen characters. But the attempt at integrating data from dispersed spores and macrofossils is at least refreshing.

W. G. CHALONER

Nuclear Physics

Edited by V. F. Weisskopf. (Italian Physical Society. Proceedings of the International School of Physics "Enrico Fermi", Course 23, Varenna on Lake Como, August 7–August 26, 1961.) Pp. v+186. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 60*s*.

THE *Proceedings* of the Varenna Schools used to be published as supplements to *Il Nuovo Cimento*. They now appear in hard covers from the Academic Press, but the style, down to the last details of typography and the familiar group photograph, remains unchanged. Only the density of misprints seems to have increased; it is here regrettably high.

Three courses take up most of the book; Villars on the Hartree–Fock approximation in nuclear physics, Levinson on nuclear physics (deformed orbitals in shell-model theory) and Brown on collective motion and many-body techniques. The other two courses, de-Shalit on nuclear moments and Ericson on the compound nucleus and random phase approximation ('fluctuations'), are rather shorter.

Much of this is good teaching, with at least some of the hand-waving that (in moderation) is an essential part of good teaching coming through. The resulting informality makes for refreshing reading and provides a good introduction to many of the subjects covered as well as useful insights to those more familiar with them.

Of perhaps more doubtful value is the inclusion of four seminars on special topics. These are doubtless intended to be concrete examples of research related to the topics of the courses, but their teaching value in this role is limited and I feel these contributions would sit less awkwardly as research papers in the journal literature. If their exclusion had made possible a modest reduction in the price, so much the better for everybody.

J. M. SOPER

Analysis of Petroleum for Trace Elements

By O. I. Milner. (International Series of Monographs on Analytical Chemistry, Vol. 14.) Pp. viii + 128. (Oxford, London, New York and Paris: Pergamon Press, 1963.) 35*s*.

THIS is a very useful monograph. It is very well referenced, and, for each of the elements which have most frequently to be determined, the author gives a recommended method of analysis the suitability of which has been confirmed by his personal experience. The title refers only to the analysis of petroleum, but the determination of trace elements in catalysts is also covered.

The book provides a very convenient and useful collection of many modern references to methods for the determination of trace elements in the petroleum industry. For those who are inexperienced in this field, the recommended methods should be extremely valuable, but they should not be new, except perhaps in detail, to the experienced petroleum analyst.

Chapter 1, "Introduction", provides information on the necessity for trace analysis, and the first half of Chapter 2 (28 pp.), on "Principles and Practices", is devoted to matters of interest to all trace analysts, for example, sampling, contamination, reagent purity, storage of solutions, and blank determinations. The remainder of the chapter deals with decomposition techniques, separations and finishing techniques. The following eleven chapters, at an average of six pages each, deal with the determination of arsenic, chlorine and bromine, copper, iron, lead, nickel, nitrogen, phosphorus, sodium and potassium, sulphur and vanadium. The reason for the presence and the analysis of each element is discussed and applicable methods are reviewed and well referenced. The author gives his recommended method for each element and all the methods are based on chemical procedures with the intention that they can be carried out by laboratories which are only modestly equipped. Some instrumentation is necessary, of course, in the form of spectrophotometers, flame photometers, etc.

In these chapters it is perhaps a little surprising to find the author, aware of the usefulness of benzene and xylene sulphonic acids, recommending the ashing of petroleum materials with sulphuric acid for the determination of iron, nickel and vanadium. All the recommended procedures quoted are complete in themselves, apart from that for sulphur, which requires reference to another publication.

The final chapter (19 pp.) reviews methods for determining eleven elements (barium, boron, calcium, cobalt, fluorine, magnesium, manganese, molybdenum, oxygen, silicon and zinc), which are less frequently determined in trace amounts, but no recommended procedures are given. The index, considering the size of the book, is adequate.

E. THORNTON

Tsetse Flies in Northern Nigeria

A Handbook for Junior Control Staff. By Howell Davies. Pp. 211. (Zaria, N. Nigeria: Gaskiya Corporation, 1963.) 20*s*.

THIS book has been expressly written as a pocket instruction manual for African tsetse control staff, and as such is outstandingly good and a model of its kind; Howell Davies has clearly given a great deal of thought to how a large body of information can be simply and attractively presented to his junior African reader, not only in a way that is easily readable but also easily memorized.

Although primarily concerned with Northern Nigerian species, this book should commend itself to any layman wanting to get quickly to the facts about tsetse flies in general, for a wealth of information is given which is easily tapped by means of the excellent index. Each fact is given very clearly in concise numbered paragraphs, and almost every aspect of tsetse flies is covered—including their recognition, bionomics, disease transmission, control and how to carry out tsetse surveys. The main text is augmented with useful glossaries giving the local Hausa names of trees and animals often associated with tsetse in their natural environment, and simple drawings help the reader to picture things not easily described in words. Sketches of the spoor of large game animals are included, perhaps rather optimistically in face of the extreme rarity of such animals as elephant and giraffe in Northern Nigeria. Altogether an admirable little book, but, unfortunately, very poorly bound.

R. W. CROSSKEY

Physiologie, Comportement et Écologie des Acridiens en Rapport avec la Phase.

(Colloques Internationaux du Centre National de la Recherche Scientifique, No. 114, Paris, 9–13 Avril, 1962.) Pp. 342. (Paris: Éditions du Centre National de la Recherche Scientifique, 1962.) n.p.

THIS volume presents the proceedings of the five-day colloquium on the physiology, behaviour and ecology of locusts in relation to phase held in Paris during April 1962. Eighteen speakers from widely scattered countries gave papers on a wide variety of topics within these three aspects of locust biology. M. J. Norris, M. Papillon, M. Verdier, P. Joly, J. de Wilde (and G. B. Staal) and K. C. Highnam spoke on physiology, P. E. Ellis, P. T. Haskell and C. E. E. Rungs on behaviour, and R. C. Rainey, Z. Waloff, E. M. Shumakov, P. E. Kohler and H. A. F. Lea on ecology; M. L. Roonwal, J. S. Kennedy, R. E. Blackith and F. O. Albrecht spoke on more general topics within the subject of the colloquium. The colloquium was convened by F. O. Albrecht, under the presidency of Sir Boris Uvarov.

The papers are published in the language of their delivery, with French and English summaries, except for two which appear in two languages. The discussions are entirely in French, having been translated where necessary. The colloquium has served an invaluable purpose in bringing together a large number of important papers on topics within its broad subject, and this volume will be not only of great value to all those engaged in locust research, but also useful to workers on the physiology, behaviour and ecology of insects generally.

D. R. RAGGE

Méthodes Statistiques à l'Usage des Médecins et des Biologistes

Par Daniel Schwartz. Pp. 290. (Paris: Éditions Médicales Flammarion, 1963.) 60 francs.

SEVERAL books on medical applications of statistical methods have appeared recently in languages other than English, and Dr. Schwartz's book is an elegant addition to their number. The author's treatment of basic statistical methodology is rather fuller than that in Bradford Hill's *Principles of Medical Statistics*, and includes brief descriptions of one- and two-way analysis of variance and of multiple correlation. Examples are drawn from clinical and laboratory research, but there is no discussion of vital statistics or of the problems of data collection and processing. The exposition is non-mathematical and the basic concepts are carefully explained. The book as a whole has a slightly academic flavour, but this may be because the author has deliberately restricted its scope. Discussions of clinical trials, ætiological investigations, biological assays and other topics are promised for further volumes.

P. ARMITAGE

La Chromatographie

Par Prof. L. Savidan. 2e Edition. (Monographies Dunod.) Pp. x+115. (Paris: Dunod, 1963.) 9.60 francs.

THIS extremely brief volume gives, in French, an account of all aspects of chromatography at the lowest possible level. The brevity of the treatment may be judged from the fact that the numerous techniques of paper and film chromatography are dealt with in eleven pages and the whole of gas chromatography in twenty more. The material is clearly presented but is in most instances rather dated. The only gas chromatographic apparatus illustrated, for example, dates from 1953. As an introduction to the subject the book would prove useful, although students in Britain would find considerably better value in the several introductory books available in English.

J. H. PURNELL

STRUCTURE OF HÆMOGLOBIN

AN X-RAY EXAMINATION OF REDUCED HORSE HÆMOGLOBIN

By DR. M. F. PERUTZ, C.B.E., F.R.S., DR. W. BOLTON, DR. R. DIAMOND, DR. HILARY MUIRHEAD and DR. H. C. WATSON

Medical Research Council Laboratory of Molecular Biology, Hill's Road, Cambridge

THIS article is a sequel to Muirhead and Perutz's recent X-ray investigation of reduced human hæmoglobin[1]. The structure of this form was found to differ from that of horse oxyhæmoglobin[2] in the arrangement of the two β-chains. It was left uncertain, however, whether this was a rearrangement accompanying the oxygenation reaction or whether it merely represented a structural difference between the hæmoglobins of man and horse. X-ray analysis of a newly discovered crystal form of reduced horse hæmoglobin has now removed this uncertainty.

One easily measurable parameter distinguishing human reduced from horse oxyhæmoglobin is the distance between two mercury atoms attached to the reactive cysteyl residues of the β-chains. We found this to be 37·3 Å in reduced horse hæmoglobin, in agreement with the value of 37·7 Å in the human reduced form[1] and different from that of 30·0 Å in horse oxyhæmoglobin[2]. Taken together with electron-density projections on a plane normal to the molecular symmetry axis, this result indicates that the reduced hæmoglobins of man and horse have very similar structures, and that the rearrangement of the β-chains must be due to the oxygenation reaction.

On gradual exposure of the reduced crystals to air, certain lattice changes occur, indicative of an ordered transition to the oxyhæmoglobin structure. Crystallization of a mixture of reduced and oxyhæmoglobin leads to the appearance of two different crystal forms, one having the morphology and absorption spectrum of the oxygenated, and another having the morphology and absorption spectrum of the reduced form.

Experimental

Earlier crops of reduced horse hæmoglobin, precipitated from salt-free solution, crystallized in the space group $C3_12$ and had the unit cell dimensions $a = 56{\cdot}1$ and $c = 354$ Å; the great length of the c axis made them unsuitable for X-ray analysis[3]. The present crystals were grown in a 2·5 M mixture of K_2HPO_4 and NaH_2PO_4 solution at pH 6·4, in the presence of 10^{-2} M ferrous citrate, and were mounted wet in thin-walled glass capillaries, in an atmosphere of nitrogen. The crystals are orthorhombic, and form bipyramidal prisms elongated along [001], with faces {110} and {111}. Birefringence and dichroism are weak, with the direction of least optical absorption parallel to [001]. The unit cell dimensions of the wet crystals are $a = 77{\cdot}0$ Å, $b = 81{\cdot}8$ Å and $c = 92{\cdot}7$ Å; $V = 584{,}000$ Å^3; $n = 4$. The space group is $C222_1$, which has eight general positions. The four molecules in the unit cell thus have to lie on dyad symmetry axes (Figs. 1 and 5).

Crystals of reduced horse hæmoglobin in which the two reactive sulphydryl groups had been blocked with mercuribenzoate were found to be isomorphous with the parent compound, but gave diffraction patterns with markedly different intensities. The diffraction patterns of the three principal zones of mercuribenzoate and native hæmoglobin were recorded on 9° precession photographs, extending to a spacing of 5·5 Å^{-1}. Intensities were measured with a Joyce Loebl microdensitometer. They were then corrected by the usual factors, and put on an absolute scale by comparison with horse oxyhæmoglobin[4].

Locating the Mercury Atoms

The squares of the differences in structure amplitude between mercuribenzoate and native hæmoglobin were used to calculate difference Pattersons in projection on the three principal planes (Fig. 2). The unit cell contains eight mercury atoms, one per asymmetric unit. The asymmetric unit of the corresponding three-dimensional Patterson can be shown to contain six vector peaks of equal weight which all lie in Harker sections (Fig. 3). In projection on the three principal planes these peaks, and their symmetrically related counterparts, would produce a system of single- and double-weight peaks, as shown in Fig. 4. Seen in projection on (100), for example, peak 1 at $x = 0$ and peak 3 at $x = \frac{1}{2}$ have single weight, whereas peaks 2, 4, 5 and 6, which have their counterparts generated by reflexion across (100), have double weight.

The three observed projections show the expected distribution of double-weight peaks. The single weight peaks also appear in the expected positions, but their weight is significantly above background only in the projection on (010). Fortunately, the three-dimensional vector structure can be constructed from the distribution of the double-weight peaks alone, and gives the mercury parameters as $x = 0{\cdot}0930$, $y = 0{\cdot}145$, $z = 0{\cdot}155$, and symmetry-related positions. After refinement by R. G. Hart's method[5], these changed slightly to $x = 0{\cdot}0947$, $y = 0{\cdot}146$, $z = 0{\cdot}155$. The positions of the mercury atoms are illustrated in Fig. 5.

Excluding reflexions too weak to be observed, the reliability factors:

$$R = \frac{\Sigma\Sigma\,(\,|\,\Delta F_{\text{obs}}\,|\,-\,|\,\Delta F_{\text{calc}}\,|\,)}{\Sigma\Sigma\,|\,\Delta F_{\text{obs}}\,|}$$

were found to be 0·280 for $\Delta F(hk0)$, 0·276 for $\Delta F(h0l)$ and 0·298 for $\Delta F(0kl)$. In X-ray analyses of small molecular structures these factors would be considered at least twice as large as they ought to be, but it must be remembered that we are here dealing with differences between

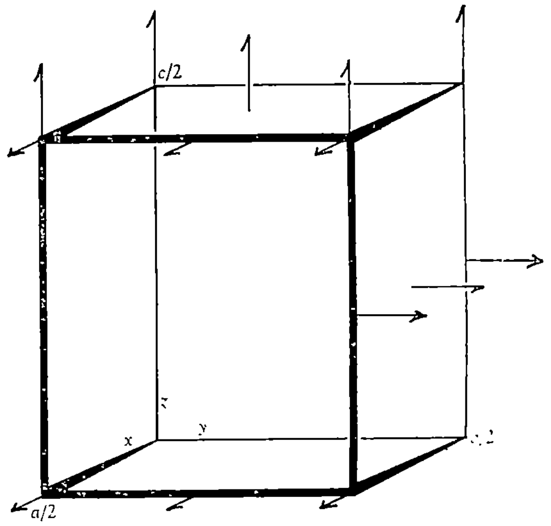

Fig. 1. Arrangement of symmetry elements in the space group $C222_1$ → dyad ⇀ screw dyad

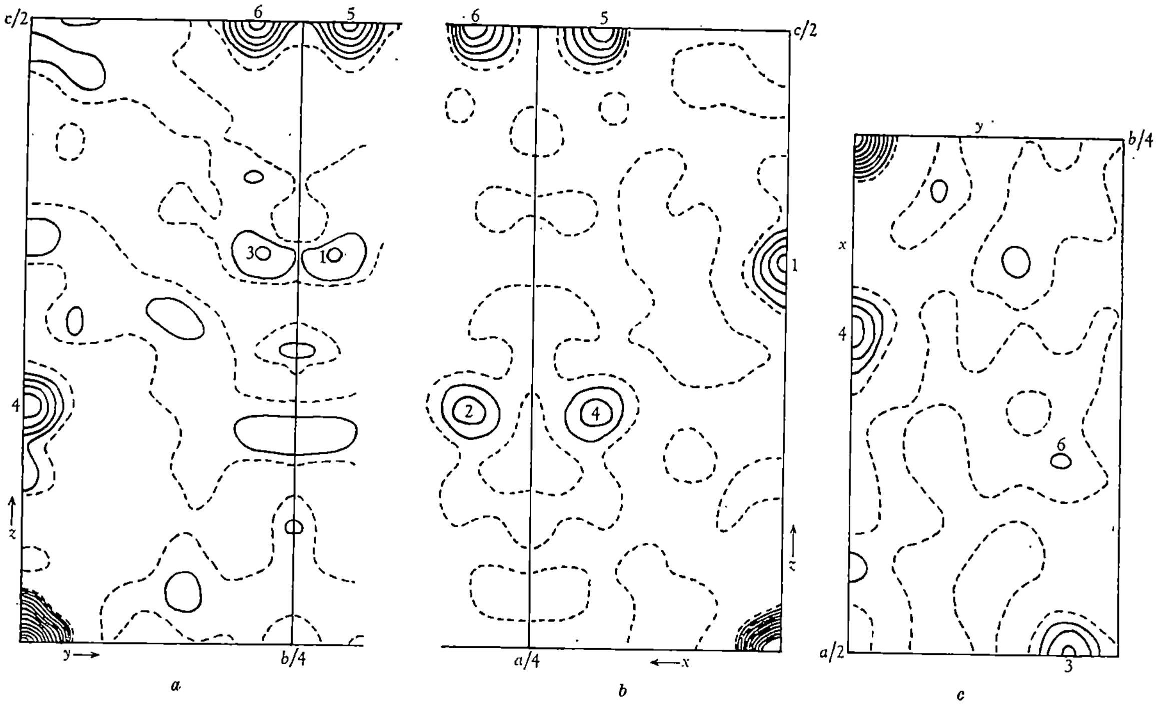

Fig. 2. Difference Patterson projections: (*a*) on (100); (*b*) on (010); (*c*) on (001). Peaks numbered as in Figs. 3 and 4

two isomorphous compounds where experimental errors are much larger. In fact, the foregoing values are as low as any obtained in previous analyses of proteins[2,6].

Orientation of the Molecules

The four hæmoglobin molecules in the unit cell may be placed in two alternative ways, either on the dyads parallel to *a* or on those parallel to *b* (Fig. 1). In the first case, peak 1 in Fig. 2*a*, and in the second case, peak 4 in Fig. 2*b*, would represent a vector between two mercury atoms attached to the same hæmoglobin molecule. Peak 1 corresponds to a mercury–mercury distance of 37·3 Å, in good agreement with that of 37·7 Å found in human reduced hæmoglobin. Peak 4 would correspond to a distance of 23 Å which has not been found in any form of hæmoglobin so far. Thus, the mercury positions indicate the arrangement of the β-chains in reduced hæmoglobin of horse to be the same as in the human form.

Ideally this should be tested by an X-ray analysis in three dimensions, but this is very laborious, and it was thought that a two-dimensional analysis might suffice provided that the molecule could be recognized in projection along its dyad axis. Surrounding each dyad, a hole passes right through the molecule and shows up as a central flat region in projection. The dimensions of this flat region are such that it forms an easily recognizable feature (Fig. 13, ref. 2).

Isomorphous substitution with mercuribenzoate was used to calculate the signs of 57 out of 85 observable *h*0*l* and of 58 out of 81 observable 0*kl* reflexions. Fourier projections on (100) and (010) were then calculated (Fig. 6). A flat central region, corresponding to the hole, is shown by the projection on (100), but not by that on (010). Hence the molecular dyads are parallel to *a*, as expected, and 37·3 Å represents the distance between pairs of mercury atoms attached to the same molecule.

So as to see if the size of the flat region fits the hole in reduced, rather than in oxyhæmoglobin, the projected electron density of models of each of the two forms was calculated. In the case of oxyhæmoglobin, all that was needed was to sum the known three-dimensional density[2] along the lines of constant *x* and *z*. The density of the reduced model was obtained by first summing the densities

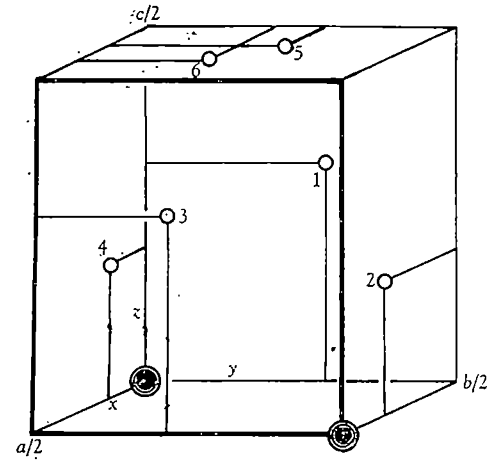

Fig. 3. Asymmetric unit of the three-dimensional vector structure for 8 atoms in the unit cell in $C222_1$

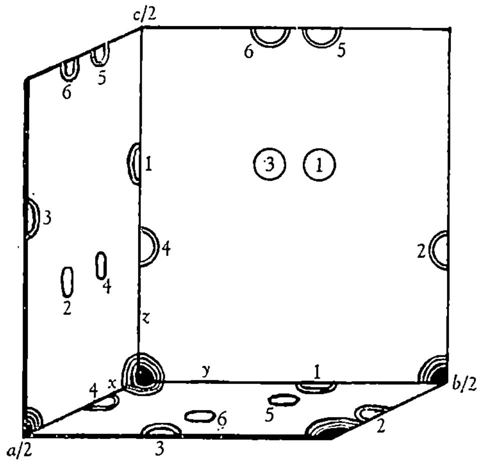

Fig. 4. Projections on the 3 principal planes of the vector structure depicted in Fig. 3. Peaks with single and double contours indicate single and double weight

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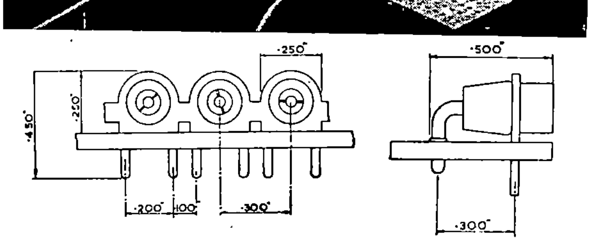

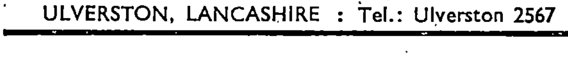

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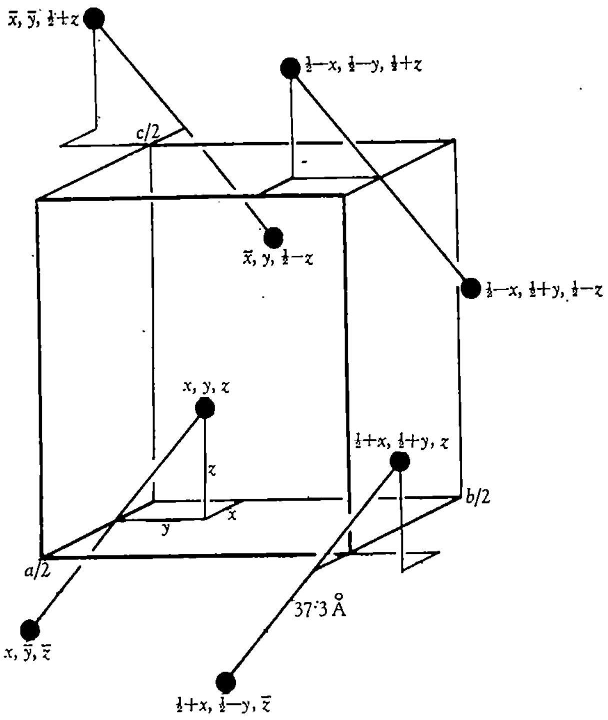

Fig. 5. Arrangement of mercury atoms deduced from Figs. 1–4. Mercury atoms attached to the same hæmoglobin molecule are connected by lines

of the α- and β-chains of oxyhæmoglobin separately; the β-chains were then shifted in opposite directions, as indicated by the structure of human reduced hæmoglobin (Fig. 10*b*, ref. 1), and their densities added to those of the α-chains.

The projected views of the two models are seen in Fig. 7. Superposition of Fig. 6*a* (horse reduced) shows that the observed flat region surrounding the dyad axis fits the hole in human reduced (Fig. 7*b*), but is too big for the hole in the horse oxy form (Fig. 7*a*). The lack of exact agreement between the calculated and observed projections of reduced hæmoglobin may be accounted for in part by the omission of 23 out of the 81 terms of the Fourier summation, and possibly also in part by slight changes in the tilt of the sub-units accompanying the shift of the β-chains.

Structural Transformations in Single Crystals

When crystals of reduced hæmoglobin are exposed to air, they generally crack up and lose most of their X-ray diffraction pattern. When the present crystals were left to become oxygenated, or oxidized, very slowly over a period of several weeks, characteristic and well-ordered lattice changes occurred. At first, extra reflexions appeared at $h + k = 2n + 1$, indicating a change from the *c*-face centred to a primitive lattice. These extra reflexions were weak and confined to spacings smaller than 10 Å^{-1}. The cell dimensions remained unchanged, except for a shrinkage of *b* from 81·8 to 79·6 Å. On further exposure to air, *b* was reduced to 76·0 Å, again without any significant change in *a* or *c*, and the lattice reverted to the *c*-face-centred form. We found that the first step of these lattice changes could be reversed by the addition of ferrous citrate, and the original diffraction pattern restored, but we have not yet succeeded in reversing the second step without breaking the crystals.

Rigorous interpretation of the two transitions would require a determination of the mercury positions in each lattice, which has not yet been done. However, their meaning is fairly obvious. The gap separating the two β-chains is almost exactly normal to *b*. The narrowing of the gap on oxygenation, or on oxidation to methæmoglobin, therefore causes the crystals to shrink along *b* without affecting the other two lattice dimensions.

The additional reflexions which appear on X-ray photographs of partially oxygenated crystals are reminiscent of the superlattice lines seen on powder photographs of copper–gold, and other alloys, after annealing, and may have a similar interpretation. At high temperatures the copper and gold atoms in these alloys are distributed

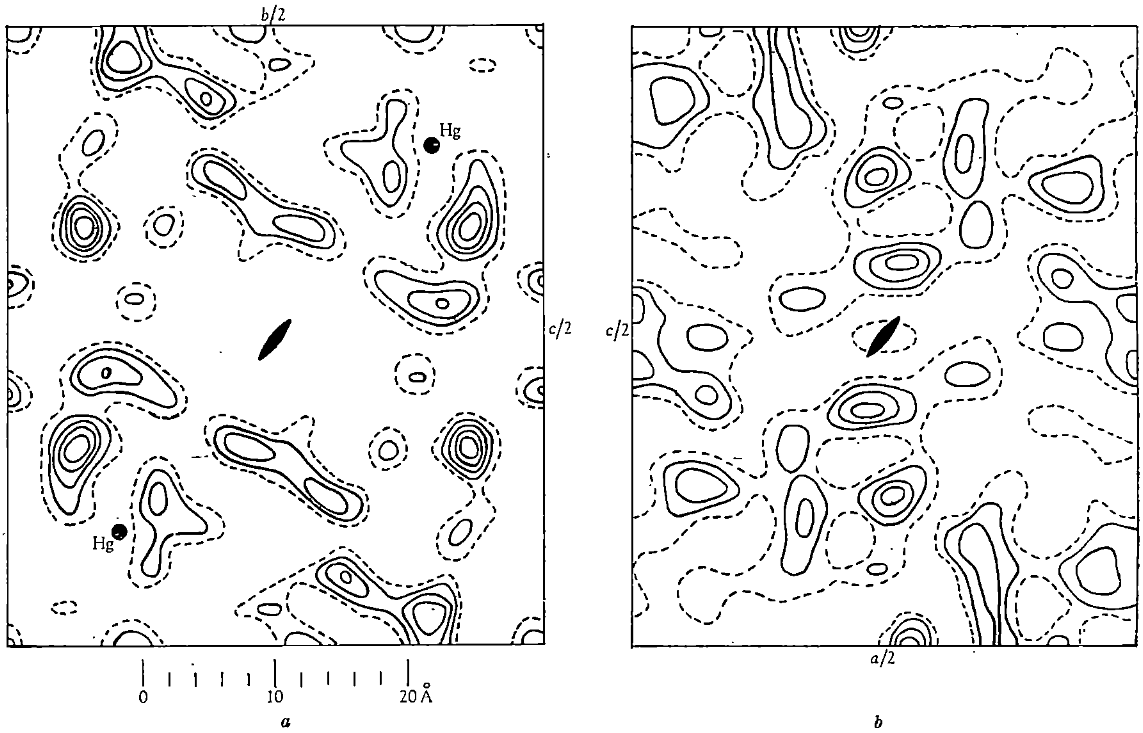

Fig. 6. Fourier projection of reduced horse hæmoglobin calculated from isomorphous replacement with mercuribenzoate: (*a*) on (100); (*b*) on (010)

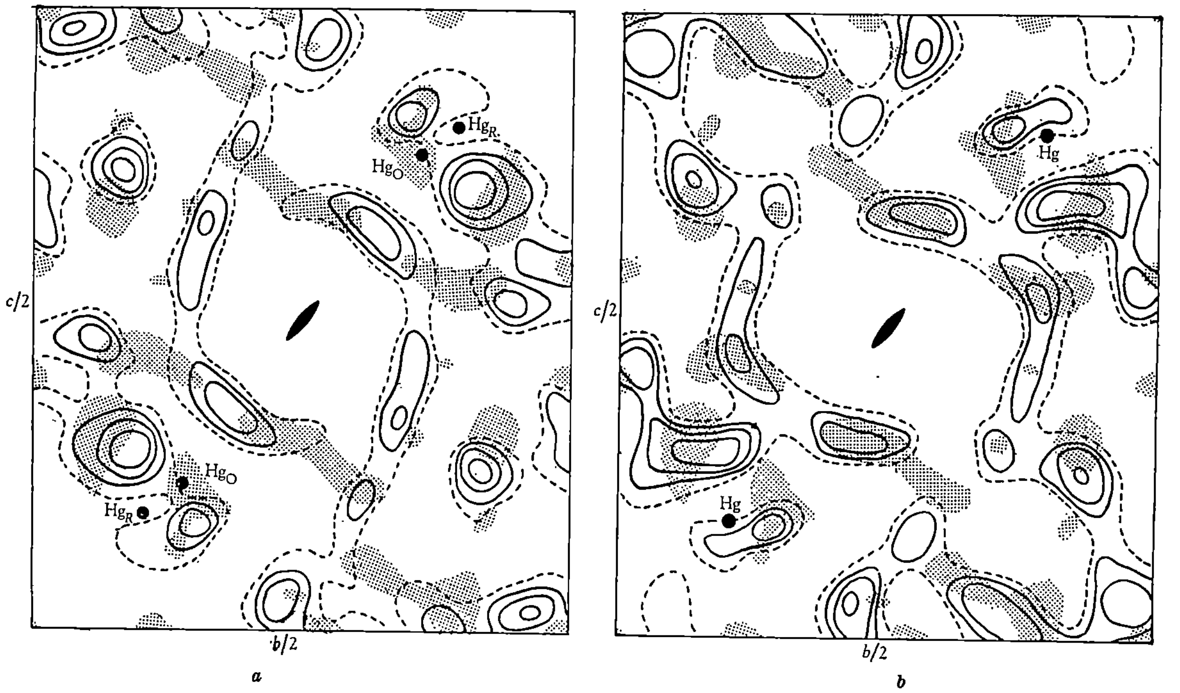

Fig. 7. Electron density projected on a plane normal to the molecular dyad axis calculated from: (*a*) the three-dimensional electron density distribution of horse oxyhæmoglobin (ref. 2); (*b*) the α- and β-chains of horse oxyhæmoglobin rearranged approximately as in the human reduced form. The shaded areas indicate the outlines of the peaks covered by the first full contour in Fig. 6*a*

at random in a cubic face-centred lattice. On annealing they segregate into specific sites, and the difference in scattering amplitude between the gold atoms at the corners and the copper atoms at the face-centres of the lattice gives rise to faint additional reflexions. In the present instance such reflexions could arise from an orderly arrangement of two kinds of hæmoglobin molecules, for example, one where oxyhæmoglobin occupied the dyads at $y = 0$ and reduced hæmoglobin those at $y = \frac{1}{2}$.

Crystallization of Mixtures of Reduced and Oxyhæmoglobin

Horse oxyhæmoglobin is less soluble than the reduced form, so that when a mixture of the two forms is added to phosphate buffer, oxyhæmoglobin crystallizes out first, leaving reduced hæmoglobin behind in solution. Occasionally the reduced form also crystallizes out, resulting in a mixture of crystals: one kind with the morphology and absorption spectrum of oxyhæmoglobin and another with corresponding properties of the reduced form. Crystals showing mixed spectra, such as would be given by the 'superlattice' described here, were not seen in fresh preparations, although they were found in preparations which had been kept for several months.

Conclusions and Discussions

(1) The distance between the two mercury atoms attached to the reactive sulphydryl groups of reduced horse hæmoglobin is 37·3 Å, in agreement with the value of 37·7 Å found in the reduced human form, and different from the 30·0 Å found in horse and bovine oxyhæmoglobin[2,11]. The dimensions of the hole surrounding the dyad at the centre of the molecule are also in agreement with the former and different from the latter structure. This suggests that the reduced forms of human and horse hæmoglobin have very similar structures, which differ from oxyhæmoglobin in the arrangement of the β-chains. Hence the structural transformation accompanying the reaction of hæmoglobin with oxygen may now be considered as proved. Its wider implications have already been discussed by Muirhead and Perutz[1].

(2) It is often asked whether the structure of proteins in the crystal is the same as in solution; old, as well as recently, published evidence suggests that this question may be answered in the affirmative[7-10]. The present results show, not only that crystalline hæmoglobin reacts with oxygen, which was known, but that the structural transformation accompanying the reaction also takes place in the crystal. Clearly the interatomic forces shifting the β-chains are stronger than those acting between neighbouring molecules in the lattice.

(3) It is interesting to find reduced and oxyhæmoglobin crystallizing out separately from a mixture as though they were different phases. An intermediate form did appear on partial oxygenation of single crystals of reduced hæmoglobin, and we cannot be sure that it did not also precipitate from solution and remained unnoticed, but the X-ray pattern given by these crystals suggests that they contain an ordered arrangement of reduced hæmoglobin molecules on one set of sites, and of oxy- or met-hæmoglobin molecules on another set of sites, rather than a single molecular species of structure intermediate between the two forms.

The formation of such an ordered arrangement has the further implication that a reduced molecule, when compressed by deformation of the lattice, due to shrinkage of neighbouring oxygenated molecules, itself becomes more susceptible to oxygenation than those molecules which are not so stressed. If this were not the case, molecules would become oxygenated at random, rather than in the selective manner necessary to produce a superlattice.

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A SURVEY OF THE SOUTH SANDWICH ISLANDS

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P. J. TILBROOK, J. F. TOMBLIN, R. W. VAUGHAN and
Lieut.-Commander C. J. C. WYNNE-EDWARDS

THE eleven volcanic islands of the South Sandwich group lie on an arc between latitudes 56° 18′ and 59° 28′ S. and longitudes 26° 14′ and 28° 11′ W. (Fig. 1). Although discovered by Cook in 1775 and visited by Bellingshausen in 1819, the Islands were not examined in detail until 1931 when R.R.S. *Discovery II* spent 21 days in making running surveys and notes on structure, volcanic activity and fauna. In more recent years brief visits, especially by R.R.S. *Shackleton*, R.R.S. *John Biscoe* and H.M.S. *Protector*[1], have indicated that the charting of the group still needs improvement while the knowledge of the geology and land biology of the Islands, on which boat landings are seldom possible, has remained very slight. The recent visit of H.M.S. *Protector* during March 5–26, 1964, was planned as a general survey and was the result of active co-operation between the Royal Navy and the British Antarctic Survey. Two geologists (Dr. P. E. Baker and Mr. J. F. Tomblin, Department of Geology, University of Oxford), one botanist (Dr. R. E. Longton, British Antarctic Survey), and three zoologists (Dr. M. W. Holdgate and Mr. P. J. Tilbrook of the British Antarctic Survey and Mr. R. W. Vaughan, Government biologist in South Georgia) made up the scientific team, while Lieut.-Commander C. J. C. Wynne-Edwards, R.N. (Hydrographic Department, Ministry of Defence), supervised a Survey programme. The two helicopters carried by H.M.S. *Protector* were used to land parties, and for aerial reconnaissance and photography, while the vessel herself carried out sounding runs. Meteorological records were kept aboard the ship throughout the period.

H.M.S. *Protector* left South Georgia on March 4, 1964, and arrived at Leskov Island, westernmost of the South Sandwich group, on March 5. The scientific and survey parties spent 4 hours ashore and vertical photographs were obtained. On March 6 a party consisting of Tilbrook (leader), Longton, and Tomblin together with a Royal Navy radio supervisor and a Royal Marine support party were established on Candlemas Island, where most of them remained until the end of the project, making a detailed geological and biological survey and carrying out a small glaciological programme. Candlemas Island was selected for intensive study because it appeared geologically the best exposed and biologically the most varied member of the island arc. H.M.S. *Protector* then sailed southwards along the island chain and, on March 8, commenced her detailed programme by landing the shipborne scientific party (Holdgate, Baker and Vaughan) together with the main Survey party, to spend three days on Bellingshausen Island in the Southern Thule group. Visits of shorter duration were paid to the other two islands of this group after which, on March 12, the ship began to work northwards landing parties on each island in turn. The Candlemas group was reached again on March 17, and Longton and Tomblin transferred to the shipborne party for visits to Vindication and Visokoi Islands. The remainder of the Candlemas party was embarked on March 22. During March 19–23, almost continuous fog greatly hampered operations, preventing landings on Zavodovski Island and reducing the utility of a visit paid to the submerged cone north-west of that island which was believed to be the source of pumice ejected in 1962[2]. In view of these conditions H.M.S. *Protector* sailed for South Georgia, where Tilbrook and Longton were landed on March 24. A brief return visit to Zavodovski Island on March 26 met with no better success and after lying offshore in thick fog all day, H.M.S. *Protector* sailed at dusk for Bouvetøya.

The work done in the South Sandwich Islands and summarized here will be published in full in *British Antarctic Survey Scientific Reports* and other journals in due course.

SURVEY

(C. J. C. W.-E.)

Persistently bad weather and the near impossibility of landing from boats have, over the years, prevented any detailed, shore-based, survey of the group. Past expeditions have landed only occasionally[1] and no observations for geographical position have been made from ashore. The only thorough examination of the Islands, by R.R.S. *Discovery II* in 1931[3], was entirely ship-based and suffers from the usual limitations of such surveys.

In March 1964, the islands were reconnoitred by helicopter during H.M.S. *Protector's* southward passage and the survey was commenced at Bellingshausen Island.

A main station was established at the summit, and 'Tellurometer' (micro-wave distance measurement system) observations made in conjunction with a second survey party, moved as necessary to other stations on Cook and Thule islands.

From Bellingshausen Island a traverse commenced northwards to terminate ten days later at Visokoi Island. This traverse by 'Tellurometer' distances and theodolite angles occupied 16 stations and covered a distance of 330 km. Stations were sited in recoverable positions where possible and marked with brass plates cemented into the rock and surmounted by cairns. When weather conditions allowed, aerial photography was carried out, the survey stations being previously marked by 20-ft.-long strips of fluorescent material. This technique was applicable particularly to Candlemas and Bellingshausen Islands, where minor triangulation schemes were established and a larger-scale survey conducted, to meet scientific requirements.

Almost continuous cloud severely restricted astronomical sightings. Brief clearances during the period allowed, however, for theodolite observations which included five azimuths, five sun shots and one curtailed set of star sights. These, linked with the traverse, should improve the existing geodesy of the islands. From the traverse alone, discrepancies of up to 5 km in relative island positions have been observed.

While the survey was in progress ashore, H.M.S. *Protector* carried out a series of sounding runs in the vicinity. As her movements were largely conditioned by the needs of the shore parties, the ship was not free to range widely; nevertheless, the general scarcity of soundings in the area gives added importance to all

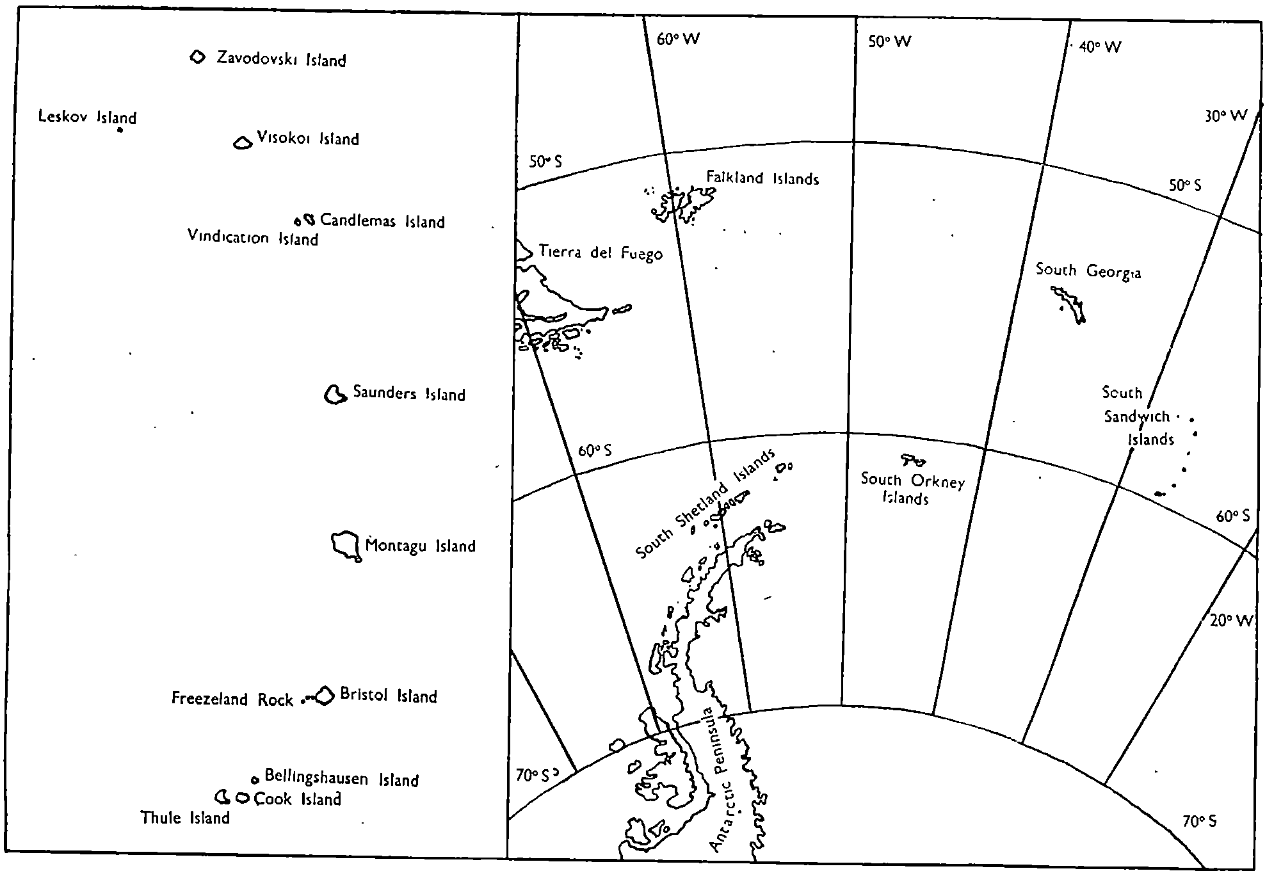

Fig. 1. The South Sandwich Islands (left) and their position in relation to South America, the Antarctic Peninsula, and the islands of the Scotia Ridge (right)

observations. H.M.S. *Protector* also obtained radar photographs to show island profiles; these will be used in conjunction with field observations and aerial photography to revise the existing topographic maps.

It is hoped to produce a revised chart of the Islands, together with large-scale maps of some islands, later in the year.

GEOLOGY

(P. E. B. and J. F. T.)

The northern part of Candlemas Island was selected for detailed geological study because of the diversity of recent volcanic features which are perfectly exposed in this relatively small area. Tomblin spent eleven working days on Candlemas, producing a geological map of the northern part on the scale of 400 ft. to the inch and making sketch maps of the accessible areas of the southern part of the island.

In the north of Candlemas, a scoria cone, rising to 760 ft. above sea-level, is surrounded by lava flows which have issued from its outer flanks and from a breached conelet to the south-west. The flows are commonly 50–150 ft. thick and form broad fans extending up to half a mile into the sea. Older flows are mantled by ash-fall deposits up to 2½ ft. thick. Younger lava surfaces are of *aa* type, with no ash cover. All lavas are basaltic-looking and those of the northern centre contain notably few phenocrysts. Present activity is concentrated in a vigorous fumarolic area occupying the whole of the western slopes of the cone, below which a steaming lake at sea-level contains water at up to 51° C. Steam issuing from the youngest lava flow suggests that this is not more than a decade or two old.

In addition to the detailed geological survey of Candlemas, geological sketch maps and extensive collections were made on Bellingshausen, Vindication and Leskov Islands. Investigations on Saunders, Montagu and Visokoi were restricted to a few small areas chosen for their good geological exposure in an otherwise ice-covered terrain. Only a few specimens were obtained from the heavily ice-capped Islands of Cook and Bristol, and a brief but informative examination was made of Freezeland Rock situated off the latter.

A variety of volcanic structures are displayed within the island group. Bellingshausen is a small youthful volcanic cone built of lavas and pyroclastics, and Leskov is evidently the relic of another small cone. Of the larger volcanoes, the most striking is on Saunders which is dominated by the 3,250-ft. ice-covered cone of Mt. Michael; vapour was billowing from its summit during the short time it was under observation. Three coalescing secondary centres form a prominent range of hills in the south-eastern part of Saunders Island. Visokoi has evidently been built about a single major volcanic centre, but several centres have probably contributed to the construction of both Bristol and Montagu Islands. Small parasitic cones were observed on Visokoi and Montagu, and the conspicuous conical hills seen on the eastern side of both Bristol and Montagu may be lateral volcanic domes.

Fumarolic activity was observed on each of the principal islands except Vindication, Montagu, Cook and Thule. The active crater at the summit of Thule reported by Holdgate[1] was not seen owing to poor visibility. Confirmation that several active fumaroles occur along the summit ridge of Leskov is of particular interest as their existence has been in doubt since Filchner's voyage in 1911[8]. Recent volcanic eruptions have been reported on

Bristol Island in 1956[4] and on Candlemas during 1953–54[1]. It is probable that a recent eruption has occurred on Saunders Island, where loose cinders are patchily distributed over the surface of the ice as on Bristol. A brief visit to the shoal believed to be the site of the 1962 submarine eruption 35 miles north-west of Zavodovski[1] revealed no significant change in the depth of 15 fathoms recorded in 1963[2]. Small samples of pumice were dredged from about 20 fathoms on the shoal and larger lumps of a similar pumice were afterwards discovered on the western coast of Bouvetøya, about 1,100 miles east of their supposed place of origin.

In contrast with rocks from the Lesser Antilles, the only other island-arc within the Atlantic basin, those of the South Sandwich Islands are in general more basaltic and less porphyritic. Only on Leskov, which lies to the west of the main arc, do andesitic types appear to predominate. Lavas with large pyroxene and olivine phenocrysts, collected from Montagu Island, appear to be oceanites, which do not commonly occur in an island-arc environment. The rocks of Freezeland Island which Kemp and Nelson[3] suggested might be sedimentary and metamorphic, proved to be wholly of volcanic origin, the highest peak consisting of stratified volcanic tuff and agglomerate and the lesser peak being formed by a basic dyke. Coarse-grained ejected blocks are rare in the South Sandwich Islands; a few specimens of gabbroic aspect were collected on Leskov and one was found on Vindication Island.

Petrological investigation of the 250 specimens collected from the South Sandwich Islands will be undertaken at the Department of Geology and Mineralogy, University of Oxford.

BOTANY

(R. E. L. and M. W. H.)

On Candlemas Island, Longton made extensive collections of bryophytes and lichens, which predominate in the flora, and surveyed the Island's vegetation. Descriptions of the relatively rich associations around fumaroles were supported by temperature measurements, while three principal plant communities were recognized in inactive areas. The most complex of these was developed on shallow peat deposits which preserved details of plant succession. The reproductive behaviour of certain mosses was examined on Candlemas Island as a part of a wider investigation of their performance in Antarctic environments of differing severity. A 12-ft.-long unworked log was found above high-water mark on the west coast of the Island.

Holdgate made a comparable, less-detailed, vegetation survey on Bellingshausen Island, undertaking temperature measurements around fumaroles. General collections and ecological notes were obtained from Leskov, Visokoi, Vindication, Saunders, and Thule Islands and Freezeland Rock, while a few specimens were also taken at Montagu and Bristol Islands.

In the South Sandwich Islands there is a marked contrast between the comparatively rich, concentrically zoned, vegetation around the fumaroles on Leskov, Visokoi, Candlemas and Bellingshausen Islands, and the generally barren conditions elsewhere. Fumarole vegetation is generally similar throughout the group, but the sparse moss and lichen communities forming the principal vegetation away from the volcanic heat and moisture vary rather strikingly from island to island. Round the large penguin colonies fouled and trampled mud supports mainly algae, particularly *Prasiola*, and areas of bryophyte peat beneath the mud on Candlemas and Vindication Islands suggest that some colonies have changed in area comparatively recently. The grass *Deschampsia antarctica* is known only from between fumaroles on Candlemas Island.

INVERTEBRATE ZOOLOGY

(P. J. T. and M. W. H.)

While on Candlemas Island, Tilbrook made general observations on the fauna as a whole; but concentrated especially on the ecology of terrestrial micro-arthropods and nematodes. Apart from giving much-needed information on the taxa and numbers occurring in the island group, this investigation formed part of a broader survey of the distribution, abundance and ecology of land arthropods of the Scotia Ridge and northern Antarctic Peninsula. All quantitative work (in which the animals were extracted from cores of known volume) was confined to areas of *Polytrichum* and *Bryum* (or *Pohlia*) mats, since these are two of the most widespread genera in these latitudes and had been sampled intensively elsewhere on the Scotia Ridge. These mosses also occurred both around and away from fumaroles, so providing comparable samples in which faunal variation might be related rather directly to a well-marked gradation of environmental factors. Extraction of arthropods on a non-quantitative basis was carried out from samples from many other habitats, including moss mats, lichen tufts, soil, seaweeds, and bird-nest material. Routine plankton and general-purpose net hauls and water samples were taken from all the freshwater bodies on the Island, but marine collecting was virtually impossible owing to the persistent very rough seas.

A less-quantitative extraction technique was used by Holdgate, working aboard H.M.S. *Protector*, to obtain micro-arthropods from Leskov, Visokoi, Vindication, Saunders, Montagu and Bellingshausen Islands. On these islands, mites and Collembola proved to occur locally in large numbers, but to be represented by only a few species. A few freshwater habitats were also sampled on Bellingshausen Island, but appeared unproductive.

VERTEBRATE ZOOLOGY

(R. W. V.)

During the project, investigations were made of the seals and sea birds found in the group. Particular attention was directed to the location of breeding colonies of fur seal, *Arctocephalus tropicalis gazella*, and a census of all seals seen on the islands visited was carried out. A small amount of fur seal skeletal material was collected. In all, five species of seal were seen; but only the elephant and fur seals are certain breeders.

Ornithological work consisted chiefly of the location of breeding colonies on the islands, and the recording of birds seen at sea in the vicinity. Most of the mapping of colonies was done from a helicopter, during flights around the islands: numbers were estimated wherever possible. Landings were made whenever possible, and a careful search was then made for breeding sites of the smaller burrowing petrels. Remains of petrels were collected in the vicinity of skua nests. Some parasites were collected on Candlemas Island, where Tilbrook made a list and census of the breeding birds, paying particular attention to the colonies of giant petrels (*Macronectes giganteus*). Altogether twenty-one species of birds were seen in the vicinity of the islands, but only twelve of these have been confirmed as breeders.

The only whales seen were a few killer whales near Candlemas Island.

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[1] Holdgate, M. W., *Polar Record*, **11**, No. 73, 394, 474 (1963).

[2] Gass, I. G., Harris, P. G., and Holdgate, M. W., *Geol. Mag.*, **100**, No. 4, 321 (1963).

[3] Kemp, S., and Nelson, A. L., *Discovery Reports*, **3**, 133 (1931).

[4] Secretaria de Marine, Republica Argentina. *Derrotero Argentino*, Parte 5, *Antartida y Archipielagos Sabantarticos Argentinos* (Buenos Aires, 1958).

ROYAL BOTANIC GARDEN, EDINBURGH

NEW HERBARIUM, LIBRARY AND OFFICES

THE Royal Botanic Garden, Edinburgh, is nearing its three-hundredth anniversary and has had a herbarium and library for perhaps half that time; yet until now they have not been housed in a building designed for the purpose. The old herbarium had once been the meeting room of the Caledonian Horticultural Society, 300 yards across the Garden from library and laboratories: all departments were hopelessly crowded and had overflowed into huts and separated storerooms. Their gathering together into a single building of adequate size cannot fail to lead to a considerable increase in efficiency. The new building is designed to accommodate normal expansion for the next twenty years.

The opening ceremony was performed by Her Majesty The Queen on June 29. The records of this occasion are a signed portrait of The Queen, a rare volume of paintings of Indian plants (1835) by Mrs. James Cookson, especially purchased for the library and autographed by Her Majesty, and a birch tree planted outside the south-west corner of the building. A copy of the first catalogue of plants grown at Edinburgh, the *Hortus Medicus Edinburgensis* of James Sutherland (1683), was presented to Her Majesty.

The building is 143 ft. long and 76 ft. wide, the long axis running east–west. It consists of a two-storey block set on top of a smaller ground floor block: there is a lower ground floor of the same size as the upper storeys. The library is on the ground floor, the herbarium and laboratories on the two upper floors, the administrative offices, storerooms and a small conference room on the lower ground floor. Except for the offices and some storerooms, the building is air-conditioned. It is designed as a framed structure in reinforced concrete with floors and roof of flat slab construction for economy and speed of erection. The external facing material is entirely prefabricated in standardized units of polished white terrazzo and the podium and terrace are finished in warm grey Heworthburn stone with inset panels of grey vitreous tiles. Windows are sealed double-glazed units fixed into black anodized aluminium window-frames. Suspended acoustic ceilings have been used. The architect was R. Saddler, Ministry of Public Building and Works, and the main contractor W. and J. R. Watson, Ltd., Edinburgh.

By using modern techniques of transplanting trees and large shrubs the new building has, within a few months, been assimilated into the Garden with remarkable success.

The library occupies the whole of the ground floor and there is a simple rectangular stack providing about 7,500 ft. run of shelving. Short folio cases form small bays with reading tables along the north wall, and there is another row of reading tables on the south side. Ancillary accommodation consists of an overflow store on the lower ground floor, a workroom and service desk on the main floor. At the entrance to the library is an open area with built-in display racks for current periodicals, reading space, catalogue cabinets, map table and atlas cases. The furnishings are uniform throughout. Bookcases have ends of American walnut which matches the panelling and built-in fixtures. Lighting over the book stack is pro-

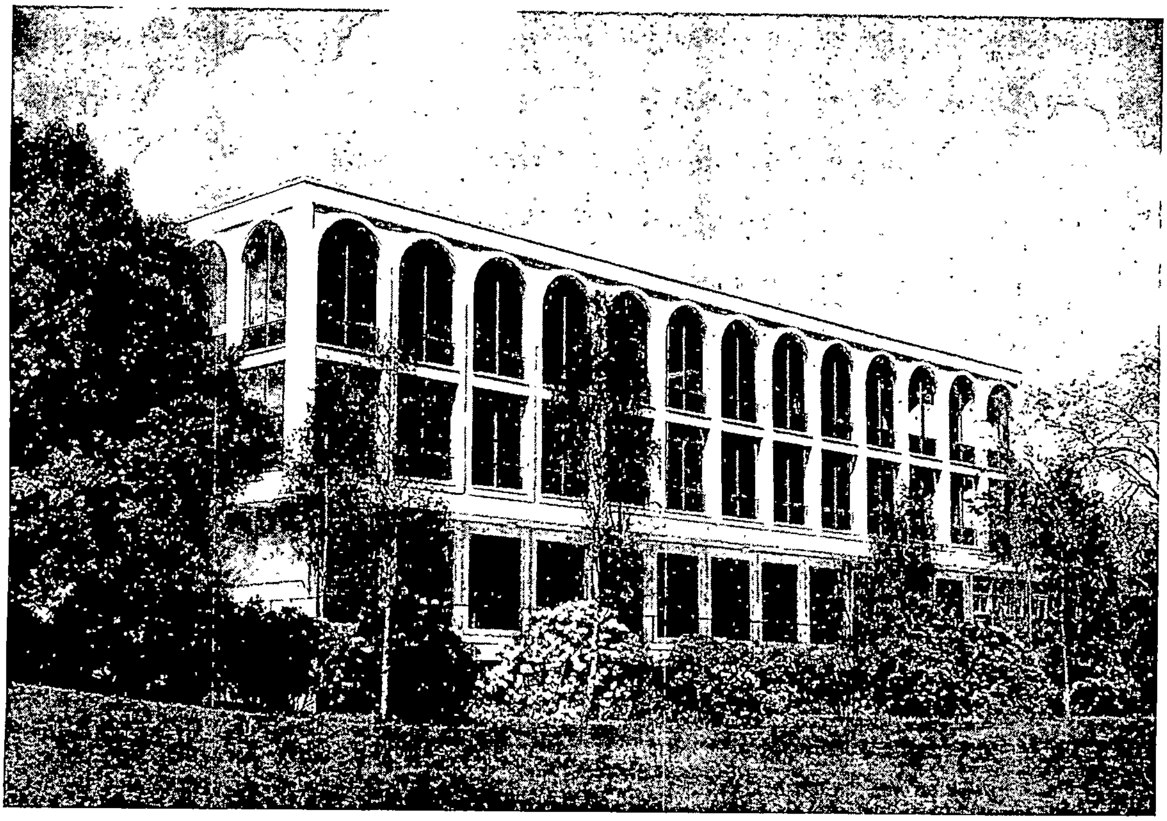

Fig. 1

vided by a suspended illuminated ceiling. The wood block floor is of Rhodesian teak.

The library at present has a stock of 30,000 volumes, two-thirds of which are periodicals. The present intake of periodicals is some 700 titles. For the first time the stock is arranged systematically, the whole library having been re-catalogued over the past seven years according to Bliss's classification. Modifications to this scheme have been kept to a minimum, but some sections have been rearranged—notably by the use of Bentham and Hooker's numbering in systematic botany, so that the arrangement of the books matches that of the herbarium.

A few of the most interesting volumes were on view at the opening ceremony and with them samples of the large collection of photographs and valuable diaries recently bequeathed to the Garden by the late Dr. J. F. Rock, whose plant collections from China are world-famous.

A 22-ft. strip all along the north side of the first floor is cut off from the herbarium to form the photographer's studio and darkroom, workrooms for the two mycologists and the cytologist on the staff, a general laboratory and a culture room. There is an independent air extraction system for this part of the building. The laboratory has working space for 5–6 technical staff and is equipped for all normal routines in mycology, cytology or plant anatomy.

The unity of the herbarium itself, which is split between two floors, is preserved by a small central well; above this there is a raised glass-brick section of roofing with underslung artificial lighting in false beams.

The herbarium is planned for maximum flexibility, with central storage of specimens and peripheral working space. Along the full length of the south side of each floor there is a free consultation bench which is not encroached on for permanent working places for staff; these are distributed around the other three sides of the top floor, and at the two ends of the lower floor.

The herbarium specimens are housed in specially designed steel cabinets: each contains two ranks of six pigeon-holes and they are stacked three high. In order to permit a simple magnetic closure each cupboard has a single door the inner flange of which is fitted with rubber containing a magnetized strip. This system gives effective dust-proofing and most acceptable silence of operation. The absence of catches or locks with moving parts will remove a constant demand for repair work. The single door demands very little more access space than the usual two half-doors, as this is largely governed not by the swing of the doors but by the depth needed to take out specimens. These cabinets are finished in grey stove-enamel and were made and installed by Roneo, Ltd. Roughly one-third of the space is at present free for future accessions.

The herbarium now contains some two million specimens. Among the most interesting of the old collections is that of Paul Dietrich Giseke (1745–96), a pupil of Linnæus; his specimens include several from the garden at Uppsala and one that evidently came from Linnæus's own herbarium and bears his handwriting. Another very valuable herbarium is that of the great Edinburgh botanist Robert Kaye Greville (1794–1866); it contains specimens received from many of his famous botanical contemporaries and is especially rich in cryptogams. It was, however, the collections made in south-west China by George Forrest that gave a new impetus to active taxonomy at Edinburgh, and from these collections there stemmed the work on *Rhododendron*, *Primula* and other Sino-Himalayan genera which brought the Royal Botanic Garden international fame.

Since the Second World War, China has been virtually closed to Western botanists and Edinburgh activity has tended on the one hand towards work on the flora of western Asia (stimulated by P. H. Davis's large Turkish collections), on the other towards the study of special groups.

On the day of the opening ceremony a few small exhibits were put up; one showed the work of G. Forrest, already mentioned; others indicated the travel made by members of the staff in furtherance of present investigations. During the past seven years these have included collecting trips in Turkey, Afghanistan, Pakistan, Burma, Malaya, Sarawak and New Guinea. These were all illustrated by interesting specimens, photographs and living plants—for the introduction of new materials to cultivation in the Garden has been an important aim of these expeditions.

On view in the library were preliminary models and plans of the new greenhouses shortly to replace the existing front range, part of which has already had to be closed to the public for reasons of safety. This is the next phase of modernization at the Garden and will provide better facilities for research collections and even greater incentive to the search for new introductions and for advances in methods of presenting the plant world to students and to the general public.

OBITUARY

Dr. W. O. Howarth

Older members of the University of Manchester and friends throughout Britain will have read with regret of the death at his home in Southport, on June 27, of Dr. W. O. Howarth, formerly senior lecturer in botany in the University of Manchester. He was in his seventy-fourth year and is survived by his wife and son.

Dr. Howarth was at one time a student of the University of Manchester, graduating in 1913. Thereafter he was successively assistant lecturer in botany at University College, Cardiff, and the University of Reading, with a break between during the First World War. He joined the staff of the Botany Department, Manchester, in 1919, and served successively under Profs. Weiss, Drummond, Ashby and Harland, until his retirement in 1956, in all completing upwards of forty years of academic teaching and research. During this period he contributed to the general courses, in particular to plant anatomy and ecology but, especially in his later years, he was responsible for all course work in botany and pharmacognosy for pharmacists. This he did with wide knowledgeability and devotion. In 1925 he was appointed an examiner for the Pharmaceutical Society of Great Britain, and continued in this capacity until after his retirement.

Dr. Howarth, who belonged to a generation of enthusiastic field botanists, was one of the best, his knowledge of the Lancastrian flora, especially of the interesting coastal and adjacent regions around Southport, being unrivalled. In his research he was a recognized authority on some of the genera of grasses. His name is familiar to a very wide circle of botanists through the successful re-issue of Lowson's *Textbook of Botany* (written jointly with the late Dr. L. G. G. Warne). This volume, which has gone through several editions in Britain, was successfully adapted, by the judicious introduction of local examples, for use in India, Pakistan and Ceylon. Howarth also collaborated in the production of a *Practical Botany* for home use and one for use in tropical countries.

To those who knew him well in his daily life, he was greatly liked for his unassuming modesty, an ever-willing helpfulness and quiet, warm friendliness, and for a deep sincerity of purpose in his work. C. W. Wardlaw

NEWS and VIEWS

Director of Observatories Branch, Canadian Department of Mines and Technical Surveys :

Dr. J. H. Hodgson

DR. J. H. HODGSON has been appointed director of the Observatories Branch of the Canadian Department of Mines and Technical Surveys. He has been chief of the Observatory's Seismology Division since 1952. Since joining the Observatory staff in 1949, Dr. Hodgson has specialized in research on the nature of the Earth's crust in the Canadian Shield and investigations of the mechanism of earthquakes. In 1958 he was elected a Fellow of the Royal Society of Canada. He is a member of many national and international organizations, and recently was elected president of the Unesco Intergovernmental Meetings on Seismology and Earthquake Engineering and president of the International Association of Seismology and Physics of the Earth's Interior. Dr. Hodgson was born in Toronto in 1913, and was educated in Ottawa public and secondary schools, and at the University of Toronto. He received a B.A. in applied mathematics in 1940, M.A. in geophysics in 1946, and Ph.D. in geophysics in 1951.

Dominion Astronomer : Dr. R. M. Petrie

DR. R. M. PETRIE has been named Dominion Astronomer in recognition of his outstanding contributions in the field of astrophysics. He joined the staff of the Observatories Branch of the Canadian Department of Mines and Technical Surveys in 1935 and, except for service with the Royal Canadian Navy during the Second World War, has been engaged in astrophysical studies. A large part of his research has consisted of measurement of line-of-sight speed of stars and its application to problems of stellar motion, and the calculation of orbits and dimensions of double stars. Dr. Petrie was educated at primary and secondary schools of Victoria, B.C., and the University of British Columbia, where he graduated with honours in physics in 1928. At the University of Michigan he obtained his Master's degree in 1929 and Ph.D. (astronomy) in 1932. He is a former president of the Royal Astronomical Society of Canada and former vice-president of the American Astronomical Society.

Physics in the University of Manchester:

Prof. J. C. Willmott

DR. J. C. WILLMOTT, reader in physics in the University of Liverpool, has been appointed professor of physics in the University of Manchester, from a date to be arranged, in succession to Prof. E. B. Paul (*Nature*, 189, 876; 1961). Dr. Willmott was an undergraduate in the Imperial College of Science and Technology, obtaining a B.Sc. in 1942, with first-class honours in physics, and becoming an associate of the Royal College of Science in the same year. He then completed his military service, from 1942 until 1946, as a regimental officer in the Royal Electrical and Mechanical Engineers before commencing two years postgraduate study at the Imperial College of Science and Technology on infra-red spectroscopy, for which he was awarded a Ph.D. in June 1949. Dr. Willmott joined the academic staff of the University of Liverpool in 1948 as an assistant lecturer in physics, being promoted to lecturer in 1950, to senior lecturer in 1958, and to reader in 1963.

Applied Mathematics in Queen's College, Dundee:

Prof. D. S. Jones

PROF. D. S. JONES has been appointed to the newly created Baxter chair of applied mathematics at Queen's College, Dundee. Prof. Jones was educated at Wolverhampton Grammar School, from which he went to Corpus Christi College, Oxford, with an open scholarship. His scholastic career was temporarily interrupted by war-time service with the Royal Air Force, during which time he was mentioned in despatches in 1943 and awarded the M.B.E. in 1945. Returning to Oxford, he graduated with first-class honours in mathematics in 1947, and in the same year, having been elected to a Commonwealth fellowship, went to the Massachusetts Institute of Technology. In 1948 he was appointed to the staff of the Mathematics Department at the University of Manchester and in 1954 was given the post of tutor for science. He remained there until 1957 when, at the age of thirty-four, he was appointed to the chair of mathematics at the University College of North Staffordshire, now the University of Keele. Prof. Jones is well known for his work in the field of electromagnetism, in particular the theory of diffraction, and has recently written a book on the subject. He has also written a book on electrical and mechanical oscillations and has published papers on such topics as asymptotic expansions of multiple integrals and theory of unsteady motion of aerofoils. In 1957 he was awarded a D.Sc. by the University of Manchester. In the session 1962–63 he paid a second visit to the Institute of Mathematical Sciences, New York, as the visiting research professor. He has recently been elected a Fellow of the Institute of Mathematics and its Applications. Prof. Jones will take up his appointment on January 1, 1965.

Physiology in the University of Birmingham:

Prof. S. M. Hilton

DR. S. M. HILTON has been appointed to the Bowman chair of physiology in succession to Prof. I. Bush. Dr. Hilton will take up his appointment on January 1, 1965. Dr. Hilton is a member of the scientific staff of the Medical Research Council, and has been in the Division of Physiology and Pharmacology at the National Institute for Medical Research since 1952. Dr. Hilton was born in 1921; he was a senior scholar at St. Paul's School, London, and from there he won an Exhibition to Jesus College, Cambridge. He completed Part I of the Natural Sciences Tripos in 1941, and Part II in 1948. He took the degrees of M.B., B.Chir. in 1944, and Ph.D. in 1956. After qualifying in medicine he held various house appointments at Guy's Hospital and was resident medical officer at Preston Hall Hospital, near Maidstone. His period of national service was spent at the Royal Air Force Institute of Aviation Medicine, during which time he was also a research assistant in the Department of Physiology at the Royal College of Surgeons. In 1950, after leaving the Royal Air Force, he worked for two years in the Department of Physiology at Cambridge and was in receipt of a personal grant from the Medical Research Council. Dr. Hilton's main field of research has been the circulatory system, with particular regard to mechanism of the changes in blood flow occurring in organs and tissues during activity. Recently he turned from the investigation of local chemical and nervous factors involved in such reactions to that of their regulation by the central nervous system, and the integration of cardiovascular responses in the behaviour and physical activity of the conscious animal.

Royal Society Expedition to the Solomon Islands

THE Royal Society has announced its intention of mounting a scientific expedition to the British Solomon Islands Protectorate in 1965. Little is known of the arc of islands from New Guinea, New Britain, the Solomon

Islands, New Hebrides, New Caledonia to New Zealand, and it is thought that a source of information of great value, which might provide the key to problems of biological distribution and ecology in this area, could be found in the Solomon Islands. Much of these extensively forested Islands has not yet been seriously disturbed by man, but its forests are already attracting the attention of those who wish to exploit them commercially, so that it is a matter of some urgency to investigate them while present conditions last. An examination of existing knowledge indicates that the first expedition to the Solomon Islands might most profitably spend about six months in the field, and consist of a marine party of about six to seven scientists to study the shallow water and shore-line biology, and a land party of about eight botanists and zoologists to concentrate on the densely forested interiors and the altitudinal variations. Areas for intensive investigation have been selected on the Islands of Guadalcanal, San Cristoval, Ysabel and Kolombangara, and the marine party will concentrate on areas of the shores of these islands together with New Georgia and Russell Island. The plans for the expedition are proceeding. The expedition will be led by Mr. E. J. H. Corner (Cambridge), who will also lead the land party, while the marine party will be under the leadership of Prof. J. E. Morton (Auckland). The personnel will include scientists from Britain, Australia and New Zealand, some of whom have already been selected, as well as scientists from the British Solomon Islands Protectorate Government Departments of Forestry, Agriculture and Geology. The planning of the expedition is in the hands of the Southern Zone Expeditions Committee of the Royal Society and a small planning group appointed by the committee, comprising the chairman of the committee, the leader and the executive secretary of the Royal Society. The logistics and field organization of the expedition will be the responsibility of Mr. G. E. Hemmen, expeditions officer of the Royal Society, and it is expected that the expedition's boat will be the M.V. *Maroro* from Fiji, under the command of her owner, Captain S. B. Brown.

National Institute of Agricultural Engineering

An informal meeting was held on July 24 at the National Institute of Agricultural Engineering, Wrest Park, Silsoe, at which Sir Harold Wernher, chairman of the Institute, made a presentation on behalf of the staff to Mr. W. H. Cashmore in view of the latter's retirement from the directorship of the Institute on July 31. Presentations were also made to Mr. J. W. Andrews, secretary, and to Mr. R. V. Hussey, who were also retiring. Those present included the Hon. J. J. Astor, J.P., chairman of the Governing Body, and Mr. C. J. Moss, director designate.

Medical Research Council New Research Unit and Research Groups

The Medical Research Council has recently announced the establishment of a new research unit and of three new research groups: *The Cell Genetics Research Unit* will be set up in the Department of Genetics in the University of Glasgow, under the honorary direction of Prof. G. Pontecorvo. The Unit will undertake research on the use of human and other mammalian cell cultures as a tool in genetic analysis. *The Oculogenital Virus Research Group* has been established at the Institute of Ophthalmology, University of London, under the honorary direction of Prof. B. R. Jones. The Group will be engaged on research on the trachoma-inclusion conjunctivitis agents, with particular reference to the relationship between infections of the genital tract and infections of the eye. *The Research Group in Basic Immunology* has been set up in the Department of Experimental Pathology, University of Birmingham Medical School, under the honorary direction of Prof. P. G. H. Gell. The Group will be mainly concerned with fundamental research in immunology and immunogenetics, with particular reference to the activities of cells in mediating immune responses. *The Thrombosis Research Group* will be established at the Institute of Basic Medical Sciences, the Royal College of Surgeons, under the honorary direction of Prof. G. V. R. Born. The Group will be concerned with research into chemical substances which promote or prevent the adhesion and aggregation of platelets in the blood and affect the formation of thrombi.

Political and Economic Planning

The annual report of Political and Economic Planning for the year ended March 31, 1964, notes that all five major current projects are due for completion in the next few months; an investigation has been made both of the organization's methods of working and of the appropriate balance between intensive studies and policy reviews (Pp. 17. London: Political and Economic Planning, 1964). Policy review groups are being set up on fuel and world population, and a number of new major projects within the general ambit of "The Shape of the Sixties" are being considered. Under the programme of work on Britain and the European Common Market, started in September 1958, there were three publications during the year, and a book-length report on economic planning and the European economic community is being prepared. A full report on the examination of community mental health services is also in preparation, and completion of another dealing with an investigation of prisoners and their families is also expected this summer. A final report on attitudes in British industry is expected to be published by the end of the year, and the report on "Education for a Developing Region" is to be followed by one giving the findings of the investigations in the United Kingdom. Publication of the results of a three-year study, *Trade Unions in a Changing Society*, started in October 1960, was completed with the issue of three Broadsheets during the year (*Nature*, 200, 817; 1963).

Scientific American Offprints

Offprints of articles published in *Scientific American* are selling in increasingly large quantities throughout the world, particularly in the United States, where they have been adopted in many colleges and universities as supplementary material for text-books, some of which become out of date quickly. The articles are written by leading scientists actively engaged in research. Recently issued offprint booklets range in subject from fundamental particles in physics to recent research on the mechanism of the human body in conserving the oxygen supply to the brain at times of threatened asphyxia. In each the English style is clear and simple, the argument is presented logically and succinctly, and the presentation with regard to printing diagrams is of a high quality. The documentation is useful and sufficient. In the United Kingdom, offprints are obtainable from W. H. Freeman and Co., Ltd., Warner House, 48 Upper Thames Street, London, E.C.4. *Catalogue* No. 4, 1964, contains a list of 865 titles arranged under subject headings, and there is an author index. The offprints cost 1*s*. 6*d*. each and cover the life sciences, chemistry, physics, technology, psychology, the social sciences, the earth sciences and the history and philosophy of science. The booklets are to be welcomed both as aids to teachers and students, who wish to keep abreast of their subjects, and to the scientific reader who wishes to keep in touch with developments in disciplines other than his own.

Magazine of Concrete Research

With the March issue of this well-known publication of the Cement and Concrete Association (16, No. 46; 1964), it now becomes a quarterly instead of a four-monthly journal, and to mark this change it appears with a new cover design; the internal appearance of the *Magazine* remains, however, unchanged. The reasons are common to the task of many editors of scientific journals, among which are the increasing number of papers selected and recommended for publication, in this case by an independent advisory board; the endeavour to contain this material within the bounds of the *Magazine* itself; and the consequent increasing lapse of time between the receipt of a manuscript and its appearance in print. Another factor in the situation is that latterly discussions of papers published have tended to be crowded out by the pressure of new contributions awaiting publication. A further innovation in this *Magazine* is described in the editorial comment: "This ever-increasing volume of published material presents the research worker and the librarian with problems in their turn. However, to offset the extra contribution which the *Magazine* will be making in future, it has been decided to include abstracts of the articles appearing in the *Magazine*, and this issue contains the first of these abstracts. The abstracts themselves are printed on a separate sheet so that their removal from the *Magazine* will not spoil its appearance in any way". The abstracts are the authors' own summaries; they are printed on pale-green card, each comfortably contained within a delineated area of 6 × 4 in., which can easily be cut out for card-indexing. This is a feature which could well be copied in other technical journals to facilitate quick reference. The editorial advisory board, equally the Editor himself, are to be congratulated on the determination and pursuit of a policy which can only serve to enhance the prestige and long-proved value of this excellent publication.

The Museums Journal

VOL. 64, No. 1, of *The Museums Journal* is devoted to the scientific aspects of the work of museums and art galleries. In this field, science has two functions—the conservation of objects in the museum and the preservation of the apparatus used by the early scientists. Dr. A. E. Werner deals with some of the endless variety of materials now available for conservation work, and Mr. G. Thomson with the chemical and physical aspects of the changes which occur in various objects. Mr. S. Slabczynski describes a moderately sized laboratory for the restoration of paintings, and Mr. F. Greenaway gives an admirable illustrated description of the new Chemistry Gallery at the Science Museum, Kensington. Mr. N. W. Bertenshaw similarly describes the Industrial Museum at Birmingham and touches on the basic principles involved in the establishment and maintenance of such museums, while Mr. B. J. Ford details a new technique for mounting museum specimens which consists of sealing a jar by means of a solution of polystyrene in xylene. The annual report of the Council of the Museums Association for 1963–64 concludes an especially interesting number.

Man as a Subject for Science

THE sixth Auguste Comte Memorial Lecture was delivered by Prof. A. J. Ayer at the London School of Economics and Political Science on February 7, 1964, under the title "Man as a Subject for Science" (Pp. 26. London: The Athlone Press, University of London. Distributed by Constable and Co., Ltd., 1964. 5*s*. net). Pointing out that the social sciences can only muster a comparatively small stock of generally accredited and well-tested theories, Prof. Ayer suggests that their relatively poor stock of generalizations may be due to something inherent in the material with which social scientists have to work. He recognizes that this idea must be received with caution and that the issue of determinism must be decided. The important question is whether human behaviour is, or is not, entirely subject to law. In discussing this, Prof. Ayer does not accept as necessarily true the common belief among philosophers that motives are not causes and suggests that causal relations should be regarded as holding between facts rather than events—where 'fact' is understood in the wide sense in which true propositions of any form can be taken as expressing facts. He does not, however, think that the issue of determinism should be interpreted as an *a priori* question. The strength of the determinists lies in the fact that there seems to be no reason why the reign of law should break down at this point, though this argument seemed more convincing in the age of classical physics than it does to-day; that of the indeterminists lies in the fact that the specific theories which alone could indicate or give any substance to their opponents' case have not yet been more than sketched. Until such theories are properly elaborated and tested, Prof. Ayer thinks that there is little more that can usefully be said about this topic. As to whether the denial of determinism is implied in our usual ascriptions of moral and legal responsibility, in so far as this is a question of what people actually believe, Prof. Ayer is now inclined to believe that the antithesis between the claims of free will and determinism is not illusory but that our ordinary ideas of freedom and responsibility are very muddle-headed although firmly held. It would not be at all easy to estimate the social consequences of discarding them.

Man, the Social Animal

THE first part of K. W. Monsarrat's *On Human Wisdom* is concerned with man's innate ideas regarding the nature of and the structure of what he names "I myself" and "the world", including his attempts to give subjective and objective accounts of both and the value of the relative truths given to man by his validity judgments (Pp. 24. Liverpool: Henry Young and Son, Ltd., 1964. 6*s*.). The second part is concerned with man in his social relations, discussing the dependence of the stability of social groups on the quality and degree of aid which the individuals composing them have given to each other and emphasizing that, on the national level, the attainment and retention of prosperity depend on the Government being concerned for the welfare of all its members and seeking agreements on the rewards due for the various types of work they perform. He sees the pattern of Government needed as provided in microcosm by the physiological account of the function performed by the nervous sub-system in organizing the dynamic relations between the organs comprising the individual. Reviewing briefly Britain's social history and the functioning of the Coalition Government in the Second World War, Mr. Monsarrat postulates for the future Government of Britain a cessation of party conflict within Parliament; a Parliament of men whose overriding interest is the cultivation of mutual aid and friendship in all home affairs and who will call on the whole people to unite in a war-effort against war and disease in any nation, and undeterred by any alliance from including in its offers of aid and friendship the U.S.S.R., China or any other nation. Such a Parliament could plan a more promising effort to prevent global war than sending delegates to a disarmament conference. It would also implement a fair-shares-of-wealth policy, firmly based on the value of contributions to the nation's well-being and on the education and training needed for different types of work.

Leukopoiesis in Health and Disease

ARTICLE 2 of Volume 113 of the *Annals of the New York Academy of Sciences* is entitled "Leukopoiesis in Health and Disease" (By Howard R. Bierman and 124 other authors. Pp. 511–1092. New York: New York Academy of Sciences, 1964. 8 dollars). The forty original papers in this volume (one of the largest in the series) were presented at a conference in May 1963. The origin and formation of the white cells of the blood have been the subject of increasingly concentrated research in recent years, and this collection gives a cross-section of present trends. Certain widely influential notions have strongly influenced attitudes and methods in this field; for example, fluctuations in the relative or absolute numbers of white blood cells in health or disease are regarded as disturbances of an underlying homœostasis, and it is the mechanism of the latter which receives most attention. The use of radioactive tracers has given to the investigation of the formation, circulation and recirculation, and final disposal, of blood cells a new power and precision. Short-term culture of blood or marrow cells in the presence of phytohæmagglutinins has made possible chromosome analysis of normal and leukæmic cells (five papers deal with chromosome abnormalities). New surgical techniques for prolonged collection of thoracic duct lymph have made quantitative examinations of lymphocyte production possible, and the use of these, together with radioactive labelling, promises a solution of the old problem of the life history and function of the lymphocyte in the not-too-distant future (four papers deal with lymphocyte kinetics). The foregoing are some of the subjects covered by this large and varied collection of articles. Though they are, for the most part, records of new work or progress reports, and only incidentally review past work, the volume as a whole gives a good conspectus of this fertile field of research.

Architecture of the Oviduct of the Domestic Hen

According to its gross appearance, the oviduct of the domestic hen can be divided into several parts. Romanoff has described the infundibulum, the albumen secreting region (magnum) and the isthmus, while Sato, Koso and Osaki have noted a narrow part between the infundibulum and the magnum. On the other hand, the hen's egg consists of yolk, chalaziferous layer, layers of albumen, shell membrane and the egg shell. It is known that the chalaziferous layer is formed in the oviduct before the albumen secreting region, that the major part of the albumen layer is formed in the magnum, and that the shell membrane is formed in the isthmus. Consequently, it is expected that the structure, function and the product of the oviduct will present marked differences in the various segments. Histological and cytological investigations of the hen's oviduct, however, are still limited and fail to demonstrate local specialization of the oviduct. An investigation by Kazuaki Misugi and Tadashi Katsumata, of the First Department of Anatomy, Yokohama University School of Medicine, Yokohama, describes the electron microscopical characteristics of the cells composing the main segments and correlates these with the different types of proteins forming the egg (*Yokohama Medical Bulletin*, 14, No. 6; December 1963). The description is confined to the electron microscopic examination of the secretory cells in an active stage of secretion and reveals that the segments are composed of different cells and show segmental specialization. This segmental morphology may be correlated with the functions of the respective segments in the formation of the egg.

The Effect of Isoniazid on Protein Synthesis of *Mycobacteria*

Michio Tsukamura and Sunio Tsukamura of the Obuso National Sanatorium, Obu, near Nagoya, Aichi-ken, Japan, have observed the effect of isoniazid on the protein synthesis of *Mycobacteria* (*Jap. J. Tuberc.*, 11, Nos. 1–4; December 1963). Their investigations show that incorporation of radioactive glutamic acid into protein is inhibited by isoniazid at a concentration of 20 μg/ml. This means that protein synthesis in the organisms, *Mycobacterium* 'Jucho' and *M. tuberculosis*, is inhibited by isoniazid. The effect of this drug on the incorporation of amino-acids into protein is heterogeneous; incorporation of glutamic acid was inhibited, while incorporation of glycine and leucine was not inhibited. It was shown also that α-ketoglutarate is converted to glutamic acid and then incorporated into protein. The former was not inhibited, but the latter was inhibited by isoniazid. It is still not clear whether the inhibition of protein synthesis is a primary effect of isoniazid. The finding that incorporation of α-ketoglutarate into the nucleic acid fraction was also inhibited by isoniazid makes it difficult to draw a definite conclusion. It is remarkable that the pattern of distribution into cellular fractions of radioactivity from incorporated α-ketoglutarate differs significantly from the pattern of acetate. Most parts of radioactive acetate-1-^{14}C were incorporated into the lipid and protein fractions, whereas only a small part of the keto acid was incorporated into the lipid fraction. However, this pattern of incorporation of the keto acid is different in *M. tuberculosis* from that of *M.* 'Jucho'. In *M. tuberculosis* a large amount of the keto acid could be incorporated into the lipid. It is thus probable that the pattern of lipid synthesis in *M. tuberculosis* differs from that of *M.* 'Jucho'.

Australian Mineral Industry

The Australian Bureau of Mineral Resources, Geology and Geophysics, among its other official publications, issues periodically *The Australian Mineral Industry—Quarterly Review* in which reasonably up-to-date information on production of selected minerals and metals and on smelter and refinery production can be sought. A recent copy (16, No. 3; March 1964) contains appropriate data (up to February 1964) on fuels (black and brown coal and petroleum); metals: aluminium, antimony, copper, gold, iron and steel, lead, manganese, monazite, tin, tungsten and zinc. In the section on industrial minerals, are included details of asbestos, gypsum, ilmenite, nitrogenous fertilizers, phosphates, rutile, sulphur and zircon. There is a keen overseas demand for Australian opal, with Japan the principal buyer, which, in the third quarter of 1963, rose to the highest value ever recorded in a three-monthly period, nearly three-quarter million pounds sterling. Part 2 of this publication contains statistics relating to Australian production of and overseas trade in minerals and mineral products for the quarter ended September 30, 1963. In addition to the *Quarterly Review*, the Bureau also publishes an *Annual Review*; this surveys the industry as a whole and comprises a general review, review of metals and minerals, and general statistics as available for each year.

Mineralogy in Japan

Progress in research in mineralogy in Japan can be followed in periodic publications of the Mineralogical Society of Japan (Chigaku Center, Niban-chô, Chiyoda-ku, Tokyo). The *Journal of the Mineralogical Society of Japan* (*Kôbutsugaku Zasshi*) contains articles and reviews on mineralogy and allied sciences in Japanese contributed by members of the Society, and is published every four months. The *Mineralogical Journal* is a half-yearly publication, principally in English, and is therefore probably of wider appeal than the *Journal* of the Society itself; it invites contributions from everyone. In the issue recently received (4, No. 1; February 1963), H. Shirozu contributes a paper on structural changes of some chlorites by grinding, in particular the unusual sensitivity found in the orthohexagonal iron chlorites attributed to this cause; T. Murano discusses radioactive equilibrium in the Ningyotoge uranium ores, especially between ionium and uranium; A. Kawahara describes his X-ray studies on some alkaline amphiboles (arfvedsonite and riebeckite), with somewhat surprising optical results; K. Hayase, R. Ōtsuka and T. Mariko contribute an important paper on the magnetic properties of natural pyrrhotite; among other conclusions they draw from results of considerable experimental work on this notoriously variable species, is that naturally occurring pyrrhotite is a mixture of 'peak-' and 'Weiss-type' pyrrhotite grains and the magnetic properties of the mineral change with the ratio of one to the other occurring therein. A valuable addition to these papers is the inclusion of mineralogical abstracts from scientific papers published in Japan (1960–62). The English rendering throughout the *Mineralogical Journal* is generally good; the presentation of the papers with their illustrations (mostly line-drawings), while perhaps not attaining the high standard achieved by its contemporaries in mineralogy in Western countries, is none the less reasonably satisfactory.

University News: Belfast

THE following appointments to lectureships have been announced: Dr. P. M. Robinson (botany); A. Refsum, Dr. P. D. Roberts and Dr. G. Williams (electrical engineering).

Birmingham

THE following appointments have been made: *Lectureships*, Dr. M. R. Daniel (physics); C. Fraser (social psychology); Dr. G. G. Henshaw (botany); M. Jobes (physics); M. C. Scott (physics); Dr. Heidi H. Swanson (anatomy); *Research Fellowships*, J. G. Allen (anatomy); Dr. N. A. Bukhari (chemistry); A. J. Collings (medical biochemistry and pharmacology); J. D. Davies (physics); Dr. J. C. Dore (Nuffield Research Fellow in the Department of Physics); G. J. Hawkins (electronic and electrical engineering); P. Hicken (pathology); J. Insley (Senior Research Fellow in the Institute of Child Health); D. A. Jackson (physics); E. W. Taylor (zoology and comparative physiology), J. E. Williams (industrial metallurgy).

London

MR. J. D. SARGAN, reader in econometrics in the London School of Economics and Political Science, has been appointed to the chair of econometrics tenable at that School. Dr. P. H. Venables, of the Medical Research Council–Social Psychiatry Research Unit, has been appointed to the readership in psychology tenable at Birkbeck College. Titles have been conferred as follow: reader in physiology, on Mr. J. E. Pascoe in respect of his post at University College; reader in sociology (with special reference to criminology), on Dr. T. P. Morris in respect of his post at the London School of Economics and Political Science; professor of sociology (with special reference to politics), on Dr. R. T. McKenzie in respect of his post at the London School of Economics and Political Science.

Royal College of Science and Technology, Glasgow

THE annual report of the Royal College of Science and Technology, Glasgow, for 1962–63, covering the 167th session, records a total of 2,004 full-time and 2,150 part-time students, compared with 1,997 and 2,546, respectively, in 1961–62; the number of part-time students has steadily fallen from 3,241 in 1959–60 (Pp. 95. Glasgow: The Royal College of Science and Technology, 1964). In his foreword the principal, Dr. S. C. Curran, refers to the Government's announcement that the College was to be given full university status and the choice of the name, the University of Strathclyde, with Lord Todd as first Chancellor. An imperative need stemming from the developments now proceeding is that of student residences, where the College is at a grave disadvantage. Discussions with representatives of the Scottish College of Commerce are examining the possibility of amalgamating that College with the new University. Besides lists of publications, Council and heads of departments, the report includes the usual departmental reports which refer to the increasing volume and scope of the research effort. Dr. Curran refers particularly to the start-up of the reactor at the East Kilbride Research Centre in a collaborative venture with the Scottish universities and the Queen's University, Belfast, and supported by a large grant from the Department of Scientific and Industrial Research. Further details of present research work are given in a separate *Report on Research 1962–63*, which also includes lists of publications and titles of theses approved for higher degrees, arranged under Departments (Pp. 53. Glasgow: The Royal College of Science and Technology, 1964).

The Night Sky in September

NEW moon occurs on Sept. 6d 04h 35m U.T. and full moon on Sept. 21d 17h 31m. The following conjunctions with the Moon occur: Sept. 2d 13h, Mars 1° S.; Sept. 2d 16h, Venus 5° S.; Sept. 19d 11h, Saturn 3° N.; Sept. 25d 19h, Jupiter 1° N. Venus is in conjunction with Pollux on Sept. 2d 14h, Venus being 9° S., and Mars with Pollux on Sept. 5d 05h, Mars being 6° S. Mercury is a morning star, rising nearly two hours before the Sun on Sept. 20; it is visible low in the south-east before sunrise, and may be observed during the last three weeks of the month. Venus is a morning star, rising at 1h 10m, 1h 25m and 1h 55m on Sept. 1, 15 and 30, respectively. Its stellar magnitude is $-3{\cdot}8$; its distance from the Earth increases during the month from 67 to 89 million miles and the visible portion of the apparent disk from 0·516 to 0·654. Mars rises at 0h 45m, 0h 30m and 0h 20m at the beginning, middle and end of the month, respectively. Its stellar magnitude is $+1{\cdot}6$, and conditions for observation are becoming more favourable. Jupiter rises at 21h 10m, 20h 15m and 19h 15m on September 1, 15 and 30, respectively. It is near the boundary between Aries and Taurus, its stellar magnitude is $-2{\cdot}2$ and its distance from the Earth on September 15 is 415 million miles. Saturn sets at 4h 20m, 3h 25m and 2h 20m at the beginning, middle and end of the month, respectively. It is in Aquarius, moving westwards among the stars. Its stellar magnitude is $+0{\cdot}7$ and its distance from the Earth on September 15 is 822 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Sept. 1d 02h 28·8m, 3 Gem. *m* (*R*); Sept. 18d 20h 25·5m, κ Cap. (*D*); Sept. 29d 00h 20·8m, 87 B. Gem. (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The autumnal equinox occurs on Sept. 23d 00h 17m.

Announcements

THE fourth annual general meeting and conference of the Society for Folk Life Studies will be held in Cardiff during September 11–13. Further information can be obtained from J. Geraint Jenkins, Welsh Folk Museum, St. Fagans, Cardiff.

THE thirty-eighth annual conference of Aslib entitled "Looking Forward in Documentation" will be held in the University of Exeter during September 28–October 1. Further information can be obtained from Aslib, 3 Belgrave Square, London, S.W.1.

A MEETING of the British Society of Rheology will be held in the University of Exeter during September 28–30. Papers will be presented on rheometry and the teaching of rheology. Further information can be obtained from Dr. M. F. Culpin, 8 The Broadway, Pontypool, Monmouthshire.

THE fifth course in "Histochemistry", arranged by L'Institut d' Histochemie Médicale, will be held in Paris during September 28–October 10. Further information can be obtained from the Secrétariat de l'Institut d'Histochemie Médicale, Nouvelle Faculté de Médecine, 45 rue des Saints-Pères, Paris 6.

A SYMPOSIUM on "The Application of Chemical Engineering in Newly Developing Countries", organized by the Institution of Chemical Engineers, will be held in London on September 29. Further information can be obtained from O. G. Weller, Institution of Chemical Engineers, 16 Belgrave Square, London, S.W.1.

ERRATUM. In the communication entitled "Allelism of Silver, Gold, and Imperfect Albinism in the Fowl" by Prof. R. K. Cole and T. K. Jeffers, which appeared on p. 1238 of the December 31, 1963, issue of *Nature*, lines 13 and 14 should read ". . . Identical linkages (7·0 per cent) were . . . ".

A COMPREHENSIVE SCHEME FOR THE QUALITY CONTROL OF DRUGS AND MEDICATED PRODUCTS

THE following scheme for the quality control of drugs and medicated products was drawn up by a technical committee of the Pharmaceutical Society of Great Britain and deals with, in its widest aspects, the testing of drugs from the stage of their importation or manufacture to the delivery of the dispensed medicine to the patient. The report has been submitted by the Society to the Ministry of Health.

As a consequence of its earlier consideration of various aspects of quality control applicable to drugs and medicated products, the Science Committee (Pharmaceutical Analysis) of the Pharmaceutical Society of Great Britain was requested in June 1963 to prepare a model drug-testing scheme to be operated in the United Kingdom. It seemed expedient and proper to consider a more comprehensive scheme than those now operating to ensure that all drugs supplied to the public, in whatever manner, should be controlled.

In addition to the sampling and testing of drugs (a necessary process in any system of quality control) provision is planned for inspecting, licensing and registering premises in which drugs are manufactured, compounded or stored. A proposal for controlling imported drugs is also included. To make such control effective, the Committee envisages the establishment of a Directorate having the necessary legal powers with inspection and with laboratory facilities. (Detailed considerations of the legislation required and of the financing of the project were considered to be outside the terms of reference of the Committee.)

In presenting its proposals, the Committee realizes that expediency may require these proposals to be modified for an interim period during which, for example, it may be necessary to correlate them with existing legislation and to use some of the existing machinery for testing drugs. Nevertheless, this period should be strictly limited and no modification should violate the basic principle that all drugs supplied to the public should be subject to scrutiny.

REGISTRATION AND LICENSING

The bodies over which some degree of control will need to be exercised include: drug manufacturers; medicated product manufacturers; importers of drugs both in bulk and as formulated products; drug wholesalers; hospital manufacturing and dispensing units; medical, pharmaceutical, dental and veterinary practitioners who carry out dispensing; clinics, and all retailers handling drugs. The purpose of registration and licensing is to ensure, so far as is reasonable, that any premises used for the manufacture, warehousing or distribution of drugs are satisfactory. The issue of a licence to manufacture, import or distribute will depend on the acceptance of the principle that the accredited representative of the Executive Organization has a right to enter, inspect and take samples. Re-licensing will be necessary following a change of ownership. Since the deterioration of drugs during storage is a major reason for the sale of poor-quality drugs, the adequacy of warehouse and other storage facilities requires attention.

Manufacturing Units

The registration and licensing of premises to be used for the manufacture of drugs in bulk or as formulated products, including medicated toilet preparations, will depend on the general level of hygiene and management. The maintenance of an efficient system of internal control records, open for inspection, relating to the various products is an integral part of a control operation. The existence of a scheme adequate for tracing the origin of any batch of drug or medicinal preparation should be a part of any internal record system.

Premises. These will be subject to inspection or approval before a licence to manufacture drugs is issued. The facilities available and the condition of the premises will determine the decision of the Executive Organization. The issue of a licence will be for a limited period of, say, three years, after which renewal will be necessary. As it is imperative that there be some measure of control of the raw materials, including packaging materials, right of entry to the premises must be allowed to inspectors appointed by the Executive Organization. Such inspectors must also have access to the production records and be given the powers to take any necessary action.

Personnel. The complexity of modern pharmaceutical industry necessitates that effective supervisory responsibility for drug manufacture be taken by highly qualified and experienced personnel. The manufacture of bulk drugs should be controlled by professionally qualified persons, such as chemists, pharmacists or chemical engineers, while the manufacture of medicated products in industry and in hospital practice should be the responsibility of pharmacists or chemists having appropriate experience. This supervisory staff should be listed by the Executive Organization, who would be informed immediately of any change in personnel. As an interim measure only, the Committee considers that a list of technicians and other experienced operatives, not qualified in the professional sense, be compiled. Such people may be granted responsibility for supervisory control where it can be shown that they have had the necessary supervisory experience within the industry for a period of not less than five years before the date of the inception of this scheme.

Warehouses and other Storage Units

Experience in the pharmaceutical industry has shown that there can be a considerable delay between the manufacture of a medicated product and its utilization by members of the public. The instability of certain drugs and the products containing them makes it imperative to have proper conditions of drug storage in order to avoid deterioration.

Bulk drug manufacturing units. The right to inspect storage areas is implicit in the issue of a licence to the drug manufacturing organization, since it is important that the drugs should not be subject to rodent infestation or other contamination, or to extreme moisture and temperature conditions.

Hospital manufacturing units. The quantities of bulk drugs in these units will be relatively small and storage in the pharmacy may be adequate. If it is necessary to store outside the pharmacy the factors under the foregoing "Bulk drug manufacturing units" must be taken into account.

Wholesalers' warehouses. These premises must be subject to the same control as for "Bulk drug manufacturing units". Special attention will have to be directed to the establishment of a satisfactory system designed to prevent the accumulation of old material, and to products carrying an expiry date.

Importers' warehouses. Since comprehensive inspection of foreign manufacturers' premises is not, in general, practicable, some control will need to be exercised when the product is landed in the United Kingdom. Warehouses at the docks and airports will require registration and be subject to inspection, etc. Appropriate information on the commodity is required. A separate clearance certificate will be necessary on each occasion. While bonded warehouses into which imported drugs will go pending their clearance will not normally be subject to inspection and registration, it is considered that the full co-operation of the Board of Trade in disclosing such importations will be required and that the storage conditions are suitable. The rejection of unsatisfactory imports at this stage could prevent their transfer to the importers' warehouses.

Outlets to the Consumer

As the purpose of a comprehensive quality control scheme for drugs is that a pharmaceutically satisfactory product be made available to the public, some inspection control must be exercised at this point of distribution. The distribution points are concerned with the unrestricted purchase of medicated products. Typical examples are pharmacies, drug stores, listed sellers, etc. An important contact point is that which occurs following the presentation of a prescription in either medical, pharmaceutical, dental or veterinary practice.

Sale by retail. The Committee feels some concern at the present situation where it is now possible to purchase drugs and medicated products from stores, grocers, market stalls, licensed premises, etc., where control is exercised by people with little or no knowledge of the problems associated with the drugs. The use of vending machines inadequately protected from the weather and granting easy access to drugs is to be deplored. It is therefore essential that all premises used for the sale of drugs should be registered and subject to inspection and approval.

Dispensaries. Dispensaries may be in retail shops, doctors' surgeries, hospitals or clinics. All such premises in which prescriptions from members of the medical, dental or veterinary professions are dispensed should be registered and open to inspection by the representatives of the Executive Organization.

SAMPLING AND TESTING

The object of sampling and testing is to provide an effective check on the quality of drugs being offered to the public as prepacked drugs, formulated products and dispensed preparations, etc., and to determine the source and nature of any defect. The range of products to be controlled comprises raw materials, drugs and packaging materials, formulated products, surgical dressings and sutures, medicated cosmetics and toiletries, medicated veterinary preparations, dispensed medicine and equipment for which claims for sterility are made. In the opinion of the Committee the functional testing of appliances is outside the scope of this quality control scheme. Inspectors must be empowered to sample at any stage of production or formulation and to sample the finished product at any point between the manufacturer and the consumer. By using proper control records, as stipulated, any defect that is revealed can be traced to its source. Where adequate records by manufacturers and importers exist, and are available for inspection, the extent of sampling may be reduced.

Sampling Techniques

Where a sample can be divided into replicate portions, it should be divided into three by the inspector in the presence of a responsible person. One portion should be handed to the person from whom the sample was obtained and the remaining samples taken away by the inspector. Of these two samples, one would be the subject of examination by the official analyst and the other would be retained as reference sample for independent testing should this be necessary. It is essential that each sample should be sealed, and clearly identifiable. The present sampling technique that is operated under the Food and Drugs Act would be applicable to general sampling under this scheme. For dispensed medicines, the sampling procedure detailed in Part 1 of the Seventh Schedule of the Food and Drugs Act, 1955, would possibly be suitep after the preliminary procedure specified under the Ministry of Health E.C.L. 7/61 Appendix 1, para. 6 (*a*). Informal sampling for the purposes of obtaining general information is not precluded from the scheme.

Raw materials (*chemical and packaging materials*). Evidence on the quality of raw materials (and the packaging materials) could be obtained from the inspection of manufacturers' control records. Samples will be taken where, in the opinion of the inspector, doubts as to quality exist. In this connexion the use of such terms as *British Pharmacopœia* (B.P.), *British Pharmaceutical Codex* (B.P.C.), etc., demands complete compliance with the official monograph.

Formulated products (*medical, veterinary or cosmetic*). The routine sampling of manufacturers' stocks should be unnecessary where inspection of the internal control records indicates proper supervision and regular control. Where little or no analytical control is exercised, consultant-analyst certificates would give an indication of the extent to which products might need further examination.

An indication of product stability is of importance and attention will need to be directed to an expiry date to be applied to those products for which prolonged storage is undesirable.

Wholesalers' stocks. Probably only a few samples will need to be taken from the wholesalers' stocks, although the inspectors will have to ensure that the conditions of storage are satisfactory.

Importers' stocks. Importers of bulk drugs and formulated products should provide adequate information on: (*a*) specification of the raw materials used; (*b*) details of product manufacture; (*c*) details of product specification and control directions; (*d*) product stability; (*e*) intended procedure for distribution of stocks. Importers must supply samples on request. All imports should be submitted to some form of control as early as possible and each batch of each product must have an acceptable certificate of analysis. It is envisaged that protocols of analysis from the consignors might be permitted, but the presentation of such documents does not preclude any sampling that might be considered desirable. The issue of a certificate to distribute would generally involve an examination by a consultant in Britain, registered as competent in the analysis of medicated products and pharmaceuticals. Holders of the joint Pharmaceutical Society and Royal Institute of Chemistry Diploma in Pharmaceutical Analysis are considered to satisfy this requirement.

Dispensed products. The present sampling of dispensed medicines under the National Health Service Acts is felt to be inadequate both in scope and manner. All dispensaries, whether under medical, pharmaceutical, dental or veterinary direction, should be subject to some form of control. The techniques of sampling might usefully form the basis of discussions with representatives of the professions concerned. It is proposed that the inspector be authorized to take as a sample any medicine or form of treatment, including proprietary preparations, awaiting collection for a patient (human or animal). It is implicit in this scheme that the inspector be provided with a copy of the relevant prescription. Quantities, for example, of tablets, supplied against a prescription could be checked on the spot by the inspector.

Retailers (*pharmacies, listed sellers, drug stores and retail suppliers of medicinal products*). It is expected that, as at present under the Food and Drugs Act, most samples will continue to be taken from retail establishments. It would be convenient to use the proposed scheme to exercise enforcement of Section 2 of the Pharmacy and Medicines Act, 1944, or the appropriate section of a new Consolidated Drugs Act. Pharmacists, by virtue of their professional training, will have knowledge of drug deterioration, but other retailers, having little or no experience of the problems, can unwittingly subject medicated products to storage and exposure conditions that are quite unsuitable; the use of batch identification codes is important in tracing any errors. A special example of unsatisfactory storage is the vending machine which may be exposed to a wide range of climatic conditions.

Sample Records

Bulk drugs and formulated products from U.K. manufacturers. A comprehensive quality control system can only be operated in a manufacturing unit where an efficient internal control system exists. While it is not part of the Committee's responsibility to define what system should be adopted, certain features are considered to be essential if the scheme is to work to the advantage of both the manufacturer and the public. For example, adequate specifications and control methods for both raw materials and finished products are fundamental. Manufacturers should investigate the stability of their products, and keep adequate records available for inspection; inspectors must have power to examine such records. As an interim measure only—and for such time as may be determined by the Controlling Authority—there may be some relaxation in respect of stability records, but manufacturers are urged to take serious note of the recommendation.

Supplies from importers. In the absence of satisfactory technical information submitted by the consignors or by the consignee's consultant analyst imports need to be sampled. Information will be required on: (*a*) the raw materials used; (*b*) specifications and control methods for the formulated products; (*c*) stability.

Reimbursement for samples taken. A scheme will have to be developed whereby reimbursement can be made for samples taken for examination.

ADMINISTRATION

The Committee does not propose to specify the form of the Controlling Authority, which would undoubtedly have wider responsibilities than quality control. While the Minister of Health may have governmental responsibility, the executive functions should be carried out by an independent body having the necessary legal powers. This body should be given the entire responsibility for the quality control of drugs.

Executive Organization

In the opinion of the Committee, the Pharmaceutical Society of Great Britain would be an appropriate 'Executive Organization'. The Society already has enforcement duties under existing Pharmacy Acts and its inspectors have considerable experience of conditions in retail pharmacy.

Advisory Committee

An advisory Committee—working in a voluntary capacity and including experienced representatives of the pharmaceutical industry—should be instituted to make recommendations on 'special cases' that may arise during the operation of any quality control programme, to advise on matters concerned with premises, registration, the issue of licences, etc., and to advise on general policy.

Directorate

To achieve effective quality control, a 'Directorate of Drug Testing' should be established. The Directorate would have the necessary powers of entry, inspection and sampling, and its own laboratory facilities. The director-in-charge, who should have appropriate technical qualifications, would be required to administer the scheme as a whole, and would be concerned with the day-to-day administration, inspection and sampling and with the analytical services. He should be directly responsible to the Executive Organization for all the activities of his department. The activities of inspectors and analysts involved in the control programme should be the responsibility of the director.

Inspectors

Inspectors should preferably hold a pharmaceutical qualification, but this would not preclude the appointment of other suitably qualified persons for specialist duties.

Qualifications. Inspectors must be trained in the techniques of sampling. Practical experience of manufacturing processes would be an advantage, as this would enable inspectors to act in an advisory capacity in matters related to drug control.

Functions of inspectors. The functions of inspectors will include: (*a*) inspection of premises of organizations seeking registration; (*b*) inspection on a routine basis of factories, warehouses, wholesalers' storerooms, hospital manufacturing and storage areas, dispensaries (medical, pharmaceutical, dental and veterinary), drug retailers' premises, clinics; (*c*) routine examination of control records of manufacturers; (*d*) routine examination of data sheets on importations; (*e*) the taking of samples and carrying out of local checks.

Manufacturers' analysts as inspectors. In other contexts manufacturers' analysts, under the supervision of the official inspectors, have been appointed as inspectors of their own organizations. In the proposed scheme this principle merits consideration.

Laboratory Services

A central laboratory will be required to carry out the analysis of samples submitted by the inspectors. Regional laboratories which will handle routine work may be needed since uniformity of interpretation of analytical results and of control is essential; the regional laboratories must be placed under the authority of the Central Laboratory. As an interim measure, until the establishment is at the requisite level, use of other laboratories, for example, those of public analysts and analysts holding appointments under the National Health Service Acts, might be considered. The authority of the director will, however, be paramount even where public analysts, who may be called on to sample over-the-counter sales, are concerned. The Laboratory of the Government Chemist should be used as a reference laboratory in cases of dispute.

Staffing (*personnel*). It is vital to the whole programme of a quality control scheme to establish confidence in the control exercised by the Central Laboratory. For this reason the staff must be of the highest calibre since it will be called on to conduct intricate analytical procedures and to undertake such analytical research as may be necessary to resolve problems. The senior members of the staff will be expected to advise the director on scientific and technical matters relating to the scheme. They would further be expected to participate on professional committees and collaborate on investigational work, possibly at international level.

Notes: (1) Since the testing of dressings requires special techniques, use should be made of established organizations, for example, the Manchester Chamber of Commerce Testing House and Laboratory. (2) Specialized testing of materials by bioassay techniques will involve the co-operation of established organizations capable of making such tests.

LEGISLATION

It is not the purpose of this Committee to indicate in detail what legislation is involved, but it directs attention to the following matters. Consolidating legislation would have to be introduced to correlate and extend the existing legislation under the Pharmacy and Poisons Act, 1933, the Food and Drugs Act, 1955, the National Health Service Acts, the Pharmacy Act, 1954, and to provide the powers necessary to permit the Executive Organization to conduct its affairs in an efficient and responsible manner.

Right of entry and inspection. The inspectors must be given the necessary powers to carry out their duties properly both as regards sampling and the inspection of premises and records.

Test results. A procedure must be developed whereby information on the results of the tests are transmitted to the source from which the sample was obtained.

Sanctions. In addition to the usual legal penalties the following sanctions might be applied: (*a*) withdrawal of licence; (*b*) removal of name from approved list of supervisory staff; (*c*) stock recall, impounding or destruction.

Appeals. There must be included some means of appeal against decisions of the inspectors or the director.

FINANCIAL

Whereas a detailed consideration of the financial aspects of the scheme is beyond the terms of reference of the Committee, attention is directed to the following points:

Expenditure. (1) Administrative expense of Executive Organization. (2) Administrative expense of Directorate and Inspectorate, including laboratory services. (3) Fees to outside analysts (for example, analysts responsible for bio-assays, public analysts, National Health Service analysts, Manchester Chamber of Commerce Testing House).

Income. (1) From registration and licensing fees (except dispensing areas). (2) From the Treasury.

INTERPRETATION

A drug (or pharmaceutical preparation) is any chemical or natural product or mixture of substances, including natural products, sold, offered for sale or represented for use in: (*a*) the diagnosis, treatment, mitigation or prevention of disease, abnormal physical state or the symptoms thereof in man or animal; (*b*) restoring, correcting or modifying organic functions in man or animal.

Drug in bulk. A chemical or natural product stored in bulk quantity intended directly for use in the manufacture of medicinal products.

Formulated product. A drug or mixture of drugs suitably prepared for administration as a medicine. A single chemical substance in small quantity, pre-packed for distribution.

Pharmaceutical specialty. A simple or compound drug ready for use, available under a special trade designation or in some characteristic form and containing one or more drugs which may or may not have official (that is, B.P., B.P.C., B.Vet.C.) recognition.

Proprietary preparation. A pharmaceutical specialty.

Surgical dressings, surgical sutures. Materials for application to wounds or lesions for therapeutic use or for their protection.

Dispensed medicine. Medicine dispensed for an individual patient in accordance with a prescription.

Medicated cosmetic or toilet article. A toilet article for external use on any part of the body, whether in liquid, solid or any other form and medicated so as to bring it within the requirements of the Pharmacy and Medicines Act, 1941.

THE SCIENCE COMMITTEE (PHARMACEUTICAL ANALYSIS)

The Science Committee (Pharmaceutical Analysis) of the Department of Pharmaceutical Sciences consists of: Dr. D. C. Garratt (chairman), Mr. J. Allen, Dr. A. L. Glenn, Mr. C. A. Johnson, Mr. E. I. Johnson, Dr. R. F. Milton, Dr. H. D. C. Rapson, Mr. S. G. E. Stevens and Mr. G. Sykes. Not all members are pharmacists, but all in their day-to-day activities are intimately concerned with the quality of pharmaceutical materials; some have had experience of drug testing under the present restricted schemes.

THE FISHERY LIMITS BILL

THE Fishery Limits Bill, which received its second reading in the House of Commons on June 15, establishes the fishery régime in British waters for which the Fisheries Convention resulting from the fisheries conference some months ago provides. As the Minister of Agriculture, Fisheries and Food, Mr. C. Soames, explained in moving the second reading, the Convention, which has since been signed by twelve countries including the United Kingdom, allows the coastal State a twelve-mile fishery zone measured from base-lines, and within this zone the State has power to regulate the fisheries. In the inner six miles the fishing is reserved for the fishermen of the coastal State, subject to a short transitional period for foreign fishermen who have traditionally fished within it. In the outer six miles the fishing is in principle reserved for fishermen of the coastal State and other parties to the Convention who have habitually fished in that area. In this way such foreign fishermen will be restricted to the stocks and grounds which they have already fished.

Mr. Soames emphasized that while Britain had consistently favoured the doctrine of narrow limits, the Government had concluded that some extension of limits could no longer be denied to Britain's fishermen. The Government's sole motive had been to secure more scope for Britain's fishermen in Britain's coastal waters. As regards conservation, the Government considered that the proper way to conserve fish stocks was by international action equitable to all in the conservation commissions which had been set up and in whose work Britain played a leading part. Mr. Soames said that there were some two or three countries which were not parties to the Convention but had some interest in the fisheries around

Britain's coast, although the extent of that fishing was not very great and Britain would need to consider with them what their position should be. It was hoped that the new arrangements could be brought into full operation by September.

The kernel of the Bill was in Clause 1, which provided for the extension of Britain's limits and for her control of access by foreign fishermen to the fisheries within these limits. The Convention provided that to qualify for recognition to be able to continue fishing between six and twelve miles this foreign fishing must have been carried on habitually during the ten years up to and including 1962 and that it must not be directed in future towards new grounds or new stocks of fish. In drawing up the designation orders, Mr. Soames said that Britain would, of course, need to take account of the fact that fish move round and that many species are often found on the same ground, but we would be able to distinguish between herring fisheries, fisheries for white fish and shell fisheries. The power of designation was not limited to the vessels of countries which were party to the convention, and Britain's fishery agreement with Norway provided that if Britain established a fishery zone she would be prepared to make for Norwegian vessels arrangements corresponding to those which that agreement made for British vessels off Norway. The new base-lines which Britain was drawing were in conformity with the 1958 Geneva Convention and she would be able to close bays with straight lines up to 24 miles in length, whereas at present 10 miles are the maximum lengths for a bay closing line. Until the Bill came into full operation there would be a transitional period for traditional foreign fishing between three and six miles from base lines. This was dealt with in Clause 2.

The right to enforce conservation measures on British and foreign vessels was one to which Britain attached great importance, but it did not require new powers from the House. It was simply a question of adapting the powers which existed under present legislation to apply conservation regulations to British vessels. This was done in the first schedule to the Bill. Mr. Soames emphasized that Britain now had a modern instrument for promoting conservation through international co-operation in the North-East Atlantic Fisheries Commission of which all countries fishing in the north-east Atlantic without exception were members. It was the responsibility of this Commission to take all the measures that conservation demanded. At a meeting of the Commission in June on the initiative of Britain a special committee was set up which would be concerned with making collective arrangements for enforcing conservation measures on the high seas. This Committee would apply conservation measures to foreign vessels when they were fishing within Britain's own fishery limits as well as to British vessels. Mr. M. Noble expressed the hope that members of the North East Atlantic Fisheries Commission, which included Poland and other countries not signatories to the Convention, would be able to work together so that, while Britain could enforce within her twelve-mile limit the conservation measures which she thought right, these other countries would at the same time be able to work out conservation measures and internationally the same measures could be adopted and brought into force.

NUCLEAR POWER IN BRITAIN

In the debate in the House of Lords on June 10 on the White Paper, *The Second Nuclear Power Programme* (*Nature*, 202, 1247; 1964), Lord Coleraine was concerned with the reasons for Britain losing her commanding lead in this field to the United States, which he attributed to the organization of the nuclear power programme. This he thought was far less efficiently organized in Britain than in the United States and this was partly because each power station carried an enormous load of research and development charges. He was concerned that the ordering of an American reactor system would mean that American technology would dominate the nuclear industry in Britain and that in effect she was opting out of that industry. He urged that the Government should take a hard look at the organization of the nuclear programme and particularly at the position of the Central Electricity Generating Board. He thought it was the function of that Board to provide the consumer in Britain with adequate supplies of cheap electricity, but that this function could not conceivably be combined with pioneering a new, expensive and complex industry. He did not think that a healthy nuclear industry could survive simply on the basis of copying designs. He suggested that the Atomic Energy Authority should be responsible for development until the first commercial station was in operation and proved, and then sell it to the Central Electricity Generating Board at a price which would meet the subsidy requested by the chairman of that Board. This was the system adopted in Canada.

Lord Champion agreed that Britain did not have all the information which should be available when considering a matter of this immensity and importance. Moreover, she had not yet resolved the problem of the extent to which the Board and the Authority should bear the responsibility for research and development of civilian types of reactors and the legislation required to amend the Authority's statute. Nevertheless, he thought that *The Second Nuclear Power Programme* was right in its flexibility, but Viscount Caldecote thought that it was doubtful whether technical resources had been as well used as they could have been and that the responsibilities had not always been shared in the best way among industry, the Atomic Energy Authority and the Central Electricity Generating Board. He thought it essential to see that scarce national technical resources were utilized to the best advantage, electrical power supplied as economically as possible, that there was an active research and development programme and that an efficient industry should be allowed to make reasonable profits on the capital involved. The proper use of national resources, he pointed out, required a stable policy and that there should be no duplication between industry, the Authority and the Generating Board as well as a much better delineation of responsibility. A suitable administrative set-up in the Government was required to control the Generating Board, the Authority and research and development and production work as well as the share done in industry. Viscount Caldecote suggested that the research functions of the Authority should be left under the Ministry for Science and Education and the responsibility of the Authority for development and collaboration with industry in their field reviewed. He entirely agreed with Lord Champion that Britain could not reject a system which was clearly and obviously more economic because it had been designed in the United States and not in Britain, but it was all too clear that it was very easy to have too much competition in these difficult technological and rapidly advancing fields.

Lord Stonham reiterated that not sufficient information was generally available for a proper judgment and that Britain should not reject an American system purely on the grounds that it was American. Lord Wolverton referred to the higher efficiency of the new station at Bradwell in Essex which had a thermal efficiency of 26·5 per cent compared with 19 per cent at Berkeley, Gloucestershire. He thought much more basic research was required before Britain embarked on a larger programme than that announced by the Government.

Replying for the Government, Lord Derwent pointed out that the magnox system might still show advantages in some other countries and that Britain should not take too insular a view of these developments. The building and commissioning of the present stations had involved a great partnership between the Authority and the Generating Board as well as with the Consortia, the contracting firms and the inspectorate of nuclear installations. The Government was satisfied that there was effective collaboration between the Generating Board and the Atomic Energy Authority, and there was, and always had been, close organization between the Consortia and the various firms in the contracting industry and these bodies. He agreed that the suggestion for adoption of systems and arrangements similar to that used in Canada merited careful consideration.

THE MUSEUMS ASSOCIATION

THE seventieth annual conference of the Museums Association was held in Nottingham during July 13–17 under the presidency of Dr. D. John, director of the National Museum of Wales at Cardiff.

The proceedings opened in the spacious Great Hall in the Trent Building of the University with a welcome from the Lord Mayor of Nottingham. Dr. John in his presidential address outlined the financial difficulties which beset the Association and mentioned some of the proposed measures to be taken to remedy this state of affairs. He congratulated the Area Museum Councils on the work they had achieved in the early stages of their plan to regionalize museums. He also referred to the 1963 report of the Standing Commission and to the recommendations, some of which had not been implemented by H.M. Government. In conclusion, he considered that at the present time there was a greater consciousness concerning museums among the general public and that much is moving forward in the museum world.

The general theme of the conference was that of "Public Relations" and this was introduced by Mr. C. Mann (president of the Institute of Public Relations), who gave a stimulating address on the importance of the public. He considered that the value of a work of art is in proportion to the number of people who see it, and for this reason museums should make every endeavour to attract the public to their galleries. A genuine belief that the public really matters was a curatorial necessity and he urged his audience to make more use of exciting entrance features, friendly and helpful attendants, and the creation of an atmosphere of goodwill.

Mr. C. Gibbs-Smith of the Victoria and Albert Museum followed with a paper on "The Fault . . . is . . . in Ourselves". He stated that good public relations meant plain good manners from the whole of the staff. He also felt that public relations should be the special duty of one member of staff who would be directly responsible to the director.

The theme was continued by Sir Frank Francis, director, British Museum, who said that museums though not in competition with themselves were certainly striving to gain customers in these days of more leisure and many interests. The aim of the curator should be to stimulate members of the public at all levels.

Mr. P. Johnstone (producer, B.B.C. Television) stressed the need for close co-operation between museums and the British Broadcasting Corporation. Both used visible communication methods and mutual help was essential. He advocated the setting apart of a special gallery in the museum for television work. Mr. I. Finlay, director of the Royal Scottish Museum, pertinently asked for what image was the museum striving and what public did we serve. A paper by the Rev. J. Jones-Davies describing the transformation of a small collection into a County Museum at Brecon closed the conference.

At the annual meeting, Mr. N. Cook, Guildhall Museum, London, was elected president for the year 1964–65 and an invitation to hold the annual meeting in Dublin in June 1965 was accepted with thanks.

Both the University and the City entertained the delegates at receptions, and during the meeting visits were arranged to the Newark and Derby Museums, Newstead Abbey, Boots Factory, Lincoln, Bakewell, Mansfield, Boston and other places.

F. S. WALLIS

PENDULAR MOISTURE IN PACKINGS OF EQUAL SPHERES

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EXPERIMENTAL relationships between capillary pressure, P, and saturation, s_w, often feature an 'irreducible' fluid content shown in Fig. 1 as the saturation s_w^*. In a random packing of equal spheres of radius R, the pendular rings found at the minimum saturation are not in equilibrium with each other and cover a distribution of sizes[1]. Values of PR/γ calculated from the tables given by Fisher[2] fall largely in the range 3–11 which is also the range of PR/γ over which random packings of equal-sized hydrophilic spheres desaturate (Fig. 1, curve R_0), γ being the air-water interfacial tension. Direct measurements of ring pressures by means of a micro capillary-probe showed that, for a given ring, the ring pressure is equal to the capillary pressure, P, at the time of formation, the error in measurement being not more than 5 per cent[3]. The ring pressure remains constant once the ring has formed, provided that mass transfer to or from the ring is negligible[3].

In the work recorded here, random packings of equal-sized chrome steel spheres were saturated with dilute acid and then the packing was drained in a fine-pored, fritted-glass Buchner filter to the irreducible saturation. After about 0·5 h, the spheres were clearly etched over the areas of contact with the retained fluid; this occurred as isolated or linked rings of fluid held around points of contact between the spheres. Circles, or for linked rings equivalent circles, were selected at random and from their diameter, L (inset in Fig. 1), the volume, V, and pressure deficiency of each ring were calculated[2]. The distribution of L was found for three sphere sizes (diameter, D = 3·18, 4·76, 6·35 mm) taking two samples of 125 circles for each size of spheres. The cumulative frequency distributions of L/D were all of similar shape and fell within 0·06 of each other on the L/D scale. Allowance for porosity by correlation according to hydraulic radius in place of D halved the separation of these curves.

The average ring volume, $\bar{V}$, was calculated excluding the rings held at points of contact between the spheres and the walls of the containing vessel. An average number of points of contact, $\bar{n}$, per sphere for the 6·35 mm spheres was obtained by counting. The results showed good agreement with the method of obtaining the average co-ordination number from the porosity of the packing[4], which was therefore used to obtain a value of $\bar{n}$ for the two smaller sizes of spheres. Average residual saturations (s_p^*) calculated from $\bar{n}$, $\bar{V}$, D, and the bed porosity were 4·5, 5·0 and 4·6 per cent for the three packings taken in ascending order of sphere sizes.

The presence of linked rings found for about 25 per cent of the total number counted, showed that not all the pores in the bed were empty; from Fig. 1 it appears that all pores having an entry pressure of greater than about 10·5 (for example, spaces surrounded by three or four spheres in mutual contact) remain full. This is because they become isolated[3] before their entry pressure is reached. Thus there is residual-pore moisture additional to that occurring as pendular rings. Rings which have calculated values of PR/γ considerably greater than 10·5 are probably of the type that can be seen at points of near contact between the spheres; these have volumes larger and pressure deficiencies smaller than the values calculated. These two observations partly explain why bed saturation on account of rings alone, calculated from $\bar{n}$ and $\bar{V}$, gave residual saturations ranging from 4·2 to 5·3 per cent compared with 6–7 per cent measured by air-drying. In cubic and rhombohedral packings, which have porosities of 47·64 and 25·95 per cent respectively, all residual bulk moisture will be held as completely formed pendular rings. If these are formed at the approximate theoretical entry values of PR/γ of 4·8 for cubic and 12·9 for rhombohedral packings[5], their respective residual saturations will be 4·2 and 5·5 per cent. These values, calculated for two extreme types of packing, are close to the range found for the random packings with measurement of ring volumes alone.

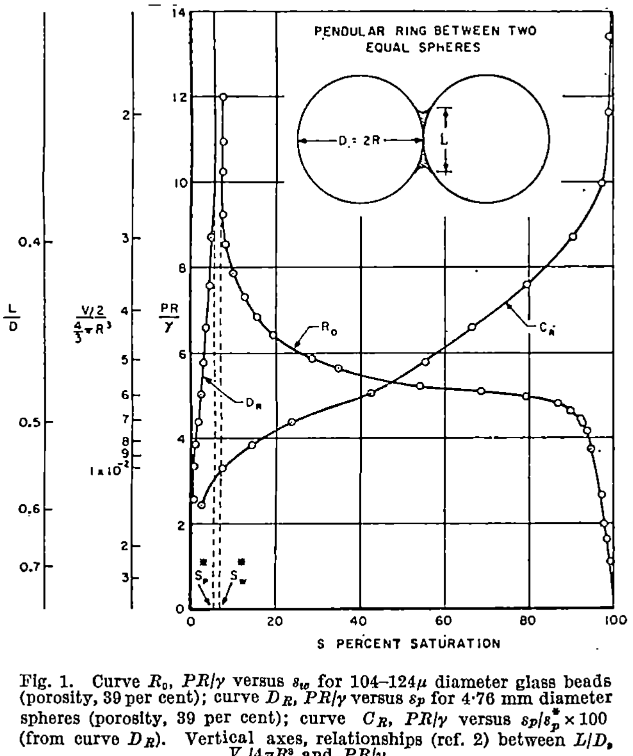

Fig. 1. Curve R_0, PR/γ versus s_w for 104–124μ diameter glass beads (porosity, 39 per cent); curve D_R, PR/γ versus s_p for 4·76 mm diameter spheres (porosity, 39 per cent); curve C_R, PR/γ versus $s_p/s_p^* \times 100$ (from curve D_R). Vertical axes, relationships (ref. 2) between L/D, $\frac{V}{2}\Big/\frac{4}{3}\pi R^3$ and PR/γ

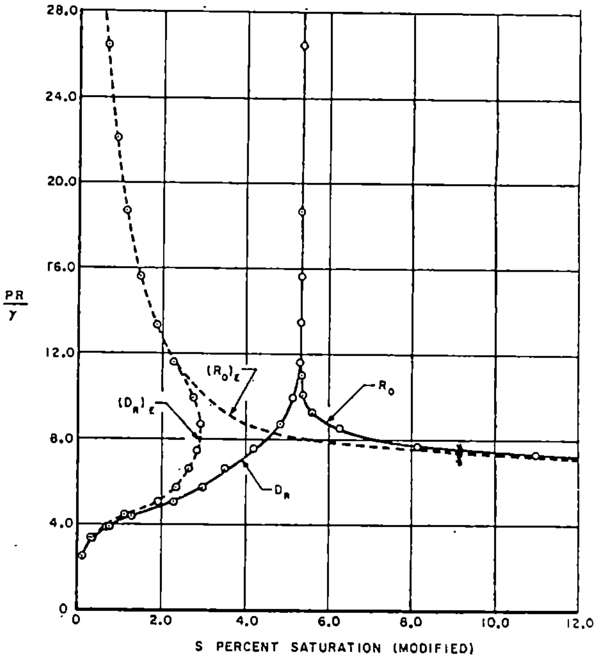

Fig. 2. Curve R_0, PR/γ versus s_w for 104–124μ diameter glass beads (porosity, 39 per cent); curve D_R, PR/γ versus s_p for 4·76 mm diameter spheres (porosity, 39 per cent); curve $(D_R)_E$ PR/γ versus s_p with pendular rings at equilibrium; curve $(R_0)_E$, PR/γ versus s_w with pendular rings at equilibrium

An approximately constant residual saturation of about 6–8 per cent is frequently reported for random packings of close-sized spheres provided that the ratio of gravitational to capillary forces is small[6]. For geometrically similar packings, the processes of desaturation and pendular ring formation maintain similarity in PR/γ; it is therefore to be expected that residual saturation is independent of sphere size. The effect of changing bed porosity is less predictable, mainly because it is not known how the amount of residual-pore moisture will vary. An increase in porosity results in fewer points of contact, but the corresponding pressure deficiency decrease for drainage causes an increase in the individual ring volumes. The approximate self-compensation of these two effects is illustrated by the closeness of the theoretical residual saturations calculated for cubic and rhombohedral packings.

In experiments with cubic packings of 6·35 mm spheres which were drained in a few seconds, it was observed that the ratio (> 1) of the size of rings held in the horizontal plane to those in the vertical decreased with increasing rate of drainage. While this effect can be eliminated by increasing the time of drainage the mechanism of ring formation is irreversible and involves as yet undetermined local pressure variations within the bed no matter how slowly the bed is drained. However, from the direct and circumstantial evidence so far available, it is believed that taking the ring pressure as the capillary pressure at the time of ring formation is a good working approximation for most of the rings and will for the present be assumed to hold for all isolated bulk moisture.

From this assumption, the accumulating quantity of moisture isolated during drainage as pendular rings is shown in Fig. 1 both as a percentage s_p of the total void space (curve D_R) and also as a percentage of their total maximum volume (curve C_R).

The relationship obtained if the pendular rings were given sufficient time to reach equilibrium with the capillary pressure, P, can be calculated by setting all formed rings (that is, those which have pressures less than or equal to the given value of P) at the ring volume corresponding to the pressure P. The curve so obtained (Fig. 2 curve $(D_R)_E$) passes through a maximum pendular saturation and then approaches the zero saturation axis asymptotically. A corresponding correction of this nature is required in order to obtain the form of truly time independent capillary pressure curves. The experimental time required to obtain such curves may be prohibitive. The change in the curve R_0 due to equilibration of the pendular rings is shown as the curve $(R_0)_E$. In order to illustrate the nature of the relationship between the two curves D_R and R_0 and their derived equilibrium relationships, residual-pore moisture has been omitted and the saturations employed have been calculated from the data shown in Fig. 1 with pore volume redefined as the sum of the moisture removed by suction and that calculated to be retained as pendular rings so that $s_p^* = s_w^*$.

The experimental curve D_R belongs to a postulated family of curves[3] relating the amount of moisture isolated as discrete quantities to the capillary pressure. This family and another for the isolated non-wetting phase are complementary to the well-known capillary pressure–saturation relationships[5].

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INVESTIGATION OF NUCLEAR WEAPON DEBRIS WITH X-RAY MICROANALYSER

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FROM 1958, investigations of the radioactive nuclear debris particles have been made in this laboratory. An autoradiographic technique has been worked out which allows identification and microscopic examination of the debris particles among all air-borne dust particles[1,2]. It was found that the particle activity is roughly proportional to its volume and that the strongest particles found, with an activity of the magnitude 10^4 d.p.m. β at an age of a few weeks, have diameters $\sim 3{\cdot}5$–5 μm. A rough estimate of the fission product content of such a particle clearly indicates that most of the particle mass must consist of some other material even if the greatest reasonable limits of uncertainty are taken into consideration.

As the X-ray microanalyser[3] is able to examine specimens of the size magnitude of μm, such an analysis could help to solve the problem of the composition of the debris particles.

High-altitude and ground–air samples from different parts of Sweden are continuously collected and autoradiographed on X-ray film. Large spots on the films indicate strong and thus[4] large particles. During the U.S.S.R. bomb test series, September–December 1961, particles of sufficient size mostly occurred in the ground–air samples. This has usually been the case. About 700 samples from a total of 1·2 kilotons of air were examined and some twenty large particles found.

As it is necessary to place the particles in the very surface layer of the preparate, the method of preparation in refs. 1, 2 and 4, therefore, was modified in the following way. With the help of the autoradiograms, small filter-pieces containing the interesting particles were punched out. The filter pieces were stirred in a viscous gelatin solution until a homogeneous suspension was obtained. The suspension was diluted and spread over a ~ 300 cm² glass plate and dried. The position of the particles was indicated by a new autoradiogram and the corresponding gelatin-layer pieces were punched out. In this way the active particles were freed from most of the accompanying dust.

The pieces of gelatin were dissolved in a few drops of water and the solution mixed with some ml. of Ilford nuclear emulsion in gel form type *G* 5 (electron-sensitive). The emulsion was cast as thinly as possible on a glass plate which was covered with a celluloid film, and allowed to dry to a ~ 10-μm thick layer. The plate was exposed for a week and developed in Ilford 'Ilfex' developer (5 min). The image was bleached away in a solution of 4 g potassium permanganate and 5 ml. sulphuric acid/l. (5 min). The stain obtained from the above bath was dissolved in a solution of ~ 50 g potassium pyrosulphite/l.; the image zones were finally cleared in a solution of 3 parts of acid-fast-fixing bath in 4 parts of glycerole (cf. ref. 2). The particles were thus indicated by being situated in the centres of clear circular zones against the, in course of time, more and more black emulsion. Care was taken to ensure that the photographic chemicals had no dissolving effect on the particles, and that they did not dissolve appreciable amounts of activity from them.

A first attempt to analyse the particles *in situ* was not successful as the electron beam caused local heating which was not led away by the glass and thus melted the gelatin and made the particle move away from the beam. Therefore, a ~ 1-mm² piece of the celluloid film with the emulsion containing the particle was cut away from the glass plate and placed upside-down on a polished copper plate. The celluloid was removed with acetone, leaving the particle in its gelatin film in a better position nearer the surface (as the particle sank to the bottom of the emulsion layer before it solidified). Still, the preparation was somewhat unsatisfactory; the gelatin persisted in making some small movements in the electron beam, and the covering gelatin, although very thin, absorbed some of the X-ray radiation, but the analysis this time could be performed at least semi-quantitatively.

The analysis was performed at the Swedish Institute for Metal Research, Drottning Kristinas väg 48, Stockholm. The results are shown in Table 1.

No element above $Z = 12$ (magnesium) not mentioned can be present to a content higher than ~ 2 per cent.

The results are in agreement with Rajewsky's examination of a 4-μ nuclear debris particle where iron and aluminium or silicon were found[5].

Mamuro and Fujita[6] report (for 7–14-μm particles) iron, aluminium and sometimes traces of silicon, although they find that the light-coloured particles have a high aluminium and a low iron content, and vice versa for the dark-coloured ones.

Calculation of the content of fission products causes some trouble, first, because the age of the particles, when measured, could be anything between 100 and 160 days, and secondly, because the composition of fission products is not the normal one, but is considerably altered by fractionation phenomena[7]. The particles were, however, individually measured in a low-background β-counter and, if strong enough, in a γ-counter, February 8–9, 1962 (at an age of 100–160 days). The two strongest particles (Nos. 6 and 11, Table 1) were also individually γ-spectrographed as well as all the particles together. The spectrograms show that 96 per cent of the γ-activity emanates from zirconium–niobium 95 and 4 per cent from other

Table 1

Particle	Colour	Optical anisotropy	Min. × max. diam. μm	Vol.‡ $\times 10^{12}$ cm^3	Feb. 8–9, 1962		Analysis (%)					
					β-Count (c.p.m.)	γ-Count (c.p.m.)	Fe	Al	Si	Pb	U	Fiss.
10	Reddish	Microcryst ?	4·10 × 4·34	38·2	38·4	19·6	40	20	10	< 2	Weak ind.	0·11 (0·07–0·22)
3	,,	Cryst.	3·76 × 4·34	32	20·3		~ 40	~ 20	~ 10	< 2	,, ,,	0·07 (0·04–0·14)
6	,,	Cryst. (or containing cryst.)	5·93 × 6·72	124	105	63·5	~ 40	~ 20	No	< 2	,, ,,	0·11 (0·07–0·22)
7	No	Cryst. birefr. green. II ord. (?)	3·31 × 3·58	21·1 †	74·6	33·6	~ 40	~ 20	~ 10	< 2	,, ,,	0·34 (0·21–0·69)
8	Red + yel.*	,,	3·76	22·7	70·5	30·3	~ 40	~ 20	No	< 2	,, ,,	0·29 (0·18–0·58)
4	Reddish	Microcryst ?	2·62 × 3·54	12·7	8·75		High	(Bad prep.)				0·06 (0·04–0·13)
5	,,	Cryst.	3·65 × 4·55	31·7 †	12·8		,,					0·04 (0·03–0·09)
1	,,	Microcryst ?	3·54	23·3	8·6			Not followed through	,,			0·04 (0·02–0·07)
2	,,	?	2·62	9·4 †	10·2			,,	,,			0·10 (0·06–0·22)
9	,,	Cryst. birefr. green. II ord (?)	2·74 × 3·42	13·4	13·2			,,	,,			0·08 (0·06–0·17)
11	No	Cryst. birefr. green.	3·3	18·8	95·8	46·6		,,	,,			0·54 (0·33–1·08)
12	Reddish	?	2·62 × 2·85	10·2	23·3	9·8		,,	,,			0·21 (0·13–0·42)

* A red and a yellow particle coalesced. † The clinging dust particle not included (see Fig.). ‡ The volume is calculated as $\pi/6$ (max. diam.) (min. diam.)^2.

γ-emitting nuclides, mainly cerium-144. A normal mixture of fission products of the age of 100 and 160 days gives respectively 63·6 and 94·5 per cent zirconium–niobium 95 γ-activity[8].

The ratio of β-counts to γ-counts did not vary much from ~ 2. Thus γ-activity of the particles too weak to measure was estimated to one-half of the β-activity. Considering the conditions above and previous experiences from large particles[7], it seems reasonable to assume a fission-product content corresponding to $\sim$ one-quarter of the calculated Zr–Nb content and an age of $\sim$ 160 days. In Table 1 the lower limits given correspond to an age of 100 days and a zirconium–niobium 95 enrichment by a factor of 4. This limit cannot be valid for the strongest particles, as it would need an unreasonable zirconium–niobium enrichment of a factor 12 to be consistent with the γ-spectra. The higher limits correspond to an age of 160 days and a zirconium–niobium 95 enrichment by a factor of 2 (ref. 7). The density of large nuclear-debris particles has been found to exceed 4 g/cm^3 by centrifugating a suspension of the particles in thallium–formiato–malonate. No heavier liquid is available except mercury (d = 13·6). It is reasonable to assume that the particles consist of oxides of the elements found and thus a density of 5 g/cm^3 may be correct within 20 per cent.

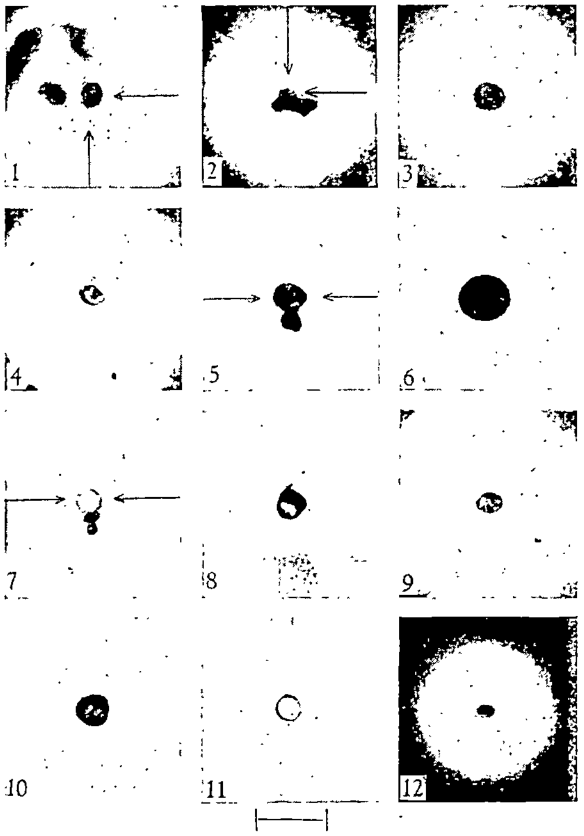

Fig. 1. Particles mentioned in Table 1

Assuming a fission efficiency in the vicinity of 20 per cent, the uranium content should be four times the fission content; thus 0·2–2 per cent by weight, which is consistent with the fact that uranium is barely detectable.

The low content of radioactive material in the particles is characteristic. A lot of nuclear debris samples (mostly the U.S.S.R. from 1958 (ref. 4) and 1961–62 (ref. 9)) with thousands of particles have been examined for correlation between particle size and activity. They give mostly about the same ratios of particle activity to particle volume as the above particles, thus indicating that only a small fraction of the particle mass consists of fission products.

The lead content can be explained by the use of lead in the tamper of the bomb[10].

It seems manifest that, although colourless particles as an average have a higher specific activity than red ones (100 × oil imm. obj. apert. 1·32) (ref. 11), the differences in colour do not indicate considerable differences in composition. The colourless particles especially do not lack iron (cf. ref. 6). Minerals with high content of iron are usually dark-coloured (hæmatite, hercynite, pleonaste, etc.).

The composition of the particles is peculiar. If the inactive material in the bomb is looked on as containment (bomb shell, construction material, the final rocket step) the ratio of containment to fissionable material seems remarkably great, apart from the case of tower shots. However, most of the U.S.S.R. shots at Novaya Zemlja occurred over the sea several miles from the west coast of the islands, and in most cases probably not below heights of $\sim$ 200 m (ref. 12). Thus, tower shots are likely to be excluded. Even if the iron is supposed to be the debris from containment, there should have been analysable amounts of alloying elements (such as manganese, chromium and perhaps titanium from rocket heads). The results of the analysis seem to indicate that most of the matter in the bomb debris particles does not emanate from the bomb itself, but from matter accidentally present in the air-volume occupied by the fire-ball. This could be particles from volcanic activity, meteorite particles, dust transported from the surface of the Earth up to the height of the nuclear blast (up to 3,600 m) (refs. 12, 13).

The apparatus used for β measurements was a 2·5-cm diameter, 7 mg/cm^2 end-window Geiger–Müller tube (General Electric *GM 4LB*) shielded with lead and an anti-coincidence arrangement, giving an efficiency of ~ 25 per cent for point-geometry, high-energy β-samples and 0·7 d.p.m. background counting. For γ-measurements: a 1·25 in. × 1 in. sodium iodide crystal, shielded by 10 cm lead, giving an efficiency for zirconium–niobium 95 (~ 0·75 MeV) of 4·8 per cent (measured with a point-geometry sample of known strength) and a background of ~ 40 c.p.m.

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APPARENT ANISOTROPY FIELD OF CONTINUOUS MAGNETIC FILMS SUBJECTED TO INHOMOGENEOUS DRIVE FIELDS

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THIN magnetic films deposited in a vacuum on to smooth substrates under the correct conditions[1] exhibit uniaxial anisotropy; that is, in the absence of a magnetic field the magnetization is stable in either of the two directions along an 'easy' axis in the plane of the film. If a homogeneous field is applied to the whole area of a film, along a 'hard' direction (perpendicular to the easy axis), the magnetization rotates towards this hard direction. The magnitude of the uniform field required to rotate the magnetization just into the hard direction against the internal anisotropy torque is known as the anisotropy field and depends on the deposition conditions and the composition of the film. One of the puzzling features of the properties of continuous magnetic films is that when the hard direction field is applied inhomogeneously by a strip conductor, the field required to rotate the film can be several times larger than the anisotropy field of the film when subjected to homogeneous fields.

The conditions affecting the value of the apparent anisotropy field were extensively investigated by Leaver and Prutton for many strip conductor widths, film thicknesses and anisotropy field values[2,3]. They showed that the excess of the apparent anisotropy field over the value measured using homogeneous fields was qualitatively consistent with an effect of demagnetizing fields: when the strip of film is activated by the passage of a current down the drive conductor, magnetic poles appear down the edges of the strip and produce a field within the strip which opposes the field applied by the strip conductor. Theoretically, the field required to rotate the film into the hard direction is increased by an amount proportional to the film thickness and inversely proportional to the mean separation of the band of poles. The measured increase, however, is far more than would be expected if the bands of poles were beneath the edges of the strip conductor. It might then be assumed that, for the case measured by Leaver and Prutton, the bands of poles were not beneath the edges of the strip but nearer the centre, giving an increased demagnetizing field. However, our experiments, using continuous films, show that in cases where the whole film beneath the strip conductor is known to be saturated (so that the bands of poles must be beneath or beyond the edges) the apparent anisotropy field is still far in excess of any value that could be accounted for by demagnetizing fields.

Explanation of apparent anisotropy field by propagation of magnetization changes in a continuous film. An alternative explanation for the anomalously high values of effective anisotropy field arose when it was found that, with a continuous film, magnetization changes could occur well beyond the edges of the strip conductor although the field was applied directly only beneath the strip[4]. This propagation is probably due to magnetostatic coupling between neighbouring regions of film, but the precise mechanism causing the propagation does not affect the present explanation. It was realized that, because the propagation occurred, larger fields had to be applied to the film to rotate the magnetization of the central strip against the internal anisotropy torque of the outer areas. This can be shown by considering a strip of film of length l beneath the drive conductor of width w. If we assume that all the magnetization within this strip rotates coherently through an angle θ_0 under the influence of the applied field, then the torque exerted on this strip of film by the field, H, is:

$$T = MflwH \cos \theta_0 \qquad (1)$$

where M is the magnetic moment per unit volume and f the thickness of the film. Now the torque which must be exerted on a segmental strip of the film parallel to the conductor and of width dx in order to maintain a deflexion θ is:

$$dT = MflH_K \sin \theta \cos \theta \, dx \qquad (2)$$

where H_K is the anisotropy field of the film. To avoid repetition of the analysis we shall anticipate the experimental result and assume that the magnetization deflexion in a segment away from the strip conductor (where no field is applied directly) is such that the hard-axis component of the magnetization decays exponentially with distance, x, away from the edge of the strip conductor:

$$\sin \theta = \exp(-x/p) \sin \theta_0 \qquad (3)$$

where p is the propagation constant. Now the total torque that is required to maintain a magnetization deflexion of θ_0 beneath the drive strip and a deflexion given by equation (3) on each side of it is obtained by integrating equation (2)*. For small deflexions, $\cos \theta \simeq \theta_0 \simeq 1$ and:

$$T = \int_{-\infty}^{\infty} MflH_K \sin \theta \cos \theta \, dx = MflH_K \sin \theta_0 \cos \theta_0 (w + 2p) \qquad (4)$$

---

* Note that there can be no net torque on the film due to any fields caused by the pole distribution. Any torque on the film at one point due to a pole at another must be exactly balanced by an opposite torque at the second point.

Because torque is not a localized quantity, we can equate the total torque required to maintain the known magnetization distribution (equation 4) and the torque produced by the applied field (equation 1), giving:

$$wH = H_K \sin \theta_0 \, (w + 2p) \tag{5}$$

For the case of a homogeneous applied field (w very large), even for large deflexions, we have:

$$H = H_K \sin \theta_0 \tag{6}$$

Equation (6) expresses the well-known line hysteresis loop for fields applied in the hard direction: when $H = H_K$ the film reaches the hard direction and the anisotropy field can be obtained from the initial slope of the plot of hard direction magnetization against hard direction field. Rearranging equation (5), we can obtain the analogous equation for a field applied inhomogeneously:

$$H = H_K(1 + 2p/w) \sin \theta_0 \tag{7}$$

Measuring the incremental slope will give the apparent anisotropy field, $H_K(1 + 2p/w)$. This is not quite the same as the field required to rotate the central strip into the hard direction ($\theta_0 = 90°$) because of the approximation that $\cos \theta$ everywhere equals $\cos \theta_0$ which will not be valid for points remote from the drive strip when θ_0 is large. In any event, however large the applied field, the strip of film beneath the drive strip could never quite reach the hard direction (when the applied torque would be zero and no torque would be required by this strip of film) because a torque is still required to deflect the magnetization of the remote regions of film.

Apparatus and techniques. The arrangement used to measure the propagation of magnetization changes is shown in Fig. 1. The conductor pattern was photo-etched from 25μ copper sheet and insulated from the film and conducting substrate by 6μ 'Melinex'. By connecting various numbers of drive strips the drive-strip width could be varied between 0·5 and 3 mm. Pulses 100 ns long and having rise times of about 1 ns were obtained from a mercury wetted reed discharge line pulse generator and matched into the low-impedance drive strips through a 50 Ω matching resistor. Each drive strip was individually terminated and the current produced by the generator was established within 3 ns in the drive strips irrespective of the number used. Equal current sharing between the strips is ensured by earthing at the far end so that there is no potential on them after the first double transit time. All the matching and terminating resistors were screened by placing them in milled recesses in the base plate of the test jig. The common return wire for the sense wires was returned near to the sense wires themselves to reduce any E.M.F. due to eddy currents induced in the conducting base plate when the drive current flowed. Any transient capacitive E.M.F. produced in the sense wires beneath the drive strips was reduced by connecting the oscilloscope to the sense wires through a 'balun' transformer[5]. With this arrangement, only differences in potential of the sense and return wires are detected. When the drive pulse begins the magnetization of the film beneath a sense wire rotates through an angle θ towards the hard direction and the flux change produces a triangular pulsed E.M.F. of height V and base-length t, such that:

$$Vt/2 = 4\pi \, Mfl \sin \theta \tag{8}$$

where l is the length of the sense wire. It was found that the value of t was about the same ($\sim$ 10 ns) for all the sense wires and so the E.M.F., V, can be used as a measure of the rotation, θ, of the film beneath the sense wire, once a slight correction for the differing lengths of the sense wires has been made. The signal/noise ratio depended on the film and the drive current but scarcely at all on the particular sense wire being used, the worst value being about four. The measurement of every point included the measurement and subtraction of the noise, the noise being the value obtained by locking the film with a large permanent magnet.

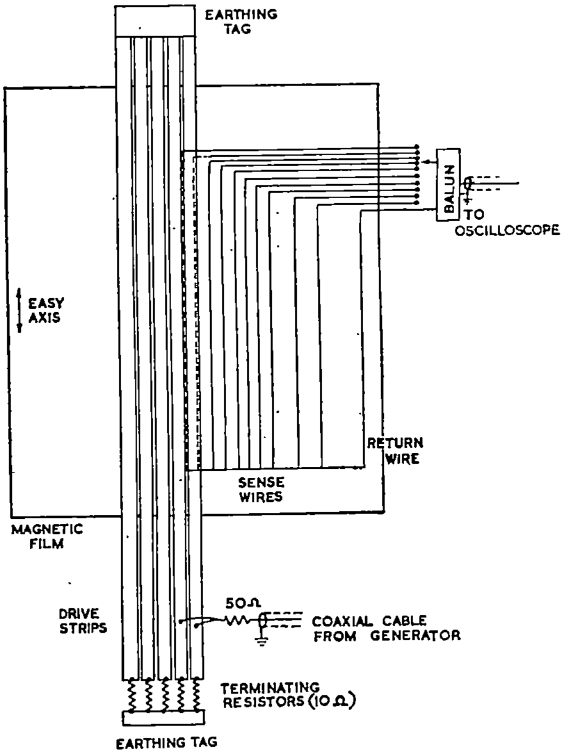

Fig. 1. Arrangement used to detect propagation of magnetization changes. The aluminium plate carrying the film fitted into a recess in the base plate, giving a continuous conducting plane. The drive strips were 0·5 mm wide separated by 0·1 mm gaps. The sense wires were 0·1 mm wide and spaced at distances of 5, 4, 3, 2·5, 2·0, 1·5, 1·0, 0·5, 0, −0·5 mm from the edge of the drive strips

The loop plotter used to measure the anisotropy field of the film under homogeneous applied field conditions produced results consistent to within about 10 per cent (this is the standard deviation of a series of measurements of the same film). The oscilloscope used to measure the drive currents could be read consistently to about 5 per cent and had a maximum sensitivity of 10 mV/cm with a rise time of 4 ns.

Experimental results. Curves were plotted of the outputs obtained from the various sense wires as a function of the drive current for various widths of drive strip and for films having a range of thicknesses and anisotropy fields. A typical result is shown in Fig. 2. It can be seen that the sense wire detecting the magnetization changes up the centre of the strip (curve 1) gives a curve with a linear initial portion flattening off as the film beneath it approaches saturation. This is in accordance with equations (7) and (8) which predict a linear increase in $\sin \theta$, and hence of V, with applied field. The rounding off of the corner, instead of a sharp bend as predicted by the equations, is probably due to dispersion in the anisotropy field of the film. The curve for the sense wire beneath the edge of the drive strip is almost identical to the curve for the wire in the centre, indicating that the magnetization changes are almost uniform in the strip of film directly beneath the drive conductor. The sense conductors (curves 3 and 4) away from the film show a reduced magnetization change increasing linearly with drive field up to about the field at which the central strip became saturated (about 24 oe) when the slope decreases. The finite slope of the second part of the curve was shown to be due to field spread from the strip conductor: this slope is approximately inversely proportional to the drive conductor width so that it is the current density which is important. As the drive current density increases, the region of uniform changes also increases, causing a corre-

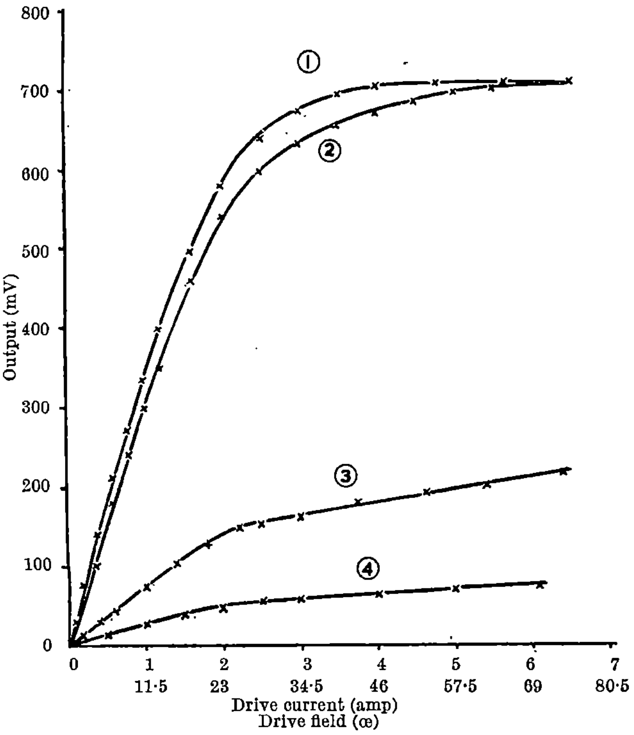

Fig. 2. Output from sense wires as a function of drive current and equivalent drive field. Drive strip width 1·1 mm, film: 2200 Å thick, H_K 8·7 œ. Sense wires: 1, at centre of strip; 2, at edge of strip; 3, 0·5 mm from edge of strip; 4, 1·0 mm from edge of strip

sponding outward shift in the magnetization distribution and giving a larger output from the sense wires. The apparent anisotropy field was measured from curves such as those in Fig. 2 by extrapolating the initial slope of curve 1 until it reached the peak output obtained at saturation. From the known relationship between drive conductor width, drive current and drive field the apparent anisotropy field can be found. Thus for the film of Fig. 2 the apparent anisotropy field was 24 ± 1 œ with a 1·1-mm wide drive conductor while the true anisotropy field measured on the loop plotter was only 8·7 ± 0·8 œ.

The distribution of magnetization changes in the film away from the drive conductor was found by measuring the output from each sense wire at a particular current below the knee of the curve. When the outputs were plotted on a logarithmic scale as a function of the position of the sense wire a straight line resulted, the slope of which appeared to be independent of both the drive field, if this was less than the apparent anisotropy field, and the width of the drive conductor. Since at any point the flux change due to the deflexion of the magnetization is proportional to the local resultant field, for this direction of measurement, it follows that this resultant field must fall off exponentially with distance from the edge of the drive conductor. The slope of the straight line provides a measure of the propagation constant, p, of equations (3) and (7). Measured values of p were between 0·3 and 1 mm. Since the curves of Fig. 2 are initially linear, the result that the propagation constant does not depend on the drive field is to be expected. The lack of dependence of the propagation constant on the drive-conductor width indicates only that the field at the film on one side of the drive conductor due to a line of poles on the other is too small to be of significance. From equation (7) we have the theoretical relationship between the apparent anisotropy field, H'_K, the true anisotropy field, H_K, the propagation constant, p, and the drive conductor width, w:

$$H'_K = H_K(1 + 2p/w) \qquad (9)$$

In Fig. 3 the values obtained by inserting the measured parameters into the right-hand side of equation (9) are plotted against the apparent anisotropy field measured directly for all the films and drive-conductor widths used. It can be seen that a reasonably good correlation is obtained, even although the films used were prepared under widely differing evaporation conditions and in three different plants. This suggests that the explanation of the increase in the apparent anisotropy field over the true value in terms of the propagation of magnetization changes is correct.

Cause of propagation. It was mentioned above that a possible cause of the propagation was magnetostatic coupling between neighbouring regions of film, but a further discussion of this is of interest. If the propagation did not occur, a line of poles would appear beneath the edge of the drive conductor and a field of the order of 10^4 œ would appear on either side of the line of poles. This field would tend to demagnetize the film beneath the drive conductor and tend to magnetize the film beyond the drive conductor, though producing no net torque on the film as a whole. Any magnetization changes produced by the hypothetical line of poles would broaden the line into a band and reduce the energy of the system. Now, the field produced by a line of poles is proportional to the thickness of the film and inversely proportional to the distance. The change of magnetization at any point in the film also depends on the relative magnitudes of the applied field and the anisotropy field. Thus it would be reasonable to suppose that the propagation constant would be greater for thicker films of lower anisotropy. Accordingly, an attempt was made to correlate the propagation constant with the film parameters. The propagation constant was found to have no distinct correlation with either the film thickness or the true anisotropy field, but there was a positive correlation with the ratio of film thickness to anisotropy field. The points obtained could be roughly enclosed in an ellipse with a ratio of major to minor axes of about three. This evidence alone cannot support the hypothesis of a linear relationship between the propagation distance and the ratio of film thickness to anisotropy field. We have as yet been unable to complete any rigorous theoretical calculation of the form of the magnetization distribution, as the integral equation describing

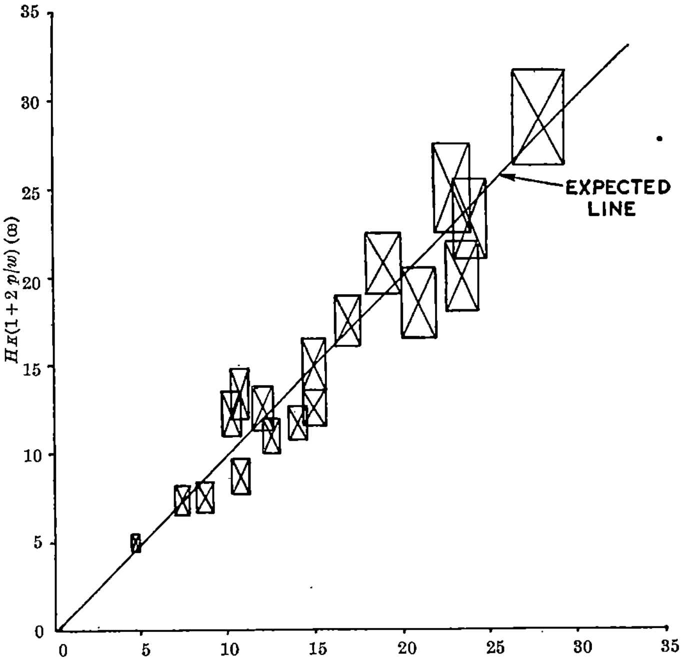

Fig. 3. Correlation between the apparent anisotropy field calculated from film parameters and the apparent anisotropy field measured directly. The points cover the ranges: true anisotropy field 3·4–10·0 œ; film thickness 700–4400 Å; drive conductor width 0·5–3·0 mm. The size of the points represents the probable random error

the equilibrium magnetization distribution requires the use of an analogue computer to obtain the solutions. An attempt to obtain an approximate solution using a digital computer was unsuccessful.

The possibility that the propagation might be due to exchange rather than magnetostatic coupling was investigated by removing a strip of film about 100 μ wide 0·8 mm from the edge of the drive conductor. The magnetization distribution was found to be unaffected by the break in the film. This appears to eliminate exchange coupling, which has a very short range and could not cross the break in the film. Magnetostatic coupling, on the other hand, would scarcely be affected, being a comparatively long-range force. If the propagation is due to magnetostatic coupling it would be expected that slight misorientation of the easy axis of the film would not affect the distribution of magnetization changes significantly. It was found that the outputs obtained from the sense wires were not affected significantly by misorientations up to 20°, larger misorientations giving a reduction in output. As might be expected from this result, there seemed to be no consistent dependence of the propagation on the angular dispersion of easy axes of the film.

Easy axis propagation. Propagation was also found to occur along the easy axis of the film when it was subjected to a hard direction bias field equal to the anisotropy field. Under these conditions the permeability along the easy direction is at a maximum, being in general about twice the normal permeability of the film in the hard direction[6]. It was found that the magnetization distribution created by the drive current applying an easy axis field was very similar to that created by the hard direction drive field: the magnetization change is nearly uniform beneath the strip conductor and falls off exponentially with distance away from the edge of the conductor. The easy axis propagation constant, p, was in general about 20 per cent less than the hard axis constant although the easy axis field required to produce a given flux change at the central sense wire was less than the hard axis field required to produce the same change (the permeability being more). There is no obvious reason why the hard and easy axis propagation constants should be similar, and the interpretation of this result must await the solutions of the relevant equation[7].

Conclusion. The increase of the apparent anisotropy field obtained with inhomogeneous applied drive fields over the true value found using homogeneous fields can be explained by the propagation of magnetization changes beyond the region of film subjected directly to the applied drive fields. The larger area of film influenced requires a correspondingly larger total torque to produce a given rotation in the region where the field is applied. The values of propagation constant found can predict the apparent value of anisotropy field to within the experimental error. The precise cause of the propagation does not affect this prediction, given the result that the magnetization changes decrease exponentially away from the edge of the drive conductor. It seems most probable that the propagation is due to magnetostatic coupling, especially since the expected dependence of the propagation distance on the ratio of film thickness to anisotropy field is confirmed experimentally. Any complete confirmation of this hypothesis must await the results of the calculations of propagation constant as a function of film parameters and comparison with the experimental results.

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GRAININESS IN THE PHOTOGRAPHIC RECORDING OF ELECTRON MICROSCOPE IMAGES

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GRAININESS can be seen in any over-enlarged photographic image. But since basically the image on the negative is simply an array of silver grains held in a thin layer of gelatin, the effect is often thought to have an obvious explanation. However, the silver grains themselves are seldom more than 1μ (10^{-4} cm) in diameter and in fine-grain material many may be beyond resolution with an ordinary microscope. So clearly graininess, which can often be seen in a ten-times enlargement, is not to be explained in a way that would make the silver grains analogous to the points of ink in a printed illustration. In fact, it results from the fluctuations in the numbers of grains in each just visible area, and the primary cause of these fluctuations does not lie in the photographic emulsion at all but in the radiation to which it has been exposed. Whatever its nature, this radiation arrives as quite independent particles in the form of quanta or electrons. A photographic negative may have been exposed to what is macroscopically a constant, uniform beam of radiation and, if the intensity is known, a simple calculation will give the mean number N of particles that fall on an area of given size in a stated length of time. But the arrival of the individual quanta or electrons is quite random, and the actual numbers that arrive from spot to spot and from moment to moment will not be constant but will fluctuate around the mean number N. Such random events conform to Poisson's law and the extent of the fluctuation is predictable; it will have a root-mean-square value of $\pm \sqrt{N}$. The fluctuation expressed as a fraction of the mean is thus $1/\sqrt{N}$. Where N is very large, $1/\sqrt{N}$ is insignificant and the radiation appears uniform and constant. But when small areas or short times are considered and N is consequently small, the fluctuations become very obvious; if, for example, N is only 100, the root-mean-square fluctuation becomes 10 per cent of the mean.

When the emulsion is exposed to an image instead of to a uniform beam of radiation, the smallest detail of given contrast that can be detected in the picture will clearly be limited by the graininess. The problem put in terms of simple information theory is that the signal (which here is the change in density on the negative caused by the object we want to detect) must be significantly larger than the noise (which is the random fluctuation in density in areas of the same size). In practice, unless the signal-to-noise ratio is greater than five, the object will not be detectable with certainty. The reason is that the amount of information conveyed from the object to the negative is not statistically significant and, since it is clear that no subsequent photographic manipulation when making

Table 1. RESPONSE OF VARIOUS PHOTOGRAPHIC EMULSIONS TO 60-kV ELECTRONS

The figures give the number of electrons per μ^2 ($=10^{-8}$ cm^2) that give a specular optical density $D=1$

Emulsion	Developer										
	Ilford							Kodak			
	ID-2 (1 : 2)	*ID*-11 (none)	*ID*-13 (none)	*ID*-19 (none)	*ID*-36 (1 : 3)	*ID*-62 (1 : 3)	*ID*-78 (1 : 3)	Microdol (none)	*D*. 19*b* (none)	*D*. 158 (1 : 1)	*D*. 178 (2 : 1)
Ilford Ilfex (X-ray film)						0·07					
,, *XK*						0·09					
,, *LN*						0·14					
,, *G*.5 (nuclear track)				0·25							
,, *N*.40	0·75	1·0	0·44	0·87	0·75	0·69	0·75	3·0			
,, special lantern contrasty	0·75	0·94	0·31	0·69	0·75	0·75	0·94	2·7			
,, *N*.30						0·75					
,, special lantern soft						0·81					
,, *N*.25						0·94					
,, *L*.4 (nuclear track)				1·6							
,, *N*.50	2·0	1·8	0·69	2·2	1·8	1·7	2·2	7·5			
,, special lantern normal						1·8					
,, *N*.60	3·6	3·5	1·4	4·9	2·9	5·1	4·1	8·1			
,, contact lantern	16	14	3·6	18	12	16	14	51			
Kodak *B*.10									0·75		
,, *B*.4										1·1	
,, maximum resolution											51
Gevaert 23 *D* 50	0·55				0·55						
,, 11 *C* 50	11				7·5						

The figures in brackets under each developer give the dilution as parts of stock developer to parts of water. Development was at 21° C for 4 min in a dish with intermittent agitation. The developers were freshly made up according to the manufacturer's published formulæ.

As explained in the text, the figures give a rough idea of exposure time in seconds when the beam gives a well-lit fluorescent screen.

enlarged prints can ever add to this information, the object will be lost in graininess, however such prints are made.

In the same way the signal-to-noise ratio on the negative can never be better than the ratio in the optical image during the time of exposure; but it may be considerably worse. Such loss of information in the photographic recording of the image will occur when each photon or electron in the image does not contribute equally to the optical density of the negative. For example, if three-quarters of the photons in the optical image pass right through the photographic emulsion without being absorbed by a grain of silver halide, they obviously contribute nothing to the photographic image. The number of effective photons is thus reduced to a quarter and so the noise (which varies with the square root of the number) is doubled with no increase in signal. The non-linear response of photographic emulsions to light complicates the rigorous application of information theory, but Fellgett[1] has estimated that a 100-fold loss of signal-to-noise ratio occurs in forming the negative with even the best emulsions available to-day.

It is well known that the resolution obtained in electron microscope pictures of biological specimens falls disappointingly short of the 5 Å resolution which can now be demonstrated with suitable test objects. The electron contrast in the ultra-structure of biological materials is so low that details smaller than 20 Å are almost always lost in graininess. In part it is true that this graininess is not photographic but exists in the film used to support the specimen or in the stains used. But if the recording of the electron image on the photographic plate was anything like as inefficient as the recording of a light image, much relevant information might be present in the electron image but lost in the photographic processes. It would then be well worth looking for more efficient ways of recording the image.

The response of a photographic emulsion to the usual 40–100 kV electron beams used in microscopy differs from the response to light in being almost exactly linear over the whole useful working range[2]. The optical density on the plate (or film) after it has been processed is proportional to the number of electrons per unit area to which it was exposed. As a consequence, a straightforward measurement gives the loss of signal-to-noise that occurs in recording the electron-microscope image. A plate is exposed in the usual way in the electron microscope but without a specimen in place. It is easy to arrange that the beam on the plate is uniform. After being processed, the plate is scanned with a recording microdensitometer and, as the aperture of the densitometer is reduced, the random fluctuations due to graininess increase. For any selected aperture area A the trace can be used to calculate the root-mean-square fluctuation in density σ and also the mean density D of the plate. If the constant k is known that relates the optical density D to the number of electrons per unit area to which the emulsion was exposed (k being, in fact, a measure of the speed of the plate to electrons), then the mean number of electrons on an area A is given by $N = AD/k$. The ratio r of signal-to-noise in the photographic image to that in the electron image is $(\sigma/D)/(\sqrt{N}/N)$ and so $r = \sigma\sqrt{A}/Dk$. For a perfect recording of all the information in the electron image r would be unity, but since in practice some information must be lost in the photographic processes $r < 1$. For exposure to light it has already been mentioned that an estimate gives r as only 0·01.

A measurement of r for an electron micrograph requires in addition to the densitometer measurements a value for k. Since there are very few published measurements of the speeds of various emulsions to electrons[3,4] we measured k for a wide range of commonly used emulsions in various developers. Each plate was exposed in the electron microscope to a 60-kV beam for an appropriate time. The electron exposures given can be measured with a Faraday cage[3], but we found it as accurate and more convenient to make counts of electron tracks on nuclear track plates (Ilford *G*.5 and *L*.4 : 10μ thick emulsions) which had been given a known fraction of the exposure. The emulsions under test were processed in the usual way with 4-min development in an intermittently agitated dish and their optical densities measured with the Joyce–Loebl microdensitometer using a large aperture. The speeds of the various emulsions were thus determined and are given in Table 1 as the number of electrons per μ^2 ($= 10^{-8}$ cm^2) that give a specular density $D = 1$. This is a convenient way of expressing speed (though it should be noted that the figures give the reciprocal of k and that a high speed is shown by a low figure), for when the fluorescent viewing screen in the microscope is made just bright enough for the image to be seen clearly it will be receiving very approximately 1 electron/μ^2/sec. So the figures in Table 1 also indicate roughly the exposure time in seconds that each emulsion will require if exposed to the image at the same intensity that is used for direct examination on the screen.

Microdensitometer traces were then taken to obtain values for σ. The densitometer aperture A was chosen to

Table 2. FACTOR r BY WHICH THE SIGNAL-TO-NOISE RATIO IS REDUCED BETWEEN THE 60 kV ELECTRON IMAGE AND THE PHOTOGRAPHIC IMAGE WITH VARIOUS EMULSIONS AND DEVELOPERS

$r = 1 \cdot 0$ (no loss)	*G*.5 (*ID*-19): special lantern contrasty (*ID*-62, Microdol): *L*.4 (*ID*-19)
$r = 0 \cdot 9$	*N*.50 (*ID*-62): special lantern contrasty (*ID*-11, *ID*-19): *B*.4 (*D*.158): 23 *D*.50 (*ID*-36): 11 *C*.50 (*ID*-36)
$r = 0 \cdot 8$	Ilfex (*ID*-62): *LN* (*ID*-62): *N*.40 (*ID*-62, Microdol): special lantern contrasty (*ID*-2, *ID*-13, *ID*-36, *ID*-78): *N*.30 (*ID*-62): *N*.25 (*ID*-62): *N*.50 (*ID*-2, *ID*-36, Microdol): *N*.60 (*ID*-2, *ID*-36): *B*.10 (*D*.19*b*): 23 *D*.50 (*ID*-2): 11 *C*.50 (*ID*-2)
$r = 0 \cdot 7$	*XK* (*ID*-62): *N*.40 (*ID*-2, *ID*-11, *ID*-13, *ID*-19, *ID*-36): special lantern soft (*ID*-62): *N*.50 (*ID*-11, *ID*-13, *ID*-19, *ID*-78): special lantern normal (*ID*-62): *N*.60 (*ID*-11, *ID*-13, *ID*-62): contact lantern (*ID*-36): maximum resolution (*D*.178)
$r = 0 \cdot 6$	*N*.40 (*ID*-78): *N*.60 (*ID*-78): contact lantern (*ID*-62, *ID*-78)
$r = 0 \cdot 5$	*N*.60 (Microdol): contact lantern (*ID*-2, *ID*-11, *ID*-13, *ID*-19)
$r = 0 \cdot 3$	Contact lantern (Microdol)

give a reasonable fluctuation in D (usually A was $50 \times 50\mu$ or $100 \times 100\mu$). The density of the plates was arranged to be close to $D = 1$. The value of r, the fractional loss of the signal-to-noise between the electron image and the photographic image, was then calculated for each emulsion from the equation given above, and the results are shown in Table 2. This clearly indicates that the photographic emulsion is an almost perfect recorder of the electron microscope image. Indeed, except for the very slowest plates, it is doubtful if in practice any of the figures would be significantly different from $r = 1$, and with several emulsions any loss of signal-to-noise was immeasurable. Thus photographic granularity in electron micrographs can be almost always equated with electron noise: so the faster the emulsion the more prominent graininess will become because for the same density the emulsion will have been exposed to fewer electrons.

If one assumes the normal contrast constants[2,5] for an electron microscope working at 60 kV with a 50μ objective aperture for various specimen weight densities ρ (approximately for most metals $\rho = 20$; for negative staining $\rho = 5$; for positive staining $\rho = 2$), then the minimum magnification of the image on the plate or film in the electron microscope to show specimen detail at any level of resolution distinctly above photographic grain can be calculated. We find approximately that the minimum magnification is given by $M = 10^8/(\rho x^2\sqrt{n})$ where x Å is the required resolution and n the number of electrons/μ^2 required by the emulsion for $D = 1$ as listed in Table 1. Obviously imperfections in the specimen or in the supporting film may in practice limit useful resolution to larger sizes than that given by this equation; on the other hand, apparent detail that is much smaller is likely to be photographic grain unless it has some regular feature (such as parallel lines in a crystal lattice) that aids recognition.

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AN ELECTRODEPOSITION METHOD FOR THE PREPARATION OF ELECTRON MICROSCOPE SPECIMENS

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IN the course of some recent investigations[1-7] concerning the cathodic deposition of various elements in the chloride, nitrate or sulphate form from alcohol plating solutions, apparatus and procedure were developed whereby thin films produced in this manner could be prepared in a form suitable for examination in an electronmicroscope of the Siemens Elmiskop I type. While our own interests were mainly directed toward various chemical compounds of uranium, the success and simplicity of the method and application to other elements are obvious.

The electrodeposition cell shown in Fig. 1 is constructed in two parts, A and C, which can be screwed together. A plastic tube D is connected by means of a nozzle to section C of the cell, and is fed by a mercury bulb provided with a tap F and a metal seal for electrical connexion. The specimen grid holder (Fig. 2) consists of a stainless-steel disk C, 28 mm in diameter, with a recessed stainless-steel projection B. The specimen grid, after cleaning, is placed in recess A. The top section of the cell is removed and the specimen grid holder placed, as shown in Fig. 1 B, in the cell base C, and held in position by replacing the top section of the cell A. The cell is now ready for flooding with mercury. This is achieved by raising the mercury bulb until the stainless-steel disk is covered by mercury and final adjustment made by regulating the flow of air into the bulb with tap F. The mercury should just cover the top of the specimen grid.

In our particular case, stock solutions of uranyl nitrate, uranium chloride and uranium sulphate were prepared having a concentration of 1 mg/ml. in distilled water. The cell was filled with 15 ml. of isopropanol and 10 µl. of the stock solution in question added during the stirring of the solution with a platinum anode having the form of

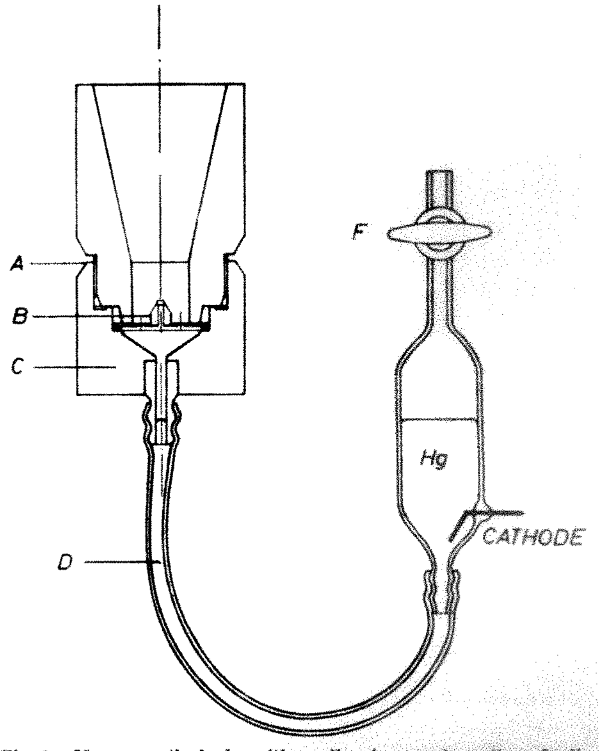

Fig. 1. Mercury cathode deposition cell. A, screw-in section of cell; B, specimen grid holder; C, base of cell; D, polyethylene tube; F, fine mercury-level adjustment tap

a flat spiral. Finally, 100 V d.c. was applied between the anode stirrer and mercury bulb. The distance between the anode and the mercury surface was adjusted for minimum current flow.

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After the completion of a 2- or 3-min run the anode is withdrawn and the plating solution siphoned off. The level of the mercury is very carefully lowered to a point just below the specimen grid and then drained out completely from the cell. Part *A* of the cell is screwed off and the specimen grid holder removed. During the early phases of the work some trouble was caused by small quantities of mercury being trapped between the deposited film and the specimen grid. However, providing some care is taken in the cleaning of such grids, no undue difficulty should arise. We found that washing the platinum grids in concentrated nitric acid followed by alcohol was satisfactory for this purpose.

Figs. 3 and 4 are some of the results obtained from specimens prepared from uranyl nitrate plating solutions. In this case the deposited material proved to be uranium dioxide and not, as was originally thought to be the case, the same compound as that dissolved in the plating solution[1]. However, recent dissociation studies involving the labelling of magnesium sulphate with radiosulphur (sulphur-35), or $Mg^{35}SO_4$, have shown that under carefully controlled conditions magnesium sulphate is plated out as such at the cathode[7]. It has also been pointed out that this could be the case with the uranium salts, but that they are later converted to the dioxide caused by the heating of the electron beam. In order to test this possibility, labelling will also be applied to uranium sulphate.

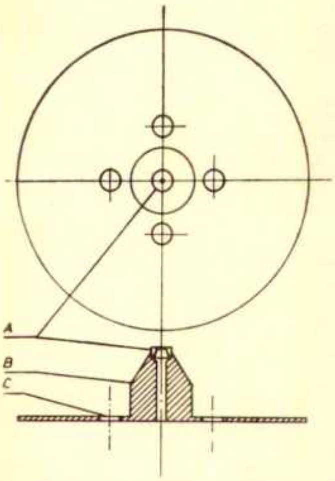

Fig. 2. *A*, electronmicroscope specimen support grid; *B*, stainless-steel grid holder; *C*, holes for admission of mercury

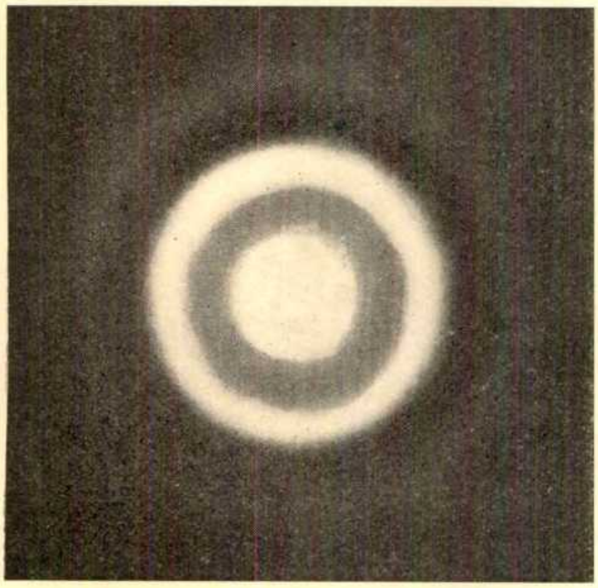

Fig. 3. Electron diffraction pattern of film deposited from uranium nitrate in alcohol plating solution shows that during some stage of preparation, or microscope examination, conversion to the dioxide has occurred

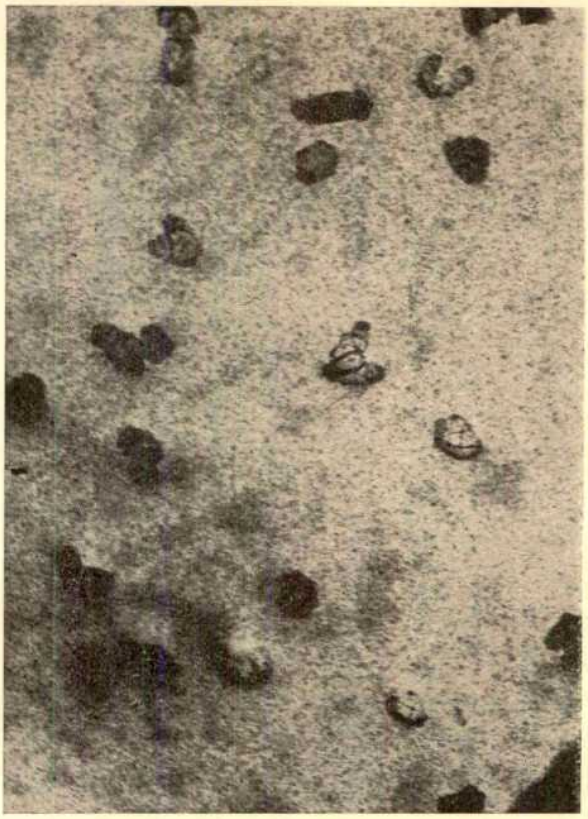

Fig. 4. Electronphotomicrograph showing surface structure of UO_2 film obtained deposited from uranyl nitrate plating solution (× *c*. 12,000)

The described plating method has been applied to a wide range of elemental compounds including uranium and thorium[2,3], the rare earths[4], cæsium, cobalt, iron, zinc, strontium and calcium[5], and plutonium[6]; it is possible that the method may find routine application in electron diffraction where one can never have too many preparation techniques available.

The possibility of using a similar technique for the preparation of thicker, self-supporting films (up to $500\ \mu g/cm^2$), having an area in the region of $1\ cm^2$, is at the present time under development[8]. Such films would find considerable application within nuclear physics.

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CYTOCHEMICAL AND IMMUNOCHEMICAL ANALYSIS AT THE ELECTRON MICROSCOPY LEVEL: OBTAINING CONTRASTING ANTIBODIES BY USE OF IODINE

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MODERN histochemistry offers various methods of histochemical analysis[1]. These methods have been developed on the basis of optic microscopy. However, limitations due to the low-resolving capacity of optical microscopes do not allow the use of these methods for investigating histochemical problems at the molecular level.

With the use of electron microscopes the only way of revealing two different neighbouring structures or substances is by contrasting. The intensity of blackening of a photographic plate (other conditions being equal) is determined by the quantity of electrons reaching each particular area of the plate. The quantity of electrons loosened by the original electron pencil passing through the object is determined by that part of the electron pencil which is scattered inelastically and elastically by the atoms of the object. The first effect is determined by the correlation between the energy of the falling electrons and the structure of electron levels of an atom. It occurs if the energy of the falling electrons is equal to or more than the energy which corresponds to characteristic frequencies of the element spectrum.

If interaction between the electron pencil and the atoms of the element is inelastic, the representation of the biological object in principle can be obtained as a mosaic which reflects the distribution of the chemical element in the preparation. In this case the change of the electron energy (for example, by a change of the accelerating voltage) and construction of an image of the object examined by inelastically scattered electrons renders it possible to obtain some photographs which reflect the distribution of different elements in the object investigated.

If one proceeds from the *K*-lines of the X-ray spectrum the excitation energy of which is closest to the energy of electrons now used in electron microscopy, a simple calculation shows that micro-elements most interesting from the biological point of view (manganese, iron, cobalt, zinc, molybdenum, silver, iodine, mercury, lead, etc.) may be detected with an electron microscope operating at 7–100 kV with sufficiently precise stabilization of the voltage (the error should not exceed 0·001 per cent), a high resolving power ($\sim$ 2 Å) and corresponding system of construction of image. At present such microscopes do not exist. However, possibilities of the method already mentioned may make the designing of such electronic microscopes very desirable.

The second effect, that is, elastic scattering, is determined in common by the size of the atom. The higher the atomic number of the element, the more intense (other conditions being equal) is the scattering of the electrons by its atoms.

This effect apparently cannot be used for identification of elements, because the additivity of scattering effect on many atoms does not allow estimation of the part brought in common scattering by every element.

At present, electron microscopy does not render it possible to distinguish between elastic and inelastic scattering. Both effects are determined by the size of atomic cross-section for electron scattering. If the atomic number is increased the cross-section for electron scattering is also increased. Owing to this it is easy to distinguish two structures containing atoms with widely distant atomic numbers, but it is difficult to do this when the atomic numbers are neighbouring. For this purpose the heavy elements (osmium, manganese, iron, uranium) are used as specific markers for increasing the contrast of objects. The specificity of a marker is highly increased by combining the iron-contained protein, ferritin, with antibodies. The possibility of obtaining antibodies against 'artificial' antigens[3,4] suggests the idea of developing a method of electron-microscopic immunochemical analysis which is highly sensitive and unique as a specific immunochemical method, on one hand, and render it possible to detect both the macromolecules and usual smaller molecules of organic substances on the other. Hence, it is evident that the method of obtaining contrasting antibodies is of great importance.

However, the method of ferritine antibodies has two significant disadvantages; for example: (1) obtaining a sufficiently pure complex molecule of antibody–ferritine is very difficult and laborious; (2) a sharp increase in the size of the immunologically active molecule may impede its diffusion to the areas of antigen localization in the cell.

To eliminate these disadvantages, attempts were made to develop a method based on combining the antibody molecule with a small organic molecule containing mercury[5,6]. This method requires a scarcely available mercury-containing organic compound and is also very laborious.

It is well known, however, that up to 20 iodine atoms may be included in the antibody molecule without losing its specific activity[7,8]. The problem is whether this amount of iodine is sufficient to make the antibody thus iodized 'black' enough, that is, contrasting in the field of an electronic microscope against a background of the neighbouring areas of the preparation.

At present there are no theoretical data allowing one to calculate precisely the dependence between the quantity of atoms with a particular atomic number and the level of scattering of electrons produced by them, that is, the value of blackening observed and the contrast in the electron microscopy. On the basis of the theoretical approach[2], Valentine[10] developed an equation making it possible to calculate as a guide the number (N) of atoms with the atomic number (Z) which must be distributed uniformly over the surface of an object or in its volume to obtain a minimally detectable increase in the contrast at the particular voltage used for electron-microscopic examination.

By using this equation, Pearse[1] carried out a similar calculation for contrasting with OsO_4. According to his results sufficient contrast may be obtained by introducing one residue of OsO_2 per 400 Å (ref. 2) of the membrane surface. Since the cross-section of the antibody is 8000 Å (ref. 2) and the atomic number of iodine (Z) is 53, it is evident that for obtaining a contrast analogous to that produced by osmium one should introduce about 40 iodine atoms into the antibody molecule.

An important factor for obtaining the contrast is the distribution of iodine atoms in the antibody molecule, determined in its turn by the distribution of tyrosine residues. Unfortunately such data are lacking.

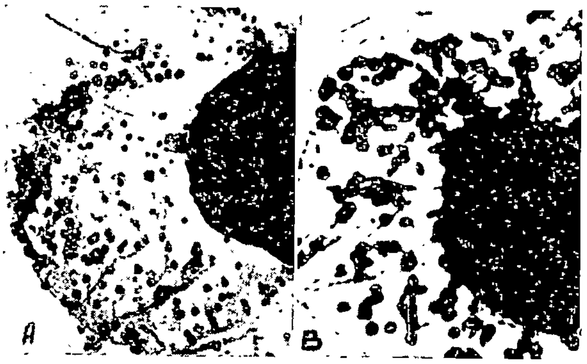

Fig. 1. Influenza virus *PR*-8 adsorbed on the chick red blood cell stroma, *A*, treated with OsO_4; *B*, treated with iodized immune γ-globulin (× *c*. 7,500)

We have attempted, however, to estimate more precisely the possibility of obtaining a sufficient contrast by iodizing. For this purpose we used the photographs taken in the course of electron-microscopic examination of thin tissue sections of the murine thyroid[11]. Thyreoglobulin contains 0·8 per cent of iodine[12]. One could hope to detect it by electron-microscopic examination. Indeed, the photomicrographs showed several types of granules with high-density levels. Although there is no direct evidence that these granules are really the granules of thyreoglobulin, their unusual character, specificity for the thyroid gland and the characteristic dynamics of their occurrence suggested the possibility of obtaining a sufficient contrast by using iodized antibodies. On the basis of the aforementioned considerations the investigations described here were performed. Influenza virus (strain *PR*-8) was used as the antigen, and hyperimmune polyvalent horse serum as the source of antibodies.

(1) An allantoic culture of influenza virus (strain *PR*-8) was purified by means of ultrafiltration[13] and differential centrifugation, as a result of which a virus suspension in 1 per cent ammonium acetate solution was obtained.

(2) γ-Globulin was prepared from hyperimmune horse serum[14]. The solution obtained was diluted with sodium bicarbonate solution, down to the γ-globulin concentration of 2 per cent and 1·5 per cent concentration of sodium bicarbonate. The solution was cooled down to 0° to + 2° C in an ice bath with constant stirring by a magnetic stirrer and gradually was added dropwise 0·2 N iodine solution in ethanol at a rate of about 0·01 ml./min. The amount of iodine added corresponded to 10 per cent of the weight of γ-globulin. The solution gradually became coloured and at the end of the iodine addition turned yellow, with a decrease of *p*H from 8·3–8·4 to 8·2–8·1. Addition of iodine lasted 2–2·5 h. Then the solution was poured into a 'Cellophane' sac and dialysed overnight against 1 per cent ammonium acetate solution, *p*H 7·0. A colourless solution was centrifuged at 8,000–10,000 r.p.m. for 20 min. The residue was discarded and the solution was used as the source of iodized antibodies.

(3) The antigen–antibody reaction was performed in two ways: (i) a sodium periodate solution was added to the virus suspension. The non-reacted sodium periodate was reduced with glucose[15]. Then the virus-containing suspension was mixed with chick red blood cells stroma, adsorbing the virus on the stroma and adding the iodized antibodies according to the method described[15]; (ii) the virus suspension was mixed with antibodies, after which the mixture was spread over a colloid membrane.

(4) The initial non-iodized anti-influenza serum and the iodized horse anti-rabic serum were used as controls.

(5) The preparations were examined with an electron microscope, model *JEM–5Y*, at an accelerating voltage of 80 kV. The expositions and the enlargements of the test and the control preparations were identical. Photographs of the materials were taken simultaneously at all stages.

Seligman[16] pointed out that it is very desirable to introduce iodine into preparations examined with electron microscopes. When iodizing was performed, we tried to introduce an amount of iodine into the antibody molecule maximal from the point of view of preserving the specific activity. The first experiments showed sufficient stability of the iodized antibodies and the amount of iodine introduced was increased up to 5 per cent. This amount is more than twice as high as that calculated. In practice, 10 per cent of iodine is added to the γ-globulin solution, since only ~ 50 per cent of iodine added is incorporated into the γ-globulin molecule. Table 1 shows the results of determining the antibody titres in the hæmagglutination inhibition reaction.

Table 1. CHANGES IN THE TITRES OF γ-GLOBULIN RESULTING FROM IODIZING

No. of experiments	Titres of the hæmagglutination inhibition reaction	
	Initial	After iodizing
1	1 : 5,120	1 : 1,024
2	1 : 512	1 : 512
3	1 : 512	1 : 128

However, since the analysis of the iodine content in the antibodies was not performed, it was difficult to say what was the real content of iodine in the antibodies. Still, the examination of the preparation with electron microscope demonstrated that, in both the first and the second

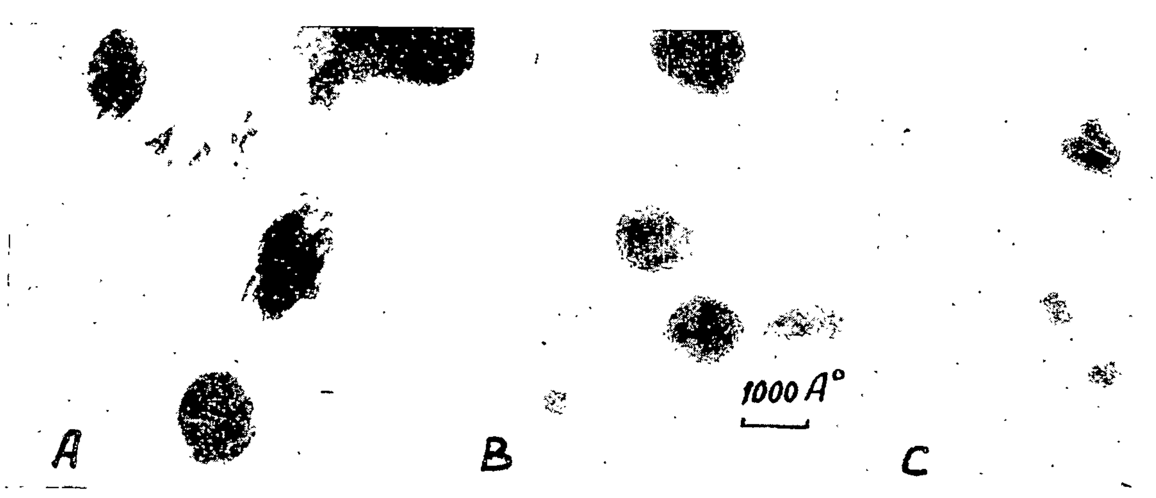

Fig. 2. Influenza virus *PR*-8 mixed with γ-globulin, *A*, with iodized immune γ-globulin; *B*, with non-iodized immune γ-globulin; *C*, with iodized anti-rabic γ-globulin (× 100,000)

variations of the reaction virus–antibodies, the contrast is observed in the presence of sufficient specificity.

Fig. 1*A* shows a preparation of influenza virus *PR*-8 adsorbed on the chick red blood cell stroma contrasted with OsO_4 according to the usual procedure. Fig. 1*B* shows an analogous preparation treated with iodized antibodies but not contrasted with OsO_4. The complex virus-antibody is clearly seen on the red blood cell stroma. Fig. 2*A* shows a preparation obtained on mixing a suspension of influenza virus *PR*-8 with iodized immune γ-globulin. A sufficient contrast of the complex virus–antibody is clearly seen.

Fig. 2*B* shows a preparation obtained in the same way by mixing a suspension of influenza virus *PR*-8 with non-iodized immune γ-globulin. The complex virus–antibody is seen, as expected, in the form of a light grey diffuse zone.

Fig. 2*C* shows a preparation, obtained with iodized anti-rabies horse γ-globulin instead of iodized anti-influenza γ-globulin. The virus particles are seen in the form of greyish diffuse spots.

The photomicrographs are demonstrating an undoubtedly sufficient level of contrast obtained with iodized antibodies. However, it should be remembered that the final conclusion as to whether iodized antibodies are applicable for cytological investigations with the use of electron microscopes may be drawn only on the basis of investigating ultra-thin tissue sections treated with similar antibodies.

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SORPTION OF WATER BY WOOL KERATIN

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In a recent communication[1], Dr. J. L. Morrison distinguishes a water-content range of 35–42 per cent (relative to dry weight) in which he proposes that water is thermodynamically bound to wool keratin. His proposal is based on the results of experiments in which water vapour is slowly withdrawn via a capillary tube from a flask holding a wool sample initially containing free water, air being absent. It is our opinion that water is not bound to keratin in the proposed range (*BC* in Morrison's notation) and that his observations result from a failure to maintain the temperature throughout his sample sufficiently uniform. Our conclusions are based on theoretical grounds and are supported by results we have obtained from an experimental arrangement similar to that described by Morrison but used with a much reduced evacuation rate to obtain more nearly isothermal conditions.

The important role of temperature in the wool–water sorption system is not always fully appreciated. Two examples may serve to emphasize the magnitude of the effect: (1) if the water content of a wool sample near saturation is changed under adiabatic conditions by 1 per cent, then the temperature of the sample will change by about 12° C. (2) In a system initially containing water vapour at saturation pressure at 20° C, if the temperature is increased by 1° C while the vapour pressure is maintained constant, then the relative pressure (relative humidity) is reduced to 94 per cent and the corresponding equilibrium water content of wool is reduced from 33 to about 26 per cent.

Owing to the presence of these strongly coupled effects the mechanisms of heat transfer are of prime importance in experimental studies of sorption. It is not easy to make observations of changes in water content which are characteristic of the wool keratin alone and uninfluenced by heat effects. This difficulty has already been considered by King and Cassie[2]. It is particularly apparent in vacuum systems where heat transfer by convection is not present.

In Morrison's experiment water is removed from the sample at an initial rate of $2 \cdot 2 \times 10^{-4}$ g/sec. Assuming the heat of evaporation as 580 cal/g, then 0·128 cal/sec must be transferred to the sample. Transfer of heat to the interior of the wool mass is effective by conduction only at the vapour pressures used. Assuming the conductivity of the sample is effectively that of the water vapour present, for which we take the value $4 \cdot 5 \times 10^{-5}$ cal/sec/cm/° C (ref. 3) and assuming a spherical sample of diameter 5 cm, it can be shown[4] that the necessary heat transfer can only take place if the centre of the wool mass is about 45° C below the surface temperature. It is clear, therefore, that either the experiment is not isothermal or evaporation is not occurring uniformly through the sample.

If evaporation occurs only from the outer part of the mass a much smaller temperature difference is required to sustain the necessary heat flow. In any actual experiment the interior temperature of the mass will fall only slightly to correspond with the rate of evaporation from the interior, which is limited by the relatively slow rate at which heat can be conducted to it. During evacuation, therefore, the source of water is essentially only the outer region of the wool mass. In the range *AB* (Morrison's notation) the free water content of the outer region becomes progressively reduced and is lower than the average for the whole sample; however, the vapour pressure is constant at the saturation value.

After point *B* is passed it is our opinion that the water content of the outer part of the wool mass is being reduced during evacuation to values progressively lower than the saturation value (that is, that usually taken as 33–35 per cent for unmodified wool keratin[5]), while in the interior of the mass free water remains present but with its boundary receding towards the centre. The vapour pressure during evacuation will fall below the saturation value (this is manifested by the fall over the range *BC* in the amount removed per 20-min evacuation). The vapour pressure of the free water inside the mass falls correspondingly because it is at the lower temperature necessary to maintain heat flow. However, in the range *BC* the average moisture content for the whole sample is above the usually accepted saturation value for keratin[5].

This appears to us the probable explanation for the anomalous region *BC*, rather than the existence of 'capillary water'.

While there is free water present throughout the sample and the temperature is uniform there is thermodynamic equilibrium and consequently no redistribution of water during the 12-h resting periods. Hence, non-uniformity in moisture distribution persists and the average moisture content of the wool mass is therefore not an indication of the condition of the outer region which is the effective source during evacuation.

After all free water has been removed from the outer regions there will be a tendency during the 12-h periods for water to be transferred from the interior of the mass to the surface, since the latter then has an equilibrium vapour pressure below the saturation value. Calculation of the time required for redistribution is difficult; some estimates indicate, however, that complete equilibrium is unlikely to occur in a 12-h period because of the thermal effects discussed earlier. Moreover, in no circumstances can the water content of the surface rise above the saturation value of 33–35 per cent. Hence, non-uniform distribution persists in this case also. We consider that these are the conditions prevailing in the range *BC* and that the progressive fall in the amount removed per evacuation is a measure of the steady depletion of the sample surface.

A further comment concerns the relative vapour pressure at the point *C*. Under the experimental conditions quoted by Morrison we believe the rate of evacuation, at least in the *ABC* region, to be almost completely determined by the resistance offered by the capillary tube and by the vapour pressure prevailing in the flask and not to any significant extent by desorption rate processes within the wool fibres. This would clearly be true while free water was present at the surface of the mass, while at high values of water content (but below saturation) the rate at which water can be released from wool fibres is several hundred times greater than the evacuation rate used by Morrison of 4–5 per cent water content per 20 min, or 4×10^{-3} per cent/sec. Thus Roberts and Haly[6] report initial desorption rates of 1 per cent water content per second, while King[7] and Nordon *et al.*[8] quote values of diffusion coefficient of about 10^{-7} cm^2/sec, corresponding to desorption times of a few seconds. Hence the vapour pressure in the flask is virtually in equilibrium with the wool and this together with the capillary resistance determines the evacuation rate. (The important point should be noted, however, that uniformity of vapour pressure within the flask does not imply uniformity of water content throughout the sample nor uniformity of temperature).

Since the capillary flow over the range of vapour pressures encountered follows Poiseuille's law, the mass transfer per second is proportional to the square of the pressure drop along the capillary. The evacuation rate in the range *AB* is 0·26 g/20 min, corresponding to saturation vapour pressure. The evacuation rate at point *C* is 0·20 g/20 min, so that the relative vapour pressure at point *C* is $(0{\cdot}20/0{\cdot}26)^{1/2}$, that is, 0·876 rather than the 0·99 quoted by Morrison. (It is not clear from his communication how the figures for relative humidity in his table were arrived at.) At a relative vapour pressure of 0·876 the water content of wool has been frequently determined[5] as about 23 per cent, rather than the value of 35 per cent quoted by Morrison. This throws doubt on the interpretation of *C* as a fibre saturation point. It appears to us that *C* could be the point at which the last of the free water is removed from the sample.

We have performed an experiment similar to that of Morrison, using a sample of wool of similar mass and shape. Our capillary tube, however, was selected to offer a much greater resistance to flow, so that the initial evacuation rate was $8{\cdot}7\times10^{-6}$ g/sec, that is, about 1/25 of that used by Morrison. The initial water content of the sample was 160 per cent. Continuous evacuation of the sample was carried on until its average water content was 50 per cent. At this stage the evacuation rate was found to have fallen to $8{\cdot}1\times10^{-6}$ g/sec, despite the slowness of the rate. Evacuation of the sample was then stopped for 60 h. On resuming evacuation it was found that the rate had returned completely to its initial value of $8{\cdot}7\times10^{-6}$ g/sec.

There is no supporting evidence for any such long-term intra-fibre sorption effect in saturated wool as would give rise to behaviour of the magnitude observed. On the other hand, the result of our experiment is consistent with the proposal that, during evacuation, evaporation does not proceed uniformly from the sample, that the outer part of the mass is first depleted of free water, and that during a subsequent long rest period water is slowly transferred from the interior of the mass to bring the surface near to the saturated condition.

The average rate of evacuation was lowered much further still, to $3{\cdot}6\times10^{-7}$ g/sec, and it was then found possible to reduce the water content of the sample to 35 per cent without appreciable fall in evacuation rate from the initial value.

Note. Dr. Morrison has kindly discussed by correspondence some of the aspects raised in our letter. This does not, however, necessarily imply his agreement with our arguments.

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OLIGOMYCIN AND ACTIVE TRANSPORT REACTIONS IN CELL MEMBRANES

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THE formation of adenosine triphosphate (ATP) by oxidative phosphorylation in mitochondria is a complex process involving a high-energy phosphate intermediate that has been partly characterized[1]. The antibiotic, oligomycin, appears to inhibit ATP formation by blocking some step in the transfer of high-energy phosphate through this intermediate to ADP[2]. The question arises whether ATP utilization for the active transport of sodium and potassium ions at cell membranes also involves a phosphate intermediate which is affected by oligomycin. Active transport intimately depends on the enzymatic hydrolysis of ATP, and it is now well established that sodium and potassium ions are required together for optimum rates of transport and of adenosine triphosphatase (ATPase) activity. There have been some indications that these ions influence the turnover of a phosphate compound which is formed during this kind of enzymatic activity[21–23,31]. It is the purpose of this article to show that oligomycin is a potent inhibitor of active transport, and that it inhibits the breakdown of a phosphate intermediate which is formed from ATP in cell membranes of kidney cortex. It seems likely that this reaction is responsible for the transformation of chemical energy into osmotic work.

Previous authors have described some effects of oligomycin on membrane transport and its associated ATPase activity. A clear inhibition of potassium uptake was found with liver slices[3]. On the other hand, with red blood cells, Van Rossum[3] found no inhibition, while Glynn[4] found partial inhibition. As regards the membrane ATPase, which is activated by sodium and potassium inhibition by oligomycin, has been shown in red blood cells[5], brain microsomes[5–7] and the electric organ of the electric eel[4,6]. Correlation of the effects on transport and ATPase, activity have not been made, however, and the question has been raised whether the antibiotic inhibits active transport directly, or indirectly by inhibiting the supply of ATP[3–5]. In investigating reactions that might be associated with membrane transport, it is important to conduct experiments with intact cells as well as with fragmented membranes. Intact membranes are, of course, required for active transport to be demonstrable, and red cells and kidney cortex slices have been used in this investigation to monitor ion movements; at the same time, metabolic activity was also measured. A membrane preparation was used to follow the fate of the terminal phosphate of ATP under conditions similar to those occurring in cells.

Red Blood Cells

ATP in these cells is derived entirely from the anaerobic conversion of glucose to lactate, and oxidative phosphorylation is not involved. Since oligomycin is generally believed to inhibit oxidative phosphorylation[2], it was decided to investigate its effect in human red blood cells, to see how it affects other processes.

Sodium and potassium transport. The potassium influx (in µmoles/ml. cells/h) was measured[8] with potassium-42, and was lowered from 2·15 in control cells to 0·75 with oligomycin at a concentration of 20 µg/ml. medium. This represented maximum inhibition of the active influx because 100 µM ouabain caused a fall to the similar value of 0·65. The oligomycin concentration that would give half maximal inhibition would be 1–2 µg/ml. Sodium transport was determined from net movements, and oligomycin and ouabain each prevented the extrusion that was found in the control cells. Controls lost (in µmoles sodium/ml. cells/3·5 h incubation) 3·4, whereas gains of 2·0 and 2·6 occurred in the presence of ouabain and oligomycin, respectively. These results agree with those of Glynn[4], but not those of van Rossum[3]. It is evident that oligomycin is analogous to ouabain so far as sodium and potassium transport is concerned.

ATP hydrolysis. The membrane ATPase activity was measured[9] by determining the orthophosphate (P_i) released from ATP by a preparation of fragmented membranes[20]. The activity (in µmoles P_i/ml. original cells/h) had a value of 1·6 in the presence of sodium and potassium ions, which are required together for optimum activity[10,11]. The hydrolysis was lowered some 60 per cent either by the omission of potassium or by the addition of ouabain or oligomycin (Table 1). The inhibitory effects of oligomycin and ouabain are not additive, because the two together cause only the same degree of inhibition as either alone (Fig. 1).

Comparison of potassium influx in intact cells with ATPase activity in fragmented membranes revealed a parallel inhibition over a range of oligomycin concentrations (Fig. 2). A decrease in ATPase activity went hand in hand with a fall in potassium influx, showing that an effect on one process is accompanied by a similar effect on the other process. The linear relationship in Fig. 2 is derived from comparing the behaviour of intact cells with fragmented membranes, and there are too many objections and assumptions involved to warrant a quantitative comparison of potassium influx/ATP hydrolysed. These results suggest, nevertheless, that oligomycin, like ouabain, inhibits active transport through an action on a membrane ATPase in human red cells.

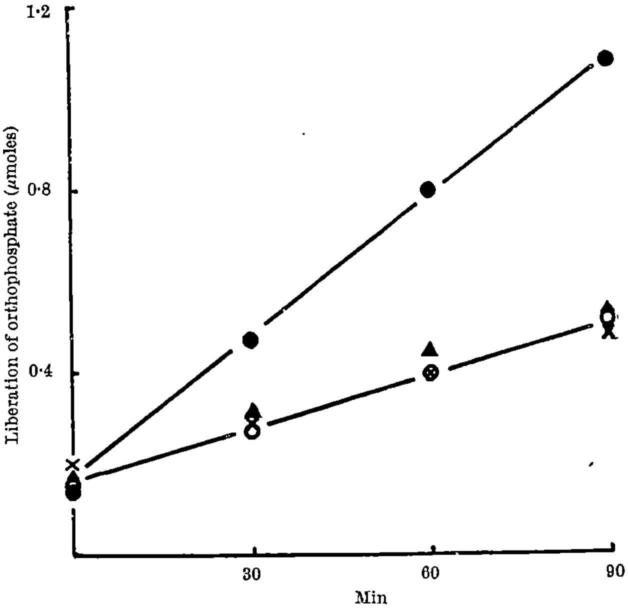

Fig. 1. Effect of oligomycin on the ATPase activity of fragmented erythrocyte membranes. Fragmented erythrocyte membranes (3 mg dry weight) were incubated in a medium of composition: 3 mM ATP; 100 mM NaCl; 25 mM KCl; 1 mM $MgCl_2$; 10 mM *tris*-HCl, *p*H 7·5. Oligomycin was added as a solution in ethanol (1 mg/ml.) and an equal volume of ethanol was added to the other experimental tubes. Controls with no inhibitor are shown (●), with 20 µg/ml. oligomycin (▲), with 0·1 mM ouabain (○), and with both 20 µg/ml. oligomycin and 0·1 mM ouabain by (×)

Lactate production. Oligomycin appears to act on the membrane ATPase and should therefore cause a consequential fall in lactate production at least as great as that due to omission of potassium from the medium[12]. Table 1 shows that lactate produced from glucose (in µmoles/ml. cells/h) was lowered from 3·05 in control cells to 2·55 and 2·60 in the presence of oligomycin or on the omission of potassium, respectively. The fall due to oligomycin was not significantly greater than that caused by omission of potassium. Since potassium ions activate the ATPase and lactate production from outside the cells[12–15] it would appear that oligomycin inhibits the same ATPase. These results do not show whether oligomycin acts on the internal or external surface of the cell membrane—only that it inhibits the mechanism that external potassium

Table 1. INHIBITORY EFFECTS OF OLIGOMYCIN ON CATION MOVEMENTS AND LACTATE PRODUCTION IN INTACT ERYTHROCYTES, AND THE ATPASE ACTIVITY OF FRAGMENTED ERYTHROCYTE MEMBRANES

	Net sodium ion movement	Potassium ion influx	ATPase activity	Lactate production
	← µmoles/ml. packed cells/h →			
Control	−1·0 (3)	2·15 (3)	1·6 (5)	3·05 (10)
0·1 mM ouabain	+0·6 (3)	0·65 (2)	0·5 (5)	—
20 µg/ml. oligomycin	+0·7 (3)	0·75 (2)	0·5 (5)	2·55 (10)
Potassium free medium	—	0	0·45 (3)	2·60 (4)
Incubation medium	10 mM KCl 115 mM NaCl 10 mM *tris*-HCl *p*H 7·5 10 mM glucose	10 mM KCl 120 mM NaCl 10 mM *tris*-HCl *p*H 7·5 10 mM glucose	25 mM KCl 100 mM NaCl 10 mM *tris*-HCl *p*H 7·5 4 mM ATP 1 mM $MgCl_2$	10 mM KCl 120 mM NaCl 10 mM *tris*-HCl *p*H 7·5 10 mM glucose

Figures in brackets show the number of determinations. For ATPase activity and lactate production the values are the means from two experiments. Incubations were performed at 37°.

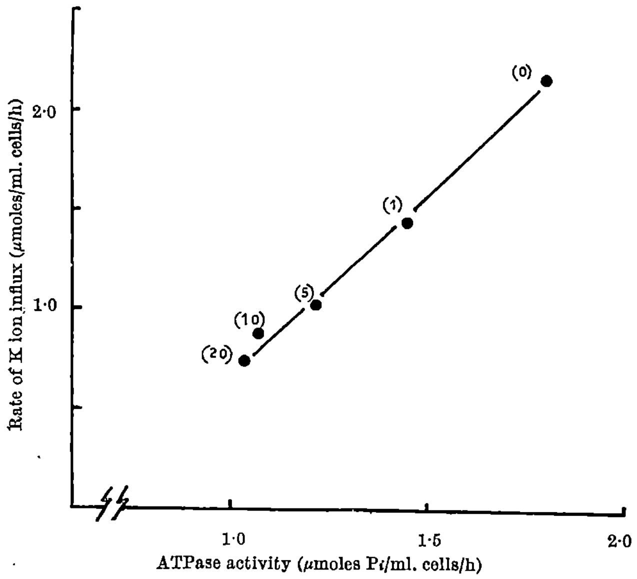

Fig. 2. Comparison of the ATPase activity of fragmented erythrocyte membranes with the rate of potassium ion influx into intact cells in the presence of oligomycin. Concentrations of the antibiotic (μg/ml.) are shown in brackets. The co-ordinates of each point are the means of at least two experimental values

activates, namely, the membrane ATPase. However, the evidence with the kidney membrane preparation (see later) shows that oligomycin is like ouabain in inhibiting the second step of the ATPase reaction, and as this is the one requiring potassium it looks as if oligomycin probably acts at the external cell surface. These results confirm that cessation of active transport, which may be due either to direct attack on the membrane (by ouabain or oligomycin) or to deprivation of external potassium, has a pace-maker effect on energy production manifested by a fall in lactate production.

From knowledge of the glycolytic pathway, it may be taken that each molecule of lactate produced is accompanied by the genesis of a molecule of ATP from ADP. The extent of the stimulation of the lactate production by external potassium is therefore the same as the stimulation of ATP production. If this amount of ATP is that which is utilized for active transport, the ratio of potassium transported/ATP hydrolysed can be calculated: the value is $(2\cdot15 - 0\cdot75)/(3\cdot05 - 2\cdot55) = 2\cdot8$. For net sodium actively transported/ATP hydrolysed the value is $\left(\frac{3\cdot4+2\cdot6}{3\cdot5}\right)\Big/(3\cdot05 - 2\cdot55) = 3\cdot4$ (Table 1). This means that the oligomycin-sensitive components of transport on one hand, and of lactate production on the other hand, yield a value of $2\cdot8 + 3\cdot4 = 6\cdot2$ for the ratio of sodium + potassium transported/ATP hydrolysed. The value is only approximate and is based on several assumptions. Nevertheless, it is remarkably close to that calculated by Sen and Post[16] by a different method. This work with red blood cells shows that all the effects of oligomycin can be explained by an inhibition of processes in the cell membrane without the need to postulate direct effects on metabolism.

Kidney Cortex Slices and Membranes

It was decided to find out if oligomycin affected kidney cells in a way that could also be ascribed to inhibition of transport reactions in cell membranes, rather than by a direct effect on energy metabolism in mitochondria.

Rabbit kidney cortex slices were incubated in a physiological saline medium containing glucose, and their potassium content and rate of oxygen consumption measured[17]. In a typical experiment, oligomycin in a concentration of 10 μg/ml. medium caused a fall in Q_{O_2} (μl. O_2/mg dry wt./h) from 12·6 to 8·7. This is similar to the fall due to cessation of active transport produced either by ouabain or by omission of sodium ions[17]. Comparable falls were found when malate or α-oxoglutarate were metabolites instead of glucose. (Neither oligomycin nor ouabain abolished the response of slices to 2 : 4-dinitrophenol, which caused a stimulation of oxygen consumption.) The maintenance of a high tissue potassium content was also prevented. Thus, in control slices a value (in μmoles/g wet-wt.) of about 50–60 was found, whereas with oligomycin or ouabain it was only about 20. To check that the inhibition was of potassium uptake and not just an increased rate of leakage, slices were first suspended in ice-cold medium in order to deplete them of potassium. The change in potassium content was then found after incubation for 1 h at 37° C. The net uptake of potassium in the controls was 25–50 and was abolished by oligomycin as it was by ouabain. Inhibition of potassium uptake in liver slices has been previously reported[3].

These effects made it worth while to investigate the effect of oligomycin on the membrane ATPase activity. Membranes of renal cortex are sedimented with two of the fractions of a homogenate the nuclear and the microsomal fractions[18–20]. The ATPase of the nuclear fraction was activated about 100 per cent by adding sodium and potassium ions together, and this component of the enzymatic activity was 80 per cent inhibited by oligomycin[20] (20 μg/ml.). The antibiotic did not inhibit the (sodium + potassium)-insensitive ATPase. In the same way, the microsomal fraction was sensitive to oligomycin, the (sodium + potassium) ATPase activity being lowered to the same extent as with ouabain (Fig. 3). The inhibition by oligomycin of the ATPase activity in both the nuclear and the microsomal fractions suggests that it inhibits the same ATPase as ouabain.

Phosphate intermediate in ATP hydrolysis. A valuable way of deciphering transport reactions is to trace the pathway of the terminal phosphate of ATP before it appears as P_i, and several investigators have described the formation of labelled protein when ^{32}P-γ-labelled ATP is incubated with fragmented membranes[21–23]. We prepared γ-labelled ATP (ref. 24) and incubated it with the kidney microsomal fraction, in a similar way to that of Charnock and Post[22]. After 10 sec the ATPase reaction was stopped with trichloroacetic acid (TCA) and the mixture spun down. The radioactivity due to phosphorus-32 in the supernatant fluid was measured in an extract of isobutanol[30]; this gave the $^{32}P_i$ liberated and hence the overall hydrolysis. The sedimented protein was washed several times with a solution of non-radioactive ATP and P_i, and the precipitate then assayed for radioactivity.

Fig. 3 shows that some tracer phosphate was not washed away with cold P_i and became attached to the TCA precipitate. It is perhaps premature to refer to the compound as phosphoprotein, although total phospholipid extraction[22] shows that it is unlikely to be phospholipid. We prefer, as Pressman[25] does in a similar situation, to refer to the intermediate as protein-bound phosphate.

The striking feature of the labelling is that it is dependent on the sodium and potassium concentrations. In the presence of sodium alone, the labelling was some 5–15 times greater than when potassium was present. Put another way, potassium prevents the labelling promoted by sodium. This feature is the converse of the hydrolysis that was taking place, because potassium stimulated hydrolysis some 200 per cent at the same time that it abolished labelling. Very little labelling was found when potassium ions were present without sodium. Ouabain had a slight effect on the formation of the protein-bound phosphate with sodium alone. However, with sodium plus potassium, ouabain caused the labelling to increase about 10-fold. These results are similar to those of Charnock and Post[22] in demonstrating the formation of a phosphate intermediate in the fission of ATP by a kidney-membrane preparation. The extent of the forma-

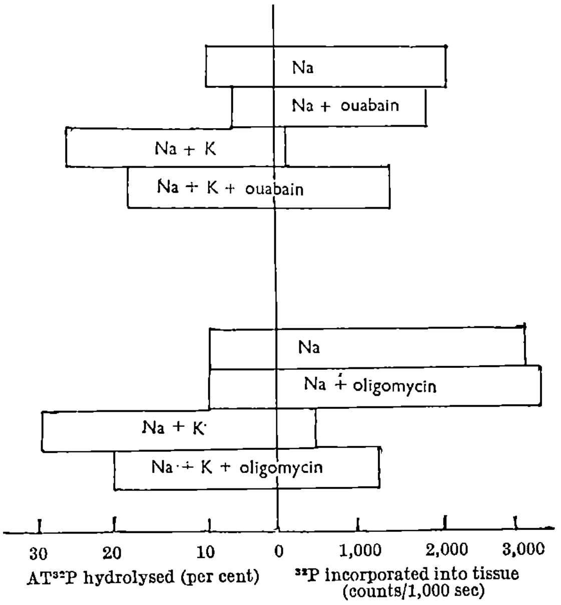

Fig. 3. Similarity between oligomycin and ouabain in opposing the action of K^+ during the ATPase reaction. Samples of the microsomal suspension (prepared according to the method of Charnock and Post[22]) were incubated for 10 sec at 37° in a medium containing 50 μM-ATP (labelled in the γ-position with ^{32}P: sp. activity 1·2 μc./μmole); 64 μM $MgSO_4$; 120 mM NaCl; and 40 mM imidazole, adjusted to *p*H 7·6 with 0·5 N HCl. The medium was modified by the addition of KCl (16 mM), ouabain (0·2 mM), or oligomycin (ethanolic solution: 10 μg/ml.) as indicated; ethanol (1%) was added to the appropriate controls. The reaction was stopped by adding cold TCA solution (final concentration, w/v, 5%) to the incubation mixture, and the precipitated protein spun down after cooling in ice. In order to follow the hydrolysis of the ATP, the TCA supernatant solution was decanted into a solution of acidified ammonium molybdate and the phosphomolybdate complex formed was then extracted in isobutanol/benzene (ref. 30). The radioactivity of samples of the latter extract was measured. The precipitated protein was washed 3 times by resuspension in a cold solution of TCA (5% w/v), *tris*-phosphate (2 mM) and ATP (2 mM). The washed precipitate was then dispersed in a solution of scintillant containing 5% (w/v) 'Hyamine 10*X*' and the radioactivity measured. All measurements of radioactivity were carried out using a liquid scintillation technique. Background counts were about one a second and the ATP added to each tube was equivalent to about 29×10^4 counts/100 sec. In the experiment presented here, the zero time values for the washed precipitates were about 1,500 counts/1,000 sec: these values have been subtracted from those for the incubated samples. Other experiments, using (^{14}C)-labelled ATP and inorganic phosphate, have shown that the labelling of the tissue which is sensitive to Na^+ and K^+ is not due to the incorporation of ATP, or $^{32}P_i$ released into the medium

tion of the protein-bound phosphate is the inverse of the overall degree of hydrolysis, as shown by the effects of potassium and ouabain.

In view of the similarity of the effects of oligomycin and ouabain described here, the influence of the antibiotic on the formation of protein-bound phosphate was examined. In each respect its action was like that of ouabain (Fig. 3). It did not interfere with activation by sodium, and it promoted labelling by counteracting the effect of potassium. Again, the extent of labelling was opposite to the degree of ATP hydrolysis, the greater the hydrolysis the lower the labelling and *vice versa*. Attempts to demonstrate similar effects with red cell membranes have so far been unsuccessful. These results show, however, that in kidney cortex, as in red cells, oligomycin and ouabain have very similar effects on a number of membrane reactions.

Regulation of Respiration and Glycolysis

These results add to earlier ones in suggesting that the rates of lactate production and oxygen consumption may be regulated by the activity of membrane ATPases concerned in active transport. Lactate production has previously been thought to be unaffected by the rate of active transport in human red blood cells[26]. However, it now appears that a small fraction, equal to about 0·5 μmole lactate/ml. cells/h, is sensitive to cessation of active transport[12]. The same fall in lactate occurs whether transport is stopped with ouabain, oligomycin or by omission of external potassium. The mechanism by which these agents regulate lactate production would appear to be through their effect on the membrane ATPase. Thus, sodium and potassium transport requires ATP, and when it is stopped there will be changes in the balance of ATP, ADP and P_i, and perhaps in the ratio of NAD and NADH. The fluorometric and spectrophotometric observations of Aubert, Chance and Keynes[27] in the electric organ of *Electrophorus electricus* show a metabolic response to electrical activity which suggests that glycogenolysis may be stimulated as a result of increased active cation transport. This would be similar to the effects with red blood cells.

The results with kidney slices also show that the effects of oligomycin on oxygen consumption can be attributed to inhibition of the ATPase of active transport. A part of the oxygen consumption of the slices appears to be regulated by the activity of the membrane ATPase[17,28], and it is particularly striking that oligomycin inhibits the enzymatic activity to the same extent as ouabain.

Mode of Action of Oligomycin

The effects of oligomycin described here leave no doubt that it inhibits transformations of energy from ATP into osmotic work in cell membranes. Oligomycin acts like ouabain on cell membranes, but the two compounds are not the same in their action on mitochondria because ouabain appears to have no effect on mitochondrial metabolism[28]. There appear to be at least two steps in the reaction coupling ATP hydrolysis with osmotic work - one requiring sodium and the other potassium. By analogy with work with red cell ghosts[13,14] and intact cells[15,29,31], sodium would act from inside and, as a cofactor, facilitate the formation of the protein-bound phosphate. It seems likely that the intermediate might have a high-energy character because no osmotic work is associated with this step, the intermediate still being formed in the presence of ouabain or oligomycin. Potassium would act from outside and promote the breakdown of the intermediate. It is this latter reaction which may be the key to the active transport mechanism, since the energy transformation is associated with this reaction; P_i is liberated to the inside of the cell, and sodium migrates outwards and potassium inwards. The reactions may be represented thus:

$$\text{ATP} + \text{protein} \xrightarrow[\text{internal Na}]{\text{activated by}} \text{protein-bound phosphate} + \text{ADP}$$

$$\text{Protein-bound phosphate} \xrightarrow[\substack{\text{inhibited by}\\ \text{ouabain and}\\ \text{oligomycin}}]{\substack{\text{activated by}\\ \text{external K}}} \text{protein} + P$$

$$\text{ATP} + \text{internal Na} + \text{external K} \dashrightarrow \text{ADP} + \text{internal K} + \text{external Na} + \text{internal } P_i$$

The effects of oligomycin on membranes of red cells and kidney cortex raise the possibility that there may be similarities in the kind of intermediate implicated in energy transformation in mitochondria for ATP formation, and in cell membranes for ATP utilization. The results recorded here do not support the view that oligomycin affects active transport indirectly by an inhibition of energy supply in mitochondria. It should be borne in mind that most of the previous work on oligomycin has been performed with mitochondria, from which its mechanism of action has been deduced. The present work directs attention to three points in this connexion. First, our results show that oligomycin has other inhibitory effects in cells and is not a specific inhibitor of a mitochondrial transphosphorylation reaction. Secondly, although the toxic action of the antibiotic is said to be due to its inhibition of oxidative phosphorylation, the present results pose the question whether some of its toxic action might also arise from poisoning of active transport in the body cells generally. This would cause a leakage of potassium from cells into the circulation and might so raise the plasma potassium concentration as to stop the heart contracting. Thirdly, in work on the mechanism of action of oligomycin in mitochondria, complications will arise unless the preparations used are free from fragments of cell membranes. These are the wider implications of the present work. The general conclusion is that oligomycin acts at two sites in cells—the cell membrane and mitochondria—and that its effect at both sites is to inhibit the transformation of energy. We have shown that key reactions in active transport are the formation and breakdown of a phosphorylated intermediate derived from ATP. Sodium ions are required for its formation, whereas potassium ions catalyse its hydrolysis; ouabain and oligomycin are alike in inhibiting this hydrolysis. Work on the chemical characterization of this intermediate is in progress.

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ELECTRON HISTOCHEMICAL EXAMINATION OF OXIDATIVE ENZYMES AND MITOCHONDRIA

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HISTOCHEMICALLY, mitochondria have been shown to possess oxidative enzymes such as succinic dehydrogenase (SDH) and dihydronicotinamide-adenine dinucleotide diaphorase (NADH–D)[1–6]. The work recorded here deals with the electron microscopic demonstration of heterogeneity among mitochondria in terms of the oxidative enzymatic activity and the fine structural localization of enzymatic activity in mitochondrial cristæ, using tetrazolium salts as electron acceptors.

Small pieces (< 1 mm^3) of rat heart muscle were fixed in 6·25 per cent hydroxyadipaldehyde[7], in 0·1 M cacodylate buffer, *p*H 7·6, containing 8 per cent sucrose for 30–60 min at 0°–4° C. After fixation, these were washed in cold cacodylate buffer thoroughly, rinsed in 0·1 M phosphate buffer, *p*H 7·6, and incubated in substrate media (0·3 ml. of 250 mg per cent tetra nitro-blue tetrazolium chloride (TNBT), 1·5 ml. of 0·1 M sodium succinate, 1·2 ml. 0·1 M phosphate buffer, *p*H 7·6, and 0·25 g of sucrose for SDH; 0·3 ml. 250 mg per cent TNBT, 0·3 ml. 0·1 NADH, 1·0 ml. 0·1 M phosphate buffer, *p*H 7·6, 1·4 ml. of distilled water and 0·25 g of sucrose for NADH–D) for 10–30 min at 0° C or room temperature. After incubation, the blocks were fixed in cold 1 per cent buffered osmium tetroxide for 1 h, dehydrated through graded alcohols and propylene oxide, and embedded in a mixture of 'Epon–DDSA–DMP-30'. Ultra-thin sections were cut by an 'LKB' ultratome, mounted directly on grids and examined with an R.C.A. 'EMU-3F' electron microscope without post-staining.

Homogenates of rat heart muscles were also made and the mitochondrial fractions were obtained by the method of Luck[8]. Portions of the fractions thus obtained were negatively stained to demonstrate elementary particles[9–12]. Other portions were also incubated in substrate media for SDH, fixed in hydroxyadipaldehyde, post-fixed in osmium tetroxide, ruptured in 1 mM phosphate buffer, *p*H 7·2, or de-ionized distilled water, and spread directly over 'Formvar'-coated grids for the electron microscopic observation. Pellets were also made following incubation, fixed in both hydroxyadipaldehyde and osmium tetroxide and treated in the same manner as blocks of heart muscle. Substrate-free media as well as media containing inhibitors (sodium malonate and iodoacetic acid) were used for controls.

Amorphous electron opaque formazans of TNBT, which were absent in the control specimens, were localized at or near the sites of enzymatic activity. Mitochondria could be classified into three varieties depending on the distribution of reaction: completely reactive ones containing deposits of formazan over all the cristæ (Fig. 1), partially reactive ones containing formazan deposits over some or few of the cristæ (Fig. 2), and unreactive ones containing no deposits of final product (Fig. 1). It was not uncommon to find a mitochondrion positive for this reaction lying adjacent to a mitochondrion which was completely unreactive histochemically (Fig. 1). These findings not only occurred for mitochondria situated between myofibrils but also for those in the peripheral sarcoplasm. This heterogeneity of enzymatic activity among individual mitochondria of the same regions of heart muscle was also observed in the moderately intact mitochondrial fragments obtained in the fractionated specimens.

Although these findings may be due to uneven penetration of the ingredients of the substrate media into the blocks, this phenomenon was repeatedly observed in areas close to the peripheral surface of the blocks where penetration of the ingredients of the substrate media are moderately even. The same findings in pellets of

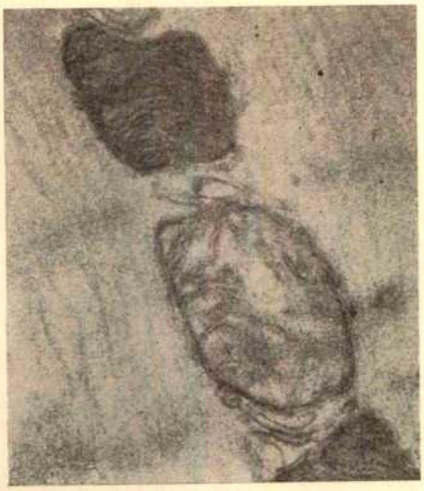

Fig. 1. Rat heart muscle mitochondria, SDH activity, showing a completely unreactive mitochondrion between two mitochondria showing reaction on all the internal membranes. (× *c.* 47,000)

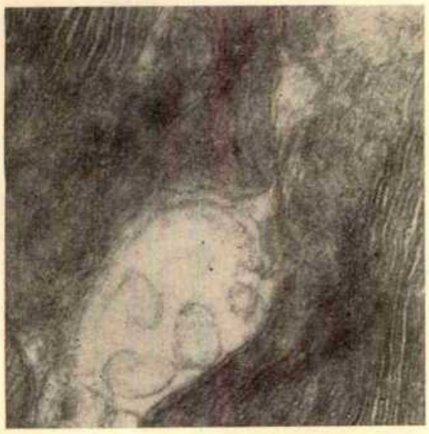

Fig. 2. Rat heart muscle mitochondria, NADH–D activity, showing spotty reaction confined to only some of the cristæ. (× *c.* 44,000)

mitochondrial fractions further substantiate the finding in blocks. Furthermore, since those mitochondria in pellets which were positive for enzymatic activity revealed formazans over the entire area of mitochondria, uneven penetration of substrate media is unlikely. The partial reaction confined to some of the cristæ in some of the mitochondria in blocks was probably due to partial inhibition during the process of fixation in hydroxyadipaldehyde. The heterogeneity among mitochondria may be explained by difference in functional stages or in the age of the mitochondria.

In mitochondria positive for oxidative enzymatic activity, the formazan deposits were confined to inner mitochondrial membranes and cristæ. Under high magnifications, formazans appeared localized as or on particles contained within osmiophilic layers of the cristæ. The size of particles contained in cristæ was in the range 60–90 Å. This particulate appearance of formazans in the cristæ was more clearly seen in the enzymatically active mitochondria contained in the pellets (Fig. 3). Formazans were also observed as or on spherical particles, 50–85 Å in diameter, in the cristæ of ruptured mitochondria, giving a linear, beaded appearance.

Mitochondria from fractionated material, negatively stained with phosphotungstic acid (PTA), revealed elementary particles, 60–90 Å in diameter, in or associated with mitochondrial membranes (Fig. 4). This finding was in good agreement with findings of Smith[9] and others[10–12], but the negatively stained particles were histochemically unreactive. However, when ruptured mitochondria were first incubated in the substrate media fixed in hydroxyadipaldehyde, and then negatively stained with PTA, particles in poor alignment are observed, some of which were dense and others were negatively stained (Fig. 5). Dumb-bell-like structures or particles attached to mitochondrial membranes by stalks were never observed in any of the specimens except those that were negatively stained. The dumb-bell-like structures or particles attached to membranes by stalks[9,12] may be artefacts obtained during the process of negative staining. They may be formed by separation of lipoprotein layers of cristæ by the high degree of dehydration[13,14].

Elementary particles isolated biochemically by Green[15], which were presumed to contain oxidative enzymes, were in the order of 150 Å in diameter and are approximately twice as large as the particulate material observed in the present investigations. This difference in size may be due to differences in types of cells[15] or in the methods used *per se*, or indeed the large particles may be agminates of smaller units. These results corroborate the suggestion that the mitochondrial membranes are composed of

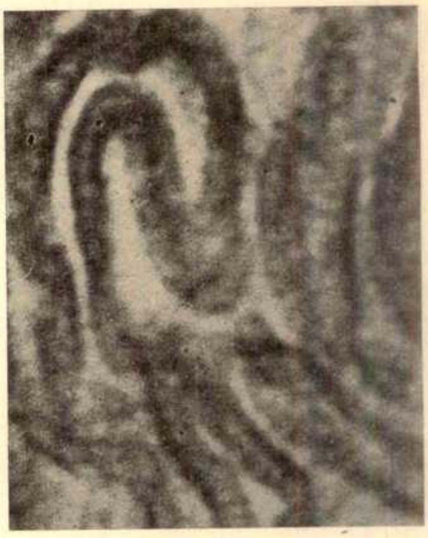

Fig. 3. Mitochondria from pellet showing SDH activity. Formazans are deposited as or on particles, 60–90 Å in diameter, in the osmiophilic layer of the membranes. (× *c.* 273,300)

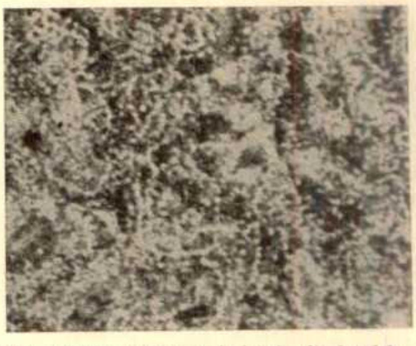

Fig. 4. PTA negative staining of isolated mitochondria. So-called elementary particles occur in membranous ribbons, sheets, free or attached to central ribbons or axes. (× *c.* 134,000)

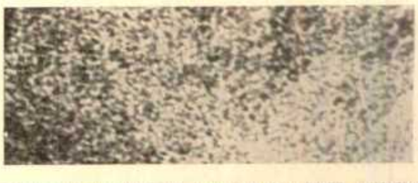

Fig. 5. Isolated mitochondria reacted for SDH activity, fixed in hydroxyadipaldehyde and negatively stained with PTA. Both electron opaque and negatively stained particles can be seen. (× *c.* 166,600)

a mosaic of sub-units containing some dehydrogenase and the electron transport system[16].

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GROWTH-RETARDING EFFECT OF ORAL L-PENICILLAMINE ON SARCOMA-180

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THE action of pyridoxamine dihydrochloride in accelerating the growth of ascitic and solid sarcoma-180 in CF_1 mice has shown the B_6-avidity of this tumour system and pointed to the existence of an exploitable biochemical difference of the tumour from normal host tissue[1]. This difference could be demonstrated *in vivo* by causing retardation of tumour growth with pyridoxine-deficient diet and by reversing this effect with pyridoxamine[1]. Furthermore, the tumour-retarding action of pyridoxine-deficient diet was found to be duplicated with a chemical antagonist of vitamin B_6, L-penicillamine (β-mercaptovaline). It is of interest that another isomer, D-penicillamine, has been in clinical use as a chelating agent for treatment of hepatolenticular degeneration. Subcutaneous injection of L-penicillamine significantly retards the growth of solid form sarcoma-180 in mice maintained on a complete diet, and especially so on pyridoxine-deficient diet[1]. The L-, D- and DL-forms of penicillamine were all found to be active as sarcoma-180 growth-retarding agents, the L-form being the most active. Growth retardation of solid form sarcoma-180 was produced by L-penicillamine whether treatment was started 24 h after tumour implantation or was delayed for 96 h (ref. 1).

This report concerns an investigation of the effectiveness of orally administered L-penicillamine in retarding the growth of solid and ascitic forms of sarcoma-180 in CF_1 mice.

L-Penicillamine was administered for long periods of time via the oral route by admixture with the diet and by addition to the drinking water, and for a short period of time (one week) by daily stomach intubation. Techniques of transplantation and methods of evaluation of tumour growth were as previously described for solid and ascitic sarcoma-180 (ref. 1). Two types of diet were used, that is, a complete diet consisting of standard mouse pellet (Rockland Farms, Inc.) and a pyridoxine-deficient diet (Nutritional Biochemical Corp.) of the following composition, both fed *ad libitum*: vitamin-free test casein 18 per cent, sucrose 68 per cent, vegetable oil 10 per cent, U.S.P. XIII salt mixture No. 2, 4 per cent; vitamins/100-lb. diet: vitamin A (200,000 units/g) 4·5 g., vitamin D concentrate (400,000 units/g) 0·25 g, α-tocopherol 5·0 g, ascorbic acid 45·0 g, inositol 5·0 g, choline chloride 75·0 g, menadione 2·25 g, *p*-amino benzoic acid 5·0 g, niacin 4·5 g, riboflavin 1·0 g, thiamine hydrochloride 1·0 g, calcium pantothenate 3·0 g, biotin 20·0 mg, folic acid 90·0 mg, vitamin B_{12} 1·35 mg.

Retarding effect of oral L-penicillamine on solid sarcoma-180. Ninety 20-g white Swiss CF_1 male mice previously maintained on complete diet each received subcutaneous implantation of 1×10^6 ascitic sarcoma-180 cells. The mice were divided into six groups, and were fed with either complete or pyridoxine-deficient diets (Table 1). L-, D- and DL-penicillamine were incorporated separately, mixed well with pyridoxine deficient diet, and then given *ad libitum*.

The daily oral administration via the drinking-water of 2–2·5 mg L-penicillamine/mouse/day to sarcoma-180-implanted mice for a period of 14 days caused 69 per cent tumour inhibition without mortality (Table 1). Tumour size and weights of the untreated control group of mice on the same diet, on the other hand, were approximately triple that of the L-penicillamine-treated group. The feeding of pyridoxine-deficient diet alone to sarcoma-180-implanted mice caused 48 per cent tumour inhibition, while addition of L-, D- and DL-forms of penicillamine to the deficient diet further increased the degree of tumour inhibition to 71, 70 and 68 per cent, respectively, for the respective isomeric forms. The greatest degree of tumour retardation was produced by the feeding of pyridoxine-deficient diet containing 0·5 mg L-penicillamine/g of diet. Subcutaneous injection of the above isomers of penicillamine to solid sarcoma-180-bearing mice had been reported to cause 55, 41 and 22 per cent tumour inhibition for the respective isomeric forms[1].

During storage of L-penicillamine-enriched pyridoxine-deficient diet, a dark greyish discoloration of the diet resulted which was feared to be a chemical reaction of penicillamine with some component of the diet. In subsequent experiments, therefore, L-penicillamine was administered in the drinking-water or by stomach intubation instead.

Effect of L-penicillamine on survival of solid sarcoma-180-implanted mice. Four experiments were conducted. In experiment 1, fifty mice previously maintained on complete diet were implanted subcutaneously with standard doses of 1×10^6 ascitic sarcoma-180 cells and were divided into two groups, after which they were maintained on complete diet (Table 2 and Fig. 1). One group of 20 mice received L-penicillamine in the drinking-water (0·5 mg/ml.), each mouse consuming approximately 2–2·5 mg L-penicillamine per day (approximately 100 mg/kg/day). Thirty untreated mice remained as controls. All the untreated mice developed solid sarcoma-180 tumours within three days after implantation, which increased regularly and rapidly in size. All mice in this group were dead within 33 days and in every case each mouse bore tumours until death, some as large as its host. The median survival time for this untreated group

Table 1. RETARDING EFFECT OF ORAL PENICILLAMINE ISOMERS ON THE GROWTH OF SARCOMA-180 IN CF_1 MALE MICE*

Diet	Penicillamine		Average change in body-weight†		Average tumour-weight g ± *S.D.*‡	Tumour inhibition (%)	Mortality
	mg/g diet	Average daily oral dose (mg/kg)	g	%			
Complete diet	0	0	+7·1	+36·6	4·82 ± 0·27	0	2/20
,, ,,	0·5 (L-)§	100–125	+4·5	+23·0	1·48 ± 0·73	69	0/10
Pyridoxine-deficient diet	0	0	+3·1	+16·3	2·51 ± 0·57	48	1/20
,, ,,	0·5 (L-)¶	100–125	+1·0	+ 5·3	1·37 ± 0·47	71	1/20
,, ,,	0·5 (D-)¶	100–125	+4·5	+23·0	1·45 ± 0·48	70	0/10
,, ,,	0·5 (DL-)¶	100–125	+3·2	+16·8	1·53 ± 0·35	68	1/10

* All mice received subcutaneous implantation of 1×10^6 ascitic cells/mouse at start. Solid sarcomas excised 14th day.

† Calculated from day of tumour implantation, inclusive of weight of tumour. Initial weight of mouse, 19·0–19·5 g.

‡ *S.D.* of individual tumour weights.

§ Added to drinking-water at concentration of 50 mg/100 ml. Average daily fluid intake 4–5 ml./mouse.

¶ Mixed with pyridoxine deficient diet.

Table 2. LIFE PROLONGING EFFECT OF ORAL L-PENICILLAMINE ON SOLID SARCOMA-180-BEARING WHITE SWISS CF_1 MICE MAINTAINED ON COMPLETE DIET*

No. and date of experiment	Continuous oral L-penicillamine (mg/kg/day)	No. of solid sarcoma-180-bearing CF_1 mice surviving at weekly intervals									% survival 8 weeks
		Start	1st wk.	2nd wk.	3rd wk.	4th wk.	5th wk.	6th wk.	7th wk.	8th wk.	
First (July 2–Aug. 8, 1963)	0	30	30	30	15	5	0	0	0	0	0
	100†	20	20	20	20	16	13	12	12	12	60
Second (July 2–Aug. 28, 1963)	0	10	10	10	4	0	0	0	0	0	0
	100	10	10	10	9	8	8	8	8	8	80
Third (July 9–Sept. 3, 1963)	0	10	10	10	3	1	0	0	0	0	0
	100	10	10	10	8	6	4	4	4	4	40
Fourth (July 16–Sept. 10, 1963)	0	20	20	18	14	6	4	1	0	0	0
	100	20	20	20	20	14	12	10	8	8	40

* Each mouse received subcutaneous implant of 1×10^6 ascitic sarcoma-180 cells at start of experiment, then maintained on complete diet (Rockland Farms mouse pellet diet).

† Drinking-water contained 0·5 mg L-penicillamine/ml. Each mouse consumed approx. 2 mg penicillamine/day (100 mg/kg). Initial average weight 20 g

was 21 days (Fig. 1). In the L-penicillamine-treated group, on the other hand, there was 74 per cent survival at 30 days and 60 per cent survival at 60 days. Furthermore, the solid tumours of this group were very much smaller than those of the untreated group. In more than half the treated animals, moreover, their tumours became necrotic, decreased progressively in size and were gradually extruded, until they were no longer palpable by the forty-third day of treatment (Fig. 1). Apart from slightly roughened coats at the sixtieth day, L-penicillamine-treated mice were healthy and active. At the end of this treatment period, eleven complete and three incomplete tumour regressions had occurred in 20 tumour-bearing animals. These animals were then maintained on complete diet without L-penicillamine treatment. At the time of this report, 342 days after cessation of L-penicillamine treatment, none of the tumours had recurred *in situ*.

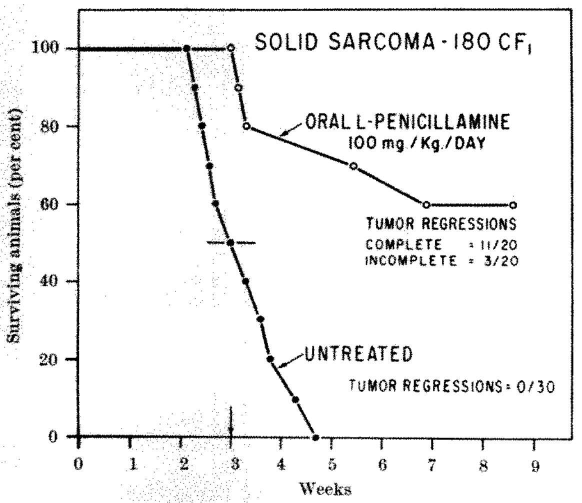

Fig. 1. Effect of oral L-penicillamine on survival of solid sarcoma-180-implanted mice maintained on complete diet. L-Penicillamine administered via drinking-water (0·5 mg/ml.) and given *ad libitum* to about 100 mg/kg/day

Exps. 2, 3 and 4 with sarcoma-180-bearing mice showed a similar life-prolonging effect of L-penicillamine (Table 2). In previous experiments, which had lasted only 14 days each, the daily subcutaneous injection of L-penicillamine had caused retardation of the development of solid sarcoma-180 but failed to reduce the size of tumour masses already formed[1]. In retrospect, we now attribute this to the short duration of treatment. Very few compounds which inhibit tumour growth cause *regression* of fully developed tumours. For example, only 6-mercaptopurine, some related antimetabolites[2,3] and pyridoxine-deficient diet[4] have been found to induce true regressions of already established sarcoma-180. The prolongation of life and regression of solid sarcoma-180 tumours in CF_1 mice, given prolonged oral therapy with L-penicillamine while being maintained on complete diet, assumes considerable importance. L-Penicillamine-induced tumour regression may be due to: (1) the blocking ability of L-penicillamine in preventing the developing tumour from obtaining sufficient vitamin B_6 needed for B_6-dependent cellular processes; (2) L-penicillamine-induced alteration of cellular metabolism; (3) immunological response of the host toward L-penicillamine-treated tumour tissue; (4) to some combination of these factors. Some support for the idea of immunological response of the host is found in the information that cortisone reduced the number of tumour regressions caused by 6-mercaptopurine without affecting retardation of tumour growth[5].

Effect of oral L-penicillamine on weight gain of tumour-free normal CF_1 mice fed with complete and pyridoxine-deficient diets. The effect on normal 22-g tumour-free CF_1 male Swiss mice of prolonged maintenance on complete and pyridoxine-deficient diets with and without oral L-penicillamine is shown in Table 3. Normal mice maintained on a complete diet gained 40 per cent body-weight after 30 days and 66 per cent in 60 days, without mortality. The addition of L-penicillamine to the drinking-water reduced this weight gain to 31 and 37 per cent over the same periods of time and the mice exhibited slightly roughened coats with 10 per cent mortality within the 60-day period. Maintenance of pyridoxine-deficient diet alone caused marked roughening of coats, but gain in weight did occur, albeit at a slower rate than with complete diet, and there was no mortality. The addition of L-penicillamine to the drinking water of the group maintained on pyridoxine-deficient diet retarded their normal weight gain still further within the first 30 days, but there was no actual weight loss. It was only during the second 30 days of oral L-penicillamine therapy with pyridoxine-deficient diet that a 15 per cent loss in body-weight and 20 per cent mortality occurred. This was attributed to the reinforcing action of L-penicillamine on the vitamin deficiency induced by pyridoxine-deficient diet. Baseline data demonstrate that within the first 30 days maintenance on pyridoxine-deficient diet, with or without daily oral treatment with L-penicillamine, does not cause either weight loss or mortality in CF_1 mice.

Table 3. EFFECT OF ORAL L-PENICILLAMINE AND PYRIDOXINE-DEFICIENT DIET ON BODY-WEIGHT OF TUMOUR-FREE NORMAL MICE

Diet	L-Penicillamine mg/kg/day	Average body-weight (g) Initial	14 days	30 days	60 days	Mortality
Complete diet	0	22·0	29·0	31·0	36·5	0/10
,, ,,	100*	22·0	28·0	29·0	31·3	1/10
Pyridoxine-deficient diet	0	22·0	24·0	26·0	28·0	0/10
,, ,,	100*	22·0	23·0	24·0	18·7	2/10

* Added to drinking water at conc. of 0·5 mg/ml. Average daily fluid intake 4–5 ml./mouse.

Effect of oral L-*penicillamine on ascitic sarcoma-180.* Maintenance of ascitic sarcoma-180-bearing mice on pyridoxine-deficient diet caused inhibition of tumour development and more so when L-penicillamine was added to the drinking-water (Table 4). These mice had received ascitic cells by intraperitoneal route. Median survival time was not prolonged significantly by either pyridoxine-deficient diet, L-penicillamine, or a combination of both.

Table 4. EFFECT OF ORAL L-PENICILLAMINE AND PYRIDOXINE-DEFICIENT DIET ON ASCITIC SARCOMA-180 IN CF_1 WHITE SWISS MICE

Diet	L-Penicillamine (mg/kg/day)	Average body-weight* (g)	Ascitic tumour inhibition	Median survival time (days)
Complete diet	0	31·6	—	10
,, ,,	100†	26·0	±	12·5
Pyridoxine-deficient diet	0	23·7	+	11·5
,, ,,	100†	21·5	+ +	11

* 8 days after intraperitoneal injection 1×10^6 ascitic S-180 cells. Initial average weight 20 g.

† Treatment started 24 h after tumour implantation, and given daily for 7 days. Drinking-water contained 0·5 mg L-penicillamine/ml. Each mouse consumed approx. 2 mg penicillamine/day (100 mg/kg).

Tumour retarding effect of L-*penicillamine on solid sarcoma-180 when administered by different routes.* Twenty-four hours after subcutaneous implantation of standard dose of ascitic sarcoma-180 in CF_1 mice, treatment with L-penicillamine was commenced via different routes, and continued daily for 7 days (Table 5). Single daily doses were administered subcutaneously, intraperitoneally and by stomach intubation. Continuous oral therapy was provided by addition of L-penicillamine to the drinking-water. L-Penicillamine proved to be equally as active as a tumour-retarding agent by all routes of administration, and the tumour-retarding effect was demonstrable at 25, 50 and 100 mg/kg daily dose-levels. Subcutaneous injection of 200 mg L-penicillamine/kg/day was toxic. More detailed effects of subcutaneous therapy of L-penicillamine on sarcoma-180 may be found in a previous publication[1]. Addition of L-penicillamine to the drinking-water proved to be the simplest method of administering the drug. The higher mortality in mice intubated with L-penicillamine as a single daily dose was attributed, in part, to instrumentation.

Table 5. EFFECTIVENESS OF L-PENICILLAMINE VIA DIFFERENT ROUTES IN RETARDING THE GROWTH OF SOLID SARCOMA-180 MICE MAINTAINED ON COMPLETE DIET

Route of administration	L-penicillamine (mg/kg/day*)	Average tumour wt. (gm ± $S.D.$)†	Tumour inhibition (%)	Mortality
None (control)	0	0·98 ± 0·32		0/10
Subcut. inject.	100	0·39 ± 0·21	60	2/10
Intraperit. inject.	100	0·60 ± 0·15	39	2/10
Stomach intub.	100	0·52 ± 0·12	47	4/10
Subcut. inject.	25	0·78 ± 0·22	21	1/10
	50	0·74 ± 0·16	24	0/10
	100	0·39 ± 0·21	60	2/10
	200	Toxic‡		10/10
Stomach intub.	25	0·74 ± 0·26	24	2/10
	50	0·57 ± 0·15	42	2/10
	100	0·52 ± 0·12	47	4/10
	200	Toxic‡		10/10
Oral (in drinking water)	25	0·72 ± 0·32	26	0/10
	50	0·63 ± 0·25	35	0/10
	100	0·53 ± 0·21	46	0/10
	200	0·55 ± 0·25	44	0/10

* L-Penicillamine treatment of 20 g CF_1 white male Swiss tumour-bearing mice started 24 h after implantation, daily for 7 days. Animals were killed on 8th day.

† $S.D.$ of individual tumour weights.

‡, Toxic, all mice dead.

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MODE OF MULTIPLICATION OF *Theileria* IN CULTURES OF BOVINE LYMPHOCYTIC CELLS

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SINCE the early work on East Coast fever by Theiler[1], Gonder[2], Koch[3] and Meyer[4], no conclusive evidence has been brought forward to establish the mode of infection and multiplication of *Theileria parva* in the bovine host. It was assumed by the early workers, particularly by Gonder[2], that the parasite, after infecting lymphocytes, multiplied by schizogony, the particles of the schizonts breaking up into merozoites which then infected other lymphocytes. Gonder believed the final stages of schizogony to be the production of gamonts which broke up into individual particles and then infected red cells. Cowdry and Danks[5] and Neitz[6] subscribed to this view. Reichenow[7], however, while agreeing with the theory of further infection of lymphocytes by merozoites produced by division of the original parasites, considered that this division was by binary fission and not by schizogony.

Prolonged cultivation of *T. parva* and *T. annulata* has provided an opportunity for detailed examination of the multiplication of the parasite *in vitro*.

Multiplication of *T. annulata* in tissue culture using spleen explants in plasma clot has been described by Tsur[8] and Brocklesby and Hawking[10]. Initial multiplication and survival of *T. parva* over a period of 15 days in tissue cultures were described by Tsur *et al.*[9] and confirmed by Brocklesby and Hawking[9]. Mass cultivation of *T. annulata* has been carried out by Tsur and Adler[11].

In this Laboratory mass cultivation of *T. annulata* has been successful over a period of four months and *T. parva* has been cultivated similarly for up to three months. During this cultivation, no signs of cytopathogenic activity by the parasite have been detected.

Evidence has been obtained to support the concept that distribution of *Theileria* into both daughter cells during mitotic division of the host cell is responsible for the increase in numbers of infected cells.

Terminology. The terminology used to describe the manifestations of the various parts of the life-cycle of the Theileridae is confusing and may be misleading. The forms of the parasite found in cells of the lymphatic system are often described as schizonts of two types: the macroschizonts and the microschizonts. We are not satisfied that division of the parasite at this stage is by schizogony and so prefer to adopt a less misleading terminology. For the macroschizont, sometimes called the 'agamont' or 'Koch's body', we use the term 'theilerial body'. By this is understood the close association of chromatin particles enveloped in cytoplasm which is characteristic of the early stages of the patent disease in the bovine host. For the individual particles of chromatin which make up this body we prefer the term 'theilerial particles'.

The so-called microschizont or gamont which appears in the later stages of the disease is made up of small, more compact particles of chromatin aggregated in larger numbers. Whereas these appear readily to break up into what may be termed 'merozoites', those stages described as 'theilerial bodies' here do not. In the series of tissue cultures described in this article, no gamont-like bodies appeared and, therefore, the terms 'theilerial body' and 'theilerial particle' refer only to the so-called agamont and its constituent particles.

Observations were made on *Theileria*-infected cells growing in monolayer. The cells had been isolated from bovine spleen, lymph nodes or blood from *Theileria*-infected animals and grown in association with a cell line of baby hamster kidney (B.H.K.), isolated by Macpherson and Stoker[12].

(*A*) *Chromosomal analysis of host cells.* Chromosomal analysis was used to determine whether the *Theileria*-infected cells were bovine or hamster. Cultures were incubated with colchicine (10^{-7} g/ml.) for 6 h, then treated for 5 min in hypotonic solution, fixed and stained with Giemsa. Bovine cells are readily distinguished from hamster cells on the basis of chromosome number and morphology. The hamster chromosomes are acro- or meta-centric, usually numbering 44 and rarely 88. The bovine chromosomes are smaller, rod-shaped and their number is 60. The centromere is situated at one end of the chromosome (telocentric) in all except one pair.

Slide preparations of such cultures showed large numbers of cells in mitosis. Theilerial particles could be recognized as pale red particles in between the chromosomes. They were found only in bovine cells and not in hamster cells; 98–100 per cent bovine cells were infected with *Theileria* in established cultures.

(*B*) *Observations on mitotic division of* Theileria-*infected cells.* Cell suspensions were fixed immediately after centrifugation to obtain all phases of mitotic division. Drop preparations were stained with Giemsa. Many mitotic figures were examined for the distribution of theilerial particles. Representative examples are shown in Fig. 1.

Prophase: Theilerial particles were found in the cytoplasm between the chromosomes.

Metaphase: The parasite was found almost invariably in close association with the chromosomes as a distinct theilerial body, the most typical location being in between them, thus disturbing the symmetry of the equatorial plate.

Anaphase: The particles were aligned in one or two, rarely more, threads along the fibrils of the spindle; in each of the threads the particles were associated in a strand of coherent cytoplasm.

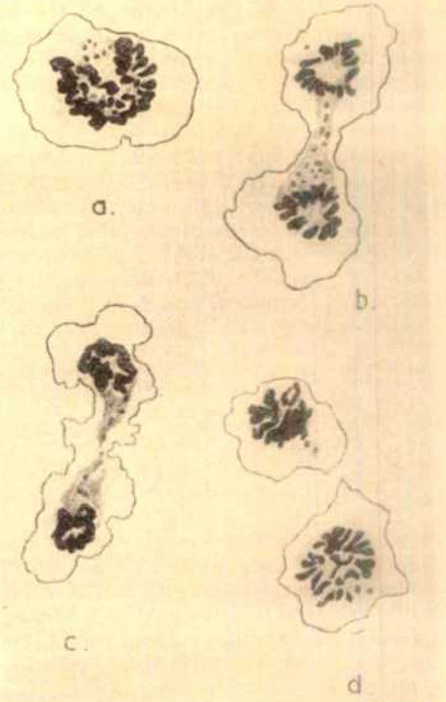

Fig. 1. Stages in the Division of *Theileria*-infected cells. (*a*) Metaphase with theilerial body; (*b*) telophase with one strand; (*c*) telophase, theilerial body separating into two; (*d*) reconstruction, both daughter cells with theilerial body

Telophase: The thread-like theilerial bodies were stretched by the dividing cell but still existed as one unit. In a few cases the thread was disrupted in one place, generally near the middle.

Reconstruction: Complete separation of the cells and the theilerial bodies had taken place.

Such a distribution of theilerial particles into both daughter cells was seen in 146 out of the 147 late mitotic phases observed and only on one occasion was a pair seen in which one of the daughter cells contained no parasite.

(*C*) *Number of theilerial particles per cell.* The number of theilerial particles per cell was counted in 100 infected cells in preparations made over a period of three months from one line of *T. annulata* isolated on Sept. 12, 1963*. The data when plotted gave a typical Poisson distribution. The averages and standard deviations are given in Table 1. In the last column the percentages of cells with more than one theilerial body are listed.

Table 1. NUMBER OF THEILERIAL PARTICLES PER CELL DURING PROLONGED CULTIVATION. DATE OF ISOLATION SEPT. 12, 1963

Days of cultivation	Theilerial particles/cell Average	*S.D.*	% cells with more than one theilerial body
28	14	12	6·5
35	16	11	8·0
54	17	17	9·0
77	15	10	14·5
95	14	8	6·5
117	14	10	8·5

During the three months of observations the average numbers of theilerial particles was found to be constant within the limits of 14 and 17. The number of cells containing more than one body varied from 6·5 to 14·5 per cent but no progressive increase occurred during this period.

(*D*) *Effect of prolonged exposure to colchicine.* Preliminary experiments had suggested that colchicine, at

* The Tova strain of *T. annulata* kindly provided by Dr. Tsur of Israel and which had been serially passaged by blood inoculation in calves.

concentrations of not more than 10^{-7} g/ml., while inhibiting the mitotic division of the bovine host cells did not prevent the multiplication of theilerial particles.

Colchicine in a final concentration of 3×10^{-8} g/ml. was added to cultures of *Theileria*-infected cells. Samples were taken at 0, 24 and 48 h. Control cultures without colchicine were examined at the same times. Theilerial particles in each cell were counted.

The results are given in Table 2. After 24 h the average number of particles per cell in a control culture was 17·6 and in a colchicine-treated culture 38·8. After 48 h the average rose in the latter to 111·2, with a very wide distribution; signs of a toxic effect on the host cells, such as giant cell formation and foamy changes of cytoplasm, could be observed.

Table 2. EFFECT OF COLCHICINE ON NUMBER OF THEILERIAL PARTICLES PER CELL

Colchicine conc.: 10^{-8} g/ml.

Time (h)	Colchicine		Control cultures	
	Average	*S.D.*	Average	*S.D.*
0	—	—	14	11
24	39	17	18	11
48	115	65	16	14

Discussion. *T. annulata* and *T. parva* were cultivated *in vitro* on a large scale in bovine cells isolated from lymphatic tissue in association with B.H.K. cells. Chromosomal analysis showed that the host cells of *Theileria* were bovine cells and that of these cells in established cultures 98–100 per cent were infected with *Theileria*. Massive propagation of *Theileria*-infected cells in tissue culture was maintained, however, for months. During the continued multiplication of infected cells, distribution of theilerial particles into both daughter cells regularly took place. The theilerial body was associated with the mitotically dividing cell in such a way that it generally formed one thread attached to the spindle fibres and this chain was pulled apart in late mitosis. In a number of cells more than one thread was aligned along the spindle fibres. The percentage of such mitotic cells was in the same range as the percentage of resting cells infected with more than one theilerial body.

Reichenow[7] described the foregoing mechanism of distribution of theilerial particles into both daughter cells during mitosis in infected cattle, but he assumed this to be a peculiarity of a few cells only. On this observation he based the assumption that intracellular theilerial bodies were agglomerations of single organisms which could dissociate freely during host cell division.

We have so far not found any signs of such dissociation in their cultures. In these investigations, the mechanism described here was observed in 98 per cent of all mitotic figures, and since there were scarcely any uninfected bovine cells, it has to be assumed that, in these tissue cultures at least, a clone of infected lymphoid cells was produced and that theilerial particles multiplied intracellularly. Disintegration of infected lymphoid cells and the release of single theilerial particles (merozoites) and infection of new cells were not observed.

The rate of multiplication of theilerial particles seemed to be in balance with the rate of multiplication of their host cells, as over several months the average number of particles per cell was constant between 14 and 17. Additional evidence was obtained from the experiments with colchicine. The single theilerial particles seemed to divide into two. Twin particles closely associated with one another within the theilerial body could be observed in the resting cell and during all stages of mitosis. All the evidence was against simultaneous division of the particles within one cell, nor did there exist evidence of the division of theilerial particles synchronously with the mitotic division of their host cell.

In the *Theileria*-infected animal the very rapid enlargement of lymph nodes has been shown to be due mainly to multiple mitotic division[13]. This suggests that *Theileria* might stimulate the host cells to divide at an increased rate, possibly by means of an association with the Golgi apparatus.

Observations made on many thousands of lymph-node biopsy smears from animals infected with *T. parva* have failed to produce convincing evidence that multiplication of the parasites in their lymphocytic stage takes place by the infection of fresh lymph-node cells by merozoites liberated from the disintegrated theilerial bodies. On the other hand, we have often observed cells in which theilerial bodies were associated with the mitotic division of the host cell in the same way as described here for tissue culture. Further work is, however, necessary to elucidate the whole picture of the life-cycle of *Theileria* in the bovine host.

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MAST CELLS AND HYALURONIC ACID IN THE BOVINE ENDOMETRIUM

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RECENT literature concerning mast cells is abundant but somewhat confusing[1–3]. The pluri-potential functions of mast cells in the dynamics of the connective tissue seem to be related to the chemical substances heparin, histamine, hyaluronic acid and serotonin. It appears that sufficient data are available to confirm the presence of heparin and histamine in the mast cell granules[3], but as regards serotonin and hyaluronic acid the question is not settled[1,2]. Whether these substances are merely stored in the mast cells or stored and synthesized is not yet clear[2,3].

In previous work[4], the acid mucopolysaccharides of the bovine uterine wall were located by histochemical techniques, and it was found that the colour intensity of the staining reaction of acid mucopolysaccharides (AMPs) varied during the sexual cycle. A biochemical investigation indicated 3 fractions of acid mucopolysaccharides present in the bovine uterine wall: fraction 1, hyaluronate; fraction 2, chondroitin sulphates and heparin monosulphate; and fraction 3, heparin. Inspection of the levels of fractions 1, 2 and 3 showed that the greatest variations occurred in the titres of fraction 1 (hyaluronate). The

Fig. 1

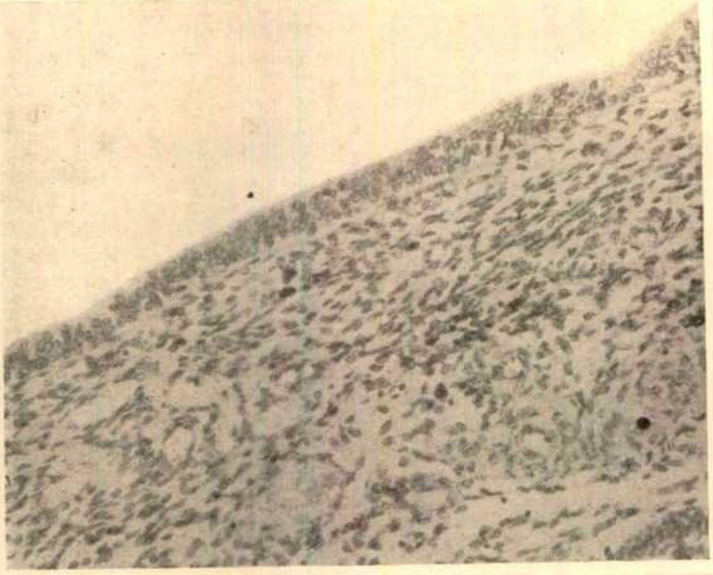
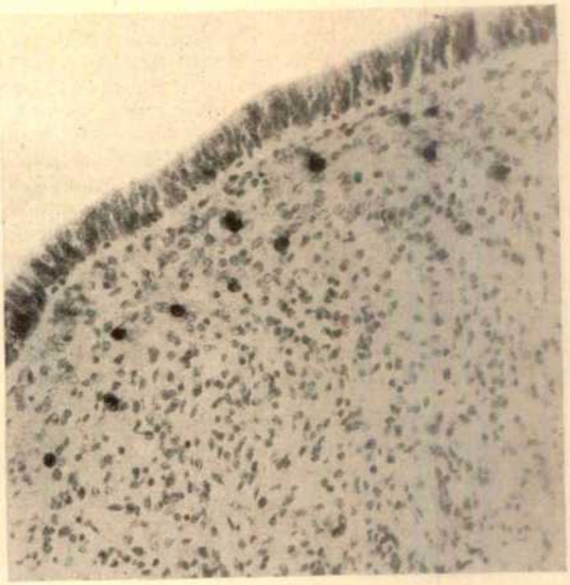


Fig. 2



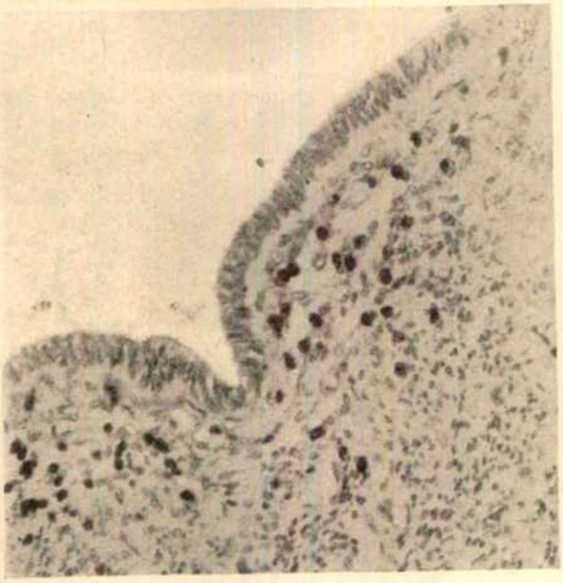


Fig. 3

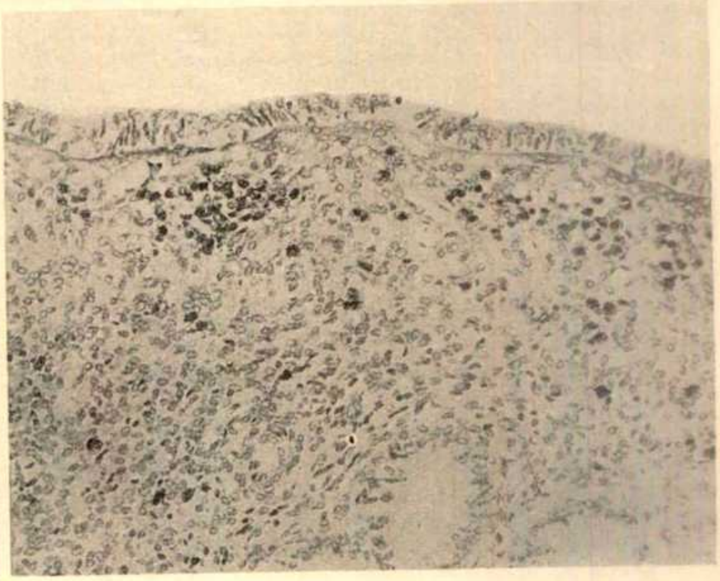


Fig. 4

Figs. 1–4. Cross-sections of bovine endometrium at different stages of the sexual cycle, stained with toluidine blue for mast cells (all × c. 225)

Fig. 1. Few mast cells; metœstrus

Fig. 2. Fair number of mast cells; diœstrus

Fig. 3. Many mast cells; proœstrus

Fig. 4. Many mast cells and evidence of degranulation; œstrus

average value of fraction 1 in pro-œstrus–œstrus was 881 μg/g, while in metœstrus–diœstrus it was 426 μg/g, the difference being approximately 50 per cent. The average values of fraction 2 (616 and 615 μg/g for pro-œstrus–œstrus and metœstrus–diœstrus, respectively) and fraction 3 (130 and 155 μg/g at different stages of the sexual cycle) did not show significant variations. However, histological observation of the presence of mast cells in the bovine uterine wall during the sexual cycle demonstrated cyclic changes, especially in the endometrium.

The purpose of the work recorded here was to investigate the relationship between the mast cells and the hyaluronic acid in the bovine endometrial stroma with particular reference to the sexual cycle.

Twenty fresh uteri with ovaries were collected from abattoirs with all necessary preventive measures. After the gross examination of the ovaries and uteri to establish approximately the stage of the sexual cycle[4], samples of ovaries and uteri were fixed in 10 per cent formalin with cetyl pyridinium chloride[11] (at room temperature) or in 96 per cent ethanol (at − 20° C). Uniform sampling of a piece of uterus-horn 1 cm from the bifurcation was utilized. The endometrium was separated from the myometrium and perimetrium and afterwards preserved in acetone at − 20° C for biochemical examination. For histochemical examination, paraffin sections were cut at 6μ and stained by Hale's method (*IC*) for acid mucopolysaccharides as modified by Rinehart and Abul'Haj[6], periodic acid–Schiff's method (PAS) according to McManus[7] and toluidine blue method (*TB*) at *p*H 4·1 and *p*H 1·5 as described by Hayashi *et al.*[8] Routine hæmatoxylin and eosin stains were used for definite verification of the stage of the sexual cycle, which was previously determined only approximately by gross examination. The mast cells were stained with toluidine blue pH 3 in 70 per cent ethanol. The total AMP and hyaluronic acid in a 2-g biopsy were determined biochemically by the method of Schiller *et al.*[5,9].

Histological examination of the mast cells confirmed our previous findings[4] that after œstrus the number of mast cells decreased rapidly and in the middle of metœstrus reached its lowest peak. During this stage only a few scattered mast cells were observed under the lining epithelia (Fig. 1). Later the number slowly increased, and mast cells with more distinct granules were noted (Fig. 2). In diœstrus the number was increased further and the mast cells were found throughout the endometrial stroma, particularly under the lining epithelia. In pro-œstrus they were concentrated more distinctly beneath the lining epithelia (Fig. 3) and around the uterine glands. The mast cells in this stage were stained more intensively with toluidine blue and some were also *IC*- and PAS-positive. At the end of pro-œstrus or at the beginning of œstrus degranulation of mast cells was observed. Degranulation was more noticeable during the end of œstrus and the mast cells were stained less distinctly (Fig. 4). The number of mast cells then decreased rapidly as described before (Tables 1 and 2).

The histochemical investigation located the AMPs in the endometrial stroma, particularly beneath the lining epithelia and much less around the uterine glands and in or around the walls of the uterine blood vessels (areas where the mast cells were present). The colour intensity varied at different stages of the sexual cycle (Table 1).

Table 1

Stage of the sexual cycle	Endometrial stroma PAS	*IC*	*TB* (pH 4·1)	Mast cells
Metœstrus	±	−	−	+
Diœstrus	±	±	±	+ +
Pro-œstrus	+	+	+	+ + +
Œstrus	+ +	+ +	+ +	+ + +

Histochemical evaluation: −, negative; ±, in traces; +, slightly positive; + +, strongly positive.

Incidence of mast cells: +, low incidence; + +, medium incidence; + + +, high incidence.

When sections were stained with *TB* at pH 1·5 the AMPs were always stained orthochromatically. This finding and PAS staining reaction in the same areas indicated also the presence of unsulphated acid mucopolysaccharides in the bovine endometrial stroma[6-8,10].

Biochemical examination of endometrium demonstrated that hyaluronic acid varied greatly in value during the sexual cycle (Table 2). These variations were parallel with an increase or decrease of mast cells, with the colour intensity of the histochemical determination and with the value of the total amount of AMPs (Fig. 5). The possibility, therefore, exists that AMPs and the mast cells in the bovine endometrium are regulated by the hormones of the sexual cycle.

Table 2

Animal	Stage of the sexual cycle	Mast cells	Hyaluronic acid (μg/g)	Total amount of acid mucopolysaccharides (μg/g)
1	Metœstrus	+	300	840
2	,,	±	360	880
3	,,	+	340	1,000
4	Diœstrus	+ +	352	1,300
5	,,	+ +	412	1,460
6	,,	+ +	550	1,500
7	Pro-œstrus	+ + +	529	1,700
8	,,	+ + +	503	1,630
9	,,	+ + +	675	1,670
10	Œstrus	+ + + +	812	1,600
11	,,	+ + + +	839	1,850
12	,,	+ + +	790	1,740

Incidence of mast cells in a microscopic field (objective 16): ±, less than 3; +, 3–10; + +, 10–30; + + +, 30–50; + + + +, 50 or more.

The different staining reactions of mast cells toward *IC*, PAS and *TB* indicate that mast cells are a heterogeneous population in the connective tissue. The orientation and number of mast cells have further significance from the point of view of available AMPs. Where there is a need for AMPs and the tissue does not contain sufficient amounts of ground substance of fibroblasts there is normally a higher number of mast cells present[2]. It would appear that AMPs are of local origin, and in this investigation a significant increase of fibroblasts was not noted by histological observation. While mast cells are not the only source nor even a significantly voluminous source of AMPs, they are important in the metabolism of AMPs[2]. Mast cells seem to be capable of both storage and/or synthesis, and of unloading the contents almost instantly, in contrast with fibroblasts and epithelial cells, which commonly produce a small continuous supply of AMPs during a longer period of time[2,3].

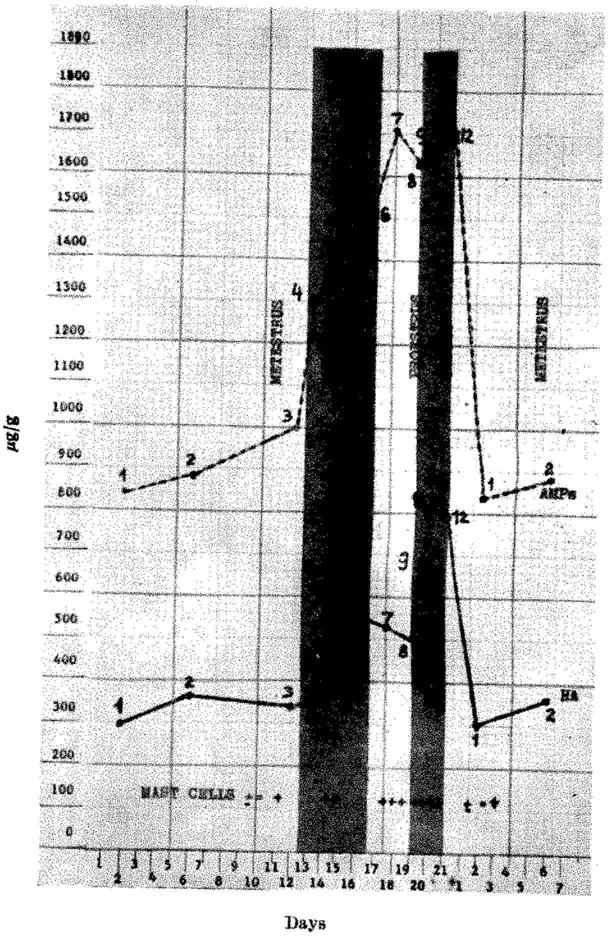


Fig. 5. Average values for hyaluronic acid (HA) and acid mucopolysaccharides (AMPs) (μg/g) and mast cells in bovine endometrium at different stages of the sexual cycle. ±, less than 3 mast cells in a microscopic field (objective 16); +, 3–10 mast cells; + +, 10–30 mast cells; + + +, 30–50 mast cells; + + + +, more than 50 mast cells

The results of this investigation support the concept that mast cells participate in the production of hyaluronic acid. These findings are in accord with Asboe-Hansen[1], who suggested that there exists a definite relationship between the mast cells and hyaluronic acid content of the tissue. In the present work, biochemical, histochemical and histological investigations of the bovine endometrial stroma demonstrate that the values of hyaluronic acid and the total amount of acid mucopolysaccharides are parallel with the numerical changes of the mast cells at different stages of the sexual cycle. Different staining reactions of mast cells at different stages of the sexual cycle suggest that the content of granules is not the same in all mast cells. The localization of mast cells, histochemical staining reactions for AMPs in these areas, and biochemical values indicate that mast cells might share in the production of hyaluronic acid in the bovine endometrial stroma.

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IMMUNOLOGICAL PARALYSIS INDUCED IN ADULT RABBITS BY SMALL AMOUNTS OF A PROTEIN ANTIGEN

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SPECIFIC immunological paralysis (tolerance, unresponsiveness) was induced in adult mice after the injection of small amounts of heterologous γ-globulin[1], an observation which has since been confirmed[2]. The earlier work suggested that the adjuvanticity of a protein antigen had an important bearing on whether or not small amounts of this antigen would induce a specific state of paralysis in adult mice. 'Adjuvanticity' has been described as that property of an antigen which leads to its recognition as being foreign or denatured[3]. Formerly it could have been argued that this phenomenon was not a general one but was restricted to bovine γ-globulin in adult *CBA* mice.

It is now known that several other mammalian γ-globulins will paralyse *CBA* and other strains of mice[4]; that *CBA* mice may have a considerable degree of paralysis induced in them by small amounts of bovine serum albumin[5]; and finally, that guinea pigs can be paralysed by bovine γ-globulin injected via the mesenteric vein[6]. It now seems more likely that the induction of paralysis by small amounts of a protein antigen lacking adjuvanticity (non-immunogenic ?) may be a general phenomenon. Paralysis to proteins possessing their own adjuvanticity, that is, proteins sufficiently foreign to initiate an immune response without the addition of an artificial adjuvant, can only be achieved by the injection of massive doses of antigen into adults (antigen overloading paralysis) or by the injection of smaller amounts of antigen into neonatal animals before the onset of immunological maturity (tolerance)[7].

The object of our experiments was to attempt to induce immunological paralysis in adult rabbits using small amounts of a protein antigen. The rabbit is generally recognized as a good producer of antisera to a wide range of proteins, including denatured rabbit γ-globulin. Therefore, we thought that a satisfactory demonstration of this type of immunological paralysis in rabbits would greatly strengthen the argument that the phenomenon was a general one and has an important bearing on the basic mechanism involved in the induction of immunological paralysis (tolerance). The classical experiments of Glenny and Hopkins[8], carried out more than forty years ago, led to the use of equine γ-globulin in some preliminary experiments. As the paralysis achieved in these preliminary experiments with this antigen was marginal indeed, we decided to use leporine γ-globulin (from *Lepus europaeus occidentalis* De Winton, the lowland hare) on the grounds that closer phylogenetic relationship might give a better chance of success. The results show that a convincing degree of paralysis was obtained with this antigen.

The rabbits used in the preliminary experiments were sandy lops (from a Southern California commercial dealer) and those in the main experiments Himalayans (from the closed colony maintained at the National Institute for Medical Research). The antigens were equine γ-globulin (EGG; Pentex Inc., Batch No. *E* 24) and leporine γ-globulin (LGG) prepared from hare serum by ion-exchange chromatography on DEAE–'Sephadex, *A*–50' coarse, using a stepwise molarity gradient. 110 ml. of hare serum was dialysed against 0·0175 M *tris*/phosphate buffer *p*H 8·0 and the DEAE was equilibrated against the same strength buffer. As the amount of protein eluted from this column (5 × 75 cm) with this buffer proved to be too small to use (it appeared to be 'pure' Y_{ss} globulin), 0·2 M buffer (0·05 M *tris*/phosphate + 0·15 M sodium chloride) was used for the next elution step. A considerable amount of protein was eluted from the column, which, after concentration by pressure dialysis, was shown by immunoelectrophoresis to contain considerable amounts of a Y_{ss}-like protein and a small amount of a Y_{1A}-like protein. Both EGG and LGG were centrifuged at 25,000–30,000*g* prior to intravenous injection. EGG was labelled with iodine-131 by the method of Wormal[1] (EGG*) and the LGG by the more satisfactory ICI method of McFarlane[9] (LGG*); both proteins received less than one atom of iodine per molecule. Rabbits injected with iodine-labelled protein were given water containing 100 mg sodium iodide/l. during the elimination of the antigen. Rabbits were bled from the marginal ear vein into tared tubes and the counts per g of whole blood expressed as a percentage of the amount of antigen in the circulation 24 h after intravenous injection (assuming that most, if not all, the label was still attached to the antigen).

Fig. 1 illustrates the results obtained with EGG as the antigen in the preliminary experiments. It can be seen that:

(*A*) There is a marked primary response to an intravenous injection of 50 mg of EGG*. This is probably of importance when compared with the lack of response to a similar injection of LGG*.

(*B*) There is an easily detectable difference between the primary response and a secondary response after prior immunization with complete Freund's adjuvant containing 100 mg EGG (Freund's/EGG).

(*C*) Rabbits injected intravenously with 100 mg centrifuged EGG 8 days before challenge with Freund's/EGG show a response intermediate between the primary and secondary responses and in most cases significantly less than the secondary response control.

(*D*) Two rabbits given the same treatment as (*C*), but also injected intravenously with 10 mg EGG 17 days earlier, showed an even greater elimination response than secondary response controls. An equivalence test, however, showed them to have somewhat less circulating antibody. It is clear from these experiments that EGG contains its own adjuvanticity in rabbits. The small number of rabbits apparently paralysed by Glenny and

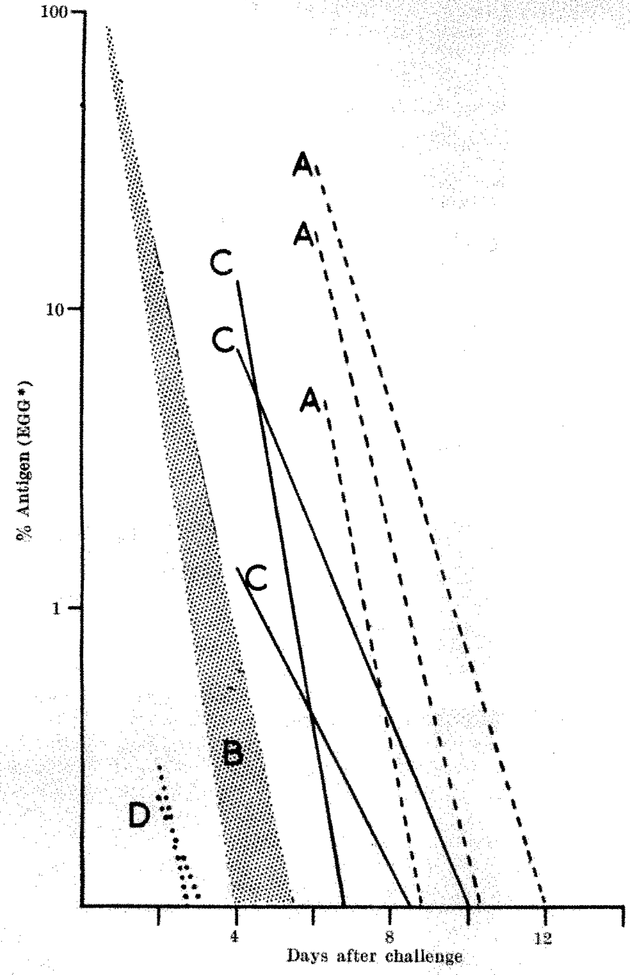

Fig. 1. Simplified graphs of the elimination of EGG*. Key to groups *A*, *B*, *C* and *D* in the text

Hopkins[8] may easily have been due to a combination of genetic differences in those rabbits with a low level of overloading paralysis.

In the experiments using LGG several rabbits were tested more than once with LGG*. Table 1 lists the animals used, the nature and dates of any treatment given and, where relevant, the approximate nature of the antigen-elimination curve with the approximate half-life of the antigen in the circulation during elimination. Fig. 2 illustrates the elimination of LGG* from the circulation of: (*a*) normal rabbits, Nos. 1 (injected twice, curves 1*a* and *b*), 2, 3; (*b*) rabbits previously injected with alum-precipitated LGG admixed with *Bordetella pertussis*, Nos. 4, 5; (*c*) rabbits previously given a series of injections of *Proteus vulgaris* coated with LGG (in the form of hare antibody prepared against this organism), Nos. 8, 9; (*d*) rabbits previously injected with Freund's/LGG, Nos. 6, 7, 10, 11. Only those animals previously injected with the antigen emulsified in complete Freund's adjuvant showed immune elimination of LGG when tested. The half-life of LGG* in normal animals varied between 2·5 and 3·9 days with an average of 3·3 days.

Fig. 3 illustrates the effect of injecting LGG as a solution prior to immunization with Freund's/LGG (Nos. 1, 2, 3, 12, 13, 14 and 15). The results show that a considerable degree of paralysis was obtained even when the intravenous injection of 16 mg LGG was as long as 3 months before challenge with Freund's/LGG.

Serial dilutions of serum samples obtained one week after the end of elimination experiments were tested against a 1 per cent solution of LGG in Ouchterlony gel-diffusion plates. These showed that rabbits 6, 7, 10 and 11, which showed immune elimination of LGG*, had precipitin titres of from 1/8 to 1/16; rabbits with weak immune eliminations or non-immune eliminations had no detectable antibody to LGG in their serum.

Although on a small scale with regard to numbers of animals our results are compatible with conclusions drawn from earlier work with mice[1], where it was concluded that there was a progressive loss of paralysis after a given dose of paralytogen; that with increased doses of paralytogen the state of paralysis would be longer lasting (Table 1, rabbit 1); and that further injections of paralytogen before a state of paralysis had been lost could prevent

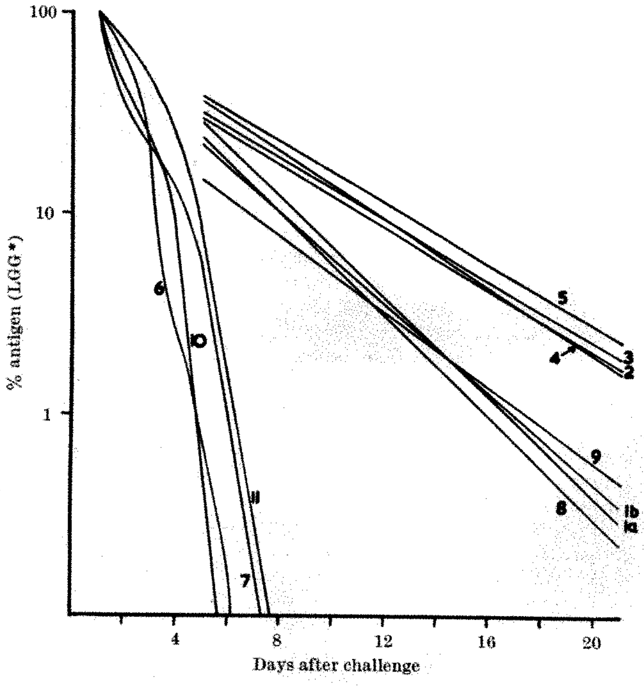

Fig. 2. Graph of the elimination of LGG*. Rabbits 1, 2, 3, 4, 5, 8 and 9 failing to show an immune response. Rabbits 6, 7, 10 and 11 showing a secondary response after prior challenge with Freund's/LGG

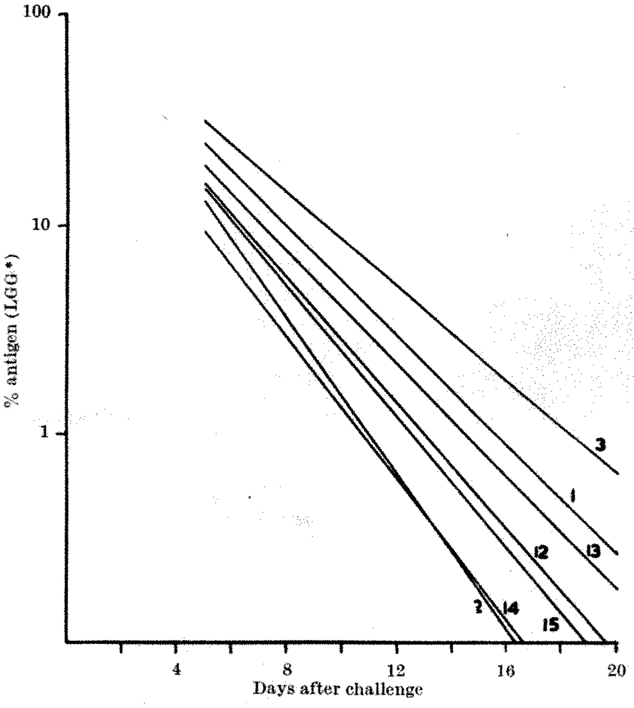

Fig. 3. Elimination of LGG* in rabbits previously paralysed (made tolerant) by injections of LGG as a solution, followed by Freund's/LGG

Table 1

Rabit, sex and No.	Date of treatment	Antigen dose	Route	Approx. $T_{1/2}$* in days	Type of response	See fig.
♂, 1 born 11.10.63	Nov. 5, '63	16 mg LGG*	i.v.	2·5	Non-immune	2
	Dec. 10, '63	16 mg LGG*	i.v.	2·7	Non-immune	2
	Mar. 4, '64	8 ml. Freund's/LGG (12 mg)	i.m. + s.c.	—	—	–
	Mar. 31, '64	16 mg LGG*	i.v.	2·3	Non-immune	3
	Apr. 27, '64	16 mg LGG*	i.v.	2·2	Non-immune	–
♂, 2; ♂, 3 born 11.10.63	Dec. 10, '63	16 mg LGG*	i.v.	3·8; 3·9	Non-immune	2
	Mar. 4, '64	8 ml. Freund's/LGG (12 mg)	i.m. + s.c.	—		–
	Mar. 31, '64	16 mg LGG*	i.v.	1·6; 2·7	Partially immune;	3
	Apr. 27, '64	16 mg LGG*	i.v.	1·5; 3·1	Non-immune	–
♂, 4; ♂, 5 born 11.10.63	Nov. 19, '63	10 mg alum ppt. LGG + 10^{10} *Bordetella pertussis*	i.m. + s.c.	—	—	–
	Dec. 10, '63	16 mg LGG*	i.v.	3·5; 3·6	Non-immune	2
	Jan. 7, '64	15 mg alum ppt. LGG + 2×10^{10} *Bordetella pertussis*	i.m.	—	—	–
	Feb. 18, '64	25 mg LGG*	i.v.	2·7; 3·2	Non-immune	–
♂, 6; ♂, 7 born 10.10.63	Dec. 20, '63	8 ml. Freund's/LGG (9 mg)	i.m. + s.c.	—	—	–
	Feb. 18, '64	25 mg LGG*	i.v.	0·4; 0·6	Immune	2
♂, 8; ♂, 9 born 30.11.63	Jan. 22, '64 to Feb. 16, '64	4 injections of 5×10^9 *Proteus vulgaris* coated with LGG (anti *Proteus* Ab)	i.v.		—	
	Feb. 18, '64	25 mg LGG*	i.v.	2·5; 3·5	Non-immune	2
♂, 10 born 25.12.63	Mar. 4, '64	8 ml. Freund's/LGG (12 mg)	i.m. + s.c.	—	—	–
	Mar. 31, '64	16 mg LGG*	i.v.	0·5	Immune	2
♂, 11 born 25.12.63	Mar. 4, '64	1 ml. Freund's/LGG (2 mg)	i.m.	—	—	–
	Mar. 11, '64	1 ml. Freund's/LGG (2 mg)	i.m.	—	—	–
	Mar. 31, '64	16 mg LGG*	i.v.	0·5	Immune	2
♂, 12; ♂, 13; ♀, 14; ♀, 15 born 3.1.64	Mar. 17, '64	25 mg centrifuged LGG	i.v.	—	—	–
	Apr. 1, '64	8 ml. Freund's/LGG (12 mg)	i.m. + s.c.	—	—	–
	Apr. 27, '64	16 mg LGG*	i.v.	1·8; 2·0; 1·7; 1·9	Partially immune	3

* Estimated from day 3 to 5 in immune animals and from 5 to 15 in all others.

a break from paralysis (for example, Table 1, rabbits 2 and 3).

Further comparisons between protein-induced paralysis in rabbits and mice now seem possible. The minimum dose of bovine γ-globulin (BGG) capable of inducing a reasonably complete degree of paralysis in adult *CBA* mice is 20–200 μg, although as little as 2 μg gives a measurable effect. As the mice used weighed about 20 g, the minimum effective dose was about 10 mg/kg body-weight. For LGG in rabbits a considerable effect was seen 3 months after a dose of 16 mg given to animals weighing 900–1,100 g. It has been estimated that 10^{11}–10^{13} molecules of BGG must remain in the mouse for a state of paralysis to be maintained[10]. A similar calculation based on the half-life of antigen in the circulation can be made for rabbits injected with LGG. This gives a figure of 10^8–10^9 molecules required to maintain a degree of immunological paralysis in a rabbit, that is, about 100 times less than the number of molecules apparently required to maintain paralysis in the mouse. This does not take into account the difference in size between the two animals and presumably a difference in the number of cells requiring antigen to remain in a state of paralysis. Such differences would increase the discrepancy between the two figures. We think that this discrepancy illustrates that the half-life of antigen in the circulation is not a good measure of the half-life of antigen in the animal as a whole. If taken at face value the figures would imply that intracellular antigen is destroyed at a slower rate than the same antigen is removed from the circulation; much more slowly in the case of LGG in rabbits (half-life in the circulation 3·3 days) than in the case of BGG in *CBA* mice (half-life in the circulation 6·5 days). Calculations of the number of molecules of antigen persisting in relation to a state of paralysis, based on half-lives of antigen in the circulation, probably have little significance.

There is a fundamental difference between the immunological response to EGG and many other proteins and the response to LGG in rabbits; also, between serum albumins and mammalian γ-globulins in mice and possibly guinea pigs. In each case the former proteins can elicit an immune response without any aid from adjuvants, whereas the latter rarely elicit an immune response on their own and, in fact, elicit a specific state of immunological paralysis. An immune response to these paralytogens can be stimulated in normal animals by the use of an adjuvant, for example, Freund's adjuvant containing the appropriate antigen. The first response of an animal to injected material may not be an immunological one, but a failure or non-failure to recognize this material as foreign as was originally proposed by Burnet and Fenner[11]. If it is recognized as foreign the material is fed into a pathway which can lead to a specific immune response, while failure to recognize it as foreign (either because it is not foreign or because it is sufficiently closely related structurally to self-components to be mistaken for self) will fail to open up the immune pathway. Several things could be implied from this postulated mechanism. First, that the specificity of the mechanism for the recognition of self is not as accurate as the specificity of the immunological paralysis mechanism. Secondly, that there is no reason to think at present that unrecognized foreign material is not metabolized in the same way as self-components. The catabolic pathway must therefore give access to the paralysis mechanism, and it would seem that the mechanism of induction and maintenance of paralysis are not basically different. Thirdly, experiments with adjuvants indicate that the immune pathway can be opened up non-specifically, which would imply that the mechanism of immunological paralysis must operate to prevent general auto-immunity. Exceptions to this would be lens protein, nervous tissue and perhaps thyroid, where, in some cases, a lack of adjuvanticity together with a lack of ready access by antibody-forming cells prevents auto-immunity to them and, almost by definition, the failure to induce or at least maintain any state of paralysis (tolerance) to these tissues.

Antibody production to many protein antigens can be suppressed in adult animals by the repeated injection of large amounts of antigen (antigen overloading paralysis), for example, 0·5 mg/g body-weight/day for several weeks[12]. This high level of antigen seems to be necessary to suppress antibody production in cells already induced or being induced to produce antibody. With LGG in rabbits and γ-globulins in mice the amount of antigen required to elicit paralysis and that required to stimulate immunity (aided by an adjuvant) seem to be of the same order of magnitude. This clearly indicates that paralysis is not a by-product of over-saturation by antigen (unless the antigen is recognized as foreign, and the induction of immunity ensues in some cells), but, as was previously suggested, is a result of the failure of one of the steps in the pathway to an immune response.

Recent investigations of rabbit γ-globulin allotypes[13] have shown that antigenic γ-globulins of maternal origin may be found for a time in the circulation of young rabbits. When the young are genotypically different from their mothers it seems reasonable to expect that immunological paralysis to the foreign allotypes will be induced and maintained for a time, although so far as we know this has not yet been tested.

The preliminary experiments were carried out by one of us (D. W. D.) in the Division of Biology, California Institute of Technology, and thanks are due to Prof. R. D. Owen. We also thank Dr. J. H. Humphrey for advice and Miss Jeanette Dilley and Mr. D. Wilson for their assistance.

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FŒTAL EXCLUSION BY TRANSUTERINE LIGATION OF THE UMBILICAL CORD

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IN the course of investigations in the albino rat on the mechanism of protection by advanced pregnancy against parathyroid hormone-induced cardiorenal necrosis and calcification[1], it appeared of interest to explore the effects of the placentas in the absence of the fœtuses. Removal of the fœtuses was carried out by Caesarean section which required individual surgical entry into all uterine beads. It was noted that even with most careful and gentle handling, interference with the integrity of the surviving part of the placenta was unavoidable, since removal of the fœtus and loss of amniotic fluid destroyed the physiological tension of the amniotic sac and created a void which could not be readily abolished by contraction of the extremely thinned-out myometrium. Although most placentas survived until term in fairly good condition when fœtal extirpation was carried out on the 19th day of pregnancy, the decrease in protection against parathyroid hormone poisoning was difficult to interpret. It could have been caused by the removal of a fœtal factor. But it might also have been caused by diminution of a maternal protective mechanism, due either to placental damage or hormonal alterations resulting from removal of the fœtus or the premature loss of uterine distension[1].

To avoid these pitfalls, a more physiological means of fœtal exclusion was sought. This led to the development of a simple procedure consisting principally in the ligation of the umbilical cords of rat fœtuses by passing a needle and thread around the umbilical cord through the intact uterine wall, leaving the conceptus and myometrium otherwise virtually undisturbed. This procedure proved not only highly successful for the intended purpose, but also appeared to offer interesting additional applications in maternal–fœtal interrelations and in studies on the placental barrier. This article deals with a description of the technique and its application for the purpose for which it was originally developed.

Virgin albino rats of the Sprague–Dawley strain, 3–4 months old, raised in our own laboratory on standard food, were used. The date of impregnation was established by limiting contact with male rats to a 24-h period. Females afterwards found not to have been impregnated by this contact served as non-pregnant controls.

As in earlier investigations[1], surgical intervention was carried out on the 19th day of gestation. Under ether anæsthesia, a midline abdominal incision 2 in. long was made and one uterine horn at a time was gently 'delivered' through the abdominal opening, starting at the vaginal insertion and proceeding towards the tip of the horn. The pregnant horn was placed on saline-soaked gauze, and covered with a second moist packing except for the bead to be operated on. The sequence of ligations was begun at the cephalic end of the horn.

In the majority of uterine beads, a loop of the umbilical cord was clearly visible through the thinned-out myometrium. In some a portion of the cord could be readily brought into view by gentle manipulation of the fœtus. As one proceeds caudad, the operation tends to become increasingly difficult because of the decreased manœuvrability of the horn near its vaginal fixation and because of the reduced transparency of the thicker uterine wall. Under these conditions a good binocular loupe and trans-illumination have proved of great help in localizing the umbilical cord.

Under careful visual control, a number 720–20 (Torrington) half circle gut needle, threaded with number 4–0 silk, was passed through the uterine wall around the umbilical cord and out again. The tip of the needle was guided so as to emerge near the point of entry into the myometrium. Care was taken to avoid puncture injury of the fœtus, the placenta, or large uterine vessels. The two ends of the ligature were then grasped and gently tied, thus occluding the umbilical cord and fastening it to the uterine wall. Puncture of the amniotic sac causes some oozing of fluid and jelly-like material which ceases, however, immediately after the knot has been completed. The free ends of the ligature were clipped close to the knot and the procedure repeated with the next fœtus until all cords had been tied in the same fashion. Knotting of the ligature is followed almost immediately by a distinct twitch of the fœtus which may serve as a sign of successful ligation. There are no further movements or convulsions of the fœtus following ligation of the cord.

With some experience a skilled operator can complete the ligature of an umbilical cord in less than 1 min. After completion of all ligations the uterine horns were gently eased back into the body cavity, the abdominal incision was closed, and the animals were permitted to continue to full term (22nd day).

Surgical extirpation of live fœtuses on the 19th day of gestation was effected through individual entry into all uterine beads as outlined previously[1]. Care was taken to minimize disturbing the placentas during this manœuvre. Each uterine incision was closed with a purse-string silk suture.

For the production of the acute hyperparathyroid state, the subcutaneous injection of 'Parathyroid' (supplied by Eli Lilly and Co., Indianapolis, Indiana, through the courtesy of Dr. Glenn W. Irwin) was started immediately following completion of the operation and continued in a dose of 150 units twice daily for three days, as done previously[1]. All rats were killed by decapitation in ether anæsthesia, 72 h after the start of parathyroid hormone injections.

Every animal was subjected to complete post-mortem examination. In pregnant rats, the placentas and fœtuses were removed individually, weighed on a microbalance and then carefully inspected. Histological sections of heart, stomach, kidneys and representative groups of placentas and fœtuses were examined in hæmatoxylin-eosin and von Kossa calcium stains. Samples of these same tissues were, in addition, homogenized for extraction and determination of calcium, magnesium, inorganic phosphate, sodium and potassium. These electrolytes were also determined in terminal samples of maternal serum.

It was found that fœtal exclusion by trans-uterine ligation of the umbilical cord on the 19th day of gestation was remarkably well tolerated by the pregnant rat since it induced no extended disability or signs of discomfort in the remaining days of pregnancy. Near term, the animals were indistinguishable from gravid controls in alertness, activity and food consumption. Distinct retardation of gain in maternal body-weight compared with pregnant controls was ascribed to death of the fœtus.

At autopsy the uteri of rats with tied umbilical cords exhibited the shape, colour and configuration expected of a normal organ near term, except that the individual uterine beads were smaller in size. Each bead contained a dead, slightly macerated fœtus which was smaller than a live fœtus at the time of ligation. The placentas were normal in size and configuration, though slightly lower than normal in weight. They seemed to have suffered little ill-effect from the ligation procedure. This was in marked contrast to our observations with surgical extirpation of the fœtus. There was in particular no evidence of infection, premature placental ablation, or uterine hæmorrhage.

As can be seen from Table 1, trans-uterine ligation of the umbilical cord had no noticeable influence on protection provided by advanced pregnancy against cardiorenal necrosis and calcification as a result of poisoning with parathyroid hormone (group 4). In fact, protection had remained as good as in control pregnant rats (group 2). This finding is in distinct contrast to surgical extirpation of fœtuses, which resulted in conspicuous loss of protection against cardiorenal injury (group 3). These findings militate against the participation of fœtal factors in the antagonism to parathyroid hormone poisoning. They also eliminate any possible role of the fœtus as a reservoir into which a deleterious calcium/phosphate overload of maternal tissues can be readily drained off. The decrease in parathyroid hormone antagonism following fœtal extirpation is thus believed more likely due to interference with maternal protective mechanisms by disturbance of the integrity of the placenta and the uterus. The histological evaluation of tissues and the electrolyte pattern of fœtuses and placentas of the various experimental groups will be reported elsewhere.

The advantages of the method of trans-uterine ligation of the umbilical cord are obvious. The procedure is simple and can thus be readily carried out by anyone who possesses the routine skills of the experimental surgeon. Fœtal exclusion is accomplished without fœtal extirpation and thus with a minimum of disturbance of the pregnant uterus. Preservation of the fœtus maintains the distension stimulus which is believed to be of importance in the continuation of hormonal homeostasis of pregnancy. Finally, the maternal part of the placenta remains apparently anatomically and functionally intact and there is thus no reason for premature ablation.

Table 1. COMPARISON OF UMBILICAL CORD LIGATION WITH FŒTAL EXTIRPATION ON PROTECTION BY LATE PREGNANCY AGAINST CARDIORENAL INJURY OF ACUTE HYPERPARATHYROIDISM

Group No.	Type of rat	No. of rats	Incidence of Myocardial necrosis	Incidence of Nephrocalcinosis
1	*Non-pregnant* Control	30	100%	100%
2	*Pregnant* Intact	20	0	60%
3	*Pregnant* All fœtuses extirpated on 19th day	10	30%	100%
4	*Pregnant* All umbilical cords ligated on 19th day	10	0	70%

All groups received parathyroid hormone, 150 units twice daily subcutaneously for 3 successive days. In pregnant rats, injections were started on 19th day of gestation.

The method lends itself to a variety of additional applications. A few examples may suffice to illustrate this aspect. It is possible, for example, in the acute experiment to 'thread' any desired number of umbilical cords in the described manner without tying the ligatures immediately. Such preparation can serve for transfer investigations across the placental membrane with timed ligations of the threaded umbilical cords at predetermined intervals following intravenous injection of the agents under examination into the maternal circulation. By this arrangement an equilibration curve of the agent between fœtal and maternal blood and tissues could be obtained by elective surgical extirpation of fœtuses immediately following ligation of their umbilical cords for blood and tissue analysis. It is also feasible by this procedure to examine the effects of timed short interruptions of the blood supply on the development of the fœtus when compared with undisturbed control fœtuses of the same mother. This can be accomplished by gentle transient compression of the threaded umbilical cord and subsequent removal of the thread, permitting pregnancy to continue to full term and delivery of live or dead fœtuses, depending on the duration of compression[2].

By ligating umbilical cords at different stages of advanced gestation, one can examine the influence of fœtal death on the physiology of the pregnant state. Moreover, by chemical and histological analyses of fœtuses the exact time of death of which can be predetermined and are carried by the pregnant rat to term, or for a shorter pre-selected span of time, one can obtain information on speed and sequence of resorptive processes and perhaps also on mechanisms of resorption.

Recently, we succeeded in decapitating 19th day fœtuses by a similar trans-uterine approach with good survival of the fœtal bodies until birth[2]. Details of the procedure and of experimental findings will be reported elsewhere.

Application of the method of trans-uterine ligation of the umbilical cord for our particular purpose made it possible to establish that factors of the live fœtus are not essential for antagonism against parathyroid hormone poisoning in advanced pregnancy.

This work was supported by grant *HE*–00890–13 from the National Heart Institute, U.S. National Institutes of Health, and contract *U*–1078 from the Health Research Council of the City of New York.

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CARBON DIOXIDE EFFLUX FROM THE HUMAN CORNEA *in vivo*

By Prof. IRVING FATT, Dr. RICHARD M. HILL and Dr. GORDON H. TAKAHASHI

College of Engineering and School of Optometry, University of California, Berkeley

THE average carbon dioxide tension in human body tissue is about 60 mm mercury[1]. This is also the gradient between the skin and the ambient atmosphere because air normally has a carbon dioxide tension of only 0·3 mm mercury. Under these conditions an efflux of carbon dioxide from the skin is predicted. The converse uptake of oxygen from the air by the skin, because the average oxygen tension in the body is about 80 mm mercury below that in air, has been observed by Hill and Fatt[2].

Carbon dioxide discharge by the skin cannot be an important part of the total-body discharge because the skin has a much lower area than the lung and is much less vascular. Discharge from the cornea, however, may be important in the normal function of the eye because the cornea is a highly specialized tissue. The human cornea is a thin tissue, 0·53 mm thick and 1·3 cm^2 in area, stretched over a chamber containing an aqueous solution of salts and protein and with a carbon dioxide tension of about 60 mm mercury[3]. Under conditions encountered in diving, flight, or more commonly, when wearing contact lenses, interference with normal carbon dioxide discharge from the cornea may cause physiological disturbances in the eye. The carbon dioxide efflux rate from the human cornea measured *in vivo* is reported here.

Redslob and Tremblay[4] measured the carbon dioxide discharge from the entire eye of the rabbit by passing carbon dioxide-free air over the eye and analysing the effluent gas mixture. They obtained a discharge of 24 µl./h for the whole rabbit eye, averaged from eight experiments, and 53 µl./h for the human eye from one experiment. Their procedure is not easily adaptable for measurements on the cornea alone, particularly on human subjects. Smelzer[5] measured the *p*H of a bicarbonate buffer withdrawn from under a contact lens on a human subject. This measurement can in theory give carbon dioxide efflux from the cornea, but in Smelzer's experiment too many uncertainties, particularly the volume under the lens, prevent a useful calculation from his data.

We have used two methods to measure carbon dioxide efflux from the cornea. In the first method the equipment shown in Fig. 1 (top) was used. The contact lens assembly has been described previously[6]. The electrode assembly was a Beckman model 40279 glass electrode and a silver wire (1 mm diam.) both cemented with epoxy resin into a 'Lucite' rod ($\frac{3}{8}$ in. diam.). The O-ring provided a liquid seal so that the electrode assembly could be pushed into place after the lens was on the subject's eye. The projecting tip of the silver wire, about 1 mm long, was coated with silver chloride by electrolysing for a few minutes at 3 V in a 1-molar sodium chloride solution. The inlet and outlet ducts on the lens assembly allowed new solution to be added while the lens was on the eye. Temperature in the lens reservoir was recorded continuously from the thermistor.

The *p*H of the reservoir solution was observed on a Beckman model 160 physiological gas analyser and recorded continuously, together with the temperature, on Sanborn dual channel model 297 recorder. The Beckman analyser has a *p*H range of only 6·8–8·0. To make readings outside this range a potentiometer, across a 1·34-V mercury cell, was placed in series with the silver chloride electrode lead. This potentiometer circuit provided a variable bucking voltage to bring the observed readings on scale. By this procedure only changes in *p*H were recorded. The absolute *p*H in the lens reservoir at the beginning of an experiment was measured by another, wide-range *p*H meter.

Solutions to be run into the lens reservoir were kept at 35° C in a thermally insulated bottle and run into the lens at the beginning of each measurement. The long, flexible line from bottle to lens, necessary to give the subject freedom of motion when inserting the lens, did allow the solution to cool somewhat, down to 30° C in some cases. This was not a serious problem because temperature in the lens reservoir was always known. All *p*H measurements were corrected to a base temperature, taken to be the steady-state temperature in the reservoir (Fig. 2) through use of the nomograph supplied with the glass electrode by the manufacturer. The reservoir volume of the lens in place in the subject's eye was 0·50 ml. for the assembly in Fig. 1 (top).

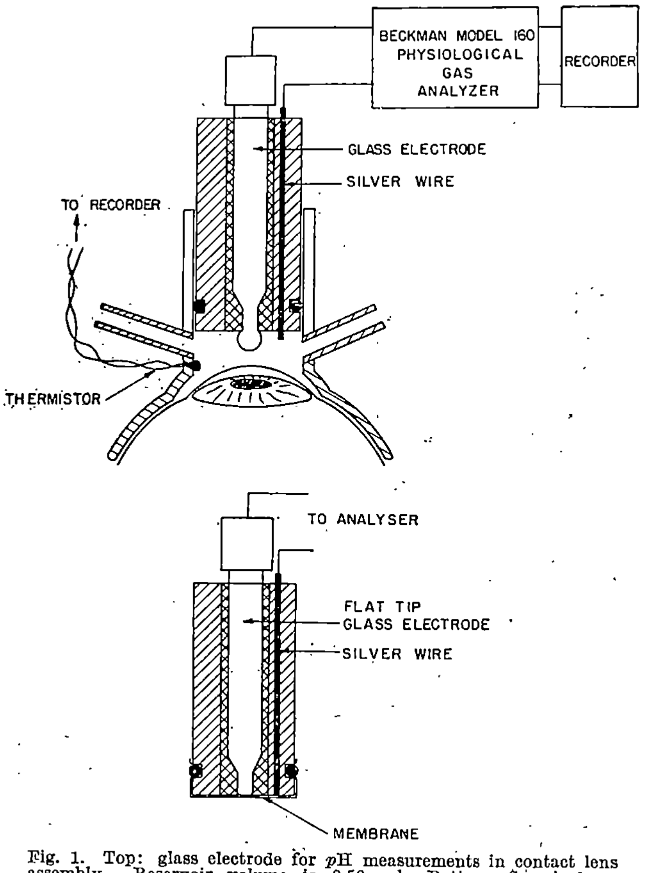

Fig. 1. Top: glass electrode for *p*H measurements in contact lens assembly. Reservoir volume is 0·50 ml. Bottom, Severinghaus carbon dioxide electrode; reservoir volume with this electrode in contact lens assembly is 0·33 ml.

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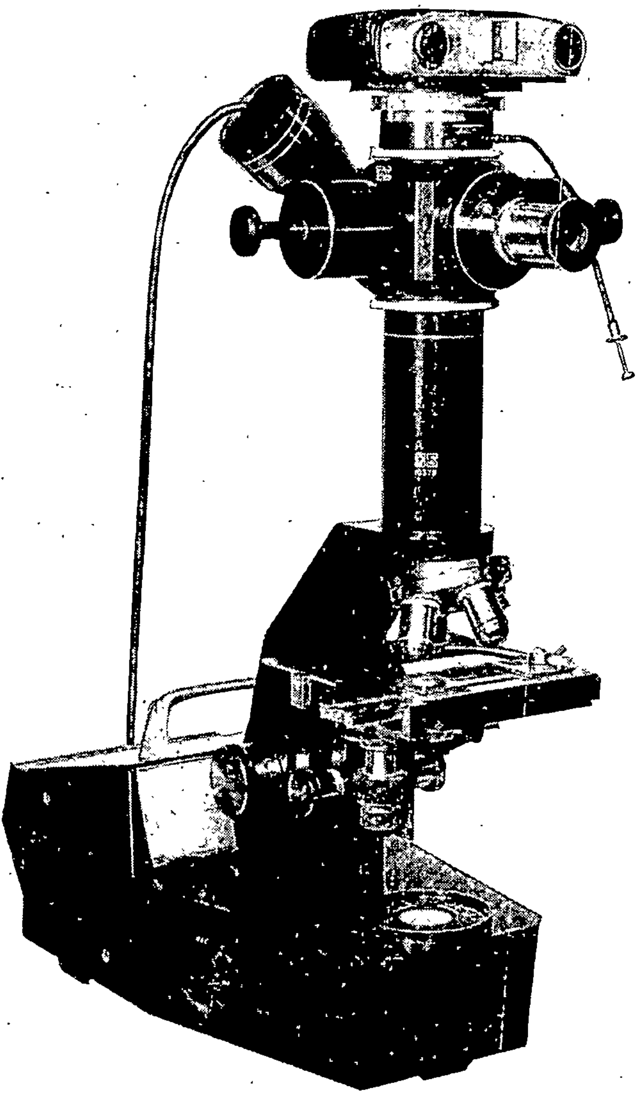

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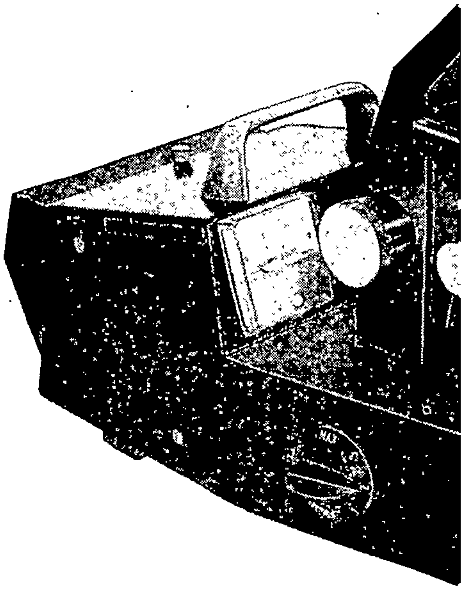

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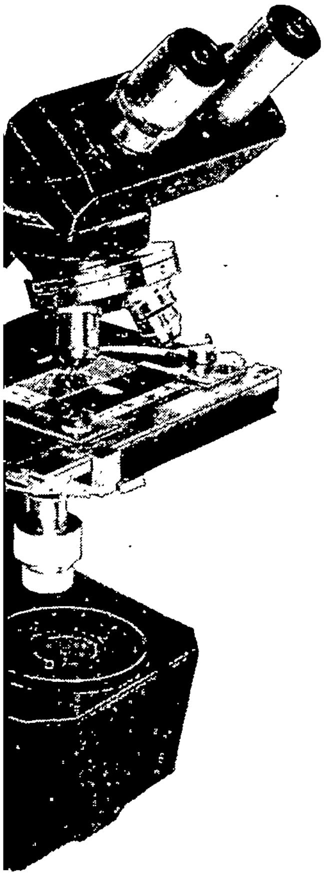

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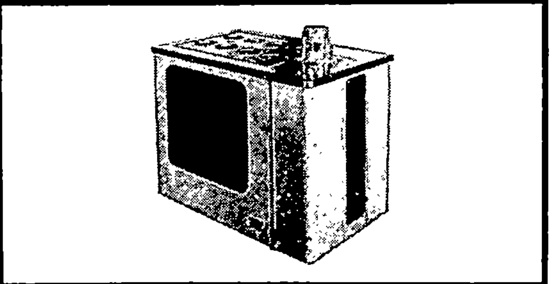

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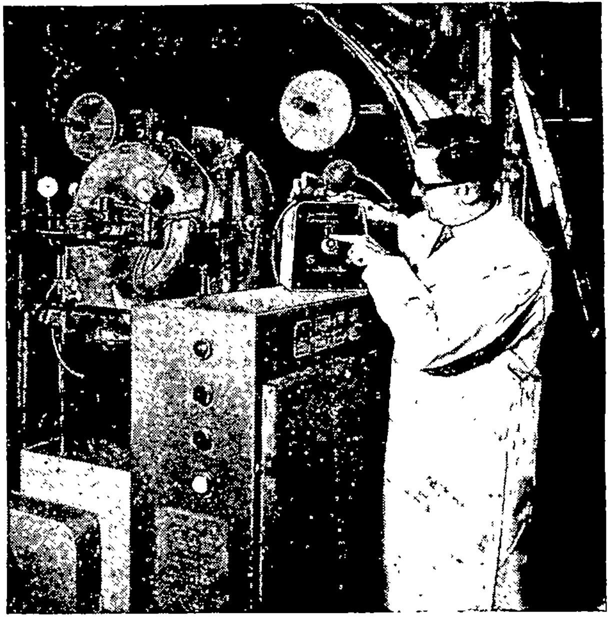

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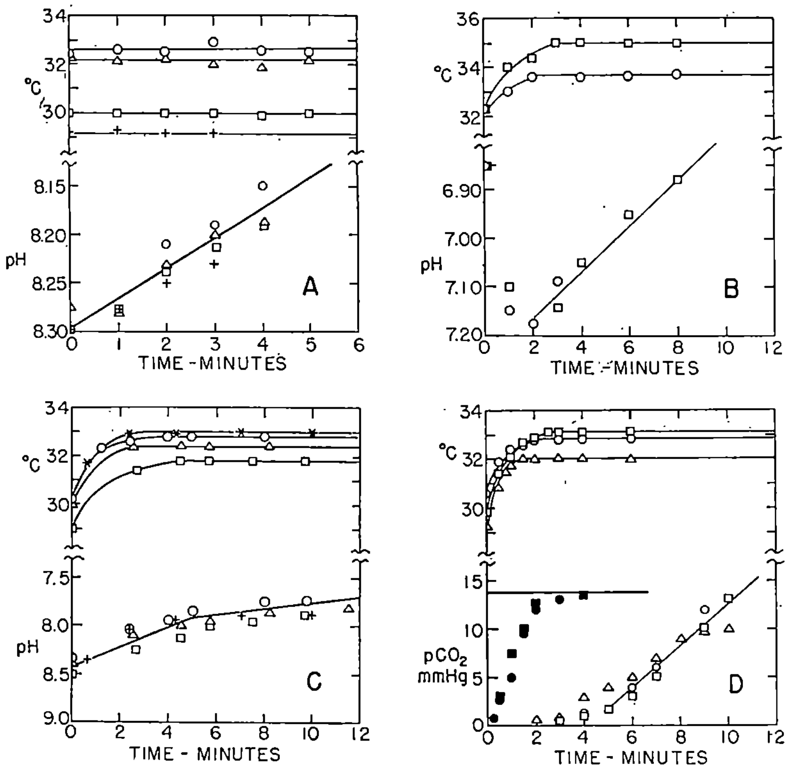

Fig. 2. pH and carbon dioxide tension as a function of time for various solutions in lens reservoir: A, pH in 0·0336 molar bicarbonate; B, pH in isotonic saline (no bicarbonate); C, pH in 0·01 molar bicarbonate. All bicarbonate solutions have sodium chloride added to make solution physiologically isotonic in total ions. D, shows increase in carbon dioxide tension in isotonic sodium chloride using carbon dioxide electrode of Fig. 1 (bottom). Upper curves are temperature record during measurements. Solid data points in D show electrode response to step increase in carbon dioxide tension

The response time of the pH electrode assembly in Fig. 1 (top) was less than one second, as observed by quickly moving the electrode from a pH 8·5 buffer to one at pH 7·0.

The reaction occurring when carbon dioxide from the cornea enters the solution in the lens reservoir is $CO_2 + H_2O = H^+ + HCO_3^-$. The mass action equilibrium equation for this reaction can be differentiated with respect to time to give:

$$\frac{d([CO_2] + [HCO_3^-])}{dt} = (K[H^+] + 1)\,\frac{d([HCO_3^-]_1 + [HCO_3^-]_2)}{dt} - 2{\cdot}3K[H^+]\,[HCO_3^-]\,\frac{dpH}{dt} \quad (1)$$

where the left-hand side is the total change in carbonate content in the lens reservoir and K is the association constant for bicarbonate and hydrogen ion ($0{\cdot}21 \times 10^7$ l./mole at the usual temperature in the lens). Subscript 1 refers to bicarbonate introduced by reaction of carbon dioxide and water, subscript 2 refers to bicarbonate ion coming directly from the cornea by diffusion. No subscript on a bracket indicates total bicarbonate.

When the solution put into the lens reservoir has the same ionic composition as the aqueous humour, 0·0336 molar in bicarbonate ion and 0·10 molar in chloride ion, then there is no flux of bicarbonate ion, $\frac{d[HCO_3^-]_2}{dt}$ is zero, and equation (1) reduces to:

$$\frac{d([CO_2] + [HCO_3^-])}{dt} = -\,2{\cdot}3[H^+]\,\frac{dpH}{dt}\,\{K[H^+] + K[HCO_3^-] + 1\} \quad (2)$$

Numerical values of the terms on the right-hand side of equation (2) are available from Fig. 2A. These data are for four runs on a human subject using an air-equilibrated solution of ion composition already mentioned in the lens reservoir. These data give a carbon dioxide efflux from the cornea of 21 μl./h. The diffusion coefficient for carbon dioxide in the cornea can now be calculated assuming the interior of the eye at 60 mm mercury carbon dioxide tension and the exterior at 0·3 mm mercury. The dimensions of the cornea are taken as given above. The diffusion coefficient is then $0{\cdot}50 \times 10^{-5}$ cm^2/sec.

In a second set of measurements using the pH electrode of Fig. 1 (top) the solution in the lens reservoir was 0·01 molar in bicarbonate ion, 0·10 molar in chloride ion, and air-equilibrated. Under these conditions there is a flux of both carbon dioxide and bicarbonate ion. The bicarbonate ion change in the lens reservoir is then given by equation (3):

$$\frac{d[HCO_3^-]}{dt} = \frac{1}{K[H^+]}\,\frac{d[CO_2]}{dt} + 2{\cdot}3\,[HCO_3^-]\,\frac{dpH}{dt} \quad (3)$$

At zero time we can take the bicarbonate ion concentration as 0·01 molar and the carbon dioxide flux as that determined from the previous experiment. The slope of the data in Fig. 2C at zero time gives $\frac{dpH}{dt}$. The bicarbonate ion flux across the cornea is then calculated from equation (3) and found to be $0{\cdot}75 \times 10^{-2}$ mM/h. From the cornea dimensions given above and the imposed bicarbonate ion concentration gradient, the diffusion coefficient for bicarbonate ion in the cornea is calculated from this flux to be $0{\cdot}38 \times 10^{-5}$ cm^2/sec.

From the diffusion coefficients for carbon dioxide and bicarbonate ion in the cornea we can calculate the accumulation of bicarbonate in the lens reservoir when it is filled with 0·01 molar bicarbonate solution at zero time. At the end of 8 min the bicarbonate concentration rises to 0·0118 molar. When this bicarbonate concentration and the observed pH at 8 min is used in equation (3) together with a bicarbonate flux calculated for this concentration, the predicted slope in Fig. 2C is 0·028 pH/min. A line of this slope is drawn through the experimental data at 8 min and is a good fit.

In a second procedure to measure carbon dioxide efflux from the cornea an isotonic saline solution was placed in the lens reservoir and carbon dioxide tension increase in this solution was measured directly. pH measurements were also made separately for isotonic saline solution (Fig. 2B).

The carbon dioxide tension was measured with a Severinghaus type electrode[7] (Fig. 1). The glass electrode, provided on special order by Beckman, Inc., Fullerton, California, had the same dimensions as their model 40279, but had a flat tip. This glass electrode and a silver wire were cemented into a 'Lucite' rod as was done with the round tip electrode except that a flat surface was obtained. A drop of solution containing 0·1 molar potassium chloride with 0·01 molar sodium bicarbonate solution was placed on this surface and then covered by a 12μ thick sheet of polyethylene held in place by the O-ring. Air bubbles were carefully squeezed out before installing to O-ring.

The carbon dioxide electrode was connected to the pCO_2 input of the Beckman analyser. The analyser was adjusted to read pCO_2 directly by placing the electrode first into air-saturated water ($pCO_2 = 0{\cdot}3$ mm mercury) and then into water equilibrated with a carbon dioxide–nitrogen mixture ($pCO_2 = 13{\cdot}7$ mm mercury). The output of the analyser is then directly, on a logarithmic scale, the carbon dioxide tension of any solution into which the electrode is placed.

The response of the carbon dioxide electrode when moved quickly from a solution at 0·3 mm mercury carbon dioxide tension to one at 13·7 mm mercury is shown for two tests by the solid data points in Fig. 2*D*. This relatively slow response requires that the observed build-up of carbon dioxide tension, when the electrode is in the subject's eye, be corrected to give the true build-up rate. This correction is made by the numerical evaluation of the convolution integral as is conventional in instrument control theory.

The change in carbon dioxide tension in the lens is given by;

$$\frac{d[CO_2]}{dt} = K[H^+]\,\frac{d([HCO_3^-]_1 + [HCO_3^-]_2)}{dt} - 2{\cdot}3[CO_2]\,\frac{dpH}{dt} \qquad (4)$$

The first term on the right-hand side of equation (4) can be calculated from data previously obtained and the second term from Figs. 2*B* and *D*. Equation (4) predicts a carbon dioxide tension change of 2·55 mm mercury/min at 8 min. The slope of the uncorrected data in Fig. 2*D* is 2·0 mm mercury/min at 8 min, and corrected for finite response time of the carbon dioxide electrode is 3·0 mm mercury/min. From this result we conclude that the data collected by *p*H measurements in bicarbonate solutions, as used in equation (4), are valid.

Figs. 2*B* and *D* require further comment. The very slow rise in carbon dioxide tension during the first 4 min in Fig. 2*D* is attributed to the reaction of carbon dioxide with water to give mostly bicarbonate ion during this time-interval. After 4 min the *p*H falls, as shown in Fig. 2*B*, so that carbon dioxide entering from the cornea tends to remain as dissolved gas.

In Fig. 2*B* the increase in *p*H during the first minute is attributed to the combined effect of efflux of bicarbonate ion from the cornea and the formation of bicarbonate ion from the carbon dioxide.

All the calculations made here are based on the assumption of steady-state diffusion. The validity of this assumption can be easily demonstrated by noting that, for the cornea dimensions and diffusion coefficients, Fatt[8] has shown steady state is reached in 3 min. For each run shown in Fig. 2 the cornea was bathed in the test solution for at least this length of time before measurements were made.

The assumption of constant composition in the anterior chamber is not easily verified. The highly vascular nature of the ciliary body probably maintains constant composition in spite of the efflux across the cornea.

This work was supported in part by U.S. Public Health Service grant *HE* 06796 to one of us (I. F.) from the National Institutes of Health, U.S. Public Health Service.

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SURVIVAL OF EHRLICH'S ASCITES CARCINOMA CELLS AT HIGH ULTRA-CENTRIFUGAL FORCES

By DR. NORMAN MOLOMUT, DR. MORTON PADNOS, VIOLET SATORY and LEO GROSS

Waldemar Medical Research Foundation Inc., Woodbury, New York

WE have found that ultracentrifugation will not sediment all cells in a suspension but that a small number of tumour or bacterial cells will remain in the supernatant even after prolonged centrifugation. This was discovered while attempting to confirm the work of Tolnai *et al.*[1], who reported Ehrlich ascites tumour induction from cell-free supernatants of the ascitic fluid obtained by centrifugation up to 105,000*g* for 1 h.

We repeated Tolnai's experiment with Ehrlich ascites carcinoma, adding ultracentrifugation of the ascitic fluid for 105,000*g* for intervals up to 5 h. We similarly obtained Ehrlich ascites tumour induction from the supernatant fractions.

Injection of such supernatant fractions in mice into several different anatomical sites, including intracerebral, resulted in a rapid induction of tumour morphologically typical of Ehrlich carcinoma cells. The identity of these induced tumours was confirmed by quantitative immunological means described previously[2].

Additional data on the physical properties of the reported[1] inducing agent appeared to be more consistent with the presence of whole cells rather than subcellular particles. We therefore repeated the differential centrifugation, instituting rigid tests to ascertain whether the resulting supernatants of 105,000*g* for 1 or more hours were indeed free of cells.

Ehrlich ascites carcinoma, originally obtained from Dr. Sugiura in November 1960, is carried routinely in C_3H mice in our laboratory and was in the 127th passage on April 20, 1964. This carcinoma grows in all mouse strains. Approximately 100 ml. of ascitic fluid was collected from the routine passage of Ehrlich ascites carcinoma in C_3H mice. The pooled ascitic fluid was subjected to differential centrifugation at 5° C. The supernatants were carefully pipetted away and the sedimented pellet from each step was discarded. All supernatants were removed from the centrifuge with a rigidly supported pipette, with care taken to avoid possible re-suspension of the pellet. The steps were 8,000*g* for 10 min, then 12,000*g* for 10 min both in a Lourdes model *L* refrigerated centrifuge, followed by 105,000*g* for 1 h, then at 105,000*g* for an additional 4 h both in a Spinco model *L* ultracentrifuge.

The supernatants of the 105,000*g* 1-h and 5-h centrifugations were divided into two portions. One was filtered through a 02 Selas filter, the pore size of which is 1·7μ;

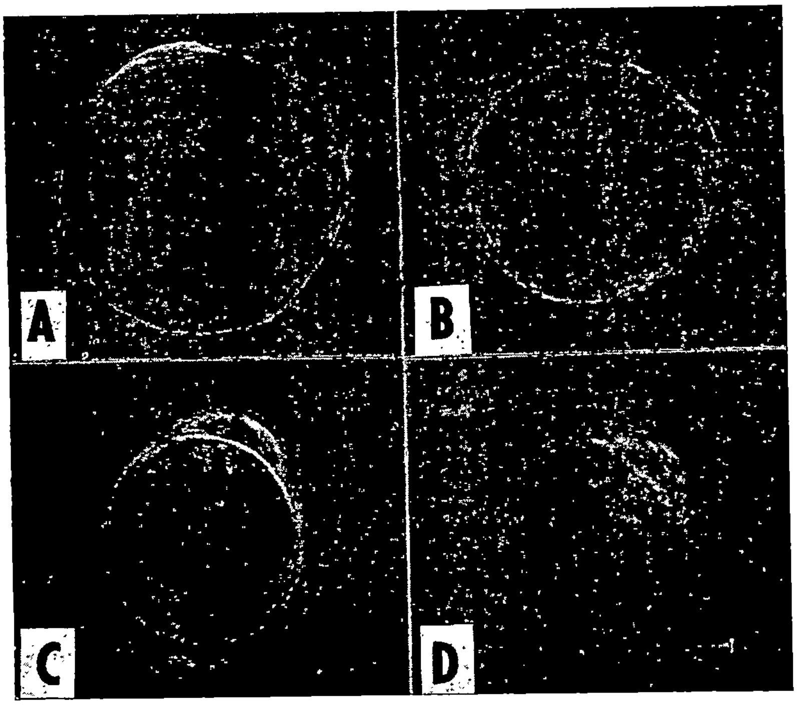

Fig. 1. *A* and *B*, individual Ehrlich ascites carcinoma cells observed in a supernatant centrifuged at 105,000*g* for 1 h. Note that the nuclei are causing distension of the cell membrane. *C* and *D*, cells obtained from a supernatant centrifuged at 105,000*g* for 2 h reveal the progressive evagination of the cell membrane

a second portion was left unfiltered. Both filtered and non-filtered portions were then injected intraperitoneally in 0·25 ml. volumes into different inbred strains of mice.

Both unfiltered 105,000*g* supernatants of the 1-h and 5-h centrifugation induced Ehrlich ascites tumour in 15–25 days in mice of several inbred strains. In repeated trials, supernatants were found to vary in tumour-inducing activity in mice of the same strain, sex, age and weight (Table 1). Filtered supernatants were completely inactive in all mice tested and observed for a period of 90 days.

In addition, we found that active supernatants were inactivated by freezing at – 20° C for short periods followed by thawing prior to testing. These data suggested that the tumour-inducing agent obtained from the unfiltered supernatant might be cellular since cells are killed by freezing and thawing.

A careful search was made for residual tumour cells in the supernatants following 105,000*g* centrifugation. Samples were taken from three different levels of the supernatant in the centrifuge tube, and 0·5 ml. of the unfiltered supernatants distributed in 0·02 ml. drops on thoroughly cleaned microscope slides were examined with phase objectives at times 100 and times 200 magnification without a cover slip. Small numbers of vacuolated cells with intact or deformed nuclei and some with the nuclei in an evaginated protrusion of the cell membrane were observed near the air–liquid interface of the drops. Approximately the same number of cells were seen in the 1-h and 5-h samples, the 5-h exposure causing greater evagination of the portion of the cell membrane in contact with the nucleus (Figs. 1 and 2).

An additional test involving five 1-h serial centrifugations at 105,000*g* was performed. After each 1-h centrifugation only the middle portion of the supernatant was removed and transferred. At the end of the 5th hour we still observed these deformed cells by direct phase microscopy. The concentration of suspended cells remained relatively unchanged at 250–300 cells per ml. (Table 1).

The number of cells required to kill 50 per cent of their hosts (LD_{50}) is approximately 40 Ehrlich ascites carcinoma cells in Swiss mice as indicated in Table 2. Since a comparable number of vacuolated cells produce similar results, they apparently survive after ultracentrifugation. They appear to be as viable and lethal to their hosts on transplantation as vacuole-free cells that were never subjected to centrifugation.

A few vacuolated tumour cells varying in size are normally present in peritoneal aspirates obtained from mice with progressing Ehrlich ascites tumour and can be readily observed in stained smears (Fig. 3). Cell vacuolation is not a special characteristic of the Ehrlich ascites carcinoma, but has been observed consistently in our laboratory for other transplantable ascites tumours, for example, sarcoma I, and *Ca* 755 mammary adenocarcinoma.

We were able to obtain tumour induction from ultracentrifugates of the *Ca* 755 ascites adenocarcinoma and have observed similarly the presence of deformed vacuolated tumour cells in the unfiltered supernatants.

Table 1. SURVIVAL OF DIFFERENT MOUSE STRAINS FOLLOWING INTRAPERITONEAL ADMINISTRATION OF 0·25 ML. ALIQUOTS OF EHRLICH ASCITES UNFILTERED SUPERNATANTS CENTRIFUGED AT 105,000*g*

Trial No.	Time of centrifugation (h)	Mouse strain (No. dead/total)				Conc. (cells/ml.)
		A/He	Swiss	C_3H	$C_{57}BL_6$	
1	1	10/12	7/12	3/12	1/12	
2	1	5/12	5/12	0/12	0/12	
3	1	11/12	10/12	10/12	3/12	
4	1	2/12	4/12	1/12	0/12	200
5	1	7/12	7/12	3/12	0/12	280
5	5		7/12			250

Table 2. SURVIVAL OF SWISS MICE FOLLOWING INTRAPERITONEAL ADMINISTRATION OF EHRLICH ASCITES TUMOUR CELLS

Total *E.A.* cells administered intraperitoneally in 0·25 ml.	Dead/total	Average No. of days survival
5×10^5	12/12	10
5×10^4	12/12	12
5×10^3	12/12	17
5×10^2	12/12	20
1×10^2	8/12	22
50	7/12	25
5	0/12	—

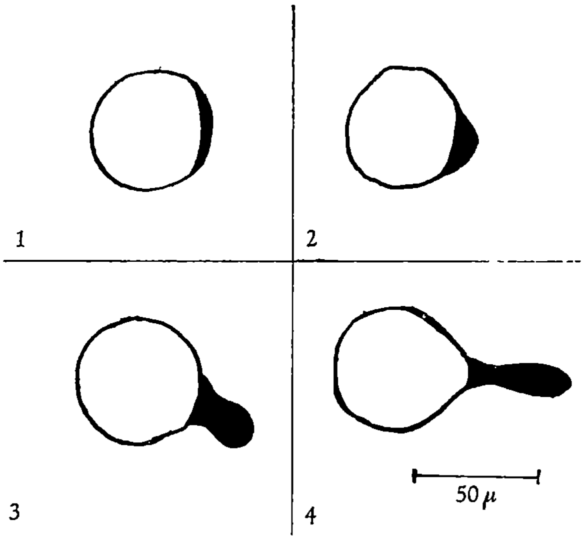

Fig. 2. Line drawing of progressive protrusion of the cell membrane by the nucleus in vacuolated cells following centrifugation at 105,000*g* for 1 h (1), 2 h (2), 4 h (3) and 5 h (4)

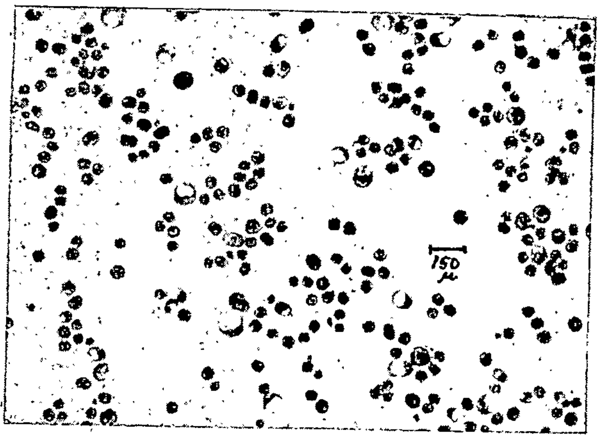

Fig. 3. Hæmatoxylin and eosin stained smear of Ehrlich ascites carcinoma aspirated from C_3H mice at nine days after transplantation showing approximately 10 per cent vacuolated cells

It is apparent that the cells found floating in the ascitic fluid after prolonged ultracentrifugation are of a specific gravity comparable to that of the ascitic fluid. The presence of vacuoles in these cells results in an overall specific gravity smaller than that of the more populous cells which are heavier and sediment.

The possibility that Selas filters may absorb a filterable agent, if present, was controlled by filtration using 290 mμ 'Gradocol' membranes, which absorb minimal protein. The injected filtrate did not induce tumours in 90 days in the most susceptible mouse strain (Swiss mice), confirming results obtained by Tolnai *et al.* with filtered ascitic fluid[3].

In summary, rigidly controlled conditions indicate that centrifugation at 105,000*g* for 5 h in one run or serially for five 1-h runs does not sediment all suspended cells of Ehrlich ascites carcinoma, and other tumours in the ascites form.

It is therefore vital that all claims for subcellular tumour-inducing agents obtained solely by high-speed centrifugation should be carefully reviewed for the definite absence of cells.

Our data indicate that vacuolated viable cells, though deformed by the high centrifugal forces, are found in the supernatant and are capable of survival, transplantation and progressive growth in susceptible mice. Tolnai *et al.*[1,3] have observed these suspended cells, and report that they are confined only to the lipid layer, which they skim off the supernatant. Our work shows that vacuolated cells can be found throughout the supernatant. Another claim of tumour induction from ultracentrifugal supernatants[4] is probably due to suspended vacuolated cells. In virulent tumours for which a low LD_{50} exists, the small number of cells required to induce tumour escape detection in unfiltered ultracentrifugates.

The observed vacuolated cells remain in suspension after centrifugation because of their apparently lowered specific gravity. The nucleus, which is denser than the cytoplasm, is drawn down into contact with the cell membrane and progressive centrifugation increases the distortion and evagination of the membrane. The recuperative powers of the Ehrlich cells so treated must be quite high, since they exhibit approximately the same virulence (LD_{50}) as cells not centrifuged.

We thank Dr. J. S. Murphy, of the Rockefeller Institute of New York, for his advice. This work was supported in part by the U.S. National Institutes of Health, grant *CA* 07114, and Mr. Wilfred P. Cohen of Great Neck, New York.

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VARIATION OF THE RELATIVE SENSITIVITY OF CLOSELY RELATED NEOPLASTIC CELL LINES IRRADIATED IN CULTURE IN THE PRESENCE OR ABSENCE OF OXYGEN

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THE examination of the differential effect of various treatments in modifying the relative radiosensitivity of closely related cell lines which have genetically or biochemically well-defined differences proved to be a fruitful experimental approach to obtain information about the mode of action of radiation[1]. In accordance with this approach, and in an extension of previous investigations[2,3], a series of experiments was performed in which cultures of an Ehrlich ascites tumour and its selected sublines were used as the cellular material, and sensitization by oxygen was used as the modifying treatment. This article reports the results of these experiments and some of the conclusions which can be drawn from them.

The Ehrlich tumour of this work was a hyperdiploid cell line, denoted *ELD*. One of the sublines, *ELT*, represents the cloned derivative of a predominantly hypertetraploid population, isolated from *ELD*. Another subline, clone *g*, has been derived from a serially irradiated line of *ELD*, denoted *SELD*. The details of the isolation of the clones and several characteristics of the cell lines, including certain growth characteristics, chromosomal variation, radiosensitivity, DNA and sulphydryl content, have been examined and are described in previous publications[4].

In vitro cultures of these lines were established from ascitic material. Freshly withdrawn ascites of the respective tumour line were dispensed in 0·1 ml. amounts in several stoppered 'Pyrex' tissue-culture vessels. The medium contained Eagle's solution[5], pregassed with carbon dioxide and supplemented with 15 per cent fœtal calf serum and antibiotics. After a period varying between 7 and 18 days, multiple foci of vigorously growing colonies became visible which were firmly attached to the bottom of the culture flasks. The colonies were detached and dispersed by gentle trypsin treatment and, afterwards, the cells were cultivated in serial passage by transfers of 10^4 cells at weekly intervals, and by medium changes on each second or third day. The plating efficiency, expressed as the percentage of cells which grew into macroscopic colonies in about 10 days, varied between 25 and 100 per cent (cf. cols. 5 and 6, Table 1). In order to protect against major alterations, prone to occur in cell strains

Table 1. THE SLOPE CONSTANT AND EXTRAPOLATION NUMBER OF SURVIVAL REGRESSIONS AND THE MEAN LETHAL DOSE, (D_0), CALCULATED BY LEAST-SQUARE ANALYSIS FROM THE MEAN OF SURVIVING FRACTIONS OF IRRADIATED CULTURES WHICH ARE ILLUSTRATED IN FIG. 1

The number of separate experiments and individual determinations of survival, used for computation, and data on the plating efficiency of the particular cell lines are also presented

Cellline 1	Atmosphere during irradiation 2	No. of separate experiments 3	No. of separate determinations of survival* 4	Plating efficiency†		Slope constant ± standard error ($b \pm \sigma_b$) elog units/100 r. 7	Extrapolation No.		D_0§ (r.) 10
				Mean 5	Range 6		Arithmetic value 8	$-\sigma$— + σ‡ 9	
ELD	Argon	24	43	71	40–100	−0·425 ± 0·013	0·93	0·80–1·07	235
ELT	Argon	18	52	35	26–58	−0·359 ± 0·010	1·01	0·89–1·15	279
Clone *g*	Argon	27	71	47	26–76	−0·343 ± 0·010	1·11	0·98–1·25	292
ELD	Oxygen	8	21	77	37–100	−1·096 ± 0·052	2·23	1·81–2·74	91
ELT	Oxygen	9	25	40	26–62	−1·003 ± 0·065	1·86	1·45–2·39	100
Clone *g*	Oxygen	13	38	31	25–44	−1·087 ± 0·030	2·30	2·01–2·62	92

* Determinations based in each case on the mean values from three irradiated and six control replicate cultures.

† Expressed as percentage of cells which grow into macroscopic colonies in 10 days.

‡ Determined according to formula (1) when $X = 0$.

§ Reciprocal of the slope constant in col. 7 indicating the increment of radiation dose which reduces the surviving fraction by 37 per cent.

maintained in prolonged serial cultures *in vitro*, the chromosomal constitution of the cell populations was examined at intervals of about 10 successive passages, as described previously[2,4]. Whenever shifts were noted in the modal chromosome number or the chromosomal markers identified in previous investigations[2], the propagation of the line was discontinued, and new cultures were established from the original ascitic material preserved in the frozen state.

Reproductive integrity of the cells, as judged by their clone-forming ability, was used as the measure of radiosensitivity. Suspensions of single cells were prepared from cultures growing in non-confluent monolayers which readily dissociated on treatment with trypsin. After counting in a hæmocytometer and diluting as necessary, the appropriate number of cells was added to a series of 6-cm Petri dishes, containing 4–6 ml. of nutrient medium. The number of cells explanted (2×10^2–6×10^5) was chosen with respect to the size of the expected surviving fraction after a particular treatment. The dishes were incubated at 38° C in a moist atmosphere of carbon dioxide in air to maintain a *p*H of 7·0–7·2. After incubation for 3 h the cells were irradiated and re-incubated for 10 days, with medium changes taking place at 3–4 day intervals. Afterwards, clones fixed and stained *in situ*, comprising more than fifty cells, were counted and the number of clones was taken to represent the surviving fraction. In each experiment, mean values were calculated of three (irradiated cultures) or six (unirradiated controls) replicate dishes. The standard error was 8 and 5 per cent respectively, as determined from a total of 170 randomly chosen individual observations. The fraction of cells surviving irradiation was calculated as a percentage of unirradiated controls in the same experiment.

Exposure to X-rays was made at room temperature in an air-tight plastic chamber of 3 l. volume. The lids were removed from the culture dishes, and the nutrient medium was withdrawn to such an extent that a layer of only about 1 mm fluid was left to cover the cells. For 20 min prior and during irradiation, the chamber was gassed with either oxygen or argon, both containing 1·25 per cent carbon dioxide, at a flow rate adjusted to 6 l./min. Control cultures were subjected to the same procedure as irradiated ones. The plating efficiency of the controls equilibrated with the oxygen–carbon dioxide mixture did not differ significantly from those gassed with the argon–carbon dioxide mixture (cf. cols. 5 and 6, Table 1). The gases were led through pre-equilibrated water warmed slightly above room temperature. Argon was cleaned in the factory by burning out its oxygen contamination by hydrogen. Its oxygen content was less than 3 p.p.m., as measured according to the method of Tödt[6]. Radiation was generated in a Siemens X-ray unit at 190 kV and 15 m.amp, was filtered by 1 mm aluminium, and had half-value layer of 0·95 mm copper. The dose rate, measured by means of ionization chamber, was 235 r./min at the bottom of the culture dishes at a distance of 43 cm from the focus.

The mean surviving fraction of several *ELT*, *ELD* and *SELD* clone *g* cultures, irradiated with different X-ray doses in either oxygen or argon, is illustrated, together with the 95 per cent confidence limits, in Fig. 1. The regression lines, calculated by the method of least-square analysis from the means of the respective tumour populations, are also shown. The 95 per cent probability limits of survival after different radiation doses are indicated by shading round the line, and were calculated, based on statistical theory[7], according to equation (1):

$$V(\log \hat{Y}) = s_{y,x}^2 \left(\frac{1}{n} + \frac{(X - \bar{X})^2}{\sum_{i=1}^{n} (X_i - \bar{X})^2} \right) \qquad (1)$$

where $V(\log \hat{Y})$ is the variance of the mean surviving fraction after treatment with a certain X dose of radiation; $s_{y,x}^2$ is the variance from regression; n is the number of observations; $\bar{X}$ is the mean dose of radiation used; and $\sum_{i=1}^{n} (X_i - \bar{X})^2$ is the sum of the squares of the

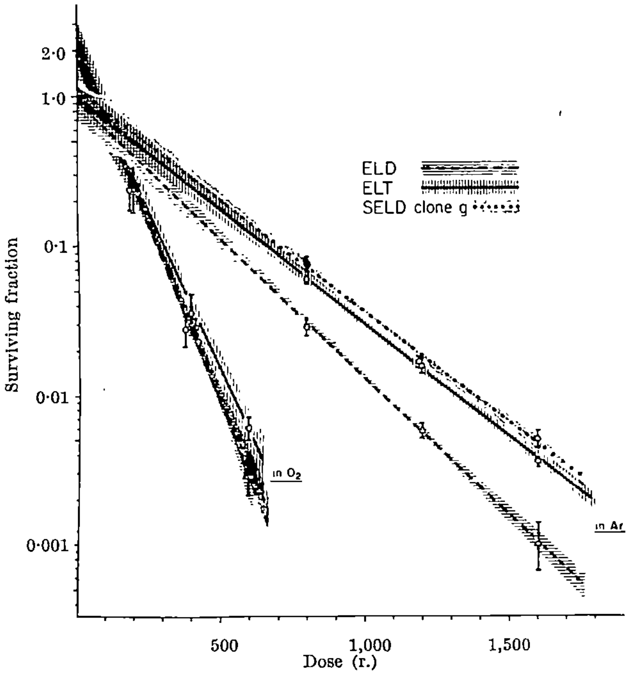

Fig. 1. Survival of cultures of the *ELD* ascites tumour line and of its *ELT* and *SELD* clone *g* sublines after exposure to different doses of X-rays in oxygen or argon. Open circles with vertical lines illustrate the means of surviving fractions in different experiments with the 95 per cent confidence limit. Oblique lines with shading indicate the statistical regression of survival with 95 per cent probability

deviations from the mean of the radiation doses used. Significant differences are apparent between the survival of *ELD*, on one hand, and of *ELT* or clone *g* on the other, after irradiation in argon, while the survival of the three cell lines is closely similar after irradiation in oxygen.

The numerical values of the slope constants and their standard error are presented in Table 1 (col. 7) together with the number of separate tests (col. 3) and the number of separate determinations of survival (col. 4) used for computation. By means of co-variance analysis[7] it can be calculated that the regression of *ELD* survival differs significantly ($P < 0{\cdot}01$) from the regression of *ELT* and clone *g* survival, respectively, after treatment in argon, but the differences are statistically insignificant after treatment in oxygen. The reciprocals of the slope constants are given in col. 10, and indicate the increment of radiation dose (D_0) which reduces the surviving fraction of the respective population by 37 per cent. The extrapolation numbers[8] with standard errors are tabulated in cols. 8 and 9. Using *t*-test analysis, it can be calculated that the extrapolation number of any of the cell lines after oxygen treatment differs significantly from the respective extrapolation number after treatment in argon ($P < 0{\cdot}001$ for *ELD* and clone *g*, and $P < 0{\cdot}05$ for *ELT*). The differences between different lines with regard to the extrapolation numbers in oxygen and in argon, respectively, are statistically not significant.

The results of the tests reported here are concordant in indicating that, in agreement with previous assays *in vivo*[2], differences in radiosensitivity do exist between the *ELD* strain and its selected sublines when irradiated under anoxic conditions. It can be calculated from the relationship between slope constants (col. 7, Table 1) that the sensitivity of clone *g* and *ELT* is 24 and 19 per cent less, respectively, than the sensitivity of the original *ELD*. The decreased sensitivity of clone *g* may be attributed to the protective effect of an increased cellular concentration of intrinsic, low molecular sulphydryls in this line, which has been previously estimated to be also 24 per cent higher than in *ELD*[9]. The reduction of the sensitivity of *ELT*, which has a sulphydryl concentration of the same magnitude as *ELD*[9], can be associated with the duplicated genome of this subline in comparison with *ELD*[2], and is in accordance with observations on the decrease of the sensitivity in other tumours with doubled chromosome sets[12,13]. The possible requirement to inactivate a larger number of sensitive sites has been considered to account for radio resistance in these cases[2].

The differences between the radiosensitivity of the cell strains demonstrated in anoxia were shown to be reduced to statistically insignificant variations when exposure to X-rays took place in oxygen. A reduction of sensitivity differences in oxygen, relative to those under anaerobic conditions, has been previously noted also when our[2] or other cell strains[10] were investigated in different test systems *in vivo*. It is conceivable that a similar 'masking' of the sensitivity differences by oxygen may explain the generally uniform survival of unrelated cultures treated in media equilibrated with air[12], that is, under conditions when cells exhibit full oxygen sensitivity[13]. If, as suggested by the data, the proportionately increased sulphydryl concentration accounts for the radio resistance of clone *g* in anoxia, then the disappearance of sensitivity difference between this strain and *ELD* in oxygen would be in agreement with the observation[14] of a considerable decrease of sulphydryl protection in an oxygenated medium.

The differences between the cell lines with regard to the relative radiosensitivities under aerobic and anaerobic conditions imply a differential enhancement of the effectiveness of radiation by oxygen. For *ELD*, *ELT* and clone *g*, oxygen enhancement ratios of 2·58, 2·79 and 3·16, respectively, can be calculated from the slope-constants. It can be inferred that oxygen does not act as a dose multiplying factor only, but there is also a qualitative difference in the lethal damage caused by irradiation in the presence or absence of oxygen. It has previously been suggested[2] that, in killing *ELD* and *ELT*, a genetic injury may prevail in connexion with anoxic X-irradiation and, conversely, a mainly non-genetic mechanism may be operative in connexion with aerobic treatment. This suggestion is in agreement with the postulate[1,15], based on observations made in experiments with micro-organisms, that DNA is more heavily involved in killing by X-radiation under anoxic as compared to aerobic conditions.

The extrapolation number of survival regressions, presented in cols. 8 and 9, Table 1, can be viewed as further evidence supporting the supposition of differential mechanisms of the radiation effect under aerobic and anaerobic conditions. Extrapolation to numbers larger than unity, found after treatment in oxygen, implies a sigmoid type of survival curve. This can be regarded as indicating that two or more damaging events, of the same or of different kind, must be accumulated for any lethal effect[8]. Conversely, extrapolation close to unity and the corresponding nearly exponential dose-effect relationship observed after treatment in oxygen-free argon denotes that a single damaging event may be sufficient for the loss of reproductive capacity. It would thus seem that the dose-effect relationship after anoxic treatment with sparsely ionizing X-rays is similar to that after treatment with densely ionizing radiations[13,16]. A reduction of the extrapolation number of the survival curve of cells treated by X-rays in an anoxic medium, as contrasted to treatment in a medium equilibrated with air, has been noted in our previous study[3] and by Humphrey *et al.*[17]. The opposite result has also been reported[18]. However, the significance of the difference between extrapolation numbers has yet to be established in these cases. Since the extent of recovery from radiation damage in connexion with fractionated X-ray treatment is related to the extrapolation number of the survival curves[19], such treatment of anoxic cells can be expected to result in a strong decrease or, possibly, absence of recovery, as in the case of densely ionizing radiations[13,16]. This expectation has been fully verified in experiments to be reported elsewhere, involving the exposure of *ELT* to fractionated X-ray doses in the presence or absence of oxygen.

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LETTERS TO THE EDITOR

PHYSICS

Propagation of a Gaseous Discharge in a Crossed Magnetic Field

THE application of a crossed magnetic field to an electron swarm moving under the influence of a uniform electric field enabled Townsend[1] to derive the high electron drift velocities, using a steady-state d.c. method without recourse to pulse techniques or oscilloscopes. From the measurement of the electron angular deflexion, θ^-, and making use of the expression, deduced by Townsend, $v_{\mathrm{T}}^- = \frac{E}{B} \tan \theta^-$, in which E and B are the crossed electric and magnetic fields, respectively, the drift velocity, v_{T}^-, was obtained in an elementary way. In this communication the early treatment is extended to include the numerous practical cases in which Townsend's secondary ionization coefficient comes into play in the full development of a gaseous discharge.

The mode of charge movement together with the crossed electric and magnetic field directions are shown in Fig. 1. An electron, or bunch of electrons, starts off instantly at point 0. As the electrons move in the transverse ($\bar{E}$) direction, they are deflected in the perpendicular ($e\bar{v} \times \bar{B}$) direction with velocity $v_{\perp}^-$. On reaching the point A (or A', A'' or A''', which corresponds to the end of the discharge region), sufficient positive ions have been produced which drift towards the cathode, in turn are deflected, though to a lesser extent on account of their mass, and strike the cathode surface to release a further burst of electrons. Thus, due to successive cycles, the discharge propagates in the perpendicular direction. Analysis of Fig. 1 shows that the propagation velocity, $\vec{v}$, is given by:

$$\vec{v} = v_{\mathrm{T}}^+ (\tan \theta^- + \tan \theta^+) \qquad (1)$$

in which v_{T}^+ is the transverse positive ion velocity and θ^- and θ^+ are the angular deflexions of the respective particles. No specification has been made as to whether the motion is governed by a few individual collisions or by multiple processes, and an important aspect is that the propagation velocity in this régime is independent of the discharge-length.

It has been observed[2] that the emission of an electron from the cathode on positive ion bombardment may be delayed for a time τ_s, called the site time, which may be comparable in duration with the ion transit time, τ_+. Taking this effect into account, the above expression becomes:

$$\vec{v} = v_{\mathrm{T}}^+ (\tan \theta^- + \tan \theta^+) \frac{\tau_+}{\tau_s + \tau_+} \qquad (2)$$

Thus the propagation velocity may vary over a wide range and is influenced by the properties of the gas and the nature of the electrodes.

In certain discharges, such as the column region, the regenerative process can be considered to take place continuously rather than in a stepped fashion, and a reconsideration of equation (1) shows that for this régime the propagation velocity is given by $v_{\perp}^-$ or $v_{\perp}^+$, depending on which ion component dominates the discharge. Thus, in general, the propagation velocity of a discharge will fall within the range:

$$v_{\perp}^- > \mu_{\perp}^+ (\tan \theta^- + \tan \theta^+) > v_{\perp}^+ \qquad (3)$$

in which the limiting velocities can be associated with columnar processes and the middle range corresponds to the cathode fall condition. The large differences between electron and positive ion velocities would lead to charge separation, and as this continuous mode may only be active in columnar regions, the presence of space charges can no longer be neglected and the concept of ion coupling[3] may have to be evoked.

It can be shown[4] for an electron swarm in hydrogen with a mean energy, $\bar{\varepsilon} = 3$ eV, that $v_{\perp}^- = 1{\cdot}5 \times 10^5 . \frac{E}{p} . \frac{B}{p}$ m/sec and $\tan \theta^- = 40 \frac{B}{p}$ for weak magnetic fields; also $v_{\mathrm{T}}^+ = \mu^+ \frac{E}{p}$ in which $\mu^+ = 96 \frac{\mathrm{m}}{\mathrm{sec}} \frac{\mathrm{cm}}{\mathrm{volt}}$ mm mercury[5], yielding $\vec{v} = 3{\cdot}84 \times 10^3 . \frac{E}{p} . \frac{B}{p}$ m/sec. Thus, the electron columnar velocity is 40 × larger than the stepped velocity of the discharge, which could correspond to that of the cathode fall. For $pd = 1{,}000$, $\left(\frac{E}{p}\right)_s = 20$ (ref. 6) and for $B = 0{\cdot}1$ Wb/m² and $p = 1{,}000$ mm mercury, $\vec{v} = 7{\cdot}68$ m/sec in agreement as to order of magnitude with observed gas discharge propagation velocities[7]. In the numerical derivation, the positive ion angular deflexion, θ^+, has been neglected. As no data are available for this quantity or $v_{\perp}^+$, there is a need for these to be measured, particularly as it is claimed[7-9] that θ^+ is the overriding quantity determining the propagation of an arc discharge. Useful information should also be gained from an investigation of the propagation velocity of a Townsend and glow discharge.

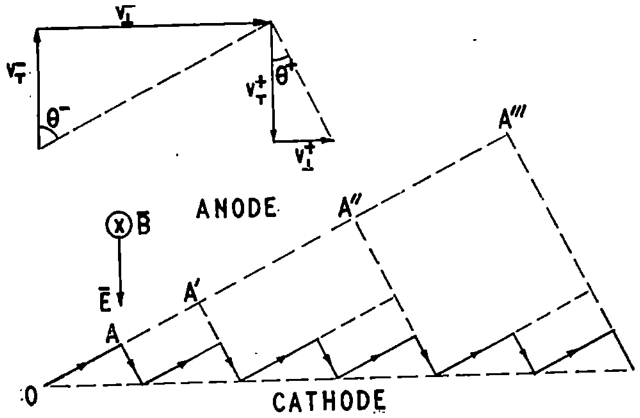

Fig. 1. Movement of electrons and ions of a Townsend discharge in a crossed electric and magnetic field

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Porous Electron Emitters

In a recent publication[1] it was shown that the electron emission at zero field from a porous cathode was very dependent on the size of the pores and their emitting apertures. In particular a reduction in the slope of the Richardson plot, with increasing temperature, is to be expected because the space charge is constrained and can only exist near to the walls of the cavities. The resulting electron emission through apertures in the cavities is then in the form of 'quasi hollow' beams. Curvature of the Richardson plot commences when:

$$\frac{D}{2} = d = 1{\cdot}54 \times 10^{-2} T^{\frac{3}{4}} j_0^{-\frac{1}{2}} \mu\text{m}$$

where D is the diameter of the apertures, d is the distance from the emitting surface for which $\rho = \rho_0/4$; ρ being the charge density at a point distance r from the centre of a spherical cavity, ρ_0 the charge density at the cavity surface and j_0 the emission current density at zero field from the emitting surfaces. For spherical cavities:

$$R \gg d;\ \rho = \rho_0 \frac{\pi^2}{4} \frac{d^2}{(R + dc^{\frac{1}{2}})^2} \sec^2\left(\frac{\pi}{2} \frac{r}{R + dc^{\frac{1}{2}}}\right)$$

$$R \ll d;\ \rho = \rho_0$$

where R is the radius of the spherical cavities.

For normal oxide cathodes with a pore diameter of about 3 μm (ref. 2) $R \gg d$ at high temperatures. The emission from the cathode is considerably lower than would be the case from cathodes with a larger number of smaller pores having smaller emitting apertures. Therefore, the porosity of porous cathodes should, if possible, be controlled so that the condition $R \ll d$ will hold at the operating temperature, and the maximum possible emission should then be obtained.

Since the emission current density from an aperture is then the same as that which would be obtained from an equivalent area of exposed uncontaminated surface it is important that the exposed surface area of the cathode (including apertures) should be maximized. The additional emitting surfaces within the pores do not contribute to the overall emission from the cathode. The main advantage of a porous cathode is the fact that the emitting surfaces are more protected from contamination. If the coating is too thick then the temperature gradient existing in the matrix will make it necessary to heat the cathode base to a higher temperature for the same emission than would be the case for a thinner coating. On the other hand, the matrix should contain sufficient material so that evaporation effects do not unduly shorten the life of the cathode.

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GEOPHYSICS

Shock Wave Propagation from a Nuclear Blast

Since the American nuclear explosion on July 9, 1962, at Johnston Island, independent reports from different parts of the world[1-5] have become available relating to the time of onset of some of its magnetic and earth-current effects. Apparently, in some places, the first impetus of the signal, almost instantaneously with the detonation, has not been noticed for some reason or other. The more intense impulse registered after 2 sec after zero time has been, perhaps, thought to be the first. At some other places, no mention is made of anything other than that received almost simultaneously with the blast. This note is to point out a substantial possibility of a hydromagnetic shock propagation due to the blast giving rise to almost simultaneous signal at different parts of the globe. Both the impulses, one at zero time, and the other 2 sec later, are treated from this point of view. It is, incidentally, pointed out that some of the auroral luminosity reported on this occasion might be attributed to the interaction of shock waves with the magnetosphere.

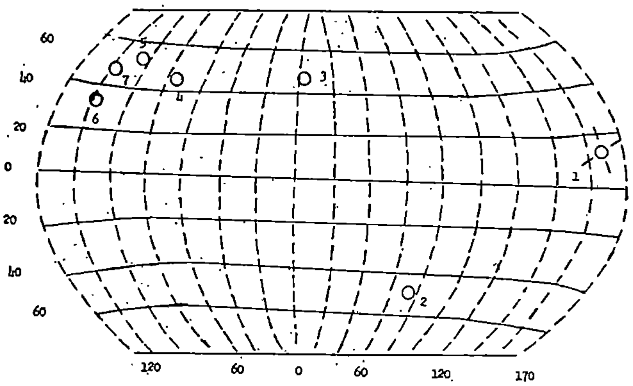

Fig. 1. Approximate locations of the different stations recording magnetic or earth-current or both effects due to the blast: 1, Johnston Island; 2, Kerguelen Island; 3, Chambon-la-Forêt; 4, Ottawa; 5, Prince Albert; 6, Los Angeles; 7, Penticton

Fig. 1 shows the approximate locations of the several stations of observation over different parts of the globe. From these places, either magnetic or earth-current effect or both have been reported. The reported times of onset of the effects at the respective stations are shown in Table 1. At some places, such as Prince Albert, Canada (53·2° N, 105·9° W.), mention is made only of the signal after 2 sec or so after detonation. In the Antarctic French station, Dumont d'Urville, the supposed first impulse has also been noticed only after 2 sec after zero time. However, other French stations (for example, Chambon-la-Forêt, France) did notice both the impulses referred to. The interesting feature is that, besides the first signal at almost zero time, the second, seen about 2 sec later, is also simultaneous at different parts of the globe. It may be recalled that, for *Argus* III explosion, the onset of some of the earth-current effects was registered within 1 sec interval over a range of 120° in longitude[6-8].

Table 1. Time of Onset of Signal due to Blast at Different Stations of the Globe

(Detonation time 09 : 00 : 09 U.T.)

Station	Signal arrival time (U.T.)	Ref.
	h min sec	
S.T.L., Los Angeles, U.S.A.	09 : 00 : 09 (±0·1 sec)	1
Penticton, Canada	09 : 00 : 09·1 (±0·1 sec)	3
Chambon-la-Forêt, France	09 : 00 : 08·8 (±0·2 sec) Also a second strong impulse 2 sec later	2
Kerguelen Island, South Indian Ocean	09 : 00 : 08·7 (±0·5 sec)	2
Prince Albert, Canada	~2 sec after detonation	5
Christchurch and Lauder, New Zealand (earth-potential)	0·2 ~ 0·5 sec after detonation	4
Ottawa, Canada (Rb vapour magnetometer)	09 : 00 : 12 (±2 sec)	3
Amberley, New Zealand (magnetic effect)	09 : 00 : 12 (±1·5 sec)	4

Considering the very small time in which the signal due to the blast is propagated over the globe, one would be led to think of either electromagnetic propagation, or a hydromagnetic shock, among others (for example, very fast neutron propagation[9]). We now consider the case of a hydromagnetic shock in the following.

For a shock front, it is known that the Rankine–Hugoniot equation must be satisfied, namely:

$$\frac{\rho}{\rho_0} = \frac{P(\gamma + 1) + P_0(\gamma - 1)}{P(\gamma - 1) + P_0(\gamma + 1)}$$

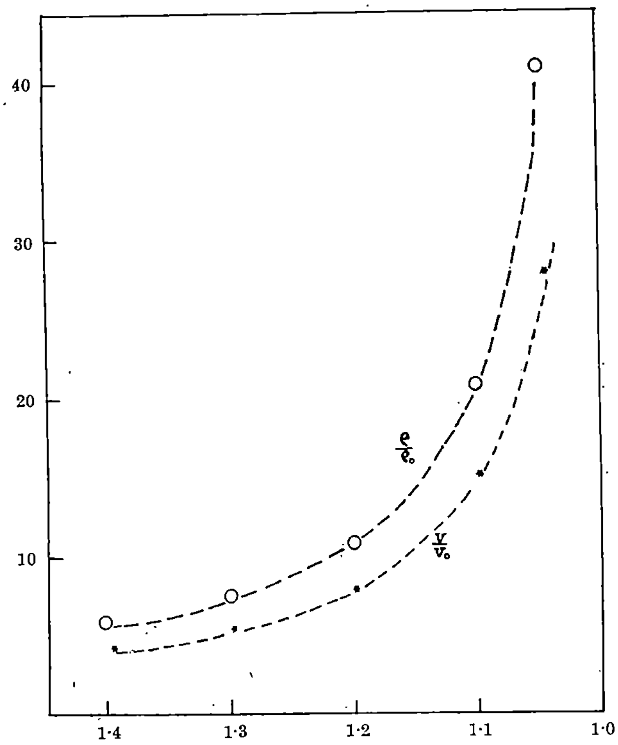

Fig. 2. Variation of shock Mach with the ratio of specific heats

γ is the ratio of specific heats; P_0, ρ_0, refer to ambient pressure and density respectively, before the arrival of the shock; and P, ρ are those behind the shock. For an explosion, $P \gg P_0$, and the Rankine–Hugonoit equation gives:

$$\frac{\rho}{\rho_0} \simeq \frac{\gamma + 1}{\gamma - 1}$$

Furthermore, it is known[3,10] that when a shock develops:

$$\frac{V}{V_0} = \frac{\rho}{\rho_0 \sqrt{2}}$$

where V_0 is the Alfven velocity in the gas at the front of the shock and V is the velocity behind the shock. Fig. 2 shows the ratios $\frac{V}{V_0}$ and $\frac{\rho}{\rho_0}$, for different values of γ. The ratio $\frac{V}{V_0}$, or the shock Mach, increases considerably as γ reduces towards unity. The ratio $\frac{V}{V_0}$ (or the shock Mach) can be considerably large depending on the intensity of the blast. Shocks arriving at the vicinity of the Earth having their origin, for example, in the solar blasts have a very high Mach number[11].

The hydromagnetic wave propagation speed at the heights of explosion, around 400 km, is of the order 150 km/s[12,13]. Even the crudest calculation on this basis gives up to 4,000 km/s for the shock propagation. Accordingly, it is obvious that a distance of the order of the diameter of the Earth can be traversed in 3-4 sec. It is interesting that this order of time is seen actually from the observations of the second impulse simultaneously at different places of the globe (stations 3 and 5, Fig. 1, for example).

The Alfven velocity can be as great as 500 km/s around 1,000 km altitude[12,13]. Simple calculations of shock speeds from this (without assuming any ducted propagation[14]) could give up to about 1/15 of the speed of light. If such a high-altitude propagation is possible at all, the simultaneity of the signal over different parts of the globe, almost instantaneously with the blast, is apparent. Auroræ have been observed[4] on this occasion at altitudes around 700 km. This is evidence in favour of the possible high-altitude propagation of the shock effect. In fact, it is possible that the observed auroral luminosity, in part at least, is by the interaction of the shock and the magnetosphere in a manner suggested earlier by Singer[11].

In conclusion, it is possible that both the first impulse due to the blast at almost zero time, and the second after 2 sec, could be attributed to shock propagation from the blast site. The second is perhaps due to propagation near the altitude of blast in space.

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GEOLOGY

Magnetic Survey off the North Coast of Ireland

The geological relationships between the Pre-Cambrian and Caledonian folding and faulting systems of Scotland and Ireland to a large extent remain unresolved because of the unknown geology beneath the North Channel. A small but detailed (0·25–0·5 mile line spacing) total field magnetic survey off the coast of Donegal shows the presence of two faults which may well be of importance in the evaluation of these relationships. A simplified map of the magnetic results is shown in Fig. 1.

A strong scarp interpreted as Fault *A* is of some 500–600 gammas in amplitude, and a simple interpretation of this feature suggests that it is largely due to the almost vertical boundary of a large magnetic body at a depth to the top of 2–3,000′ below sea-level. The geographical linearity of the feature is indisputable and strengthens the fault interpretation, particularly as it is parallel to the known Caledonian faults of surrounding areas. Extension of the observed lineation to the north-east would take it west of Colonsay to south-east Mull, and to the south-west, into Mulroy Bay (marked MB on the inset map, Fig. 1). It is not suggested that this is necessarily a fault of major importance but it does indicate that the fault 'complex' of Donegal is probably continued to the north-east.

The fault marked *B* is in a complementary direction and divides two areas of particularly high magnetic values. Again a steep scarp is seen on the western group of anomalies following the line of the suggested fault, although the eastern group is less precisely bounded by this same direction. A detailed bathymetric survey (unpublished) reveals a linear scarp feature corresponding with the projected line and confirming its geological reality. The direction of movement of this fault can be suggested if it is assumed that the western and eastern groups of anomalies are equivalent, in which case the movement is dextral with a tear displacement of 5 miles. This is very similar to the fault mapped by McCallien in Inishowen and referred to by Pitcher, Elwell *et al.*[1]; nevertheless correspondence of the two groups is by no means certain.

Detailed sections show that in general there are two types of anomaly present, the large wave-length anomaly

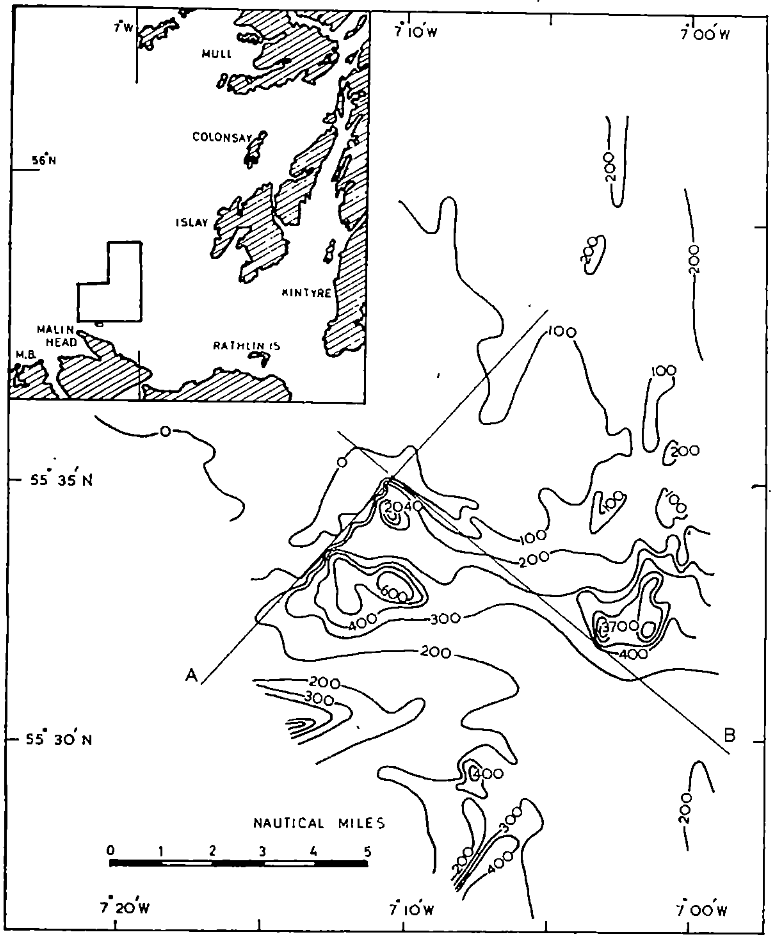

Fig. 1. Simplified total magnetic field contours of the survey area (regional removed). Values are given in gammas, zero value being the absolute value of the field at Eskdalemuir Observatory. Inset map shows the location of the survey area

which has been interpreted as referred to above, and narrower high-amplitude anomalies which are judged to be the product of smaller shallower basic bodies. The two types of anomaly have a similar geographical extent and the rocks are probably closely associated geologically, although there is some suggestion of a differential response to the north-west–south-east fault. The absence of any evidence of reversed magnetization gives preference to a non-Tertiary age for the rocks, but any closer identification is impossible at this early stage.

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CHEMISTRY

Proton Magnetic Relaxation Times of Aqueous Tetra Alkyl Ammonium Halide Solutions

The effect of ionic solutes on the structure of water has been of considerable interest to electrochemists for a number of years[1-3]. Especially intriguing has been the anomalous behaviour of tetra alkyl ammonium salts. In order to account for the apparent molal heat capacities[2] and large negative entropies of hydration[1], Frank and Wen[2] have proposed that the apolar groups of these solutes exert a net 'structure-making' influence on surrounding water molecules in addition to the structural changes (that is, primary hydration) associated with the electric field of the ions. If the extent of structure-making is significant it might be expected that the correlation time, τ_c, for fluctuations of the local magnetic environment and hence the spin-lattice relaxation time, T_1, for water protons would alter appreciably[4].

In this communication we report spin-lattice relaxation times for water protons in solutions containing tetramethyl and and tetraethyl ammonium bromide over a wide concentration range. Relaxation times were measured by the spin-echo method and the individual T_1's are accurate to 5 per cent. Both solutes are observed to decrease T_1 with increasing concentration with the largest rate of decrease noted for the tetraethyl compound. A plot of the relaxation enhancement, $E = \frac{1}{T_1} - \frac{1}{T_1^0}$, versus molality is given in Fig. 1 and shows that the molal relativity, defined by the slope of the linear portions of the curve, is considerably greater for tetraethyl ammonium bromide 0·0825 sec^{-1} mole^{-1} kg than for tetramethyl ammonium bromide 0·0358 sec^{-1} mol^{-1} kg, and alkali halides[5,6]. A deviation from linearity in the E versus m plots is also noted to occur at lower concentration for tetraethyl ammonium bromide.

An enhancement of the relaxation rate in solutions of tetra alkyl ammonium salts may arise from three effects: (1) relaxation by the nitrogen-14 quadrupole; (2) intermolecular dipolar interaction with protons on the apolar groups; (3) increased intra- and intermolecular dipolar contributions due to structural changes of the solvent.

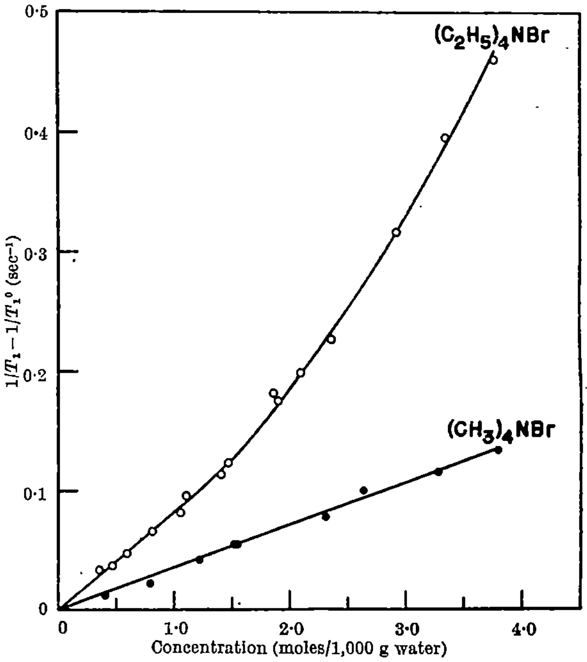

Fig. 1. Relaxation rates of water protons in solutions of tetramethyl- and tetraethyl ammonium bromides at 23° C. T_1^0 (pure water) = 3·10 sec; T_1 = relaxation time of water in solution

Of these the first possibility is unlikely because of steric hindrance by the alkyl groups while the second, although leading to a T_1 change in the correct direction, would not give rise to the large changes observed. The third and more likely possibility is an increase in the correlation times for rotation and diffusion of the water molecules with a resultant increase in both the intra- and intermolecular components of the relaxation rate[4]. Such an increase in τ_c would result if a significant fraction of the water molecules are partially immobilized in the vicinity of the apolar groups either by an increase in the 'ice-likeness' of water near the apolar groups or by the formation of partial clathrate hydrates about the apolar groups[7,8].

A qualitative interpretation of the E versus m curves can be given in terms of a simplified model in which the protons are assumed to occupy three distinct sites; that is, clathrate (or 'ice-like') water, hydrated Br^- ion and bulk water. The observed relaxation rate, $1/T_1$, is then given by the weighted average of the contributions from these sites and a preliminary calculation[5,9] shows that approximately 30 water molecules are involved in the ordered water layer about the $(C_2H_5)_4N^+$ ion. The ordering is accompanied by a ten-fold increase in the correlation time more than that in bulk water. In comparison, tetramethyl ammonium bromide is a much less effective structure-maker orienting less than 5 water molecules about the $(CH_3)_4N^+$ ion. The deviations from linearity observed in the E versus m curves at higher concentrations indicate that the three-site model is inadequate under these conditions.

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Detection of Divalent Sulphur Compounds on Paper and Thin-layer Chromatograms

Recently a communication has been published by Thompson *et al.*[1] which describes a sensitive qualitative test for sulphoxides on paper chromatograms. The method is based on the reaction of sulphoxides with hydriodic acid to form iodine. C. De Marco[2] essentially extended the method of Thompson to certain other classes of partially oxidized sulphur compounds like sulphinic acids and sulphones. However, as explicitly stated in De Marco's communication, the Thompson test is of no direct use for the detection of other classes of organic sulphur compounds, especially the large number of substances containing divalent sulphur.

The publication of the two aforementioned papers[1,2] induced us to report a method used in this laboratory, which makes it possible to detect just those classes of organic sulphur compounds giving no reaction with hydriodic acid. Our method is based on the well-known oxidation of divalent sulphur compounds by periodic acid to sulphoxides, sulphones, sulphinic acids or derivatives thereof.

A positive reaction with neutral aqueous potassium (*meta*) periodate solution is given by aliphatic thiols, sulphides, disulphides, thioketones, thiamides and thioureas[3]. The aromatic thiols, sulphides, and disulphides usually do not react under these conditions[4]. However, these aromatic sulphur compounds will give satisfactory positive results with periodate in acetic acid solution, since an increase in the acidic strength of the oxidant favours the oxidation reaction[5,6].

The oxidation of compounds on paper chromatograms with periodate and the subsequent development of the unconsumed periodic acid with benzidine was originally used by Viscontini *et al.*[7], in carbohydrate chemistry, where the method indicates glycol groups. In this laboratory, the application of Viscontini's method for the detection of sulphur compounds was first observed while working with sugar–amino-acid complexes, the amino-acids having been DL-methionine and L-cystine. The method was later extended to aliphatic and aromatic thiols, sulphides and thioketones. As expected, thiophene and its derivatives give no reaction, the reason being the aromatic character of these compounds.

The technical details of the periodic acid oxidation of sulphur compounds are very similar to those given in the original application by M. Viscontini. The paper or the glass-plate is sprayed with a 0·1 per cent solution of sodium (*meta*) periodate in water or in 8 N acetic acid, respectively. After 4 min the colour is developed by spraying the paper with a 0·5 per cent solution of benzidine in *n*-butanol/acetic acid (4 : 1 v/v). Where oxidizable substances are present on the paper, the periodate is consumed and, afterwards, the colourless benzidine is not oxidized to the dark blue compound as on the rest of the paper. The final chromatogram shows white spots on a blue background.

The procedure is readily reproducible and sensitive. In the case of methionine the sensitivity is comparable to that of ninhydrin (5–10 µg). With aromatic sulphur compounds, the sensitivity is somewhat lower and depends on the aromatic residue (20–30 µg).

A preparative periodate oxidation of methionine was performed under the conditions of the development of the chromatogram. The main reaction product was methionine sulphoxide, with a minor amount of the sulphone present.

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Production of Gallium by Continuous Electrodeposition from Acidic Solutions

As a means of production, electrolysis of gallium from only alkaline and not acidic solutions has been reported[1]. The principal difficulties reported using acidic solutions are: (*a*) poor current efficiencies; (*b*) formation of solid gallium oxychloride[2].

Reports of acidic gallium electrolyses[3,4] have shown it to be feasible, and the simple considerations leading to successful acidic gallium electrodeposition useful for large-scale production are outlined in this work.

Electrolysis of an aqueous solution of $GaCl_3$ using a molten gallium cathode and a graphite anode results in the sequence of events here.

(*a*) Aqueous dissolution of $GaCl_3$ yields an acidic solution (hydrolysis of a salt of a strong acid and weak base).

(*b*) Electrolysis begins with little or no hydrogen produced at the cathode with increasing amounts being generated as the process proceeds.

(*c*) The *p*H of the solution increases and, finally, GaOCl is precipitated and the gallium deposition current efficiency approaches zero.

(*d*) Addition of hydrochloric acid to $GaCl_3$ solutions reduces the gallium deposition current efficiency finally to zero.

For a 2 molar solution of $GaCl_3$ which has a *p*H of about 1, one calculates the following approximate values[5]:

$$E_{\text{Ga deposition}} \simeq 0 \cdot 51 \text{ V}$$

$$E_{\text{H}_2\text{ discharge}} \simeq 0 \cdot 50 \text{ (at molten gallium)}$$

Thus the reduction of Ga^{+3} and H^+ occurs with about equal ease and roughly in proportion to their relative concentrations. At first $[Ga^{+3}] \gg [H^+]$, but as the process proceeds this ratio is decreased and eventually is reversed when the *p*H is sufficiently high, causing the precipitation of GaOCl. The ratio is also decreased by the addition of hydrochloric acid.

A simplified overall reaction may be written:

$$(1 + \chi)GaCl_3 + \chi H_2O \xrightarrow{\text{electrolysis}} Ga + \chi H_2 + \frac{3 + 2\chi}{2} Cl_2 + \chi GaO^+ + \chi Cl^-$$

where, if χ becomes sufficiently large:

$$\chi GaO^+ + \chi Cl^- \rightarrow \chi GaOCl$$

In order to conduct a continuous electrodeposition of gallium from an acidic solution the Ga^{+3} concentration must be replenished and the acidity controlled to prevent the precipitation of GaOCl or the excessive formation of hydrogen at the cathode.

The Ga^{+3} can be replenished by the addition of a concentrated stock solution of $GaCl_3$. A measure of the amount of hydrochloric acid which must be added is the departure of the $[Cl^-]$ to $[Ga^{+3}]$ ratio of the solution from 3. The excess water may be removed by evaporation.

Utilizing the principles outlined, electrodeposition of gallium from about 1·5 l. of a 2 m $GaCl_3$ solution has been carried out indefinitely, producing 200–250 g of gallium in 6–7 h at current efficiencies of 60–80 per cent.

Many variations in automatic equipment are conceivable, but certain features are desirable:

(1) The graphite anode and molten gallium cathode areas should be such as to have current densities of 0·3–1 amp/cm² at each, and horizontally mounted parallel to each other at a distance that passes the required current (50–100 amp) at a potential of 12–16 V. It is advisable to perforate and channel the anode to facilitate the escape of chlorine gas.

(2) A cooling arrangement to keep the operating temperature within the range of 70°–90° is desirable.

(3) The only materials to be used that will come in contact with the gallium (III) chloride solution should be 'Pyrex', quartz and 'Teflon'. Other materials such as the 'Tygons' and 'Kel-*F*' are badly attacked, as are most metals.

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Reaction between Molybdenum Disulphide and Water

MOLYBDENUM disulphide, MoS_2, undergoes oxidation in bulk at temperatures above 450° C, which may be demonstrated[1] by gravimetry. However, the substance must ordinarily be covered with a surface layer of oxide, since heating samples in a good vacuum leads to the evaporation of molybdenum trioxide. It is possible that such layers are involved in the frictional transients observed[2] when molybdenum disulphide is used as a lubricant in wet atmospheric environments. It is germane to inquire as to the origin of the oxide layers, and we wish to comment on the possible extent of the hydrolytic decomposition of molybdenum disulphide to yield hydrogen sulphide and molybdenum dioxide. Haltner found[2] that when an MoS_2 film, supported on certain metals, and particularly copper, was exposed to rubbing and shearing forces in a wet atmosphere, hydrogen sulphide could be detected. If no metal is present, however, the reaction appears to be different, for Cannon[3] found that the gravimetry of adsorption of water on fine particle molybdenum disulphide showed a strong and extensive irreversible interaction at 0° C. The latter also found that for very finely dispersed samples, if the specific surface area, rather than the mass of the solid, were taken as the extensive variable, the observed gravimetry conformed most closely with the reaction:

$$MoS_2 + H_2O \longrightarrow MoOS_2 + H_2$$

rather than a hydrolysis. While no search for hydrogen evolution was made in the earlier experiments[1–3], an examination of the thermal behaviour[3] of the putative oxysulphide indicated that a temperature of 350° C could be attained before any appreciable loss in weight was observed, and that this loss appeared to be associated with the formation of sulphur dioxide rather than hydrogen sulphide. It thus seemed likely that the reaction of molybdenum disulphide with water, studied in the absence of metals under conventional high vacuum conditions, was not a hydrolytic process of sulphide to oxide plus hydrogen sulphide, but a net oxidation. We have now extended the work to higher temperatures, with the following interesting results. The water adsorption work was repeated at 180° C, 20μ water vapour pressure, in quartz microbalance equipment like that described before[1,3]. The surface-area limited reaction with a permanent weight increase proportional to the specific surface area was seen again. 20 g samples of molybdenum disulphide ($\Sigma = 15$ m²/g) were then exposed to 20 mm water vapour at progressively higher temperatures between 200° and 500° C. The experiments were conducted in a quartz envelope to permit pre-degassing the solid. Pressures were monitored through the seven-day holding period of each of the experiments and gas samples were withdrawn for mass spectrometric study after one day each at various temperatures. The results are summarized in Table 1.

Table 1

Exp. No.	T° (C)	H_2O charge	Gas yield (mass spectrometric analysis) (S.T.P. c.c.) Condensable at 77° K	 Non-condensable at 77° K
1	200	5·5	Trace	Large amount of H_2
2	300	4·8	Trace	Large amount of H_2
3	400	4·0	0·29 (SO_2)	Trace
4	500	2·9	1·5 (SO_2)	Trace

No hydrogen sulphide was formed at any time during the sequence, and measurable amounts of sulphur dioxide only at temperatures in excess of 300° C. The first gas to be evolved from the samples was actually hydrogen, which was found in quantity in the gases withdrawn after a day at 200° C. The validity of the proposed equation is thereby supported.

It is also evident that any hydrolytic reaction which does occur must proceed through a mixed sulphide or at least involve the accommodation of the reactive metals cited (copper and iron) as ions in the molybdenum disulphide crystals.

These results confirm the interpretive extrapolation of the previous work[2], namely, that the action of water on MoS_2 is surface limited, and, in the absence of metals, consists primarily of an oxidative rather than a hydrolytic step.

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A Kinetic Approach to the Nature of Ferrous Ions in Aqueous Solution

Ion association involving ferrous ions is difficult to detect by the usual techniques: direct spectrophotometry due to the low extinction coefficients of Fe(II) species and the possible presence of trace Fe(III) species with high extinction coefficients; conductivity due to the high concentrations of anions needed; electrometric measurements due to the easier formation of complex species involving Fe(III) ions. In these circumstances, kinetics can be useful in providing information on complex formation, if a suitable reaction can be found. Such a reaction to study the nature of Fe(II) ions is that of Fe(II) with hydrogen peroxide: with $[\mathrm{Fe(II)}]/[H_2O_2] > 2$, the reaction is first order in [Fe(II)] and first order in $[H_2O_2]$ (refs. 1 and 2):

$$\mathrm{Fe(II)} + H_2O_2 \rightarrow \mathrm{Fe(III)} + \cdot OH + OH^- \qquad (1)$$

$$\mathrm{Fe(II)} + \cdot OH \rightarrow \mathrm{Fe(III)} + OH^- \qquad (2)$$

We have found that the reaction is independent of ionic strength, independent of hydrogen ion concentration below *p*H 3 using perchloric acid and that the rate of the slow step (1) is dependent on the charge on the Fe(II) species. This allows the determination of the concentrations of free and of associated Fe(II) from the observed rate: and ionic strength effects must be due to the existence of equilibria between various Fe(II) species.

Hardwick[2] has shown, using ferrous sulphate in aqueous sulphuric acid, that the rate constant appears to depend on the concentration of sulphuric acid added when the latter is high. We have confirmed this, and carried out an investigation of the effect of added sulphate ions on the reaction of ferrous perchlorate with hydrogen peroxide in aqueous perchloric acid, following the rate spectrophotometrically by the formation of the Fe(III) species. Under these conditions, with added $[HClO_4] = 4 \times 10^{-3}$ M and ionic strength μ adjusted to 1·0 with added sodium perchlorate, the observed bimolecular rate constant k_0 increases with the concentration of added sulphate ions until a limiting value of k_0 is reached which is independent of the sulphate ion concentration, as shown in Table 1. This Table also shows that increasing the concentration of perchloric acid reduces the rate constant to the original value obtained in the absence of sulphate ions.

Table 1. Variation of k_0 with Sulphate Ions added at $\mu = 1·0$ and 25°C

$[SO_4^{2-}]$ M added	$[SO_4^{2-}]$ M corrected	$[HClO_4]$ M added	k_0 moles^{-1} l. sec^{-1}
—	—	0·0040	49·5
0·025	0·024	0·0040	53·0
0·050	0·0482	0·0040	57·9
0·075	0·0733	0·0040	60·0
0·10	0·0975	0·0040	65·7
0·125	0·122	0·0040	68·2
0·15	0·147	0·0040	73·0
0·20	0·197	0·0040	74·4
0·30	0·299	0·0040	72·5
0·35	0·347	0·0040	73·0
0·10	0·094	0·010	58·0
0·10	0·073	0·050	54·0
0·10	0·054	0·100	53·5
0·10	0·009	0·700	48·8

These results can be interpreted as follows:

$$SO_4^{2-} + H_3^+O \overset{K_b}{\rightleftharpoons} HSO_4^- + H_2O \qquad (3)$$

$$SO_4^{2-} + Fe^{2+} \overset{K_c}{\rightleftharpoons} FeSO_4 \qquad (4)$$

$$Fe^{2+} + H_2O_2 \xrightarrow{k_1} Fe^{3+} + \cdot OH + OH^- \qquad (5)$$

$$FeSO_4 + H_2O_2 \xrightarrow{k_2} FeSO_4^+ + \cdot OH + OH^- \qquad (6)$$

$$\mathrm{Fe(II)} + \cdot OH \rightarrow \mathrm{Fe(III)} + OH^-$$

with $k_2 > k_1$: Fe^{2+} is assumed to be hexahydrated ferrous ion and all other ionic species are assumed to be hydrated. When sulphate ions are added the species $FeSO_4$ is formed via equation (4), which produces an increased rate through equation (6). The presence of a possible species $FeHSO_4^+$ is rejected under these conditions due to the effect observed on increasing the acidity, as recorded in Table 1. If [Fe(II)] is the total concentration of ferrous species,

$$\begin{aligned} d[\mathrm{Fe(III)}]/dt &= 2k_0[\mathrm{Fe(II)}][H_2O_2] \\ &= 2k_1[Fe^{2+}][H_2O_2] + 2k_2[FeSO_4][H_2O_2] \end{aligned}$$

and it can be shown that:

$$k_0 = \frac{k_1 + k_2K_c[SO_4^{2-}]}{1 + K_c[SO_4^{2-}]} \qquad (7)$$

where $K_c = [FeSO_4]/[Fe^{2+}][SO_4^{2-}]$. If $K_c[SO_4^{2-}] < 1$, this reduces to:

$$k_0 = k_1 + k_2K_c[SO_4^{2-}]$$

When k_0 is plotted against $[SO_4^{2-}]$ a straight line is obtained (Fig. 1*a*): $[SO_4^{2-}]$ was corrected for the formation of HSO_4^- ions by using a value of K_b at $\mu = 1$ interpolated from the data of Reynolds and Fukushima[3]. If the reactive species in the presence of sulphate ions is $Fe(SO_4)_2^{2-}$, a plot of k_0 against $[SO_4^{2-}]^2$ should give a straight line: Fig. 1*b* shows that such a plot is a curve, and therefore $Fe(SO_4)_2^{2-}$ is not formed under these conditions. From the slope of Fig. 1*a*, using the value of k_2 obtained from the limiting value of k_0, an approximate value of K_c can be obtained. This value was substituted back into the full expression (7) and $k_0(1 + K_c[SO_4^{2-}])$ plotted against $[SO_4^{2-}]$, and a further value for K_c obtained from the slope of the straight line (Fig. 2). By re-substituting this

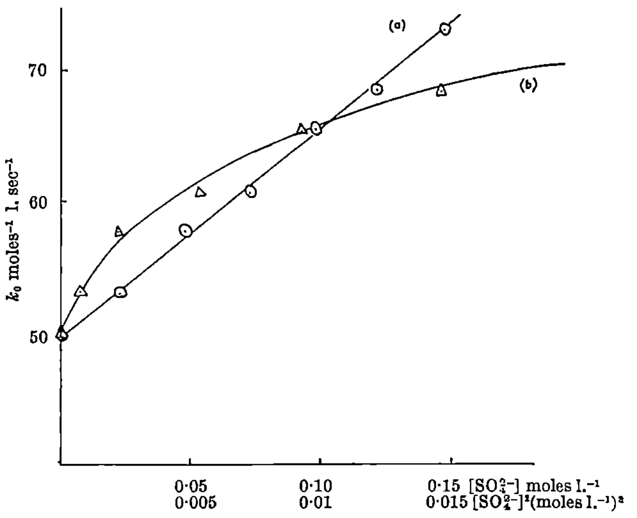

Fig. 1. Plot of k_0 versus (*a*) $[SO_4^{2-}]$ and (*b*) $[SO_4^{2-}]^2$

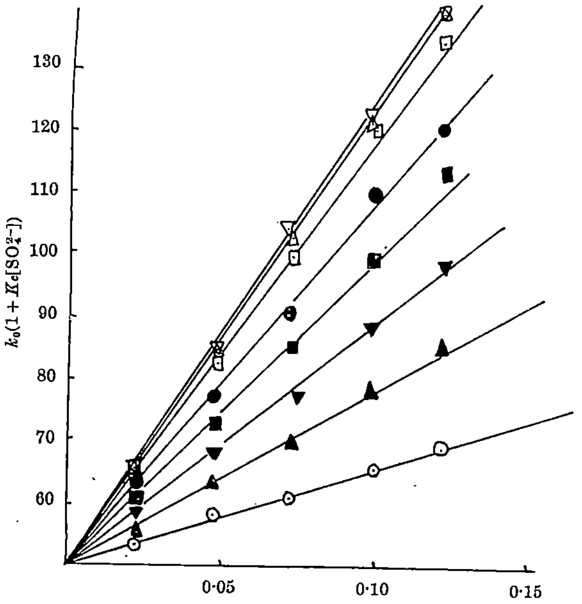

Fig. 2. Plots of k_0 $(1+K_c[SO_4^{2-}])$ versus $[SO_4^{2-}]$ with converging values of K_c as follows: ⊙, 2·06; ▲, 3·92; ▼, 6·60; ■, 8·10; ●, 8·75; ⊡, 9·30; △, 9·90; ▽, 10·20

value and replotting in this way until successive plots converge an accurate value was obtained for K_c: these plots are shown in Fig. 2. By this method for $FeSO_4$, $K_c = 10{\cdot}2$ moles^{-1} l. at $\mu = 1{\cdot}0$ and 25° C. These results can be compared with $K_c = 1{\cdot}1$ at $\mu = 1{\cdot}0$ and 30·5° C suggested by Huffman and Davidson[4] to explain their kinetic data on the autoxidation of Fe(II) in aqueous sulphuric acid and $K_c = 0{\cdot}61$ for $FeHSO_4^+$ and $K_c = 1{\cdot}7$ at room temperature used by Beukenkamp and Herrington[5] to explain their ion-exchange data involving ferrous ions in sulphuric acid without control of ionic strength.

Table 2. Values of k_2 and K_c at 25° C

Fe(II) species	μ	k_2 moles^{-1} l. sec^{-1}	K_c moles^{-1} l.
Fe^{2+}	1·0	$(k_1=)$49·5	—
$FeCl^+$	1·0	68·7	4·45
$FeSO_4$	1·0	74·4	10·2
$FeHP_3O_9$	1·0	91·5	9·42
$FeP_3O_9^-$	1·0	123·2	13·4
$FeCl^+$	4·0	66·2	10·3

Similar detailed kinetic investigations of the reaction of ferrous perchlorate with hydrogen peroxide in aqueous perchloric acid with added chloride ions and trimetaphosphate ions show in each case an increase in k_0 to a limiting value independent of the concentration of the anion. These results have been interpreted in an analogous manner to the above for the $FeSO_4$ species, and values of k_2 and K_c for the species $FeCl^+$, $FeP_3O_9^-$ and $FeHP_3O_9$ are given in Table 2. These data, when combined with that for $FeSO_4$ and k_1 for Fe^{2+} ions in perchlorate media alone, indicate that, as expected on simple electrostatic considerations of electron transfer, the ability to abstract an electron from Fe(II) is increased by a decrease in the positive charge, with a further increase on changing to a negative charge, but comparison of values of k_2 for $FeSO_4$ and $FeHP_3O_9$ shows that the size of the anion has some influence. The association constant K_c increases with an increase in the negative charge on the anion, as expected on a simple electrostatic picture. Experiments with $[ClO_4^-] = 8$ M and $[NO_3^-] = 5{\cdot}2$ M indicate that, in these conditions, the association of ferrous ions with perchlorate ions and nitrate ions does not occur. The data for $FeCl^+$ in Table 2 show, as expected, that an increase in ionic strength has no effect on k_2 but changes K_c.

This work will be described in detail elsewhere, together with further work on other anions and the effects of the variation of temperature. One of us (M. A. S.) thanks the Colombo Plan for a scholarship.

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BIOPHYSICS

Electron Paramagnetic Resonance in Single Crystals of Cupric Insulin

The binding of divalent metal ions to insulin has been the subject of several investigations, both chemical and physical in nature[1-6]. In describing here the application of electron paramagnetic resonance (EPR) spectroscopy to this problem, we wish to emphasize the richness of the data obtainable from orientation investigations of single crystals. This information is of the following kinds: (1) the number of distinct magnetic centres; (2) the orientation with respect to the crystal axes of the symmetry axes of the magnetic centres; (3) the symmetries of the protein environments of the magnetic centres, and the ground-states of the transition metal ions; (4) the delocalization of the electrons in metal ion–protein bonds; (5) identification of protein atoms bound to metal ions when the former have nuclear magnetic moments; (6) an estimate of the distance between metal ions.

Single crystals of ferrimyoglobin and hæmoglobin have been investigated by EPR[7,8], but no cuproprotein crystal has.

Orientation investigations require the crystal to be mounted in a resonant cavity so that knowledge of the directions of the crystal axes is retained; then spectra are taken at many angles of the magnetic field with respect to the crystal axes. Protein crystals pose special problems. They are difficult to prepare in large size and are magnetically dilute, whence a sensitivity requirement is imposed. The crystals are fragile, which makes handling difficult. If the protein must be cycled between room and cryogenic temperatures in order to re-position the crystal, the EPR spectra progressively deteriorate.

We have developed a fused-quartz mount which automatically determines the initial orientation of the crystal. A gear train permits remote orientation of this mount and hence, in conjunction with rotation of the magnet, provides access to all orientations of crystal relative to field and eliminates temperature cycling and remounting. The resonant cavity, a totally enclosed, gold-plated, fused-quartz structure (half-wave-length), is immersed directly in the refrigerant. A 100-kc/s field modulation is used.

The low noise and high sensitivity of this apparatus are demonstrated by the photographically reproduced record of Fig. 1*A*, taken from a single cupric insulin crystal (at 90° K) containing 5×10^{16} spins. The signal-to-noise ratio of this spectrum is about 50 : 1 for a 1 sec integration time and a modulation field of two gauss. The number of detectable spins for a signal of width ΔH is 1×10^{11} ΔH at optimum modulation field strength[9]. The practicality of the apparatus is indicated by the fact that a complete orientation investigation (more than 50 spectra) was performed at 90° K within 48 h without removing or disturbing the crystal (though rotating it through many angles).

Cupric pig insulin crystals were prepared and analysed at Novo Terapeutisk Laboratorium A/S, Copenhagen, by the method of Schlichtkrull[5]. These rhombohedral crystals

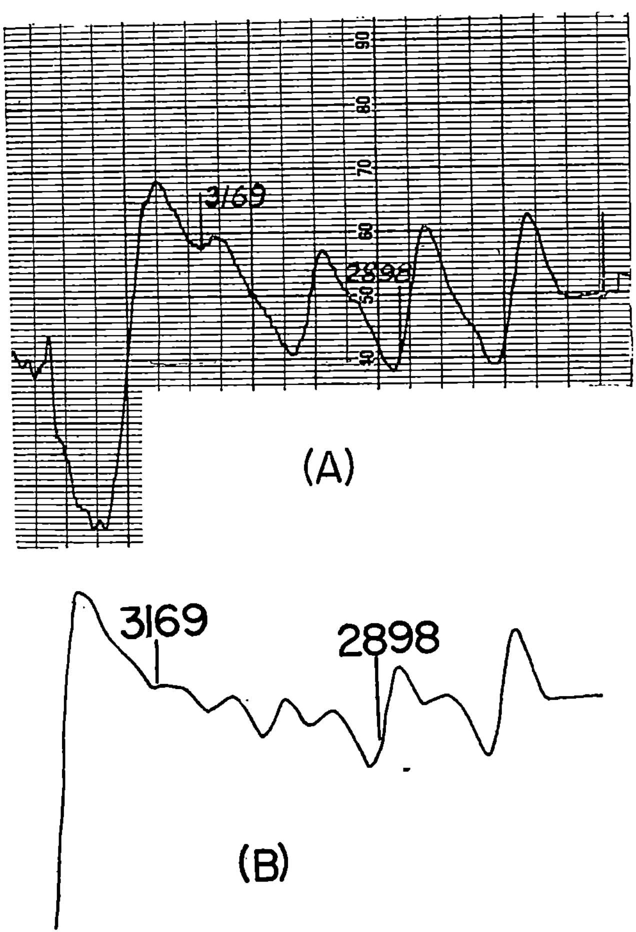

Fig. 1. Cupric insulin crystal at 90° K. (*A*), $\varphi=0°$, $\theta=56\cdot6°$, $\nu=9\cdot494$ Gc/s; (*B*), $\varphi=-20°$, $\theta=55\cdot0°$, $\nu=9\cdot541$ Gc/s

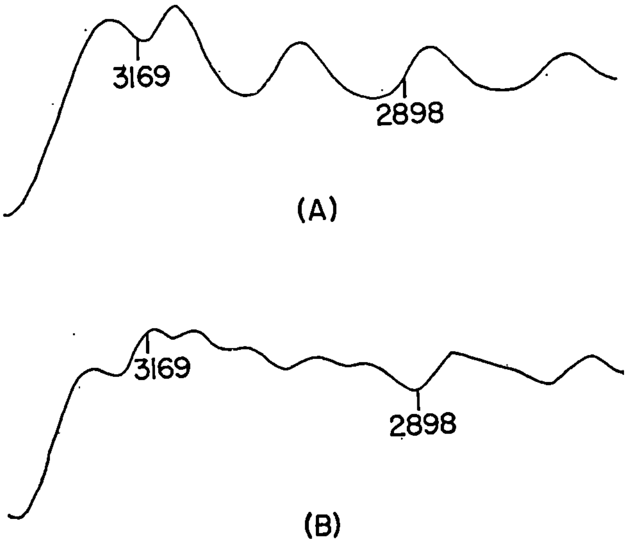

Fig. 2. Cupric insulin crystal at 295° K. (*A*), $\varphi=0°$, $\theta=56\cdot6°$; (*B*), $\varphi=-20°$, $\theta=56\cdot6°$; $\nu=9\cdot492$ Gc/s in (*A*) and (*B*)

contain two cupric ions per 36,000 molecular-weight unit cell (6 Sanger units), and, along with insulin crystals similarly prepared with other divalent metal ions of the 3*d* group, are isomorphic with the diamagnetic zinc crystals.

Spectra from a single Cu^{++} insulin crystal at room temperature are shown in Fig. 2. θ and φ are spherical co-ordinates, with the three-fold symmetry axis of the crystal the polar axis. For θ = 90°, φ = 0°, *H* is perpendicular to a crystal edge. The spectrum of Fig. 2*A* was taken with *H* in the direction of the highest *g* value observed (peaks appearing at minimum *H*). There are two other directions giving the same spectrum. The spectra do not change very much as θ is varied from this position, but become quite different—peaks are only partially resolved (Fig. 2*B*)—as φ is changed. When only room temperature spectra were available, we believed this direction and the other two like it to be the $g_{\max}$ principal axes of three magnetic sites.

The spectra in Fig. 1 were taken at 90° K with the crystal in the same orientations as in Fig. 2. Fig. 1*A* is similar to Fig. 2*A* but for a reduced line-width. The direction shown in Fig. 1*B* now shows well-resolved peaks. The crispness of the spectra taken at 90° K makes it possible to observe the movement of the separate peaks as φ is changed and so see that the directions originally believed to be $g_{\max}$ of three ions were each actually a superposition of peaks of (at least) two ions. Careful investigations indicate that the maximum *g* values of these ions are identical and lie at φ = about ± 15° from the original directions.

Due to the large line-widths and overlapping lines present even at 90° K, the detailed magnetic characteristics of Cu^{++} insulin must be determined by a digital computer programme we have developed. Although the computer analysis of the room temperature and 90° K spectra is not complete, some general features of our data are clear:

(1) The spectra are completely reproducible from crystal to crystal.

(2) The magnetic symmetries exhibited by the crystals are consistent with the known space group of insulin, $R3$ (ref. 1).

(3) Although there are 2 Cu^{++} per unit cell (on the average), there are 6 magnetic sites per unit cell. These sites appear to differ only in the orientation of the ligands about the ions. While the existence of 6 sites has not been predicted by physical chemical measurements[3,4], it is consistent with the data of Schlichtkrull, which show

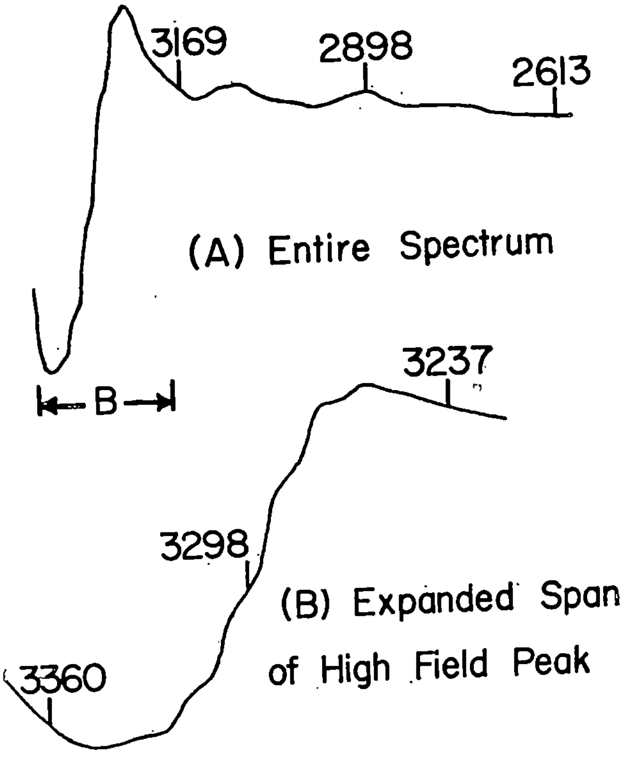

Fig. 3. Cupric insulin crystal at 90° K. $\varphi=135°$, $\theta=88\cdot0°$, $\nu=9\cdot532$ Gc/s

that a maximum of 6 atoms can go into a 36,000 molecular-weight unit[6].

(4) In certain directions (Fig. 3) the high-field absorption is observed to be modulated by five sub-peaks. This is the interaction of the cupric ion with its ligands. In terms of co-ordinating groups available in the protein, the only likely possibility is that the metal ion is bound to two magnetically equivalent nitrogen atoms (nuclear spin = 1), in agreement with the suggestion of Tanford and Epstein[3] and contrary to the implication of Marcker[4].

When the computer analysis is complete and results are available from present experiments at 20° K, a complete report will be submitted for publication.

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Molecular Geometry of Androsterone

THE structure of androsterone, $C_{19}H_{30}O_2$ (Fig. 1), the first male sex hormone to be discovered, was determined recently by High[1] by vector coincidence methods. The space group of this compound is $P2_1$; the unit cell constants are $a = 9{\cdot}56$ Å, $b = 7{\cdot}90$ Å, $c = 11{\cdot}78$ Å, $=111{\cdot}36°$, $Z = 2$, and the measured density equals 1·164 g cm^{-3}. High's atomic positional parameters have been used to calculate the quantitative geometry of the androsterone molecule as follows. The final monoclinic fractional co-ordinates (x, y, z) were converted to Cartesian co-ordinates in Å (p, q, r) using the expressions $p = a_0x + c_0z \cos \beta$, $q = b_0y$, and $r = c_0z \sin \beta$, where a_0, b_0 and c_0 are taken in the Donnay setting ($a_0 = 11{\cdot}78$ Å, $b_0 = 7{\cdot}90$ Å, $c_0 = 9{\cdot}56$ Å). The Cartesian Å co-ordinates were used, in turn, to compute a series of 'best' planes (Table 1) to which various geometrical features of the molecule could be referred, namely, the root mean square distances of atoms from the 'best' planes (Table 2), the perpendicular distance of individual atoms from the 'best' planes (Table 3), and the angles between the 'best' planes and various sub-portions thereof (Table 4). The 'best' plane calculations were performed on an IBM 1620 computer using the 'best' plane 'Fortran' programme of Harris and Harker[2].

Table 1. COEFFICIENTS a, b, c, d IN THE EQUATION ($ax+by+cz=d$) OF THE 'BEST' PLANES OF ANDROSTERONE*

	a	b	c	d
Entire molecule	−0·347	−0·055	0·936	0·354
Steroid nucleus plus angular methyls	0·324	0·126	−0·938	0·011
Steroid nucleus	−0·312	−0·021	0·950	0·756
A	0·458	0·025	−0·888	0·538
*A*1	0·833	0·072	−0·548	5·348
*A*2	−0·174	0·008	0·985	2·129
*A*3	0·853	0·077	−0·516	4·502
B	−0·337	−0·069	0·939	0·199
*B*1	0·812	0·120	−0·572	4·273
*B*2	−0·036	−0·039	0·999	2·173
*B*3	0·757	0·086	−0·648	2·423
C	−0·275	−0·019	0·961	0·789
*C*1	0·727	0·051	−0·685	2·037
*C*2	0·029	−0·000	0·999	2·017
*C*3	0·761	0·052	−0·646	1·067
D	−0·141	−0·049	0·989	1·019
*D*1	0·623	0·165	−0·765	1·037
*D*2	0·059	−0·010	0·998	1·408
*A*3*B*1	0·837	0·100	−0·538	4·413
*B*3*C*1	0·743	0·068	−0·666	2·212
*C*3*D*1	0·681	0·106	−0·725	0·903

* In the Cartesian co-ordinate system described in the text.

Table 2. ROOT MEAN SQUARE DISTANCE ($\sqrt{\lambda}$) OF THE ATOMS OF ANDROSTERONE FROM THE 'BEST' PLANES

	$\gamma\lambda$ (Å)
Entire molecule	0·773
Steroid nucleus plus angular methyls	0·625
Steroid nucleus	0·269
A	0·227
*A*1	0·000
*A*2	0·001
*A*3	0·000
B	0·233
*B*1	0·000
*B*2	0·015
*B*3	0·000
C	0·232
*C*1	0·000
*C*2	0·008
*C*3	0·000
D	0·197
*D*1	0·000
*D*2	0·055
*A*3*B*1	0·030
*B*3*C*1	0·021
*C*3*D*1	0·077

The degree of planarity, as indicated by the root mean square (r.m.s.) distance of atoms from the 'best' planes (Fig. 2), improves as one considers smaller and smaller portions of the androsterone molecule. A clear demonstration of this is given by the gradual decline of the r.m.s. distances of atoms from: (1) the entire androsterone molecule ($\sqrt{\lambda} = 0{\cdot}773$ Å); (2) the steroid nucleus plus the angular methyl groups ($\sqrt{\lambda} = 0{\cdot}625$ Å); (3) the steroid nucleus by itself ($\sqrt{\lambda} = 0{\cdot}269$ Å).

Table 3. PERPENDICULAR DISTANCE (Å) OF INDIVIDUAL ATOMS FROM ONE OR MORE OF THE 'BEST' PLANES OF ANDROSTERONE*

	Entire molecule	Nucleus plus angular methyls	Nucleus	*A*	*B*	*C*	*D*	*A*2	*B*2	*C*2	*D*2	*A*3*B*1	*B*3*C*1	*C*3*D*1
*C*1	0·202	−0·519	0·199	−0·239				−0·001				−0·028		
*C*2	−0·076	−0·264	−0·017	0·214				0·001						
*C*3	0·366	−0·621	0·491	−0·199										
*C*4	−0·186	0·028	−0·053	0·218				−0·001						
*C*5	0·098	−0·233	0·169	−0·241	0·252			0·001				0·033		
*C*6	−0·353	0·321	−0·270		−0·225				0·015			−0·030		
*C*7	0·086	−0·093	0·110		0·201				−0·015				−0·021	
*C*8	−0·338	0·250	−0·383		−0·210	−0·228				−0·008			0·020	
*C*9	−0·088	−0·280	0·033		0·241	0·218			0·015				0·021	
*C*10	−0·425	0·206	−0·421	0·247	−0·258				−0·014			0·025		
*C*11	−0·253	−0·020	−0·379			−0·222				0·007			−0·021	
*C*12	0·314	−0·563	0·130			0·230				−0·008				−0·078
*C*13	−0·140	−0·006	−0·315			−0·244	0·257				−0·041			0·087
*C*14	0·250	−0·317	0·147			0·246	−0·275			0·008				0·065
*C*15	0·016	0·016	−0·090				0·176				0·040			−0·074
*C*16	0·493	−0·481	0·313				−0·016				−0·065			
*C*17	0·555	−0·657	0·337				0·141				0·066			
*C*18	−1·653	1·504	−1·839			−1·777	−1·804							
*C*19	−1·963	1·731	−1·958	1·782	−1·793									
*O*3	1·810	−2·060	1·933	−1·634										
*O*17	1·108	−1·253	0·836				0·540							

* Atoms lying between the origin of the unit cell and the plane have a negative value. Those on the opposite side of the plane from the origin have a positive value.

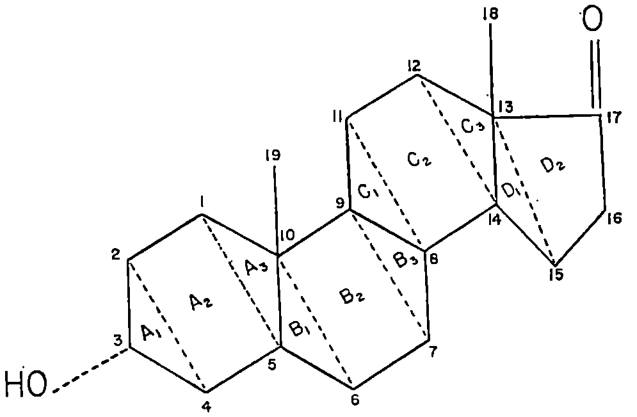

Fig. 1

If one considers the divisions and subdivisions of the steroid nucleus, it is apparent that the same relationship holds. The r.m.s. distances of atoms ($\sqrt{\lambda}$) from rings *A*, *B*, *C*, and *D* are 0·227, 0·233, 0·232 and 0·197 Å respectively. Atoms in the sub-portions of the ring system have the following r.m.s. distances: (1) those for *A*2, *B*2, *C*2 and *D*2 equal 0·001, 0·015, 0·008 and 0·055 Å, respectively; (2) those for the major-ring-division joins *A*3*B*1, *B*3*C*1 and *C*3*D*1 equal 0·030, 0·021 and 0·077 Å, respectively.

Information concerning side group configuration can be obtained from consideration of the perpendicular distances of individual atoms from the 'best' planes (Table 3). The hydroxyl oxygen attached to *C*3 is coplanar with respect to ring *A*. This is illustrated by the fact that the perpendicular distance of O_3 from the 'best' plane *A* is 1·634 Å, a distance which is nearly equal to the sum of the C_3–O_3 bond-length (1·444 Å) and the perpendicular distance of O_3 (0·199 Å) from 'best' plane *A*. The perpendicular distance of the keto oxygen (O_{17}) to the 'best' plane of ring *D* is 0·540 Å.

Table 4. DIHYDRAL ANGLES OF ANDROSTERONE

Planes	Dihedral angles (deg.)
AB	172
BC	175
CD	172
*A*1*A*2	133
*A*2*A*3	131
*A*3*B*1	175
*B*1*B*2	127
*B*2*B*3	132
*B*3*C*1	176
*C*1*C*2	132
*C*2*C*3	129
*C*3*D*1	168
*D*1*D*2	137

The C_{18} angular methyl group is axial with respect to ring *D* as evidenced by the fact that the perpendicular distance of C_{18} (1·804 Å) to the 'best' plane *D* is equal to the sum of the C_{13}–C_{18} bond-length (1·548 Å) and the perpendicular distance of C_{13} (0·257 Å) from 'best' plane *D*.

The C_{19} angular methyl group is axial with respect to ring *A* and ring *B* respectively. The perpendicular distance of C_{19} from 'best' plane *A* is 1·782 Å; from 'best' plane *B* it is 1·793 Å. In each case this distance is the sum of the C_{10}–C_{19} bond-length (1·541 Å) and the perpendicular distance of C_{10} to the 'best' planes (0·247 Å and 0·258 Å respectively).

That the angular methyls are on the same side of the molecule is shown by the agreement of signs (both negative) prefixing the perpendicular distances of the methyls to the plane of the entire molecule.

Consideration of the dihedral angles (Table 4) reveals the following additional steric information about androsterone.

(1) The *A*, *B* and *C* chairs are symmetrical as illustrated by their similar dihedral angles (*A*1*A*2 = 133°, *A*2*A*3 = 131°; *B*1*B*2 = 127°, *B*2*B*3 = 132°; *C*1*C*2 = 132°, *C*2*C*3 = 129°).

(2) The interchair which joins *A*3*B*1 and *B*3*C*1 is almost planar (dihedral angles equal 175° and 176°, respectively). The joint between chair *C* and ring *D* is less planar (*C*3*D*1 = 168°). The r.m.s. distances of atoms involved in the *A*3*B*1, *B*3*C*1 and *C*3*D*1 interchair joints substantiate these data (0·030, 0·021 and 0·077 Å, respectively).

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BIOCHEMISTRY

Optical Rotatory Dispersion of Liver Alcohol Dehydrogenase and its Complexes

WE have recently reported that the stability of liver alcohol dehydrogenase (LADH) towards denaturing agents, such as heat, hydrogen or hydroxyl ions, is greatly enhanced in the binary complexes with NADH* or NAD, and still more in the ternary complexes with coenzymes and inhibitors[1]. The reaction velocity of the free SH groups with PCMS was greatly reduced by complex formation, and the solubility of the enzyme in phosphate buffer increased considerably.

Furthermore, X-ray diffraction studies on crystals of LADH and its complexes showed definite differences between LADH or its binary complexes and the ternary complexes[2]. It was therefore of interest to study the optical rotatory dispersion of LADH and its complexes in solution, especially since the changes indicated here could very well be due to changes in α-helix content of the protein moiety. Even if the strict numerical relations between optical rotatory dispersion and α-helix content have so far been established only for polypeptides, the relative changes we are interested in here can be interpreted with considerable confidence.

The LADH used was prepared essentially according to Dalziel[3] with the omission of the chromatographic steps. The quality of the coenzymes used has been described previously[1]. The optical rotatory dispersion spectra were run on a partly modified Rudolph recording spectropolarimeter. The effective wave-length scale was recalibrated for the slit width used. The temperature was on all occasions 25·0° C. The solvent was a sodium phosphate buffer of ionic strength 0·1 and *p*H 7·0. The refractive index of the solvent was determined as a function of wave-length and the values obtained were used for calculation of Moffitt–Yang plots. The λ_0 value used was 2120 Å, which gave linear plots on all occasions.

The presence of NADH and NAD molecules respectively in the enzyme solutions produces a series of Cotton effects characteristic for the complexes formed[4]. These effects were located at the absorption maxima of the coenzymes. The effects at the NAD band at 260 mμ and the NADH bands at 260 and 340 mμ could be measured directly. Additional Cotton effects can be assumed to exist at lower wave-length. The partial rotatory contributions from these bands can be expressed as a series of correction terms. The magnitude of the contributions can be estimated and the measured values corrected, thus making it possible to calculate the Moffitt–Yang parameters. The detailed calculations will be published separately.

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* Abbreviations: NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; PCMS, *p*-chloromercuribenzene sulphonate.

Table 1. Moffitt–Yang Parameters of LADH Solutions at 25° C $\lambda_0 = 2120$ Å

	LADH in buffer	LADH 1·00% NADH 417 μM	LADH 1·00% NADH 300 μM isobutyramide 16·6 mM	LADH 1·00% NAD 330 μM pyrazole 8·3 mM
b_0	-95 ± 15	-119 ± 10	-185 ± 10	-180 ± 10
a_0	-30 ± 15	-49 ± 10	-65 ± 10	-60 ± 10

The estimated errors are mainly due to differences between different batches of enzyme. The error in b_0 values due to instrumental error could be estimated from repeated runs of the same solution. It never exceeded ± 5 units. The changes in a_0 values cannot be interpreted in detail as, in addition to the usual complications, it contains additional terms. The b_0 values can, on the other hand, be directly correlated with the protein part of the LADH. It is remarkable that the presence of NADH, which results in the appearance of the whole system of Cotton effects, has very little influence on b_0. In the case of ternary complexes, additions of pyrazole or isobutyramide, which do not introduce further Cotton effects in the long wave-length region, give rise to considerable increases in b_0 values. The magnitude of the increase is the same for the ternary complexes LADH–NADH–isobutyramide and LADH–NAD–pyrazole. Thus the evidence at hand strongly suggests that a definite change of protein conformation takes place when the ternary complex is formed. The absence of definite changes in b_0 for the binary complex does not of course imply that the protein is entirely unchanged, but only that the type of change to which b_0 values are sensitive is small or absent. As for the interpretation of the nature of the change visualized by the increase of b_0, the tentative assumption can be made that it represents an increase of α-helix content of the enzyme, or more correctly, an increase in any system of identical and weakly interacting groups[5].

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Stability of Bilirubin

CRYSTALLINE bilirubin changes with time as noted by Küster[1]. Newly recrystallized, it dissolves immediately and completely in chloroform. With aged preparations, when dissolved at room temperature, a certain amount of insoluble substance is observed which dissolves within a few seconds when boiled under reflux. This must be due to either an isomerization from bilirubin to dihydrobiliverdin—a displacement of the π electrons from the vinyl to the central methylene bridge[2]—or a transition between two crystalline phases, the less soluble having the highest thermodynamical stability.

Dissolved in pure chloroform, bilirubin is stable for hours even in diffuse daylight as a result of the intramolecular hydrogen bonds which stabilize the central methylene bridge[3].

Alkaline solutions of bilirubin, on the contrary, are very labile depending on alkalinity, the presence of metal ions and light. The intramolecular hydrogen bonds are broken and the central methylene bridge is adapted to dehydrogenation either by isomerization[2] or oxidation. This process is catalysed by metals as shown in Fig. 2 by adding zinc acetate or a metal-complexing reagent as EDTA (ethylenediamine tetraacetic acid). Metals are bonded to the pyrrolnitrogens in biliverdins[4] and consequently, in the presence of metals, the more linear bilirubin molecule is forced into the circular biliverdin (Fig. 1), that is, a double bond is introduced into the central bridge.

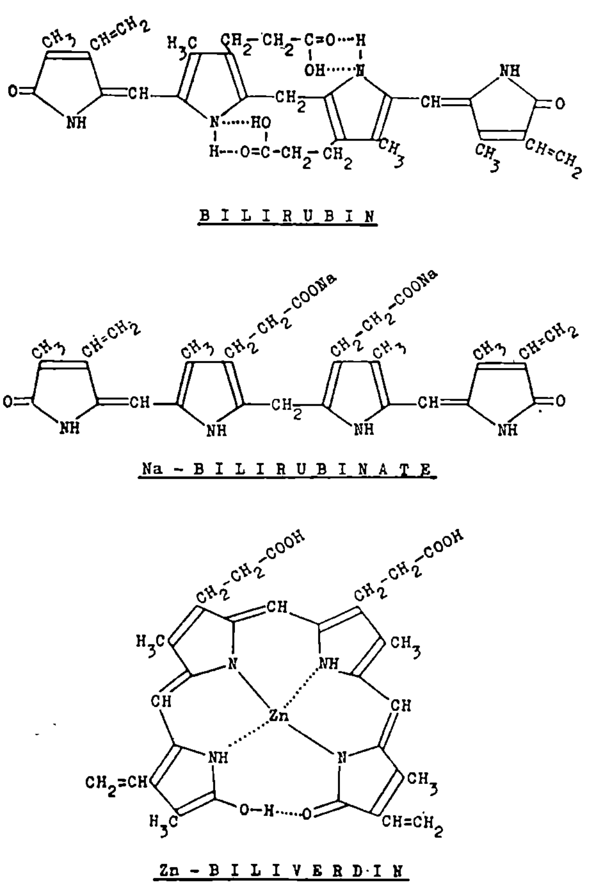

Fig. 1

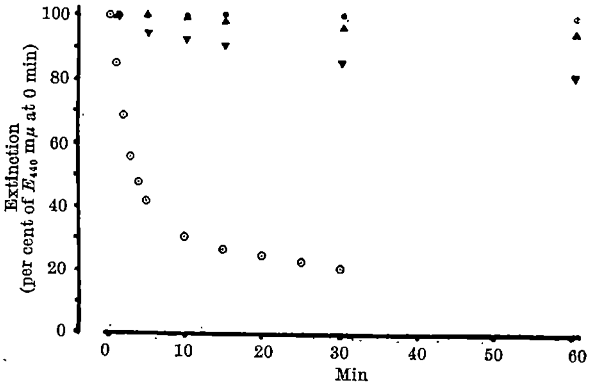

Fig. 2. ●, 2 g Na_2CO_3 and 2·5 mM EDTA/l. (pH 11·0); ▲, 2 g Na_2CO_3/l. (pH 11·0); ▼, 10 g Na_2CO_3/l. (pH 11·3); ⊙, 2 g Na_2CO_3/l. + zinc acetate (tracer quantity)

Alkaline solutions are stabilized also by human serum probably because of a re-establishment of the intramolecular hydrogen bonds as shown by the 'indirect' Hijmans van den Bergh's reaction observed with such solutions.

Daylight facilitates electron displacements and bilirubin absorbs strongly in light. In alkaline solutions the extinction coefficient is approximately 55,000 (440 mμ/M/l./cm).

Consequently, standard solutions of bilirubin in chloroform should be prepared by boiling under reflux. Alkaline standards are stabilized by adding a few mM EDTA per litre. The standard solutions should be kept in the dark. Concentrated solutions are more stable than diluted samples[5] and when human serum is added solutions of bilirubin are stable for many hours[5,6].

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Influence of the Injection of a Sucrose Solution on the Properties of Rat-liver Lysosomes

In work recorded here, rats were injected intraperitoneally with 8 ml. of a 50 per cent sucrose solution. They were killed 2 h after the injection. Some of the properties of the hepatic lysosomes were found to be deeply affected by the treatment, namely: (1) their density was reduced, as shown by a shift towards the regions of low density in the distribution curves of their hydrolases, following density equilibration in a glycogen gradient (Fig. 1); (2) their size was increased, as suggested by a fall of the sedimentation boundaries of their enzymes in the centrifuge tube, after incomplete sedimentation in a stabilizing gradient of aqueous sucrose (Fig. 2). On the other hand, the distribution of proteins, used as marker for mitochondria[1] and of particle-bound catalase, remained practically unaltered.

The observed changes may be explained by supposing that some of the injected sucrose is stored up in the hepatic lysosomes along with water required for their tonicity. After an injection of sucrose labelled with carbon-14, some of the sugar was found indeed to be taken up by the liver; using the differential centrifugation technique, according to de Duve *et al.*[2], we noticed that a significant portion of the radioactive material had sedimented with the mitochondrial fraction. Further analysis of this fraction by isopycnic centrifugation in a glycogen gradient made it clear that the distribution patterns of radioactivity and acid hydrolases were the same (Fig. 3). It appears very likely, therefore, that sucrose was indeed collected in the lysosomes.

If the rats are killed more than 2 h after the injection, the percentage of acid hydrolases present under unsedimentable form increases progressively in liver homogenates. This suggests that the filling of lysosomes by aqueous sucrose is going on for hours after the injection. By administering sucrose labelled with carbon-14, we were able to detect indeed an increase in the liver radioactivity up to 24 h after the injection. Becoming larger and larger, the lysosomes become more sensitive, too, to the homogenizing procedure.

The related changes in the properties of lysosomes are reversible. A gradual return to normal was observed during the days which followed the treatment.

These striking modifications of the physico-chemical properties of liver lysosomes are likely to be related to those observed after injection of 'Triton *WR*1339' to the rat[3,4]; like this substance, sucrose stored up in the lysosomes is probably also not affected by the lysosomal enzymes—among them no invertase has been described—so that accumulation can easily be observed; moreover, the small molecular weight of sugar greatly enhances the osmotic content of the granules and would explain their rapid swelling *in vivo* after an injection.

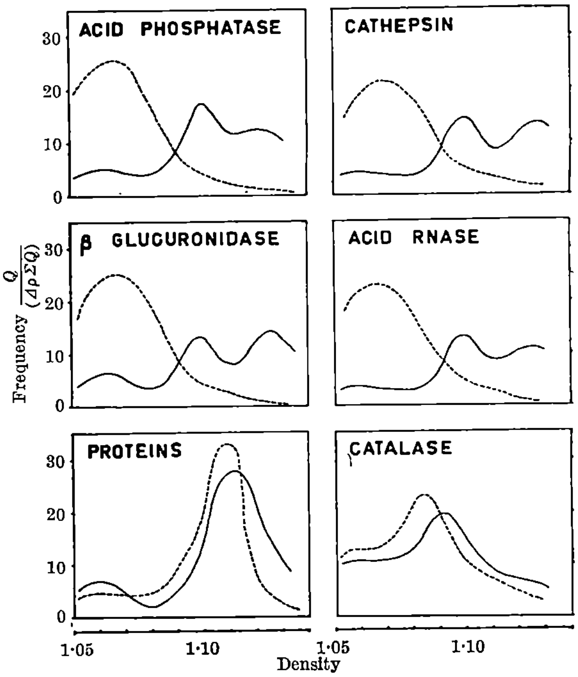

Fig. 1. Distribution of reference enzymes and proteins after centrifugation (for 2·5 h at 39,000 r.p.m. in head *SW*39 of the Spinco model *L-HV* preparative ultracentrifuge) of a mitochondrial fraction of rat liver, through a glycogen gradient extending from 0 to 20 per cent (w/w) with as solvent 0·264 molal sucrose in water. This experiment was performed according to a method similar to that followed by Beaufay *et al.* (ref. 6). Enzyme determinations were made according to the techniques of de Duve *et al.* (ref. 2) and Beaufay *et al.* (ref. 1) for the hydrolases and of Baudhuin *et al.* (ref. 7) for catalase. Proteins were measured by the method of Lowry *et al.* (ref. 8). For the drawing of the curves, the average frequency of the components was calculated for each fraction: $\frac{Q}{\Delta\rho\Sigma Q}$, where Q represents the activity found in the fraction, ΣQ the total recovered activity and $\Delta\rho$ the increment of density from the top to the bottom of the fraction; these values were plotted against density in histogram form and the smoothest possible continuous curve was fitted to this histogram. For calculation details see Beaufay *et al.* (ref. 6). ——, preparation from a control animal; – – –, preparation from an animal killed 2 h after the injection of sucrose

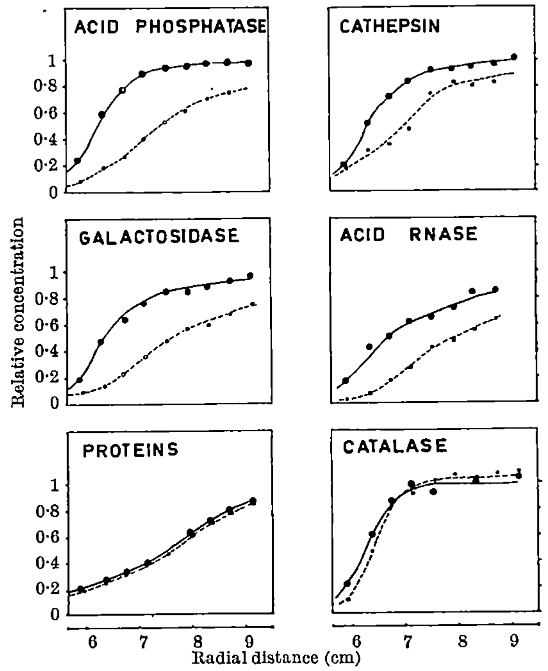

Fig. 2. Sedimentation boundaries of particle-bound enzymes and proteins after centrifugation (for 8 min at 6,000 r.p.m. in head *SW*39 of the Spinco model *L-HV* preparative ultracentrifuge) of a mitochondrial fraction of rat liver, through a linear gradient of 0·25–0·5 M sucrose in water. The experimental technique was similar to the method of Beaufay *et al.* (ref. 1). ——, preparation from a control animal; – – –, preparation from an animal killed 2 h after the injection of sucrose. The relative concentration (ordinate) is the ratio of the observed activity to that which had been found if the enzyme had been homogeneously distributed throughout the whole gradient

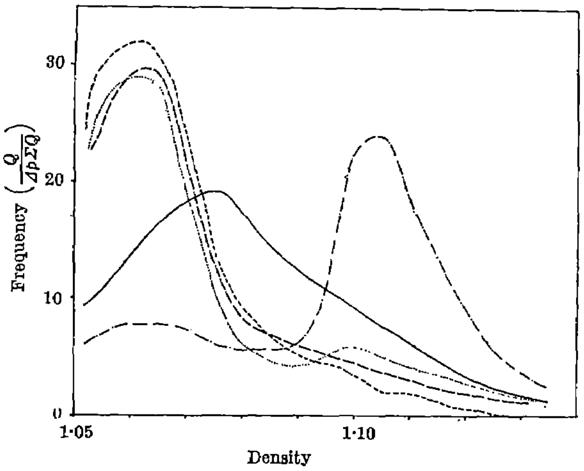

Fig. 3. Distribution of reference enzymes, proteins and radioactivity. Experimental conditions were as in Fig. 1. The mitochondrial preparation comes from a rat injected with 4 g of sucrose dissolved in 8 ml. of distilled water to which was added 20 μc. of sucrose labelled with carbon-14, and killed 2 h after the injection. Radioactivity was measured in a liquid scintillation system using an Ekco scintillator. ------, acid phosphatase; ———, acid ribonuclease; . . . ., radioactivity; –·–·–, proteins; ——, catalase

Further, taken together with the morphological observations of Brewer and Heath[5], our observations on the consequences of a hypertonic sucrose injection in the rat strongly support the hypothesis that vacuolation of the liver cell, produced by this treatment, is initiated by an accumulation of sucrose in the lysosomes.

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Effect of Glucose on Insulin Secretion *in vitro* by Rabbit Pancreas pre-incubated with β-Hydroxybutyrate

WE have shown[1] that β-hydroxybutyrate does not induce secretion of insulin *in vitro* as does glucose or citrate. Newsholme, Randle and Manchester[2] have observed a diminution of uptake of glucose (perfused heart) in the presence of β-hydroxybutyrate, acetoacetate and octonoate. They conclude that these substrates inhibit uptake of glucose by inhibiting the intracellular phosphorylation of glucose by hexokinase and the phosphofructokinase reaction. According to Passonneau and Lowry[3] phosphofructokinase is responsive to the balance between ~P expenditure and ~P formation. In view of those results we decided to ascertain the effect of glucose on insulin secretion *in vitro* by rabbit pancreas pre-treated with β-hydroxybutyrate.

A piece of rabbit pancreas weighing 250 mg was incubated in Krebs–Henseleit buffer containing β-hydroxybutyrate 1×10^{-12} M plus glucose 0·6 mg/ml. and another piece of the same weight (± 1 mg) was incubated in the same buffer containing glucose 0·6 mg/ml. for 30 min at 37° C. After washing, the pieces of pancreas were incubated for 15 min in buffer containing 3 mg/ml. glucose. Extraction of insulin from the buffer was performed according to the method of Grodsky and Tarver and estimated by the technique of epididymal fat tissue[4]. The effect of insulin was expressed as uptake of glucose and the statistical analysis of the results was performed using the paired observations method[5].

Table 1. EFFECT OF GLUCOSE (3 MG/ML.) ON INSULIN SECRETION *in vitro* BY RABBIT PANCREAS PRE-INCUBATED WITH β-HYDROXYBUTYRATE (1×10^{-3} M)

Metabolites	Mean glucose uptake (mg/100 mg tissue/4 h)
β-Hydroxybutyrate + glucose (25)	0·352
Glucose (25)	0·517
	± 0·022* $P = 0·01$

* *S.E.M*, the numbers in brackets give the number of experiments.

As can be seen from Table 1, the insulin extracted from the incubation buffer, in which was a piece of pancreas pre-incubated with β-hydroxybutyrate, is significantly less than the control. The effect of glucose as stimulus for insulin secretion *in vitro* seems to be reduced by pre-treatment with this ketone body.

We thank Dr. S. Ochoa for his advice. This work was supported in part by a grant from Eli Lilly Co.

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Co-precipitating Properties of 3 *S* Fragments derived from Horse Antitoxins

NISONOFF *et al.*[1,2] showed that precipitating fragments of rabbit antibody obtained by peptic digestion (5 *S* fragments) could be transformed by reductive cleavage into non-precipitating univalent 3 *S* fragments. These results have been taken into account for the new scheme developed by Porter[3] for rabbit γ-globulin structure.

We prepared 5 *S* fragments by peptic digestion[4–6] of specific precipitate (diphtheria toxin + β-2-type horse antitoxin) using 0·08 μg of crystallized pepsin (pH = 2·8; 0·066 M phosphate buffer, 30 min; 20° C). After neutralization and equilibration with 0·02 phosphate buffer pH = 7·8 containing 0·011 M sodium chloride, the soluble fraction is passed through a DEAE-cellulose column. The first part of the non-adsorbed fraction is collected: it is 70–90 per cent precipitable by antigen (diphtheria toxin 3. 100 Lf units*/mg nitrogen) and has a sedimentation constant of about 5 *S*. It will be abbreviated here as *P* (precipitating 5 *S* fragment). Reduction of *P* is not so easy as the reduction of rabbit 5 *S* fragment[1]. The reduction is performed with 0·100 β-mercaptoethanol for 18 h at 20° C and pH = 7·5. After alkylation (with 0·125 M monoiodacetamide) and dialysis, we obtain a fragment having a sedimentation constant equal to 3·0 *S* (value extrapolated to zero concentration of protein). It can be kept at − 15° C without alteration: the product does not aggregate or precipitate spontaneously.

The neutralizing activity is retained, being equal to 80–90 per cent of the activity of *P*, without change of dilution ratio as expressed in Cinader's notation[6]. The

* Flocculation units.

3 S fragment will be designated as i (inhibitory 3 S fragment); i inhibits the passive hæmagglutination test of antigen-coated erythrocytes at a relatively low concentration (0·01 I.U./ml.). We obtained fresh results concerning the inhibitory action of 3 S fragment 'i' on the precipitation reaction. (By itself 'i' does not give any precipitation with antigen.) We observed that the behaviour of the 'i' fragment depends on the P/i ratio. 'i' fragments have been labelled with iodine-131 (ref. 7) for these experiments (i^*).

Mixtures containing different proportions of P and i^* have been used. Toxin is first mixed with i^*, then P is added. The proportion of antigen (toxin) is equal to the sum of antibody ($P+i^*$) used. This point is an important one (see following).

It can be seen (Fig. 1) that for P/i^* lower than or equal to 0·50, one observes a total inhibition of the precipitation. This value of P/i^* can be called 'critical P/i value'. (This result is in accordance with classical observations in this field. The 'critical P/i value' found by Porter[8] for rabbit antipolysaccharide antibodies is much lower: 0·04 (calculated from Fig. 4, ref. 8).) For a ratio P/i^* between 0·5 and 1·0, there is only a partial inhibition: the total amount of precipitated nitrogen is lower than that corresponding to the precipitating antibody (P) alone present in the mixture. Nevertheless, a part of the specific precipitate contains the 'inhibitory' monovalent fragment i^*, as shown by precipitate's radioactivity. For a ratio P/i^* between 1 and 2 there is no apparent inhibition: the total amount of precipitated nitrogen is higher than the amount corresponding to the precipitating antibody (P), but the i^* fragment is only partially co-precipitated. For a sufficiently high value of P/i^* (more than 4) all the monovalent 3 S i^* fragment is co-precipitated with P. The influence of the proportion of antigen used is more apparent when a complete precipitation curve is plotted for a mixture corresponding to a P/i^* ratio, equal to 5. We have by these means observed that the presence of the monovalent 3 S fragment in the mixture has several effects. (1) It enhances the length of the inhibition zone in antibody excess (this inhibition zone is one of the characteristic features of horse's β-2-antitoxin). (2) If the amount of antigen added is lower than the amount equivalent to $P+i^*$, the co-precipitation of i^* is incomplete. (3) If the amount of antigen is sufficiently large, the whole 3 S fragment is co-precipitated. It is possible to calculate, from the curve, the minimal amount of antigen which may be used for this purpose and which can be said to be 'equivalent to $P + i^*$'.

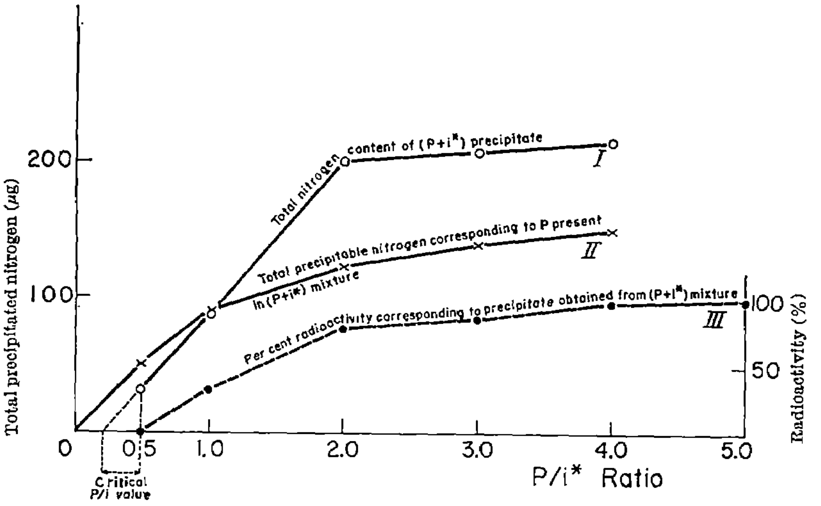

Fig. 1. Influence of the P/i^* ratio with $T=P+i^*$. Curve I corresponds to the total nitrogen found in the precipitate obtained by adding a constant amount of antigen (100 *Lf units of diphtheria toxin) to a constant amount of* $P+i^*$ (100 I.U. of antitoxin), containing variable proportions of P and i^*. Curve II is the total nitrogen which could be obtained by adding to P the equivalent amount of antigen (T) in the absence of i^*. Curve III corresponds to the per cent of radioactivity (i^*) recovered in the specific precipitate. T, pure diphtheria toxin. P, purified 5 S antibody fragment. i^*, ^{131}I-labelled 3 S fragment

These results show that it will now be possible to devise for any system a quantitative test for measuring non-precipitating fragments of antibody. This test can be probably applied to the quantitative determination of residual antibody activity of A or B chains, provided they are obtained in an aqueous soluble form.

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Stimulation of Steroid C-11β Hydroxylase Activity in Adrenal Homogenates by Cyclic 3′,5′-Adenosine Monophosphate

Steroid biosynthesis in the mammalian adrenal cortex is dependent on the activity of a number of complex enzyme systems, including several hydroxylases which require NADPH as cofactor[1]. NADPH is continuously generated from NADP within the adrenal cortex by the action of several dehydrogenases acting on appropriate substrates[2]. Of the latter, glucose-6-phosphate appears to be the most important[3]. Haynes, Koritz and Péron[4] demonstrated that adenosine-3′,5′-monophosphate (cyclic 3′,5′-AMP) enhanced the production of corticosterone from endogenous precursors by surviving rat adrenal sections. Since cyclic 3′,5′-AMP also stimulated phosphorylase activity in adrenal slices, the effect of the nucleotide on corticosteroidogenesis was apparently related to increased provision of glucose-6-phosphate[5]. Of added significance was the observation by Haynes[5,6] that ACTH resulted in the accumulation in adrenal slices of cyclic 3′,5′-AMP, which presumably was responsible for the resulting stimulation of corticosteroidogenesis. Attempts to demonstrate a stimulatory effect of cyclic 3′,5′-AMP on corticosteroidogenesis from endogenous substrates in adrenal homogenates have hitherto been unsuccessful[4,7]. In the investigations recorded here, addition of this nucleotide to rat adrenal homogenates markedly stimulated C-11β hydroxylation of added progesterone and 11-deoxycorticosterone, but appeared to have no effect on C-21 hydroxylation of progesterone.

Adrenal glands were obtained under light 'Nembutal' (sodium pentobarbital) anæsthesia from young male rats (120–150 g) of an inbred Sprague–Dawley strain. The glands were dissected free of fat and connective tissue, cut into eighths, and subjected to a preliminary incubation for 1 h in a medium composed of 109 parts 0·154 M sodium chloride and 21 parts 0·154 M sodium bicarbonate[7]. Preformed steroid hormones were removed by this procedure. The adrenals were then blotted, weighed and homogenized for 0·5 min in cold 0·154 M potassium chloride. The homogenate was diluted to a concentration of 75 mg wet weight of tissue per ml. The final incubation mixture contained 0·4 ml. of homogenate (30 mg adrenal tissue), 40 μmoles $NaHCO_3$, 7 μmoles Na glucose-6-P, and 2·1 μmoles NaNADP, made up to 2 ml. with 0·154 M KCl. The presence of varying amounts of (4-^{14}C)-progesterone or (4-^{14}C)-11-deoxycorticosterone, cyclic 3′,5′-AMP, and other additives is indicated in the results. All incubations were carried out in a Dubnoff metabolic incubator at 37·5° under an atmosphere of 95 per cent oxygen–5 per cent carbon dioxide.

After incubation, the media were each extracted three times with 6 ml. of methylene chloride. Total radioactivity recovered from the incubation mixture was determined on an aliquot of the extract by a scintillation counting procedure[8]. Purification of the steroidal products was accomplished by partition between 70 per cent ethanol and petroleum ether. Finally, the steroid residue was dissolved in ethyl acetate/methanol (1 : 1) for analysis by thin-layer chromatography. The chromatographic system most commonly used was system *L* of Lisboa[9], utilizing chloroform/ethanol (19 : 1). Plates, 2 in. × 8 in., were coated with a 250μ layer of 'Silica Gel *G*' containing 2·5 per cent zinc silicate to provide a fluorescent background against which the separated steroids could be visualized more readily by their absorption of ultra-violet light[10]. The chromatogram was allowed to develop at 25° until the solvent front had ascended 15 cm. Appropriate standards were run alongside each incubation sample. The plate was dried, photographed in ultra-violet light, then analysed for radioactivity on the Packard chromatogram scanner, model 7200. Recovery of radioactive steroids carried through the extraction procedure averaged 80 per cent[11]. Additional methods used for the separation and identification of the steroidal incubation products will be described in detail[11].

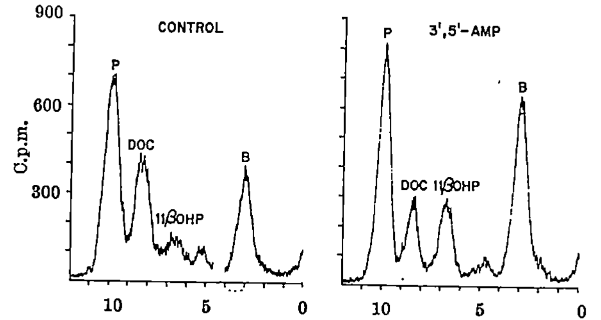

Fig. 1. Radioactivity scanning patterns of thin-layer chromatograms, showing corticosteroids formed from (4-^{14}C)-progesterone in rat adrenal homogenates. The incubation medium (2 ml.) contained $16{\cdot}2 \times 10^{-2}$ μmoles progesterone (0·05 μc.) in 0·0125 ml. of 95 per cent ethanol; 8 μmoles of cyclic 3′,5′-AMP were added where indicated. Chromatographic origin is represented by 0 on abscissa.
P, progesterone; DOC, 11-deoxycorticosterone; 11βOHP, 11β-hydroxyprogesterone; B, corticosterone

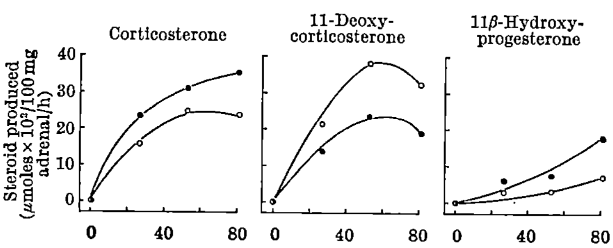

Fig. 2. Influence of cyclic 3′,5′-AMP on conversion of (4-^{14}C)-progesterone to corticosteroids by rat adrenal homogenates. In each case, 0·05 μc. of steroid was present in 0·0125 ml. 95 per cent ethanol per 2 ml. of medium. Results are based on percentage recovery of added radioactivity in each metabolite after incubation for 15 min. Open circles, controls; solid circles, 8 μmoles of cyclic 3′,5′-AMP added

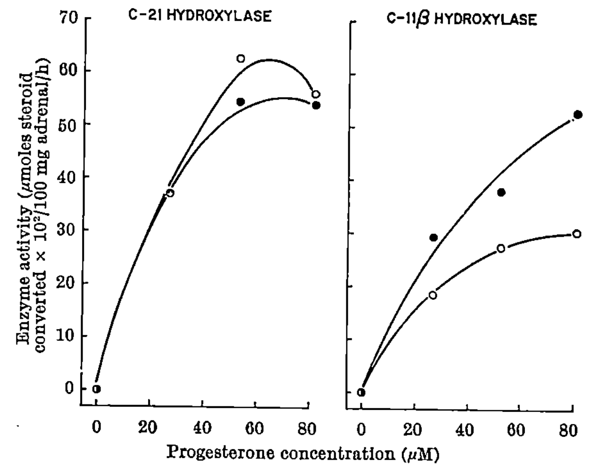

Fig. 3. Selective influence of cyclic 3′,5′-AMP on steroid hydroxylations in rat adrenal homogenates. See legend to Fig. 2 and text for explanations

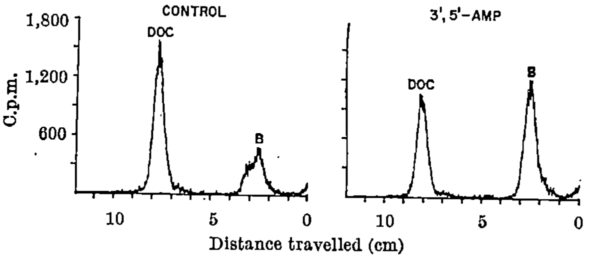

Fig. 4. Radioactivity scanning patterns of thin-layer chromatograms, showing conversion of (4-^{14}C)-11-deoxycorticosterone to corticosterone in rat adrenal homogenates. The incubation medium (2 ml.) contained $21{\cdot}0 \times 10^{-3}$ μmoles 11-deoxycorticosterone (0·05 μc.) in 0·0125 ml. of 95 per cent ethanol; 8 μmoles of cyclic 3′,5′-AMP were added where indicated. See legend to Fig. 1 for additional explanations

In incubation periods of 15-min duration the major products formed from (4-^{14}C)-progesterone were 11-deoxycorticosterone and corticosterone (Fig. 1, control). In some instances, measurable quantities of 11β-hydroxyprogesterone were also formed. The influence of varied amounts of added progesterone on the production of these corticosteroids is shown in Fig. 2 (open circles). Although the conversion to corticosterone and 11-deoxycorticosterone reached a maximum at 50 μM progesterone, the formation of 11β-hydroxyprogesterone tended to increase with additional substrate. The transformations involved may be represented by the following scheme:

$$\begin{tikzcd}
\text{progesterone} \arrow[r, "\text{C-21 hydroxylase}"] \arrow[d, "\text{C-11}\beta\text{ hydroxylase}"'] & \text{11-deoxycorticosterone} \arrow[d, "\text{C-11}\beta\text{ hydroxylase}"] \\
\text{11}\beta\text{-hydroxyprogesterone} & \text{corticosterone}
\end{tikzcd}$$

The activity of the C-21 hydroxylase enzyme system may be estimated from the combined rates of formation of 11-deoxycorticosterone and corticosterone. Similarly, overall C-11β hydroxylase activity can be measured from the combined rates of formation of 11β-hydroxyprogesterone and corticosterone. In incubation studies of longer duration than 15 min, additional products were formed, complicating estimation of enzymatic activities.

Cyclic 3′,5′-AMP had a striking effect on the pathway of progesterone utilization (Fig. 1). Corticosterone formation was markedly enhanced at the expense of 11-deoxycorticosterone; concomitantly, the production of 11β-hydroxyprogesterone was doubled (Fig. 2, solid circles). The net effect of 3′,5′-AMP was a striking and selective stimulation of overall steroid C-11β hydroxylase activity (Fig. 3). This action of the cyclic nucleotide was accentuated at the highest concentration of progesterone. No effect on endogenous steroid utilization was noted[4,7]. Under the conditions of the experiments described here, steroid C-21 hydroxylase activity appeared to be maximal at about 50 μM progesterone (Fig. 3). However, the lack of responsiveness of this enzyme system to cyclic 3′,5′-AMP was not due to saturation with substrate. Thus, even at the highest substrate-level C-21 hydroxylase activity was stimulated about 35 per cent on addition of 0·1 ml. fresh rat serum to the incubation medium. Homologous serum has previously been shown to activate C-21 hydroxylation of added progesterone in adrenal homogenates[11] and microsomes[12]. In separate experiments, conversion of (4-^{14}C)-11-deoxycorticosterone to corticosterone was enhanced by cyclic 3′,5′-AMP (Fig. 4); 5′-AMP, the degradation product of 3′,5′-AMP, was only half as effective. ATP (2 mM) plus Mg^{++} (3 mM) also stimulated C-11β steroid hydroxylations in the presence of either substrate. This finding raised the possibility that adenyl cyclase was active in adrenal homogenates.

The selective stimulation of C-11β hydroxylations in adrenal homogenates by cyclic 3′,5′-AMP may have been brought about via enhancement of glucose-6-phosphate formation from glycogen and a resultant increase in NADPH generation. Although stimulation of NADPH generation in the adrenal cortex would be expected to accentuate all steroid hydroxylations, recent investigations from this laboratory[11] have shown that concentrations of NADPH-generating system in excess of the requirement for maximal production of corticosterone from progesterone selectively enhanced C-11β hydroxylation of this substrate. The mechanism of this phenomenon is not clear but may be related to a differential requirement for NADPH by mitochondrial C-11β hydroxylase and microsomal C-21 hydroxylase enzyme systems or to some functional association between the NADPH-generating system and adrenal mitochondria[11].

These observations may help in the elucidation of the mechanism of action of ACTH. A selective action of ACTH on C-11β hydroxylations in the adrenal cortex has been reported by Grant[13]. The investigations recorded here indicate that this selective effect of ACTH on steroid hydroxylations may be associated with rapid generation of NADPH in response to elevated levels of cyclic 3′,5′-AMP.

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Relationship between Pyrophosphate Content and Oxytetracycline Labelling of Bone Salt

HEATING of bone to 325° C has been shown to induce the formation of pyrophosphate[1-3]. The quantity of pyrophosphate so formed is greater in the epiphysis than in the diaphysis and greater in the younger than in the older animal, and is therefore presumably greater in newly laid down than in older bone salt[1]. The question to be investigated is whether pyrophosphate-yielding mineral of new bone has specific chemical characteristics. There is much evidence which suggests that the tetracyclines are deposited in high concentrations in newly formed bone salt[4]. This communication demonstrates that the tetracycline labels the form of the bone salt that yields pyrophosphate in large amounts after heating.

Two New Zealand rabbits were injected with 25 mg of oxytetracycline intravenously and killed 24 h later. The long bones of the hind quarters were excised and ground in a hand coffee mill. The resulting small particles were separated into two groups by means of ultra-violet light and a dissecting microscope. The particles that fluoresced brightly were picked out with the forceps and placed in one crucible while those that had no visible fluorescence were collected in another. Each crucible was heated to 325° C for 1 h in a muffle oven. The ortho- and pyro-phosphates obtained from these samples were separated by the method of François[5]. The resin used was 'Bio-Rad *AG* 1 × 8, 200–400 mesh'. Pyrophosphate was hydrolysed by heating for 30 min in 1 N hydrochloric acid at 100° C, and the orthophosphate was determined by the Fiske–Subbarow method[6]. The results, summarized in Table 1, clearly demonstrate that the fluorescent particles contain the mineral with the greatest potential for forming pyrophosphate. This corroborates the observation that both the tetracycline reactive and pyrophosphate-yielding minerals are found in less than 1 per cent of the bone salt that is the exchangeable, reactive, labile or metabolic fraction. The exact composition and structure of this fraction are not known, but the following are possibilities that have been considered in the literature: monetite, brushite, octacalcium phosphate, hydrated tricalcium phosphate, calcium-deficient apatite. New observations with new methods may be made using the foregoing method of labelling and separation of new mineral.

Table 1. PYROPHOSPHATE CONTENT OF BONE WHICH DOES AND WHICH DOES NOT BIND WITH OXYTETRACYCLINE

Sample	Rabbit (approximate age)	$\frac{\text{Pyrophosphate} \times 100}{\text{ortho- + pyro-phosphate}}$
Oxytetracycline-labelled bone	1–2 yr.	10·3
	6 mo.	15·8
Non-labelled bone	1–2 yr.	3·6
	6 mo.	5·0

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Effect of Nicotinic Acid on Sterol Biosynthesis in *Saccharomyces cerevisiae*

In 1955, Altschul *et al.* reported[1] that large doses of nicotinic acid, but not of its amide, decreased serum cholesterol-levels in man and rabbit. This finding has been confirmed by many workers[2]. Despite several investigations of mechanisms, however, the locus of the nicotinic acid effect remains unknown[3,4]. We wish to report an effect of high concentrations of nicotinic acid on sterol biosynthesis in yeast. This effect may account for the hypocholesteremic action of nicotinic acid.

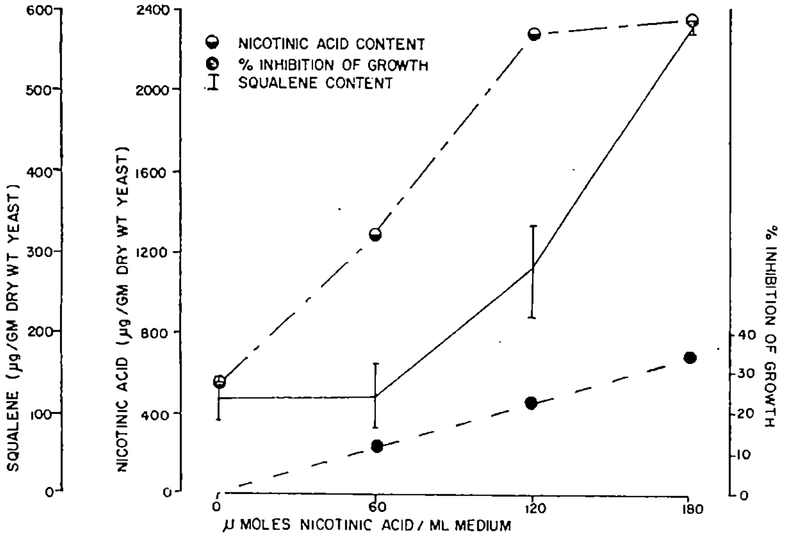

Fig. 1

Saccharomyces cerevisiae UT-139, a prolific producer of sterols[5], was grown at 25° C, with gentle stirring, in Fernback flasks containing 500 ml. medium. The medium was prepared according to Atkin[6], with the addition of 0·068γ biotin/ml. The initial *p*H of all media was 5·5–5·6. Nicotinic acid and mevalonic acid-2-^{14}C were added as in Fig. 1 and Table 1. An elevated or reduced oxygen tension was obtained by bubbling air or nitrogen into the medium and carbon dioxide was collected in 2 N sodium hydroxide.

Cells were collected at given times by centrifugation and washed three times with cold 0·85 per cent saline. Dry weight was determined on aliquots of cells dried at 85° C. Lipids were extracted from the wet cells either by repeated grinding with sea sand and Folch reagent[7] or by saponification[8]. Squalene and sterols in the non-saponifiable fraction were quantitatively determined by gas liquid chromatography. Cholestane or allopregnane-3,20 dione was the internal standard. The column, 6 ft. by 0·25 in. internal diameter silanized glass U tube, contained 2 per cent '*SE*-30' coated on 80–100 mesh 'Diaport *S*' and was housed in an F. and M. model 609 flame ionization instrument. For determination of specific activities, squalene and sterols were isolated by silicic acid thin layer chromatography, using hexane/ether/acetic acid, 80 : 20 : 1·5, as the developing solvent. The radioactivity taken up by whole yeast cells was determined in a flow-gas counter and corrected to infinite thinness. Lipid fractions and $^{14}CO_2$ in hyamine[9] were counted in a Packard liquid scintillation spectrometer. Nicotinic acid was determined by microbial assay[10].

It was observed (Fig. 1) that the squalene content of yeast cells increased almost linearly as the nicotinic acid concentration in the medium was increased. This effect was preceded by elevation of nicotinic acid levels in the cells and associated with growth inhibition. In these and other experiments (Table 1), a small depression in total sterol-levels and a marked fall in lanosterol content were noted when nicotinic acid was present in the medium. This suggested that nicotinic acid was inhibiting the conversion of squalene to sterols at the squalene oxidocyclase step[11].

To test this hypothesis further, the incorporation of acetate-1-^{14}C and mevalonic acid-2-^{14}C (MVA) into non-saponifiable lipid was studied. In the MVA experiment, cells were grown under both elevated and reduced oxygen tensions. The latter condition was used because it was thought that nicotinic acid might magnify the inhibitory effect of anaerobiosis on squalene cyclization. In the presence of nicotinic acid there was a marked fall both in total radioactivity taken up by cells and in the specific activity of the non-saponifiable lipid with little change in mass (Table 1). This result appears to be due to an effect of nicotinic acid on the uptake of mevalonate from the medium since the specific activity of whole cells, the radioactivity recovered in $^{14}CO_2$ per mg cell, and the fall in specific activity of non-saponifiable lipid were of the same order. By similar reasoning, it was concluded that reduced oxygen tensions in the absence of nicotinic acid stimulated MVA uptake. This novel blockade of mevalonate uptake by nicotinic acid seems to be independent of the effect on sterol biosynthesis. The latter, therefore, was evaluated by comparing the specific activities of given non-saponifiable lipids after correction for the penetration effect.

The results (Table 1) clearly demonstrated that in the presence of nicotinic acid and MVA, under both oxygen tensions, squalene mass and total radioactivity increased, while sterol mass and total radioactivity decreased. Similar distribution of radioactivity in lipids was noted with acetate-1-^{14}C as substrate, although with this substrate no effect of nicotinic acid on uptake by cells

Table 1. Effect of Nicotinic Acid (180 μmoles/ml. Medium) on the Uptake and Incorporation of Mevalonic Acid 2-^{14}C into Yeast Cells

Exp. No.	Growth conditions		Penetration effect Radioactivity in fractions				Biosynthetic effect Mass and radioactivity in lipids				
	Nicotinic acid	Oxygen tension	*A* Yeast cell* (d.p.m./g)	*B* $^{14}CO_2$ (d.p.m./g)	Non-saponifiable lipid		Squalene		Sterol†		Lanosterol mg/g
					Spec. act. (d.p.m./mg)	% total act.	mg/g	d.p.m./g	mg/g	d.p.m./g	
1	–	Elevated	136,000	718	2,731	39·0	0·126	459	19·3	70,368	3·6
2	+	Elevated	57,000	308	647	19·3	1·285	4,925‡	16·3	11,977‡	2·3
3	–	Reduced	821,250	1,666	24,963	41·7	2·059	162,157	11·6	246,054	4·0
4	+	Reduced	51,139	80	904	47·1	6·565	218,231‡	5·6	68,298‡	2·7

Mevalonate was present as the DBEB salt; 10 μc./500 ml. medium. Yeast cells were gathered during the log phase (controls: 15 h; nicotinic acid inhibited: 40–50 h, depending on oxygen tension).

* The efficiency factor (20 per cent) was based on the value given by 5 mg of $Ba^{14}CO_3$ ($3{\cdot}01 \times 10^{-1}$ μc./mM).

† Sterols measured include zymosterol, ergosterol, two unidentified sterols and lanosterol.

‡ Specific activity is corrected for normal penetration of isotope by the factor: $(A+B)_{\text{control}}/(A+B)_{\text{nicotinic acid}}$.

was observed. These results strengthen the view that nicotinic acid inhibits the cyclization of squalene.

The general mechanism of sterol biosynthesis in yeasts is similar to that of cholesterol biosynthesis in animals[12]. From rat liver slice experiments, Gamble and Wright concluded[13] that nicotinic acid inhibits cholesterol biosynthesis prior to MVA. However, these experiments have been criticized[4]. Since divergence of pathways in sterol biosynthesis in yeast as compared to animal tissues occurs after the formation of lanosterol[12], it is possible that nicotinic acid acts at the squalene cyclization stage rather than prior to MVA in animals. This possibility is under continuing investigation.

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Metabolism of Phenoxyacetic Acid by *Aspergillus niger* van Tiegh

A RECENT communication[1] reported the isolation of *o*-hydroxyphenoxyacetic acid as the main acidic product from the metabolism of phenoxyacetic acid by *A. niger*, using a replacement culture technique. Since these findings contrasted with our earlier work[2] in which both *o*- and *p*-hydroxyphenoxyacetic acids had been isolated, it was thought advisable to re-investigate the problem.

It has now been shown using both paper and thin-layer chromatography that while ortho-hydroxylation is predominant, all three hydroxyphenoxyacetic acids are in fact produced. While the *m*- and *p*-isomers have very similar R_F values in the three solvents used, their presence is clearly indicated by the characteristic colours of the diazo-coupled spots (*m*-isomer, bright yellow; *p*-isomer, salmon-pink). Exposure of the chromatogram to ammonia vapour changed the colour of the *m*-spot to magenta and that of the *p*-spot to bright blue. Furthermore, the relative positions of the spots are reversed in the two solvent systems used in the paper chromatographic examination.

Cultures of *A. niger* (Mulder strain, C.M.I. 31283) were grown in penicillin flasks and after 3 days the medium was replaced by a solution of phenoxyacetic acid (10^{-3} M) in aqueous disodium hydrogen phosphate (10^{-2} M). After 24 h at 26°, the substrate was poured off and concentrated in a cyclone evaporator, and the acidified concentrate continuously extracted with ether.

In a typical experiment, from 31 l. of fungal substrate initially containing 4·7 g phenoxyacetic acid, 3 g of sodium bicarbonate-soluble material was isolated. Separation was achieved by partition chromatography using silica gel as the supporting medium for the stationary aqueous phase which was buffered to *p*H 8, the mobile phase being *n*-butanol–chloroform (successively 60 : 40, 70 : 30, 80 : 20). After elution of unidentified material (0·9 g) and unchanged phenoxyacetic acid (1·42 g), the monohydroxyphenoxyacetic acids followed in the order *o*-(0·4 g), *m*-(0·02 g) and *p*-(0·1 g), details of their identification being given in Table 1.

Table 1. HYDROXYPHENOXYACETIC ACIDS

Substituent	R_F solvent A	R_F solvent B	Diazo coupling colour	λ_{max} *p*H 2	λ_{max} *p*H 11	Identification
Ortho-	0·53	0·60	Purple (orange centre)	275	290	Mixed m.p. U.V. and I.R.
Meta-	0·31	0·45	Bright yellow	273 278	286	Mixed m.p. U.V. and I.R.
Para-	0·27	0·51	Salmon pink	286	307·5	Mixed m.p. U.V. and I.R.

Solvent *A*: butanol/ethanol/3 N ammonia (4 : 1 : 5).
Solvent *B*: propanol/0·880 ammonia (7 : 3).

The difference between these findings and our earlier report[2] may reflect an improved separation technique or it may be due to a strain difference in *A. niger*. The culture used in the earlier work was Mulder strain, which had been in use at Long Ashton for some years previously; current practice is to renew the inoculum at frequent intervals from the Commonwealth Mycological Institute. The general pattern of hydroxylation appears to be relatively non-specific—resembling the metabolism of 2- and 4-chlorophenoxyacetic acids[3], but contrasting with results obtained in the metabolism of 2-naphthyloxyacetic acid[4] and with recent results obtained with 2,4-dichlorophenoxyacetic acid[5].

The metabolism of phenoxyacetic acid by a wild-strain of *A. niger* (kindly supplied by Dr. S. M. Bocks) has also been investigated. Thin-layer (propanol/0·880 ammonia (7·5 : 2·5) using 'Silica Gel *G*') and paper chromatographic examinations of acidic material obtained from four separate experiments using the replacement culture technique clearly reveal the presence of all three hydroxyphenoxyacetic acids, though the *m*- and *p*-isomers are present in a lower proportion than in the case of the Mulder strain.

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PHYSIOLOGY

Effect of Intraventricularly Injected Anti-cerebral Antibodies on the Histamine-like Substance and Potassium Content of Various Regions of the Brain of the Cat

IN an attempt to investigate the possible differential reactivity of various cerebral structures when exposed to the action of different brain region anti-sera, it has been shown that the injection of heterologous anti-caudate nucleus antibody into the lateral cerebral ventricle of the cat was followed by pronounced electrographic abnormalities, confined mainly to the caudate nucleus. Since the electrical activity of other, simultaneously investigated brain regions was not essentially modified, the alterations observed were interpreted as being strongly suggestive of the possibility that the anti-caudate antibody has an affinity for the homologous nervous tissue[1].

Pursuing this line of investigation and having in mind the well-known fact that, in general, an antigen–antibody reaction is associated with a release of histamine, 5-hydroxytryptamine and other biologically active substances, a series of experiments have been undertaken to test: (1) if a similar phenomenon occurs when the brain is attacked by an intraventricularly injected anti-brain antibody; (2) whether or not the differential reactivity of various brain structures to the action of an antibody produced against the tissue of specific cerebral region, as suggested by previous experiments, could be ascertained using as indicators the variations in the histamine and potassium contents. This communication refers to the effects of intraventricularly injected anti-caudate and anti-hippocampal antibodies on the histamine-like substance and potassium contents of the frontal cortex, caudate nucleus, hippocampus and the cerebrospinal fluid of the cat.

The methods used for the preparation of antigens, immunization and separation of γ-globulins from immuno rabbit sera were the same as those previously described[1,2]. Immune anti-caudate and anti-hippocampal γ-globulins were tested by diffusion in gel and complement fixation reaction in which saline extracts and lipids (ethanol extracted) of caudate and hippocampal tissues respectively were used as antigens. Thus, tested normal rabbit γ-globulins, obtained from the sera prior to immunization, did not show any serological activity.

Experiments were performed on unanæsthetized adult cats carrying the cannula inserted under aseptic conditions (at least five days earlier) into the same, anterior portion of the lateral cerebral ventricle according to the method of Feldberg and Sherwood[3]. Animals were divided into three groups: the first group was injected with the anti-caudate, the second with the anti-hippocampal and the third group of animals with the normal γ-globulin. Three injections of 0·3 ml. containing 40 mg of protein were administered successively with an interinjection interval of 60 min. One hour following the last injection, animals were anæsthetized ('Nembutal', 0·6 ml. of 5 per cent solution/kg), the cerebrospinal fluid obtained by means of sub-occipital puncture and immediately centrifuged at 2,500*g*. Then the animals were killed and tissue samples from the head of caudate nucleus, hippocampus and the frontal cortex were quickly dissected, taking care to obtain the equal amounts of tissue from both hemispheres of the brain.

A histamine-like substance from the brain tissue was extracted according to the method of Barsoum and Gaddum[4] as modified by Kwiatkowski[5], while that from the cerebrospinal fluid and blood was extracted according to Code's method[6]. The extracts were then assayed on the guinea pig ileum suspended in 3 ml. of oxygenated, magnesium-free, Tyrode's solution, containing $1/3 \times 10^{-7}$ of atropine. Contractions induced by brain extracts could be antagonized by adequate doses of antistin. All values for histamine-like substance refer to the base. The tissue for potassium determination was deproteinized and potassium in tissue filtrate, the cerebrospinal fluid and plasma estimated by means of an EEL flame photometer.

Table 1

Mean values

Substance injected*	N. caudatus		Frontal cortex		Hippocampus	
	Histamine-like subst. (μg/g)	Potassium (mg%)	Histamine-like subst. (μg/g)	Potassium (mg%)	Histamine-like subst. (μg/g)	Potassium (mg%)
Anti-n. caudatus γ-globulin	0·128	404	0·069	391	0·049	378
Normal rabbit γ-globulin	0·038	386	0·067	406	0·066	383
Anti-hippocampus γ-globulin	0·051	412	0·038	403	0·157	399

* Three injections of 0·3 ml. (total protein content 40 mg per cent), with an interinjection interval of 60 min, have been administered; animals were killed 1 h following the last injection.

Table 1*a*. ANALYSIS OF VARIANCE

Histamine-like substance in n. caudatus

Substance injected	Animal No. 1	2	3	4	5	6
Anti-n. caudatus γ-globulin	0·118	0·135	0·046	0·281	0·096	0·092
Normal rabbit γ-globulin	0·030	0·020	0·020	0·060	0·040	0·060
Anti-hippocampus γ-globulin	0·030	0·030	0·031	0·082	0·066	0·065

Summary of computations

Source of variation	Sum of squares	*D.F.*	Mean square	Ratio
Rows	28·345	2	14·172	7·36*
Columns	17·720	5	3·544	1·83
Error	19·239	10	1·924	
Total	65·300	17		

*P, 0·01.

Table 1*b*. ANALYSIS OF VARIANCE

Histamine-like substance in hippocampus

Substance injected	Animal No. 1	2	3	4	5	6
Anti-n. caudatus γ-globulin	0·017	0·064	0·012	0·031	0·133	0·038
Normal rabbit γ-globulin	0·022	0·091	0·115	0·053	0·060	0·055
Anti-hippocampus γ-globulin	0·251	0·100	0·201	0·152	0·120	0·121

Summary of computations

Source of variation	Sum of squares	*D.F.*	Mean square	Ratio
Rows	40·777	2	20·388	7·06*
Columns	3·271	5	654	0·229
Error	28·852	10	2·885	
Total	72·900	17		

*P, 0·01.

The results are presented in Table 1. They show, first of all, that in contradistinction to the normal rabbit γ-globulin, an immune anti-cerebral γ-globulin, when injected into the lateral cerebral ventricle of cat, produces an important increase of histamine-like substance in the brain. Furthermore, they demonstrate an apparently differential reactivity of various cerebral structures to the action of an antibody produced against the tissue of specific brain region. Thus, an intraventricular injection of anti-caudate antibody resulted in a significant increase of histamine-like substance in the tissue of the head of caudate nucleus, associated in some instances with its increase in the cerebrospinal fluid, but not (or at least not in a significant degree) in tissues of other cerebral structures investigated. On the other hand, the increased content of histamine-like substance following intraventricular injection of anti-hippocampal antibody was not evidenced in any other cerebral region investigated, but in the hippocampus itself and in some cases in the cerebrospinal fluid as well. Results were statistically tested using the method of variance analysis. The predominant increase of histamine-like substance in the caudate nucleus and hippocampus following the intraventricular injection of anti-caudate and anti-hippocampal antibodies, respectively, proved to be statistically significant (P = 0·01). Although in some of these experiments the values of histamine-like substance in the cerebrospinal fluid were remarkably high with respect to normal, the differences as expressed by the means were not found to be statistically significant. It should be added that in another series of experiments, in which the histamine content of both caudates and hippocampi was separately estimated, the increase of the amine was usually, but not necessarily, greater on the cannulated side of the brain. Potassium concentrations of investigated cerebral tissues as well as that of the cerebrospinal fluid were not essentially modified.

On the basis of electroencephalographic findings reported in a previous communication[1], the idea has been put forward suggesting the possible regional specificity of some high molecular weight substances present in morphologically and functionally differentiated parts of the central nervous system. Although the results of the present experiments challenge the discussion in many other directions, only the aspects pertaining to problems implied in the aforementioned hypothesis will be briefly commented. Despite the high degree of differentiation, various portions of the grey matter of the central nervous system could still be considered morphologically similar in that they are all composed of neurones, glia cells, unmyelinated and myelinated fibres, blood vessels, elements of the supposedly common ground substance, etc. Therefore, in view of the high specificity of serological reactions, so amply attested by Landsteiner[7], it can be assumed that immune γ-globulins used in our experiments contained a bulk of antibodies against the number of various components present in homogenates of given brain regions, including non-specific ones, common to the brain as a whole. Yet, as could be judged on the basis of variations in contents of histamine-like substance found in the present experiments, anti-caudate and anti-hippocampal γ-globulins were still able to 'recognize' the corresponding nervous structures. It could be inferred from these considerations that the predominant (if not selective) increase in the histamine-like substance content in the part of the brain attacked by the corresponding antibody indicates that in the antigen–antibody reaction are involved primarily those elements (most likely cellular, whatever they might be: neurones, glia or both) which determine the antigenic specificity of the given nervous structure.

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Possible Role of a Phospholipid in the Development of the Slow Muscle Contracting Activity of Human Plasma

THE presence of a substance in the *G*.2 fraction of human blood plasma with a slow contracting activity on plain muscle (S.M.C.) was established and the active principle has been isolated from the *G*.2 fraction and designated as *G* acid[1]. It is important to note that the same effect has been obtained from citrated human plasma and is best observed with dialysed plasma, since dialysis eliminates the quick contracting agents[2]. The chemical nature of *G* acid prompted a search for a possible precursor of this lipid-soluble unsaturated fatty acid (3-octadecenoic acid)[1].

Although the *G*.2 fraction stimulates the isolated guinea pig ileum, the γ-globulin and the albumin fractions are much less active on the same muscle preparation. The S.M.C. effect of human plasma and of *G*.2 fraction is reduced to about 50 per cent of the initial effect after the addition of calcium ions to a final concentration of M/40. The serum prepared by recalcification of the plasma is much less active on smooth muscle than the serum obtained after clotting the same plasma with human thrombin.

The formation of a slow muscle-contracting substance by alkaline hydrolysis of lecithin or by the action of phospholipase *A* present in cobra venom on lecithin[3], and the recognition of another variety of phospholipid in human serum identified as lysolecithin[4], suggested the investigation of the effect of human plasma on egg lecithin *in vitro*. The results obtained show that the addition of egg lecithin (0·25 per cent) to plasma causes a marked increase in its S.M.C. activity which is neutralized by the addition of calcium ions to a final concentration of M/40. Lecithin alone is inactive on the isolated ileum of a guinea pig at the same level of dosage. Incubation of the plasma with lecithin at 37° C or raising the *p*H of the plasma to 8 increases the slow contraction of smooth muscle produced by lecithin-plasma. Olive oil, cotton-seed oil and butter fat do not produce similar effects on smooth muscle when emulsified with human plasma and incubated at *p*H 8.

It may be interesting to note that the amount of egg lecithin which doubles the S.M.C. effect of human plasma is about 250 mg per cent of the plasma, a concentration which roughly corresponds to the concentration of the naturally occurring choline-containing phospholipids in human plasma. Parallel results were not obtained after incubation of egg lecithin with 5 per cent solution of human serum albumin in saline.

The S.M.C. effect produced by lecithin added to human plasma is not antagonized by atropine or by antazoline. Similar to the S.M.C. effect of the original plasma, the S.M.C. effect produced by added egg lecithin is not destroyed by an esterase-rich fraction of blood which destroys acetylcholine under the same conditions. The esterase-rich fraction is produced during the purification of human serum albumin by the method of Kekwick and Mackay[5].

Processing of human plasma with kaolin considerably decreases its effect on the isolated ileum of a guinea pig[6,7]. The S.M.C. effect produced by lecithin added to human plasma is completely abolished by the same treatment. The S.M.C. effect of such plasma is indistinguishable from the S.M.C. effect of kaolin-treated plasma without added lecithin.

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Brown Adipose Tissue and Thermoregulatory Heat Production in the Rat

THE role of brown adipose tissue in thermoregulatory heat production of the cold-adapted rat was inferred by R. E. Smith from the thermogenic activity of the interscapular fat *in vitro*[1] and the anatomical distribution of brown adipose tissue in the body[2]. The thermogenic role of brown fat has been directly demonstrated in the hibernating hamster by measuring temperatures at various sites in the body[3], and by measuring simultaneously the oxygen consumption of the whole animal, in the new-born rabbit[4,5].

In our experiments, copper-constantan thermocouples were inserted under light urethane anæsthesia (0·8 g/kg body-wt.) into adult rats adapted to room temperature at the following sites: (1) midbrain, 7 mm deep; (2) colon, 10 cm from the anus; (3) interscapular fat; (4) muscle,

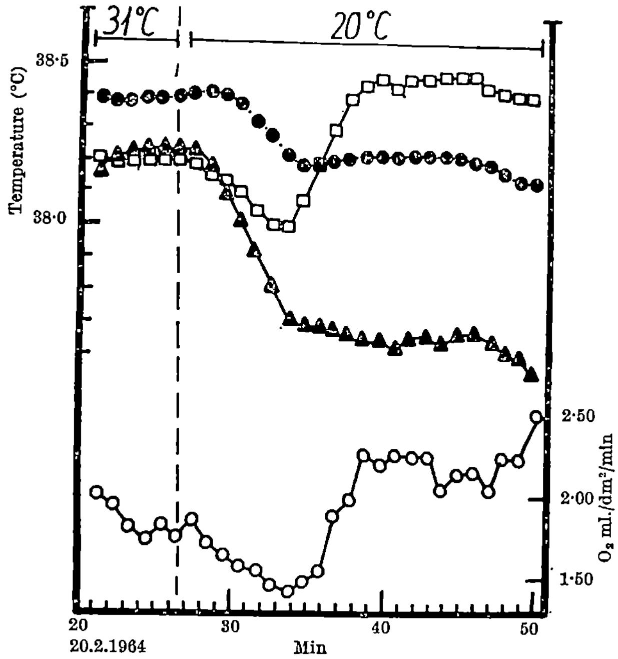

Fig. 1. Changes in colonic, brain and interscapular fat temperatures and the increase in oxygen consumption in response to transfer from a thermoneutral to a moderately cool environment in the adult rat. ●, Colonic temperature; ▲, brain temperature; □, interscapular fat temperature; ○, oxygen consumption

just beneath interscapular fat; (5) lumbar musculature. Oxygen consumption was recorded by a Noyons diaferometer permitting the registration of rapid changes[6].

Since the temperature of the lumbar musculature remained in every instance well below all the others and, in the cold, that of the thoracic muscle was found to be always less than that of the interscapular fat lying above it, these have been omitted from the figure and need not be discussed here in detail. Fig. 1 demonstrates a typical response of colonic, brain and interscapular fat temperature to exposure to a moderately cool environment. In many cases, however, the difference between colonic and interscapular fat temperature was greater in the thermoneutral environment than in this observation and brain temperature held an intermediate level between colonic and interscapular fat temperature. The increase in oxygen consumption in response to the cool environment coincided in every case with a marked and rapid increase in the temperature of the interscapular fat, and generally it became the highest in the body. Simultaneously, colonic and brain temperature ceased to fall. Following re-transfer into a thermoneutral environment, in parallel with the fall in oxygen consumption, the temperature of the interscapular fat decreased rapidly to a level below that of the colonic temperature, and in many instances also below that of brain temperature. The consecutive rise in colonic and brain temperature was followed by the interscapular fat only with a lag of several minutes. These observations seem to prove that the interscapular fat participates in thermoregulatory heat production in the adult, non-cold-adapted rat.

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Effects of Deceleration on Rats exposed to Prolonged Centrifugation

Prolonged exposures of animals to simulated high gravity environments by centrifugation have been reported to repress their normal growth and attainable body mass. Matthews[1] centrifuged rats for more than a year at 3*g* and found that the body mass of centrifuged rats was appreciably lower than corresponding control animals. Similar effects for shorter exposure periods have been noted by Wunder[2] with mice and by Smith[3] with birds. The latter workers have found that when their animals were removed from the centrifuge the weight of these animals over a period of time approached that of the non-centrifuged controls. This investigation was undertaken to evaluate the effects of both an increase and decrease in gravity on rats adapted to relatively high *g* by prolonged centrifugation.

Weanling female Sprague–Dawley rats weighing approximately 84 g, usually 4–6 per cage, were placed on a 10-radial armed centrifuge and subjected to either 2·5, 3·5 or 4·7*g* depending on the radius used. The centrifuge, which had a maximum radius of 8·5 ft., was run at a constant 40 r.p.m. Animal cages were mounted on a swinging-bucket type of assembly and were illuminated by means of individual fluorescent lights automated to simulate 12 h of day and night cycling. A detailed description of the centrifuge is in preparation and will be published elsewhere. Rats were maintained on a standard laboratory rat chow ('Purina'). Water was provided by means of an inverted water bottle tray assembly. The centrifuge was stopped an average of 20 min each day to service and weigh the animals. The overall stoppage time was approximately 1·6 per cent of the total centrifugation time.

Fig. 1 (Curve *A*) shows the changes in body-weights of 4·7-*g* adapted rats removed from the centrifuge and successively exposed to normal gravity for periods of 168, 48, 24 and 9·5 h. These off-centrifuge exposures to normal gravity were spaced no closer than 2 weeks to allow sufficient time for recovery. A characteristic rapid increase in body-weight of rats removed from the centrifuge is noted with the greatest rate of increase occurring during the first 24 h. There was a progressive rise in the magnitude of this increase with each successive exposure treatment. This increase can be attributed to an enhanced rate of food and water consumption. For example, the basal food consumption of 16·4 g/rat/day for animals on the centrifuge increased to 25·3 g/rat/day when they were removed from the centrifuge for the first time. Comparable increases were noted in the other treatments.

The precipitous fall in body-weights of these rats when returned to the centrifuge was due to inanition. Rats

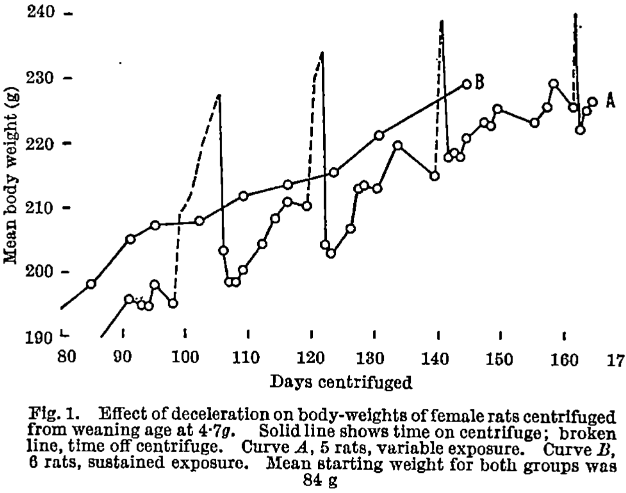

Fig. 1. Effect of deceleration on body-weights of female rats centrifuged from weaning age at 4·7*g*. Solid line shows time on centrifuge; broken line, time off centrifuge. Curve *A*, 5 rats, variable exposure. Curve *B*, 6 rats, sustained exposure. Mean starting weight for both groups was 84 g

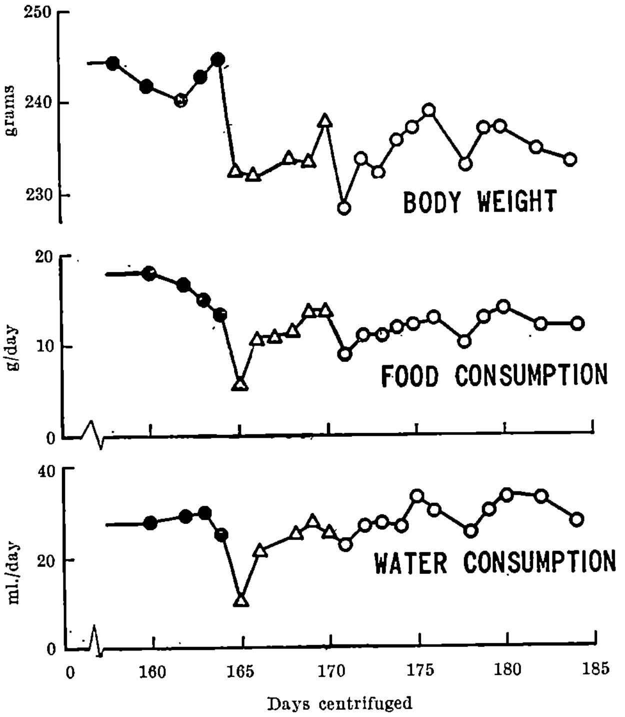

Fig. 2. Effect of increased g-loads on female rats previously exposed from weaning age to 2·5g. ●, 2·5g; △, 3·5g; ○, 4·7g. Values are means of four rats

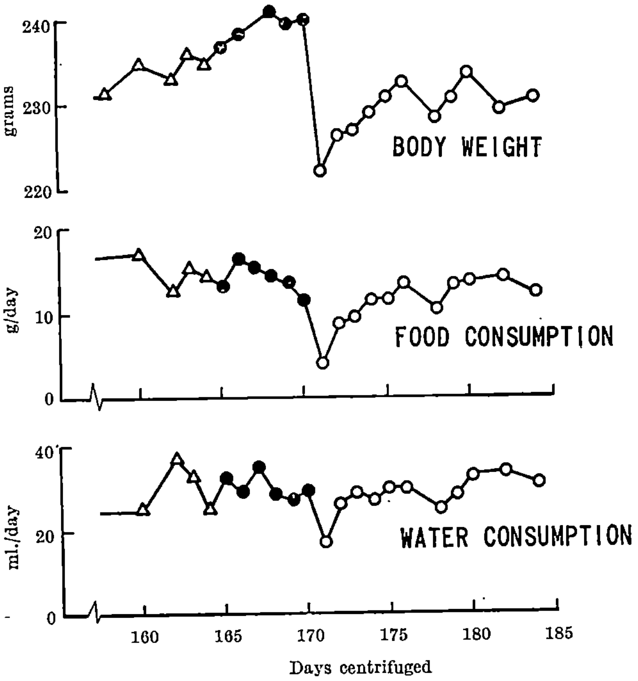

Fig. 3. Effect of decrease and increase in g-loads on female rats previously exposed from weaning age to 3·5g; △, 3·5g; ●, 2·5g; ○, 4·7g. Values are means of four rats

consumed no food or water during the first 24 h on the centrifuge following the first off–on treatment. Nine days elapsed before the food consumption of these rats was restored to the pre-treatment basal level of 16 g/rat/day. With progressively shorter exposures to normal gravity, the time required for the restoration of the basal food consumption rate was decreased proportionately. The recovery time after the 48-, 24- and 9·5-h exposure treatments was 5, 4 and 2 days respectively.

The repeated off–on treatments had no apparent effect on the overall growth rate since the growth-rate curve of these rats was similar to centrifuged rats not subjected to the off–on treatments (curve B). Preliminary growth examinations of rats exposed to prolonged centrifugation from weaning age to maturity have been reported[4] and will be presented in detail later (in preparation). Fig. 2 shows the response of rats adapted to 2·5g for 164 days and then subjected to 3·5g and 4·7g. It is observed that there is a marked decrease in body-weight, food and water consumption when the g-load is increased from 2·5 to 3·5g. A smaller decrease is noted in going from 3·5 to 4·7g. In distinct contrast, rats adapted to 3·5g for 164 days when decelerated to 2·5g show no marked change in body-weight or food and water consumption (Fig. 3). The characteristic decrement in these parameters with the imposition of a g-load is observed, however, when the rats are moved from 2·5g to 4·7g. The treatment accorded the animals in the latter experiment may be analogous to that received by terrestrial organisms when subjected to sub-gravity or weightlessness in space and then returned to Earth.

The results from this investigation indicate that deceleration is well tolerated by rats and appears not to be a stress factor. On the other hand, any sustained incremental increase in gravity has a deleterious effect on appetite and body-weight maintenance and can be considered a condition of imposed stress.

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Duplex Function in the Grey Squirrel's Electroretinogram

THE grey squirrel (*Sciurus carolinesis leucotis*) was initially considered to have an all-cone retina, based on evidence obtained by light microscopy[1,2] and electroretinography[2]. In support of this, a difference spectrum obtained by reflexion densitometry was found to peak at 535 mμ[3]. Afterwards a difference spectrum of a photopigment extracted from this retina peaked at 504 mμ, however, and resembled rhodopsin[4]. Thereafter a group of diurnal squirrels including the grey squirrel was found to have double-peaked ERG spectral sensitivity curves, suggesting that there was more than one receptor mechanism in these retinæ[5]. Similar ERG results have been reported for other diurnal squirrels[6,7]. Recently, conditioning experiments on the grey squirrel demonstrated a peak wave-length sensitivity at 502 mμ (ref. 8), and rod-like in addition to cone receptors were found by electronmicroscopy[9]. This report shows that there are at least two receptor systems contributing to the grey squirrel's ERG which behave similarly to the rod and cone components of the ERGs of duplex retinæ.

The ERG has been recorded from a corneal contact lens electrode and a stainless steel needle placed under the skin on the forehead of squirrels anæsthetized with 'Nembutal'. The eye is stimulated in Maxwellian view subtending 90° of visual angle at the pupil. The light source is a well-regulated, high-pressure xenon arc lamp, the output of which can be filtered by neutral and double interference filters both calibrated spectrophotometrically. The energies of the spectral lights have been measured by a thermocouple and galvanometer. Sinusoidally flickering stimuli are obtained by rotating a 'Polaroid'

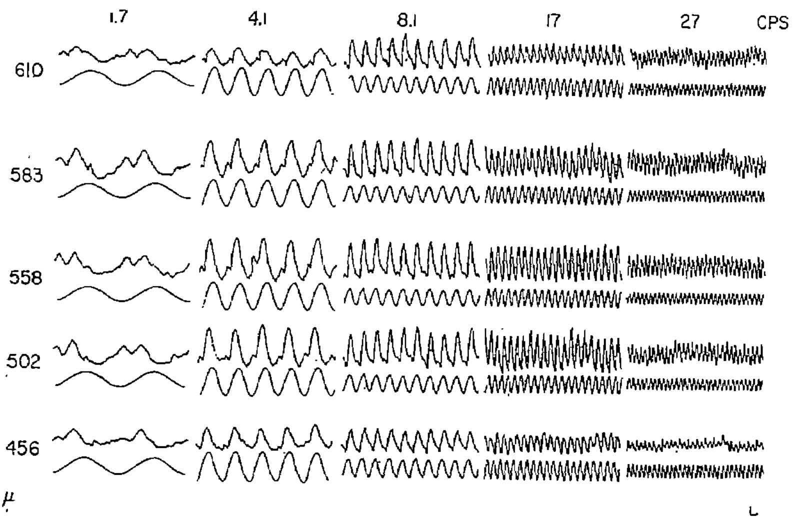

Fig. 1. ERG responses at different wave-lengths and flicker frequencies. The upper trace in each set is the ERG and the lower, the response of a photocell monitoring the stimulus. The calibration, lower right, represents 100 microvolts vertically and 100 msec horizontally. Upward deflexions indicate positivity and light on

plate in a polarized beam. The light modulation depth is 99 per cent.

Duplex function can be demonstrated by comparing the ERG responses obtained with light stimuli from both ends of the visible spectrum at different flicker frequencies (Fig. 1). With 610 mμ the ERG has a pronounced corneal positive 'off' effect and a higher peak-to-peak amplitude at 8 c/s than at lower or higher frequencies. At approximately equal response amplitudes with 456 mμ, there is neither a conspicuous 'off' effect nor amplitude peaking in the frequency response. The flicker fusion frequency is higher at 610 mμ than at 456 mμ, but if the energies in these stimuli are reduced equally, the ERG is more sensitive to 456 mμ than to 610 mμ. This can be seen in the spectral sensitivity curves obtained at low-response criteria (Fig. 2). With dim stimuli the ERG is always more sensitive to 456 mμ than to 610 mμ. With brighter stimuli the ERG response increases more rapidly at 610 mμ than at 456 mμ. With bright stimuli of high frequency the ERG becomes more sensitive to 610 mμ than to 456 mμ. This is not the result of saturation because larger responses are obtainable with wave-lengths of greater luminance than 610 or 456 mμ at all flicker frequencies.

There must be at least two receptor systems contributing to the grey squirrel's ERG, one more rod-like in the sense of determining threshold at dim light levels and being more short wavelength sensitive but slower than the other. The shift in spectral sensitivity resulting from changes in either the luminance or the flicker frequency of the stimulus parallels the Purkinje shifts of more familiar duplex retinæ.

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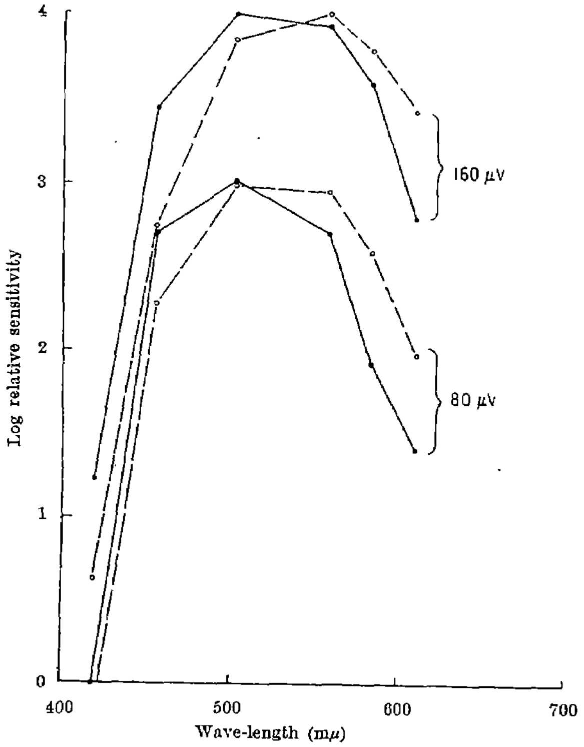

Fig. 2. Spectral sensitivity of the ERG at two response amplitude criteria and at two different flicker frequencies. The pairs of curves are displaced along the ordinate axis. ●, 4 c/s; ○, 10, c/s

HISTOCHEMISTRY

Alkaline Phosphatase in the Juxtaglomerular Apparatus of the Rat

Since the original observations by Goormaghtigh[1] on the structure of the juxtaglomerular apparatus of the mammalian kidney, and his subsequent observations on its endocrine function[2], the rat has been used as an experimental animal to provide material for studies of this organ. Thus examinations of enzyme changes in experimentally induced hypertension[3] and more recently granular cell changes following endocrine organ manipulation and hormone administration[4,5] have been carried out using the juxtaglomerular apparatus of the rat kidney.

In one respect at least the rat juxtaglomerular apparatus differs from that of many other mammals: that is, in the occurrence of non-specific alkaline phosphatase in a specialized layer of the cells which constitute the juxtaglomerular body. Activity at this site has been observed in four strains of laboratory rat following the application of two histochemical methods. The methods used were Lillie's modification of the calcium precipitation method of Gomori[6] and a modification[7] of the simultaneous coupling azo dye method of Manheimer and Seligman[8].

The activity which was observed varied with the strain of rat, and the intensities of the alkaline phosphatase visualized by the different methods did not correspond. This is shown in Table 1.

Alkaline phosphatase activity was located in a band of cells separating the myoepithelial cells of the juxtaglomerular body from the macula densa (Fig. 1). The afferent arteriole is surrounded by a zone of clear myo-

Table 1. ALKALINE PHOSPHATASE IN THE JUXTAGLOMERULAR APPARATUS OF THE RAT

Rat No. Strain	1 Suffield*	2 Suffield	3 Suffield	4 Sprague–Dawley	5 Sprague–Dawley	6 Sprague–Dawley	7 Fischer	8 Hooded	9 Hooded
Gomori method	+	–	+ +	+ + +	+ + +	+ + +	+ + +	+ +	+ + +
Azo dye method	/	/	/	+ +	+	+	±	+	±

+ + +, Strong positive; + +, positive; +, weak positive; ±, very weak positive; /, method not performed.
* Inbred line from Sprague–Dawley/Wistar cross.

epithelial cells while the cells in contact with the macula densa show an intense alkaline phosphatase reaction. The nuclear staining in the cells of the macula densa and the vascular endothelium of the afferent arteriole is attributable to the nuclear staining artefact which results with the Gomori method. Comparison of the Gomori and azo dye results shows a weaker, more diffuse reaction by the azo dye method although the intensities of the reactions observed in the proximal convoluted tubules were approximately equal in both methods (Figs. 2 and 3). The variation in intensity which was observed may perhaps be due to the occurrence of isoenzymes visualized selectively by the different methods.

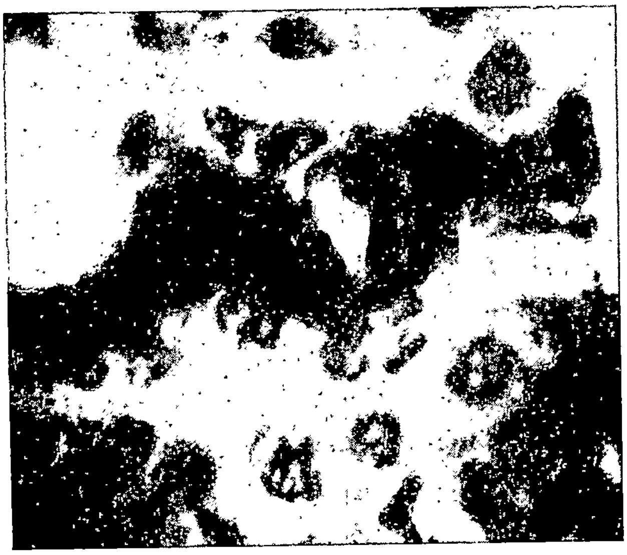

Fig. 1. Juxtaglomerular apparatus of rat. Alkaline phosphatase activity in layer of juxtaglomerular body adjoining the macula densa. Rat 4. Gomori method (×1,500)

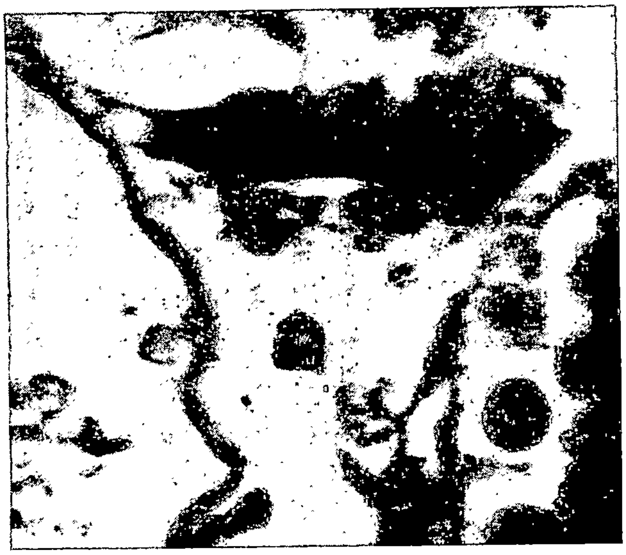

Fig. 2. Strong alkaline phosphatase reaction in the juxtaglomerular body. Rat 4. Gomori method (×1,500)

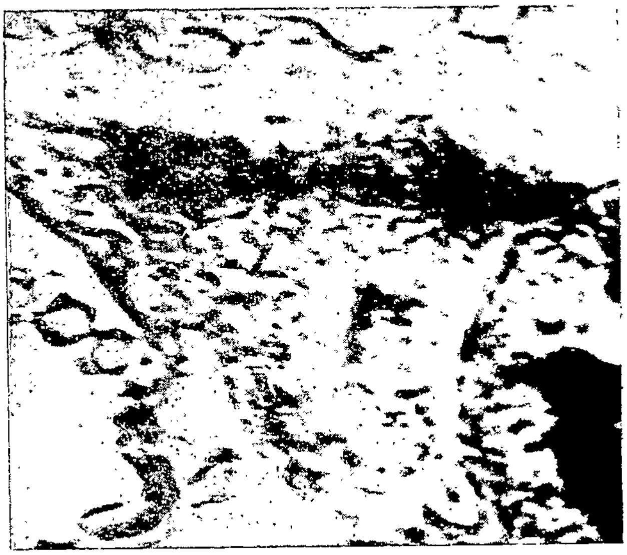

Fig. 3. Moderate alkaline phosphatase reaction in the juxtaglomerular body. Rat 4. Azo dye method (×1,500)

The occurrence of non-specific alkaline phosphatase in this location has not been observed in nineteen other species of mammal which have been examined[9]. It may be of some significance that the rat differed from the other species in the possession of well-defined alkaline phosphatase activity in the adventitia of arterial vessels. The possibility therefore exists that this specialized layer of the rat juxtaglomerular apparatus is derived from elements of the adventitia, as opposed to the myoepithelial cells derived from the media of the juxtaglomerular afferent arteriole.

It would be difficult to assign a function to alkaline phosphatase activity at this site. Perhaps this activity is concerned with the energy requirements of chemical or hormonal transfer related to the homeostatic or endocrine function of the juxtaglomerular apparatus, or perhaps more directly with the absorption of substances from the macula densa and their transference to the juxtaglomerular body.

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Histochemical Localization of Alkaline Phosphatase and Adenosine Triphosphatase in the Myoepithelial Cells of Rat Salivary Glands

The histochemical demonstration of alkaline phosphatase in the myoepithelial cells of salivary glands, sweat glands and mammary glands has been reported by a number of workers[1-7].

Leeson[5,6] distinguishes between the myoepithelial cells surrounding the gland acini which form an extensive, irregularly branching network, and those of the intercalated ducts which are smaller and less extensively ramifying. He points out that the remaining portions of the duct system in rat salivary glands are alkaline phosphatase negative, implying thereby the absence of myoepithelial cells in the large ducts.

My own investigations of the distribution of alkaline phosphatase in rat salivary gland were carried out on fresh-frozen cryostat sections fixed in formol–calcium for 10 min, using naphthol *AS–BI* phosphate as substrate and coupling with fast red *TR* (5-chloro-*o*-toluidine)[8]. The results confirm Leeson's observations with regard to the myoepithelial cells of the acini and intercalated ducts. In addition, however, they clearly indicate the presence of similar cells giving strongly positive reactions for alkaline phosphatase, surrounding the striated ducts (Fig. 1). This suggests that in the rat submaxillary salivary gland, myoepithelial cells are present also in relation to the striated ducts.

Little is known of the function of alkaline phosphatase in myoepithelial cells. Certainly, its demonstration in these cells has contributed little to the understanding of their function. Although Leeson[6] states that the function of the myoepithelial cells is primarily one of contractility, there is little direct evidence in these cells of metabolic activity normally associated with muscle contraction. Indirect evidence has been provided by the work of Linzell[9], who found by direct microscopic examination of living mammary glands that the alveoli contracted following stimulation by vasopressin, oxytocin and other agents which produce smooth muscle contraction. A silver impregnation technique was used by Richardson[10], who observed structural changes in the epithelium of contracted as compared with distended alveoli and ducts in goat mammary glands. More recently, in electron microscope investigations, Scott and Pease[11] have demonstrated fibrils in the myoepithelial cells of salivary glands which they consider to be evidence that these cells can contract.

The work recorded here was undertaken in order to determine whether adenosine triphosphatase (ATPase), which is known to be present in smooth muscle, is present in the myoepithelial cells of salivary glands. Fresh-frozen cryostat sections were treated either unfixed, or fixed in formol–calcium at – 3° C for 5–10 min, and were incubated at *p*H 7·2 for 30 min at room temperature after the method of Wachstein and Meisel[8].

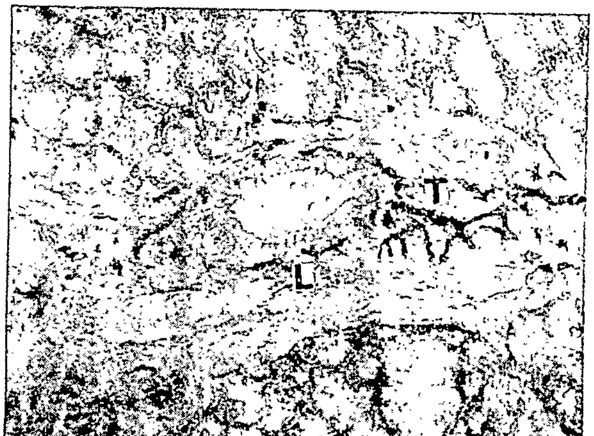

Fig. 1. Myoepithelial cells surrounding a striated duct in rat submaxillary gland. Sectioned longitudinally at *L* and tangentially at *T*. (Alkaline phosphatase; × *c*. 90)

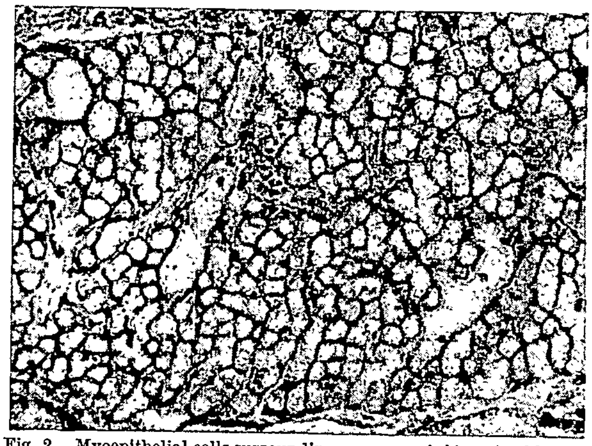

Fig. 2. Myoepithelial cells surrounding mucous acini in rat sublingual gland. (Adenosine triphosphatase; × *c*. 45)

Sections treated in this way show the presence of elongated, flattened cells which give a positive reaction for ATPase surrounding and lying in close contact with the gland acini and ducts. Their distribution is the same as that of the alkaline phosphatase-positive material in rat salivary glands and it may be assumed that they are myoepithelial cells. They form an almost unbroken ring around the mucous acini but are more sparsely located around the striated ducts (Fig. 2). Blood vessels and nerve fibres show equally strong reactions, but the acinar and duct cells themselves show no ATPase activity.

The specificity of the various histochemical reactions for ATPase has frequently been debated, as non-specific alkaline phosphatase is known to dephosphorylate ATP. The problem is discussed by Pearse[8], who concludes that, at *p*H 7·2, the *p*H involved in the Wachstein–Meisel procedure, the activity of non-specific alkaline phosphatase can be ignored. Furthermore, it was noted in this investigation that fixation at room temperature for 15 min was sufficient to obliterate any activity in these tissues, using the Wachstein–Meisel technique. As similar fixation will obviously not interfere to any marked extent with alkaline phosphatase activity in histochemical procedures, it may be assumed that the enzyme demonstrated here, using the Wachstein–Meisel procedure, is magnesium-activated ATPase and not non-specific alkaline phosphatase.

The indications are, therefore, that both alkaline phosphatase and ATPase are present in the myoepithelial cells of rat salivary glands. It is suggested that the presence of ATPase in these cells provides further evidence of their contractile nature.

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PATHOLOGY

Experimental Myopathic Syndrome associated with Pituitary Activation of Thymus

Formation of lymphoid follicles with light germinal centres is generally considered as being characteristic of myasthenia gravis[1] and is regarded as one of the manifestations of the auto-immune nature of this disease[2]. However, quite similar changes are induced in the thymus with no symptoms of myasthenia by subcutaneous injection of Freund's adjuvant[3] or through direct administration to the thymus of various antigens[4], proving only that the thymus is involved in every enhanced immunization process. On the other hand, the relationship of myasthenia gravis to thymus pathology is beyond any doubt; yet the nature of this relationship is obscure.

We have suggested that a mutual endocrine relationship does exist between the thymus and hypophysis. If, therefore, some known or unknown pituitary hormone carries a thymotropic function, implantation of the pituitary cells directly into the thymus of intact animals might lead to proliferation and drastic activation of the function of various thymic structures.

Experiments have been carried out on non-inbred albino rats (100–150 g). Minute pieces from 5–10 rat pituitaries have been surgically administered to the thymus of each animal.

More than 75 per cent of the experimental animals (22 out of 27) on the 14th–60th day after implantation have become ever more depressed, constrained and motionless and when set on the edge of a table usually drooped their heads for many hours. Aspiration was enforced. These symptoms as well as rapid onset of fatigue revealed a disease greatly similar to myasthenia. The tactile skin sensitivity was increased. The healing of wounds in the myopathic rats was longer than in normal ones. Electrophysiological examination shows neuromuscular disorders in this syndrome.

The following are additional symptoms observed in some individual rats: changes in the ratio of serum proteins; symptoms of hæmorrhagic diathesis; appearance in the blood of a great number of large immature cells with unsegmented nuclei; anæmia—the fall of erythrocytes number without changing the type of erythropoiesis.

Control animals injected intrathymically with pieces of brain, posterior pituitary or with the Freund adjuvant did not reveal any of the foregoing clinical changes.

Living grafts of the anterior pituitary were observed in the thymuses of some myopathic rats killed on the 25th day after implantation. These thymuses were almost normal in appearance with insignificant prevalence of lymphoid elements. Extreme hyperplasia of the lymphoid tissue with abundant germinal centres and with proliferation of plasma cells were noted in the spleen and lymph nodes.

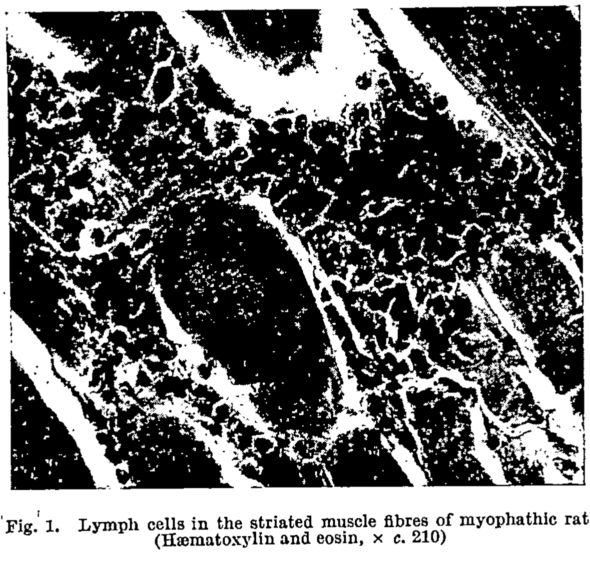

Fig. 1. Lymph cells in the striated muscle fibres of myophathic rat. (Hæmatoxylin and eosin, × c. 210)

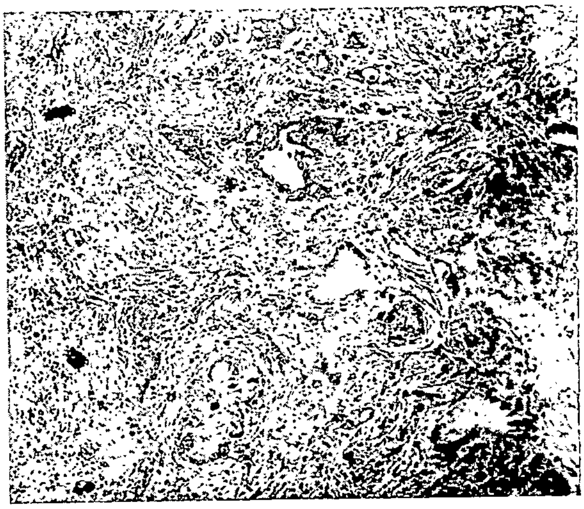

Fig. 2. Pituitary-activated thymus in cobalt-60 irradiated rat. Cavities lined with epithelium (× c. 52)

In some groups of striated muscle fibres of myopathic rats clumps of lymphoid cells and histiocytes with individual leucocytes between unchanged muscle fibres (lymph cells) were noted (Fig. 1).

In a rat with myopathic syndrome killed on the 80th day after pituitary implantation the thymus was reduced without any features of accidental involution. Ovoid lobules with nodular hyperplasia of the lymphoid tissue were noted in the upper part of the gland but without any follicles. In the lower part, lobules with thick fibrous capsule rich in mast cells, there occurred groups of epithelial and lymphoid cells surrounded by an abundance of fibrous cells. Lymph cells in the striated muscles were noted.

As to the morphological structures responsible for the myopathic syndrome, this question remains open. The lymphoepithelial thymoma-like structures previously described in this laboratory[5] have no relation to myopathic syndrome. The thymectomy of 2 rats with developed myopathia did not lead to recovery.

In some rats irradiated with cobalt-60 (200 r.) and then intrathymically implanted with the anterior pituitary unusual activation of the epithelial structure was observed which was different from that previously described[3]. The thymus was a little reduced in size, being composed only of reticular tissue in which many cavities of various size lined with monolayered epithelium were observed, some of them with pyroninophil homogeneous secretion. The whole thymus has an appearance of a secreting gland (Fig. 2). Lymphocytes were absent. Transient inhibition was observed in these rats. Such structures but less numerous were noted in control experiments with intrathymical implantation of hepatic tissue to irradiated rats. Such irradiated and pituitary activated thymuses can be used as a source for extraction of the thymus hormone. Preliminary experiments show that injection of such a thymus extract induces a marked but transient inhibition of the irradiated rats.

The foregoing experimental evidence proves the thymotropic action of the pituitary. Experiments show that this action is connected with the anterior lobe of the pituitary. Injection into the thymus of pure so far known pituitary hormones opens the possibility of deciding whether or not there actually does exist a specific pituitary thymotropin.

If the pituitary–thymus interrelation is mutual the thymus should act on the pituitary according to the feed-back principle, and experimental implantation of the pituitary into the thymus of hypophysectomized animals should not either restore or change the syndromes developing after hypophysectomy. This theory, as well as the method of stimulating implantation of one endocrine gland into another, is of general significance and may be used for the examination of any pair of organs to reveal in one of them an unknown endocrine or hormone-inhibiting function with regard to the other organ. Results of autotransplantation of the pituitary into the thymus indicate the existence of the anti-hypophyseal action of the thymus.

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Artificial Heterogenization of Tumours by means of Herpes Simplex and Polyoma Viruses

We have suggested elsewhere[1] the possibility of artificial heterogenization of malignant tumours by induction in tumour cells of new antigen determinants followed by exposure of these antigens to actively acquired or passively administered lymphoid cells or antibodies.

Experiments in this laboratory on artificial heterogenization of tumours with *Salmonella* and *Staphylococcus* antigens followed by treatment with corresponding antisera proved of low efficacy. Artificial induction of new antigens in tumour cells by means of 'infectious' and 'oncogenic' viruses is described here.

In these experiments sarcoma 237 induced in C_{57} mice by means of 7,12-dibenz-(α)-anthracene and 2–5 times transplanted to these mice was used. The tumour nodes appeared on approximately the thirtieth day after inoculation. The tumour was minced without any trypsinization. The cells were filtered through 2 layers of gauze and infected *in vitro* with the herpes simplex virus (strain *El*-2 (kindly supplied by Dr. A. I. Shatkin) with low pathogenicity to mice has previously undergone about 80 passages in chick embryonic tissue cultures, titre 10^4 PFU$_{50}$ (plaque forming units)) and then implanted in mice. The cells of grown infected tumours have once more been infected with the herpes virus and implanted in mice. This procedure was carried out three times.

Tumour cells of the third passage were once more infected *in vitro* with the herpes virus and different quantities of such virus-infected tumour cells (10^4, 10^5 and 10^6 cells) were administered at a rate of 0·2 ml. to the following groups of mice: first group was twice immunized at 10-day intervals with herpes virus alone 1 month before tumour cells inoculation; the second group was immunized with vaccinia virus according to a similar schedule; the third group consisted of non-immunized mice. The same amounts of non-infected tumour cells have been injected into immune and non-immune mice.

It will appear from Table 1 that the growth of tumours infected by herpes virus was specifically inhibited in the herpes virus immunized mice.

In the next experiment the cells of sarcoma 237 were mixed *in vitro* with *SE*-polyoma virus, containing 4,960 hæmagglutinating units per 1 ml. This strain has kindly been supplied by Dr. S. Stewart. After exposure for 2 h the cells were precipitated by gentle centrifugation and resuspended in Earle's solution and injected into mice.

Table 1. Artificial Heterogenization of Cells of Cancerogen-induced Sarcoma 237 by Means of the Herpes Simplex Virus

Mice	Herpes virus-infected tumour cells			Non-infected tumour cells		
	10^4	10^5	10^6	10^4	10^5	10^6
Immunized with herpes virus	3/14*	6/15	15/15	14/14	14/14	14/14
Immunized with vaccinia virus	14/14	13/14	14/14	nt†	nt	nt
Non-immune	14/14	12/14	14/14	12/12	14/14	13/14

* Denominator indicates number of inoculated mice; numerator, number of tumours which have developed.

† nt, not tested.

Ripe tumours were minced and repeatedly treated with the polyoma virus *in vitro*. The cells treated with polyoma virus as well as non-treated control sarcoma cells were injected in doses of 10^4, 10^5 and 10^6 per mouse to the following groups of mice: (1) twice immunized with polyoma virus at 10-day intervals; (2) immunized with vaccinia virus; (3) non-immune control mice.

Absence of antibodies to polyoma virus was tested in the sera of the latter group of mice by the hæmagglutination inhibition test.

It is clear from Table 2 that growth of sarcoma 237 infected with polyoma virus is inhibited in polyoma-immunized mice.

The immunological nature of the growth inhibition phenomenon of heterogenized tumours is obvious from its passive transmission by means of lymphoid cells.

Fresh C_{57} mice were injected with polyoma virus-treated cells of sarcoma 237 (10^4 cells per mouse) and 48 h thereafter half these mice received lymphoid cells from the C_{57} mice actively immunized with the polyoma virus (10^4 lymphoid cells intravenously + 10^6 intraperitoneally). A second group of mice received the same quantity of lymphoid cells from vaccinia-immunized mice. Tumour growth in these two groups of mice was 3/14 and 14/14 respectively. The experiment shows the possibility of subsequent immunological action on the artificially heterogenized tumour.

The foregoing experiments show that tumour cells are artificially heterogenized by herpes and polyoma viruses and that immunity acts specifically on these newly artificially induced antigens. Another alternative, namely, that tumour growth is stimulated by means of virus in non-immune mice, and that this effect is inhibited in immune animals, can probably be excluded by a comparison of growth of infected and non-infected tumours.

The result of artificial heterogenization of tumour cells by means of herpes virus complies with our previous suggestion that natural heterogenization of infected cells is a general property of viruses[2].

Inflammatory lymphoid cell infiltrates common in virus disease of birds and mammals are a host reaction on the cells heterogenized by means of virus[3].

The principal problem is artificial heterogenization of ripe tumours followed by immunological treatment. For these purposes it is necessary to examine artificial heterogenization by means of various cytotropic organisms and compounds.

In one of the pioneer papers on viral oncolysis, Pierce and Rivers[4] have pointed out that growth of Brown–Pierce carcinoma infected with virus III is to some degree inhibited in rabbits immune to this virus. They regarded this effect as a non-specific one. It might be suggested, however, that here we are dealing with a specific immunological effect on an artificially heterogenized by virus III carcinoma.

Table 2. Artificial Induction of 'Polyoma' Antigen in Cells of Sarcoma 237*

Mice	Polyoma-infected tumour cells			Non-infected tumour cells	
	10^4	10^5	10^6	10^4	10^5
Immunized with polyoma virus	0/18	4/20	19/19	18/19	20/20
Immunized with vaccinia virus	20/20	18/19	20/20	nt	nt
Non-immunized	20/20	20/20	19/20	17/19	19/20

* Legends same as in Table 1.

The resistance of polyoma immunized mice to implantation of some non-polyoma tumours as reported by Sachs[5] was probably due to casual infection with the polyoma virus and subsequent artificial heterogenization of these tumours.

Note added in proof. Recently, Sjogren[6] has shown that polyoma antigen can be induced in non-polyoma tumours by a very simple method. It was shown recently in our laboratory that vacuolating virus SV_{40} can induce strong artificial heterogenization of tumours.

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Protein Components of Amyloid

AMYLOID may be deposited in animal or human connective tissues in a variety of pathological circumstances. It consists mainly of protein, with up to 5 per cent of carbohydrates largely in the form of mucopolysaccharides. Previous attempts to characterize the protein moiety of amyloid have included : (*a*) Extraction with strong alkali followed by electrophoresis, revealing either α-1-, α-2-, and β-globulins with traces of albumin[1] : or α-globulin alone[2] ; (*b*) Extraction with strong alkali followed by analytical ultra-centrifugation, demonstrating a single component with a sedimentation coefficient of 6[2] ; (*c*) Extraction with concentrated aqueous urea followed by analytical ultracentrifugation, showing five fractions with sedimentation coefficients of 1, 6, 9, 16 and 23 (ref. 3); (*d*) Extraction with strong alkali followed by amino-acid analysis, which gave no precise protein identification but excluded collagen as a significant constituent on the basis of low hydroxyproline values[2]; (*e*) Fluorescent protein tracing in histological preparations, which is so sensitive a technique that trace contaminants would be revealed, has detected the presence of γ-globulin[4–6], β-1 complement[6], rheumatoid factor[6], and fibrinogen[6]; (*f*) Absorption of specific anti γ-globulin antiserum with purified amyloid substance, which produced no significant reduction in titre[7]; (*g*) Extraction with concentrated aqueous urea followed by immuno-diffusion and electrophoresis against specific antisera to albumin, γ-globulin, cæruloplasmin, orosomucoid, β-2 macroglobulin, rheumatoid factor, C-reactive protein, a pool of myeloma globulins, and whole serum ; none of these antisera precipitated the amyloid proteins[8]; (*h*) Extraction with strong alkali followed by preparation of an antiserum which, on immuno-electrophoresis against whole serum, gave precipitin arcs in the α-2 regions[8]. None of these methods has entirely surmounted the two main obstacles to precise identification of the protein components of amyloid. These are, first, the relative insolubility of this material in all but chemically vigorous reagents which are liable to degrade proteins and, secondly, the difficulty in obtaining amyloid free from incidental contamination by plasma proteins. The new method reported here appears to circumvent both these difficulties in the isolation of pure natural amyloid.

The technique involves cycles of : (*a*) brief ultrasonic disintegration into particles of macromolecular dimensions; (*b*) differential cold precipitation of the amyloid, which sometimes involves freezing, combined with numerous washings with neutral isotonic saline in order to remove all traces of incidentally trapped serum proteins. The substance so isolated is still in its original insoluble state and retains the tinctorial reactions (Schiff positivity, methyl violet metachromasia, positive congo red staining) characteristic of the parent amyloid deposits in the original tissues. The preparations from two human amyloidaceous kidneys were used to immunize rabbits and the antisera produced were analysed for their ability to precipitate human plasma proteins in immuno-electrophoresis.

Antiserum obtained with amyloid prepared from a patient with chronic syphilis reacted solely with 7*S* γ-globulin (Fig. 1). The antiserum produced by amyloid derived from a patient with chronic rheumatoid arthritis reacted with five plasma proteins ; very weakly with 7*S* γ-globulin, but very strongly with one α-1 and three α-2 components (Fig. 2). If the plasma proteins had been present in the amyloid preparations as a result of contamination from tissue fluids or blood they would most likely have been similar in both cases. However, since the antisera to each amyloid sample contained antibodies to different plasma proteins, the components revealed were probably integral with and peculiar to their respective amyloid substances. Neither sample induced precipitating antibodies to β-2-macroglobulin, to which class both rheumatoid factor and the Wassermann antibody belong[9], and thus these auto-antibodies were not components of the respective amyloid deposits. Hence, in opposition to Letterer's[10] belief regarding the pathogenesis of amyloid substance, it appears that neither of the amyloid samples tested contained sequestered products of an auto-immune reaction.

The present method of isolating amyloid, using only gentle physical means, seems to provide the substance pure and with its protein components undegraded. The findings with antisera prepared from such material may thus be accepted with less reserve than evidence obtained

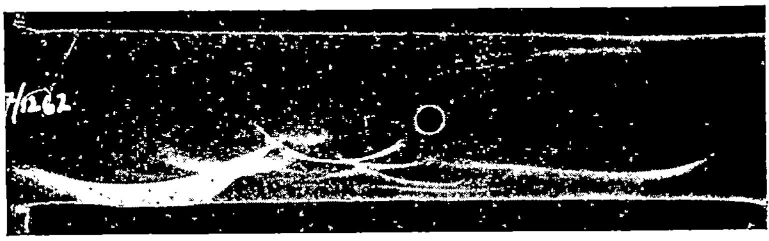

Fig. 1. *Upper trough*, antiserum to syphilitic amyloid; the only arc developed is to 7*S* γ-globulin, recognizable by its characteristic situation and curvature; *well*, whole human serum, anode to the left; *lower trough*, antiserum to whole human serum; *buffer*, borate-succinate; *p*H 8·6; M 0·05. N.B. The reduplication of the 7*S* γ-globulin lines on both sides is gel and/or Liesegang artefact

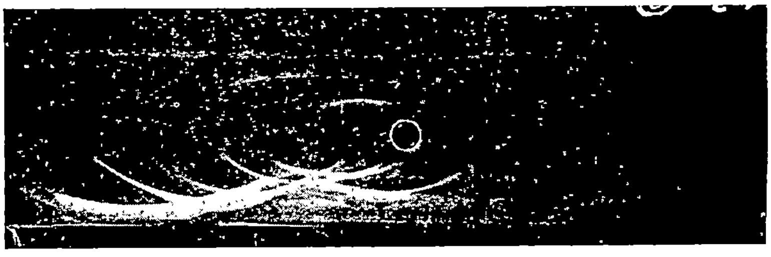

Fig. 2. *Upper trough*, antiserum to rheumatoid amyloid; the α-1 arc, two α-2 localized arcs, and an α-2 arc extending forward into the α-1 region are clearly visible. The 7*S* γ-globulin arc is too faint for photographic reproduction; *well*, whole human serum, anode to the left; *lower trough*, antiserum to whole human serum; *buffer*, barbitone; *p*H 8·2; M 0·05

by earlier techniques. While most authors are in agreement that different amyloids probably contain different proteins, there is less unanimity regarding their source. The results of the work recorded here confirm the heterogeneity of the protein components in amyloid from different sources and provide evidence that some of these proteins are derived from those normally circulating in the blood plasma.

Work is proceeding on similar analyses of other human and animal amyloids, and on the more precise identification of the alpha components revealed in the rheumatoid amyloid.

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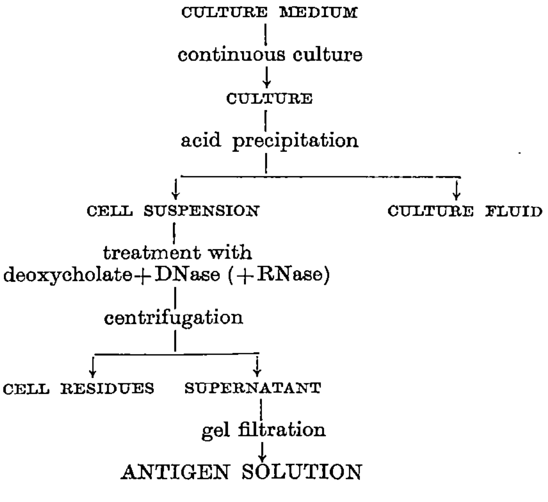
IMMUNOLOGY

Preparation of Soluble *Pertussis* Vaccine

SEVERAL suggestions have been made for isolating from *B. pertussis* the cell fraction which is responsible for immunizing man against whooping cough. Most investigators[1–4] have used some form of mechanical disintegration of the cells, but the possibility must also be considered, since the protective fraction is most probably located in the cell wall[5], that chemical solubilization of the outer layers of the cell may be a better approach.

Barta[6], using sodium deoxycholate, has shown that such an approach is possible although a reproducible process for the treatment of large quantities of cells was not considered. The process we describe here is one by which a solution of the protective antigen can easily be obtained in sufficient quantity, yielding a fairly constant starting material for further investigation.

The method is outlined as follows:



The method comprises:

(1) Continuous culture of *B. pertussis* according to the chemostat principle with a dilution rate of 0·06–0·09 h^{-1} (ref. 7). This procedure provides a suspension of cells grown under the same conditions, which is of great advantage to the reproducibility of the solubilization process.

(2) Precipitation of cells by lowering the *p*H to 3·8–4·0 as described by Pusztai *et al.*[8], followed by decantation of the culture fluids in a closed system.

(3) Treatment with 1 per cent sodium deoxycholate at 25° C and *p*H 8·0 of a cell suspension containing about 200 × 10^9 cells/ml. Barta[6], using a suspension of 20 × 10^9 cells/ml., sometimes obtained a very viscous liquid at this stage, but we have found that treatment with DNase at 25° C for 15 min reduces the liquid to a low viscosity and thus enables the next stages to be done quickly.

In some experiments addition of a very small quantity of RNase was necessary to reduce the viscosity to the desired level. An explanation of this fact may lie in variation in the RNA-content of the cell material. Unlike DNA, RNA is known to vary with dilution rate in continuous culture[9] and with age of the cells in stationary culture[10,11].

(4) Centrifugation of the treated suspension at 14,000*g* for 15 min.

Table 1. ACTIVITY OF SOLUBLE FRACTIONS COMPARED WITH THAT OF WHOLE BACTERIA

Cells prepared from culture No.	Values (I.U.) per human dose* Whole bacteria Protective antigen	H.S.F.	Soluble fraction Protective antigen	H.S.F.
117–1/13	5·4	—	1·4	—
	6·2	15·8	4·0	—
127–2/15	4·6	—	4·8	—
			13·6	--
			2·6	—
			4·4	—
			5·6	—
138–8/14	7·2	6·2	3·6	18·8
	13·4	12·6		
138–15/18	3·6	15·8	6·4	7·0
			6·2	7·2
150–2/22	2·2	3·0	7·2	18·2
	6·8	24·0	3·8	41·4
			9·4	12·4
			3·6	10
			3·2	8·4
			5·2	22·4

* The protective antigen was assayed in relation to the international standard for *pertussis* vaccine and the potency expressed in international units. The H.S.F. was also assayed in relation to the international standard according to the method of Preston[14] and the potency expressed in arbitrary units (ref. 15).

(5) Gel filtration of the clear supernatant in 0·025 molar phosphate buffer at *p*H 8·5. 'Sephadex *G*-75' has proved to be the most suitable. The filtration can be accomplished in a closed sterile system. Dialysis, as recommended by Barta[6], has certain disadvantages in that it takes longer and can be carried out aseptically only with difficulty; moreover, it is difficult to reduce the deoxycholate concentration sufficiently. In our gel-filtration procedure less than 20 mg deoxycholate per litre was left, this being the lower limit of sensitivity of the test which involves heating with 60 per cent sulphuric acid for 15 min and measuring in the photometer at 385 mμ. The column used was a modified version of that described by Porath[12], and is equipped with a double glass wall for working at constant temperature; it enabled 6 l. of the soluble fraction to be processed in one day.

A number of preparations of the soluble fraction have been tested in mice for protective antigen and histamine sensitizing factor (HSF) in comparison with whole bacterial suspensions. The results are given in Table 1. The lack of accuracy of the biological assay for protective antigen does not permit a precise estimate to be made of the yield, but it can safely be concluded that at least 75 per cent of the protective antigen of the whole cells is contained in the extract. The high concentration of HSF gives another indication of the close association of this factor with the protective antigen[3,13].

In the toxicity test according to the American minimum requirements for *pertussis* vaccine mice have shown no toxic reactions during the 7 days observation period.

The soluble fraction has been shown to retain its antigenic potency for at least 3 months when stored in the liquid state at 4° C. The fraction may be lyophilized but so far the method used has given rise to losses of activity of up to 50 per cent.

Further investigations are to be made to evaluate the product in pharmacological and immunological tests, and also to purify it still further.

We thank Prof. D. G. Evans (London School of Hygiene and Tropical Medicine) for his advice.

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Secondary Immune Response to Bacteriophage T1 in the Shore Crab, *Carcinus maenas*

Comparative investigations of the clearance of injected bacteriophage from the circulation of several species of animal have been carried out. Clearance from the chicken, frog and from certain elasmobranch, holostean and teleost fish was very rapid and was associated with the development of neutralizing antibody[1,2]. In the cyclostomes *Petromyzon marinus* and *Eptatretus stoutii* clearance was relatively slow, and little or no neutralizing ability developed in the serum of injected animals[2]. The insect *Samia cecropia* cleared 90 per cent or more of the injected dose of bacteriophage from the hæmolymph between the second and the tenth hour, but no neutralizing activity appeared to develop[3]. Accelerated secondary clearance has been demonstrated in the chicken, the frog and in some fish, but was not tested in the insect. The following preliminary experiments were carried out to investigate primary and secondary clearance of bacteriophage in other invertebrates as part of an investigation in comparative immunology.

Bacteriophage *T*1 (10^3 'phage particles) was injected into the hæmocœle of three shore crabs (*Carcinus maenas*) which were maintained in sea water at 16°–18° C. Hæmolymph samples were taken 30 min after injection and then at fortnightly intervals. In the two instances in which the bacteriophage was completely cleared from the circulation, a further dose of 10^3 particles was injected and again the clearance was followed in fortnightly samples.

Samples of hæmolymph were allowed to clot, centrifuged and the supernatant tested for viable bacteriophage by the following pour plate technique. The hæmolymph was serially diluted in nutrient broth and 0·1 ml. of one or more suitable dilutions was mixed with 0·1 ml. of a 4 h culture of *Escherichia coli B*. The mixture was pipetted into 0·5 ml. of melted nutrient agar (0·7 per cent) at 50° C which was then poured evenly over the surface of a 1 per cent nutrient agar plate. After overnight incubation at 37° C the number of plaques was counted and from this the number of viable bacteriophage particles was calculated. Each selected dilution of hæmolymph was titrated in this way 5 times and the mean of the results taken.

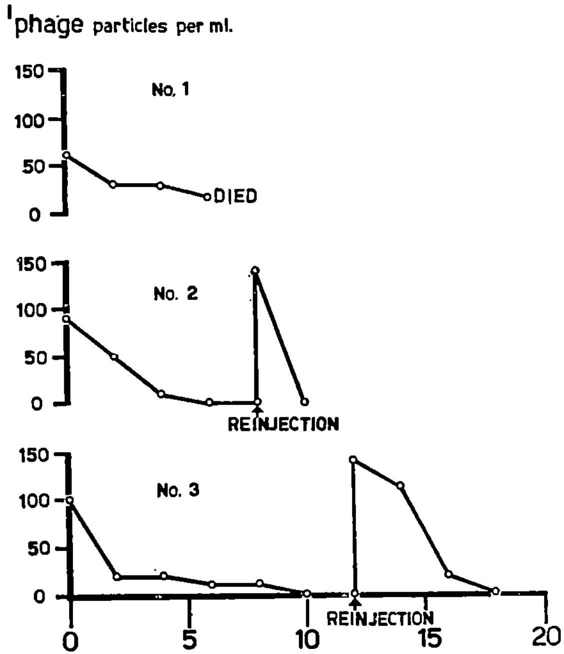

Fig. 1

Fig. 1 illustrates the decrease in bacteriophage particle counts in the three animals. Primary clearance occurred in 42 and 70 days in animals 2 and 3, respectively. Animal 1 died before complete primary clearance, but the 'phage particle counts were following a similar course to those in the other two animals. Secondary clearance was more rapid, animals 2 and 3 clearing completely in 14 and 42 days, respectively. Thus in the two instances in which secondary response was measured the rate of clearance increased by factors of 3·0 and 1·6.

Hæmolymph from crabs 2 and 3 was tested for neutralizing activity against bacteriophage *T*1 after secondary clearance of 'phage from the circulation and again 2 and 4 weeks later. No activity could be demonstrated. Although this observation suggests that the accelerated secondary clearance is mediated by a cellular rather than by a humoral mechanism, the possibility that undetected humoral factors are involved cannot be discounted.

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RADIOBIOLOGY

Soluble Liver Proteins Fractionation by Zone Electrophoresis on 'Pevikon C-870'

Liver proteins have been separated by agar-gel electrophoresis into thirteen[1] and fifteen[2] separate moieties. The use of 'Pevikon *C*-870' (PVK) as supporting medium for electrophoresis has numerous advantages[3–5], and an analysis of soluble proteins in rabbit liver separated by PVK-block electrophoresis has yielded the results reported here. PVK (Stockholms Superfosfat Fabriks Aktiebolag) blocks measuring 50·0 cm × 24·0 cm × 1·6 cm; veronal buffer, pH 8·6 (0·075 M sodium barbital and 0·015 M diethyl-barbituric acid), were used throughout.

The liver (73·0 g) of a heparinized male rabbit previously injected with homologous serum proteins labelled with iodine-131 was perfused with about 2 l. of saline through the portal vein and homogenized with an equal volume of veronal buffer, 0·15μ, pH 8·6. The homogenate was frozen at − 39° and thawed six times, and the suspension was centrifuged at 34,000*g* for 90 min. The whole procedure was carried out at 2°–4°.

Protein solutions were labelled with iodine-131 (*IBS*.3, Radiochemical Centre, Amersham) according to McFarlane[6] and were equilibrated, by dialysis, against buffer solutions. At the end of the electrophoresis, performed at room temperature with adequate cooling and buffer mixing systems, the PVK block was separated into 49–50 segments 1 cm wide. The proteins in each segment were eluted with 0·15 M sodium chloride and the eluates were estimated for their radioactive and protein contents. Proteins were estimated from 540 mμ[7] absorption in a Unicam *SP*.500 spectrophotometer.

^{131}I-TCA (trichloroacetic acid final concentration: 10 per cent) soluble radioactivity as a percentage of the original radioactivity was measured in the presence of sodium iodide and of 35·0 mg of carrier bovine serum proteins.

After concentration and clarification the protein fractions were tested by starch-gel electrophoresis[8]. Agar used for double diffusion[9] was a 1 per cent solution of 'Ionagar' No. 2 (Oxo, Ltd., London) in phosphate buffered saline, pH 7·3.

Technical details about PVK-block electrophoresis have been previously described[6]. Soluble hepatic ^{131}I-proteins (2·1 g, 80 mg/ml.; ^{131}I-TCA soluble radioactivity: 4·6 per cent) were applied into a block at 10 cm from the cathodal end and electrophoresis was carried out for 44·5 h with a gradient voltage of 4·4 V/cm (current 160 m.amp).

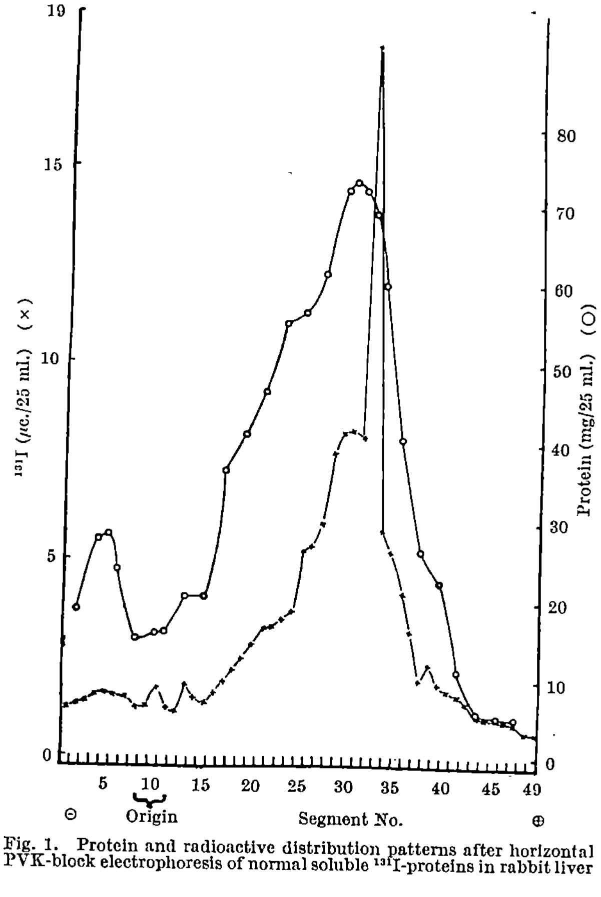

Fig. 1. Protein and radioactive distribution patterns after horizontal PVK-block electrophoresis of normal soluble ^{131}I-proteins in rabbit liver

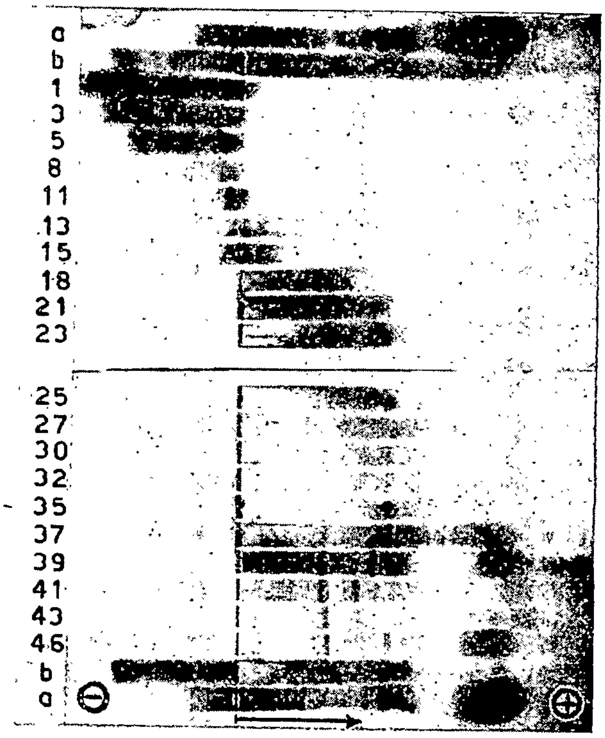

Fig. 2. Starch-gel electrophoresis (pH 8·6, 0·025 M borate buffer) of: (*a*) rabbit serum proteins; (*b*) rabbit liver soluble proteins and of rabbit liver protein fractions the numbers of which correspond to segment numbers in Fig. 1

Bromophenol blue-proteins bound had moved about 25 cm towards the anode. Fig. 1 shows the radioactive and protein contents of the PVK segments. The two profiles correspond fairly well, and therefore by measuring radioactivity the degree of separation can easily be assessed. Fig. 2 shows the electrophoretic pattern in starch gel of the separated liver protein components run compared with the original liver proteins sample and with rabbit serum proteins.

Starch-gel electrophoresis did not separate into distinct units the soluble liver proteins, whereas it helped to distinguish the separated protein fractions due to different electrophoretic mobilities, but showed in many cases the persistent complexity of the separated protein fractions. Segments Nos. 1–10 contained at least four liver protein components which moved towards the cathode, and some of these γ-globulin type proteins migrated faster than γ-globulin serum proteins, possibly because of a net positive charge under the conditions of electrophoresis. Segments Nos. 11–16 contained protein components having a low electrophoretic mobility. Segments Nos. 17–49 contained protein components all moving towards the anode. On starch-gel electrophoresis the liver protein fractions obtained from PVK segments gave at least 22 easily recognizable migrating bands. Some could be considered β-α-globulins or albumin or pre-albumin type proteins as they had the same migratory rate as corresponding protein serum fractions.

However, the soluble proteins extracted from liver should be free of contaminating blood serum proteins, because the liver, after an accurate perfusion, contained a very small amount of radioactivity due to circulating ^{131}I-serum proteins. Intracellular and extracellular serum proteins, by considering the role of the liver in plasma protein synthesis[10], could account for some of the protein bands. However, the immunological examination of rabbit serum proteins and liver soluble protein fractions, tested against a goat anti-rabbit serum, showed little, if any, contribution of serum proteins to liver protein bands.

The resolution of the liver proteins in such a high number of fractions is due, at least in part, to the possibility of large loading of protein in the PVK-block.

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Exchange Labelling of 'Lipiodol' Ultra Fluids with Iodine-131 and its Intra-lymphatic Administration with a Fistula on the Thoracic Duct

Lymphangiography of diagnostic contrast media was first reported in clinical practice by Kinmonth in 1955 (ref. 1). The therapeutic significance of this method was recognized by Fischer in 1959 (ref. 2) and is gaining increasing importance. Most authors are using radioactive colloidal gold for intra-lymphatic application[3-5] and supplementary therapy to surgical intervention in the case of microscopical metastatic infiltration of lymph nodes in malignant diseases. However, recently[6-8] iodine-131-labelled 'Lipiodol *UF*' or 'Ethiodol' is preferred for this purpose.

A new method for preparing 'Lipiodol *UF*'-iodine-131 by an exchange reaction and an improved method of administration is described here.

After intra-lymphatic administration of large quantities of 'Lipiodol *UF*', embolization of this compound occurs in the lungs, occasionally causing severe to lethal complications in the patient[9,10]. A technique was elaborated experimentally which prevents these difficulties. A polyethylene tube was introduced into the terminal part of the thoracic duct on the necks of dogs. A fistula was formed through which the lymph as well as 'Lipiodol' leaves the body of the experimental animal. Figs. 1 and 2 (1, lungs; 2, para-aortal lymph nodes; 3, popliteal lymph nodes) show scintigraphs of dogs after administration of 1 ml. of 'Lipiodol *UF*'-iodine-131/10 kg body-weight (spec. act. 1 mc./1 ml.). Fig. 1 shows a dog without the fistula on the thoracic duct, in which a high activity above the lungs can be observed. Fig. 2 shows distribution of 'Lipiodol' after application of the same quantity of 'Lipiodol *UF*'-iodine-131 (relative to body-weight) by the fistula. No activity is detectable in the lungs. Fig. 3 shows the lymphangiogram of the latter dog; the popliteal and two para-aortal lymph nodes filled by 'Lipiodol *UF*'-iodine-131 can be recognized. Radioactivity in the lungs was measured also after killing the dog. The results confirmed the conclusions from the scintigraph as well as histological examination of the lung tissues.

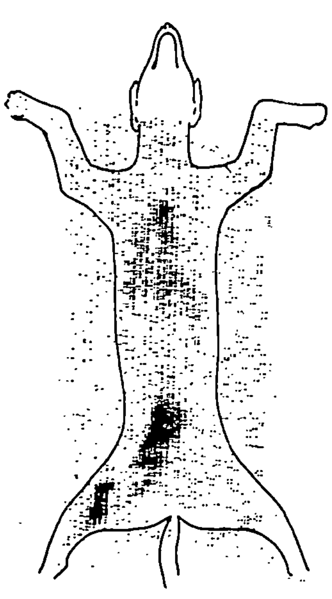

Fig. 1

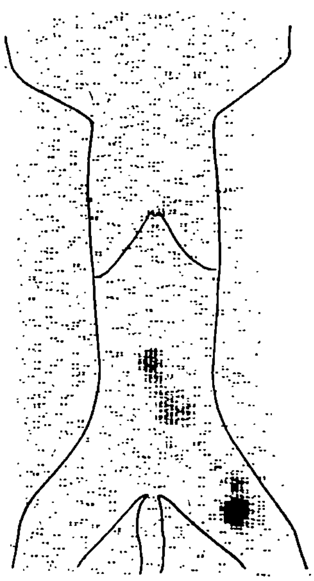

Fig. 2

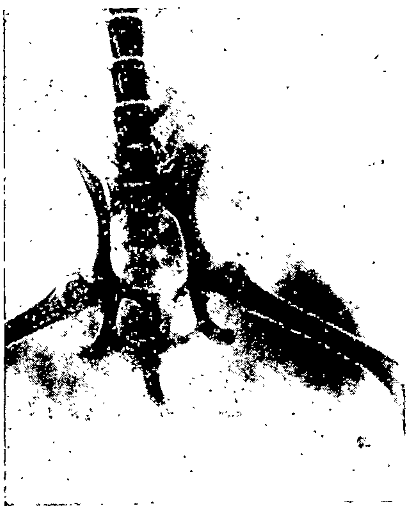

Fig. 3

Since performance of a thoracic duct fistula is simple and safe in clinical practice, this method is recommended for patients to whom large quantities of these compounds are injected.

Iodine-131-labelled 'Lipiodol *UF*' was prepared by a modified radioactive exchange method based on our previous experience with labelling of *RTG* contrast media[11] and rose bengal[12]. Two methods of exchange labelling of 'Lipiodol *UF*' (iodinated higher fatty acid ethyl esters with 38 per cent iodine content)[13] have been elaborated, the first being more advantageous.

(*a*) Exchange reaction between 'Lipiodol *UF*' and iodine-131. 0·1 ml. of 0·1 N potassium iodide, 0·1 ml. of 0·01 N sodium nitrite, 1 ml. potassium iodine-131 (10 mc.), 2 ml. carbon tetrachloride and 0·15 ml. 0·02 N hydrochloric acid are pipetted into a glass-stoppered flask and intensively stirred for 1 h. The organic phase containing the iodino-131 released is separated and washed three times with 1·5 ml. water. The iodine-131 solution in carbon tetrachloride together with a mixture of 2 ml. of carbon tetrachloride and 1 ml. of 'Lipiodol *UF*' is heated to 50°–60° in a tapered flask under reflux. The solution is then extracted twice with 2 ml. of 0·001 N $Na_2S_2O_3$, twice with 2 ml. 0·001 N potassium carbonate and twice with 5 ml. water. The carbon tetrachloride solution is evaporated *in vacuo*, dissolved in ethanol, filtered if necessary, and evaporated again. A clear oil is obtained: radioactivity 9 mc. iodine-131 (yield 90 per cent). The product is chromatographically identical with the starting material and does not contain any free iodine-131.

(*b*) Exchange reaction between 'Lipiodol *UF*' and potassium iodine-131, 1 ml. 'Lipiodol *UF*', 35 ml. of acetone and 2 ml. of an aqueous solution of potassium iodine-131 and 0·5 ml. 0·001 N potassium iodide carrier are mixed and heated under reflux for 2 h. The solvents are evaporated, 5 ml. petrol ether and 2 ml. water added and the layers separated. The petrol ether solution containing 87 per cent of the initial radioactivity is evaporated to give a colourless oil.

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BIOLOGY

Resistance to Warfarin in the Common Rat

A case of apparent resistance of the common rat to warfarin occurred in 1958 on a farm in West Scotland[1], and further evidence of resistance from the same farm was given in 1963 (ref. 2). No other report on this matter is known to me.

In 1962 some difficulties arose in controlling rats (*Rattus norvegicus*) on a couple of farms in Jutland, Denmark, and some rats caught on the farms during the winter of 1962–63 were consequently tested for warfarin resistance in the laboratory. After being fed for two or three weeks on the same diet as brown rats from the rat farm of the Laboratory, they were compared with these rats in four tests, each consisting of four rats from Jutland (*J*-rats) and four rats from the rat farm (*RI*-rats). The two groups were identical in regard to sex and rather uniform in regard to weight. They were placed singly in metal cages and supplied with water from a bottle with tube at the top of the cage.

Table 1

Dose of warfarin	No. of rats *J*-rats	No. of rats *RI*-rats	No. of rats surviving *J*-rats	No. of rats surviving *RI*-rats	Average No. of days from first portion eaten to death *J*-rats	Average No. of days from first portion eaten to death *RI*-rats
Test 1 0·025% 1 ml. for 5 days	4	4	2	1	10 (2 *r*)	4·7 (3 *r*)
Test 2 0·045% 1 g for 5 days	4	4	3	0	11 (1 *r*)	6·5 (4 *r*)
Test 3 0·049% 1 g for 5 days	4	4	3	0	6 (1 *r*)	6·5 (4 *r*)
Test 4 0·024% 1 g for 5 days	4	4	4	0	—	7·3 (4 *r*)
Total	16	16	12	1		

(-*r*) = (-rats). All the dead rats had pronounced internal hæmorrhages.

In test 1 each rat had 1 ml. of a warfarin solution dripped on a piece of white bread on each of five successive days.

In tests 2–4 each rat had 1 g of a warfarin preparation mixed up in white bread on each of five successive days. When five portions had been eaten, the rats were fed with ordinary rye bread.

In test 4 the *J*-rats were survivors from tests 1, 2 and 3.

As two groups of *J*-rats, each consisting of seven rats, were received during October–November 1963, two supplementary tests were carried out.

In test 5 the seven *J*-rats from the first group were fed a bait containing 0·05 per cent warfarin. On the first day each rat was offered 30 g of the bait as the only food and more was added the following days, the rat always having a surplus of bait to eat. After 51 days the test was broken off and the amount of bait eaten was measured. One rat died in seven days with internal hæmorrhages, the other six remained sound, eating on an average 630 g bait each in the period (504–887 g).

In test 6 four *J*-rats from the second group were fed a bait containing 0·025 per cent warfarin + 0·025 per cent sulphaquinoxaline (an antibacterial agent supposed to eliminate the bacteria in the intestinal tract of the rat, and in this way reducing the amount of vitamin K, the antidote to warfarin). These rats were compared with the three remaining *J*-rats being fed on an ordinary 0·025 per cent warfarin bait.

One of the four *J*-rats died after 11 days with no sign of warfarin poisoning (inflammation of the lungs and enteritis), the rest remaining sound for 30 days, when the test was broken off. In this period each rat had eaten on an average 410 g (358–460 g).

The surviving *J*-rats from tests 1–4 plus later received rats were placed in an empty rat pen at the laboratory in order to let them breed.

Besides showing an astonishing resistance to warfarin in the *J*-rats, the test may indicate that the vitamin K-producing bacteria in the intestinal tract are not primarily responsible for this resistance.

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Retino-hypothalamic Connexions in Cetacea

The existence of retino-hypothalamic neural connexions in mammalian forms has long been controversial. The more recent investigations of Blümcke[1] in the cat and guinea pig, of Frey[2] in the guinea pig and of Knoche[3,4] in man, dog and rabbit strongly suggest that a bundle of fibres leaves the optic pathway at a chiasmatic level and passes dorsalward into the area of the paraventricular nucleus of the adjacent hypothalamus and also into the posterior lobe of the hypophysis. Altman[5], using the Nauta–Gygax staining technique to demonstrate fibre degeneration following optic nerve section in the cat, observed fibres of retinal origin above the optic chiasm and in the lateral hypothalamus immediately adjacent to the optic tract. Giolli[6], on the other hand, using

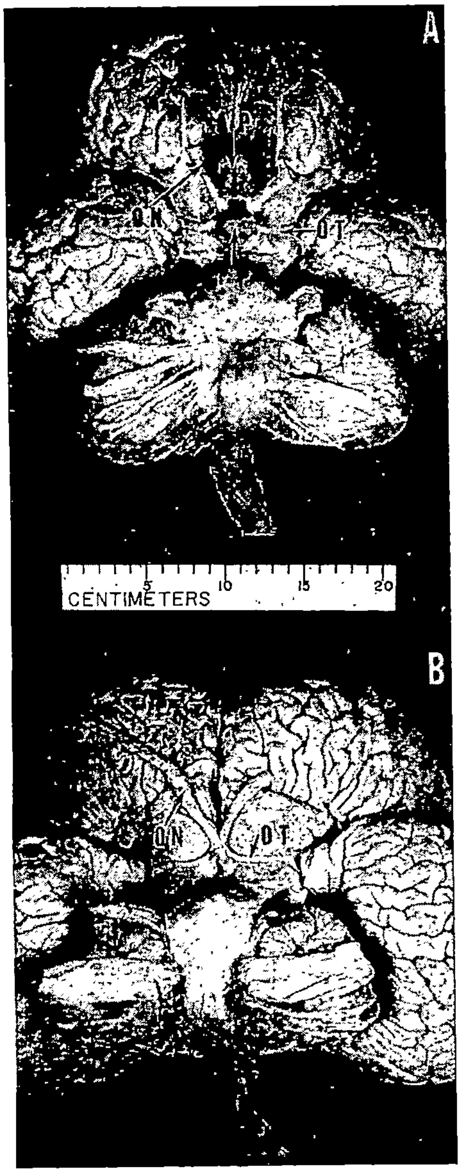

Fig. 1. Ventral aspect of cetacean brains. *A*, humpback whale (*Megaptera nodosa*) optic nerve (*ON*) and optic tract (*OT*) are indicated. Anterior lobe of pituitary gland (*AP*) reflected anteriorly from separate posterior pituitary (*PP*); *B*, sperm whale (*Physeter catodon*) depicting optic nerve (*ON*) and more slender optic tract (*OT*)

both the Nauta–Gygax and Romanes silver methods to investigate the accessory optic system in the cynomolgus monkey, found no evidence of retino-hypothalamic fibres in this form after enucleation of the eye.

In our laboratories we were impressed by the general disparity in relative size between the optic nerve and optic tract in a variety of cetacean brains. On gross observation the optic nerve always appeared to be the larger of the two structures. This feature has been observed in the sperm whale (*Physeter catodon*), fin whale (*Balaenoptera physalus*), humpback whale (*Megaptera nodosa*), Sei whale (*Balaenoptera borealis*) and in the bottlenose dolphin (*Tursiops truncatus*). This disparity in size can be clearly seen as it exists in the humpback whale (Fig. 1*A*) and sperm whale (Fig. 1*B*).

In a 401-lb. male bottlenose dolphin, with brain perfused through the aorta with 20 gallons of Windle's solution, planimetric measurements of transverse sections of the optic nerve and tract were made. Such measurements were carried out as part of the procedure[7] utilized to estimate the number of fibres in those structures. Similar data on other cranial nerves of the bottlenose dolphin[8] have been reported earlier. In this particular animal, the area of the optic nerve was $93 \cdot 0 \pm 1 \cdot 2 \text{ mm}^2$ (mean ± *S.D.*) whereas the area of the optic tract was only $74 \cdot 0 \pm 1 \cdot 9 \text{ mm}^2$. Since microscopic examination showed the fibres in both optic nerve and tract to be similar in size (Fig. 2*A* and *B*), it appeared that the increased cross-sectional area of the nerve represented a larger number of fibres.

This was confirmed by fibre counts which showed that the average number of fibres for both optic nerves in this *Tursiops* specimen was 158,435, whereas it was only 132,753 for the optic tract.

The fact that the optic nerve has a cross-sectional area exceeding that of the tract by about 25 per cent and

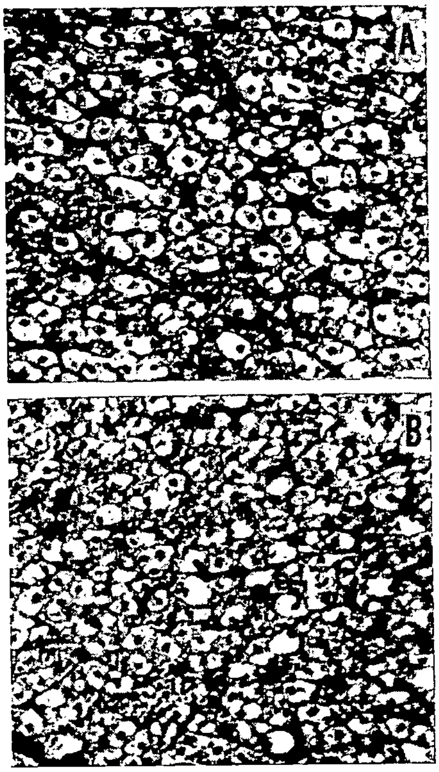

Fig. 2. *A*, optic nerve fibres; *B*, optic tract fibres. Both from bottlenose dolphin (*Tursiops truncatus*). (Alzheimer–Mann, × 440)

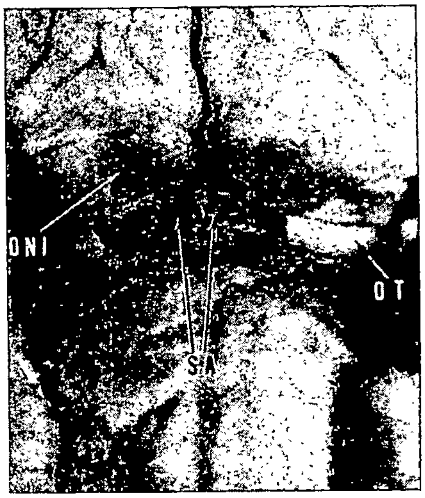

Fig. 3. Ventral aspect of chiasmatic region of bottlenose dolphin (*Tursiops truncatus*). Photograph shows the optic tracts (*OT*), severed attachment with the hypothalamus (*SA*) and impression for optic nerve (*ONI*)

contains about 19 per cent more fibres than the corresponding optic tract leaves little doubt that certain contingents separate from the visual pathways at approximately the chiasmatic level. To test this hypothesis further, the optic chiasm of one *Tursiops truncatus* was dissected from the brain base. In the process it became necessary to sever a broad tissue attachment to the overlying hypothalamus (Fig. 3).

The isolated chiasm was embedded in paraffin, sagittally sectioned at 6μ and stained by the method of Klüver and Barrera[9]. In sections through the middle third of the optic chiasm, a fibre bundle appeared to take origin from its dorsal aspect and stream obliquely upward and posteriorly into the adjacent hypothalamic area (Fig. 4*A* and *B*). As yet, chiasmatico-fugal terminations of this bundle have not been investigated in the cetacean form, but our future investigations will bear on this point.

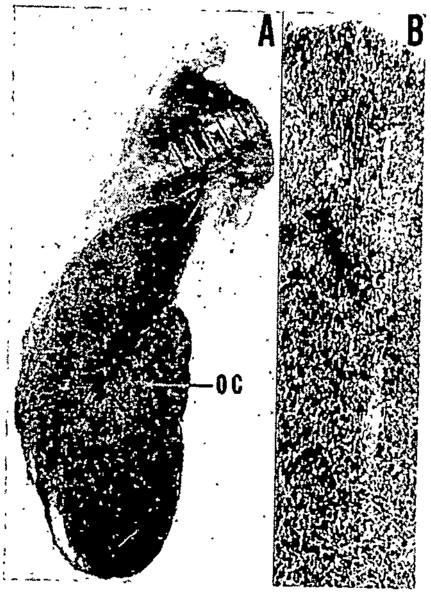

Fig. 4. Optic chiasm in bottlenose dolphin (*Tursiops truncatus*). *A*, retino-hypothalamic bundle (arrows) coming from optic chiasm (*OC*). (Sagittal section, Klüver–Barrera, × 9.) *B*, fibres of retino-hypothalamic bundle at higher magnification (× *c*. 133)

The preceding considerations strongly suggest the existence in Cetacea of direct neural connexions between the optic pathway and hypothalamus. These observations support other investigations in the literature indicating that retino-hypothalamic connexions do exist in a variety of animal forms.

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Keratinized Epidermal Derivatives as an Aid to Climbing in Gekkonid Lizards

The ability of gekkonid lizards to climb up vertical glass surfaces and to hang upside-down on ceilings is a problem which has attracted the attention of numerous workers. All of them[1-3] have referred to the presence of microscopic bristles on the undersides of the feet which are apparently associated with the animal's climbing ability. However, the exact histological structure of the bristles has not been described, and even Altevogt's[3] electron-microscope observations contain no reference to the nature and histological origin of the bristles. The lack of information on the histological structure of the squamate epidermis, for the bristles are, in fact, epidermal derivatives, precluded any precise interpretation.

Sections of the adhesive pads on the feet of *Gekko gecko* (the tokay) have been examined during examination of the structure of the squamate epidermis, and some preliminary observations regarding the structure of the bristles, with comments on the present theories of the adhesive mechanism, are presented here.

Longitudinal sections of the digits show that the adhesive pads consist of a series of lamellar scales, each of which shows a slightly modified form of the typical squamate scale[4]. The adhesive bristles arise in pairs from the surface of the β-keratin on the outer scale surface[4]. Each bristle is about 90μ long and about 10μ wide at its base. Under oil immersion they are seen to be cylindrical structures of homogeneous keratin the distal ends of which have a brush-like appearance (Fig. 1). Altevogt's[3] electron-micrograph shows that there are numerous terminal subdivisions in this region.

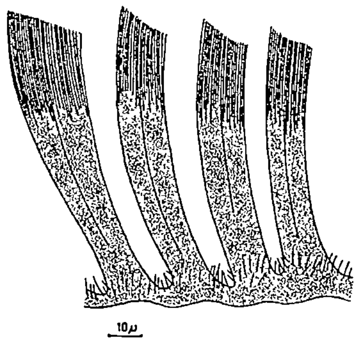

Fig. 1. Diagrammatic drawing of pairs of adhesive bristles of the foot of *Gekko gecko*

The topographical position of the pairs of bristles suggests that they are homologous with the sculpturing which characterize squamate keratin[4,5]. As has been described elsewhere[5], the sculpturings arise during keratinization by the splitting of the Oberhautchen cells: the 'split' Oberhautchen cells thus form the outer surface of the β-layer[4]. The method of formation of the adhesive bristles of the gecko's foot is more complex. The Oberhautchen of the inner epidermal generation on the underside of the foot is a very distinct cell layer the component cells of which show a striking vertical differentiation when keratinization begins (Fig. 2). The pairs of bristles are formed from without inwards, so that whereas the distal portion of the Oberhautchen cell, away from the stratum germinativum, consists of dead polymerized keratin, the proximal portion, nearest the stratum germinativum, is living, having a distinct nucleus and slightly granular cytoplasm. The bristles increase in length by the addition of new keratin at the proximal end. When the Oberhautchen cell dies and becomes fully keratinized, the bristle attains its full length (Fig. 2). It is not possible to describe the formation of the terminal sub-divisions of each bristle as they are beyond the limit of resolution of the light microscope. Oil immersion examination of the dead Oberhautchen cell body reveals thousands of even

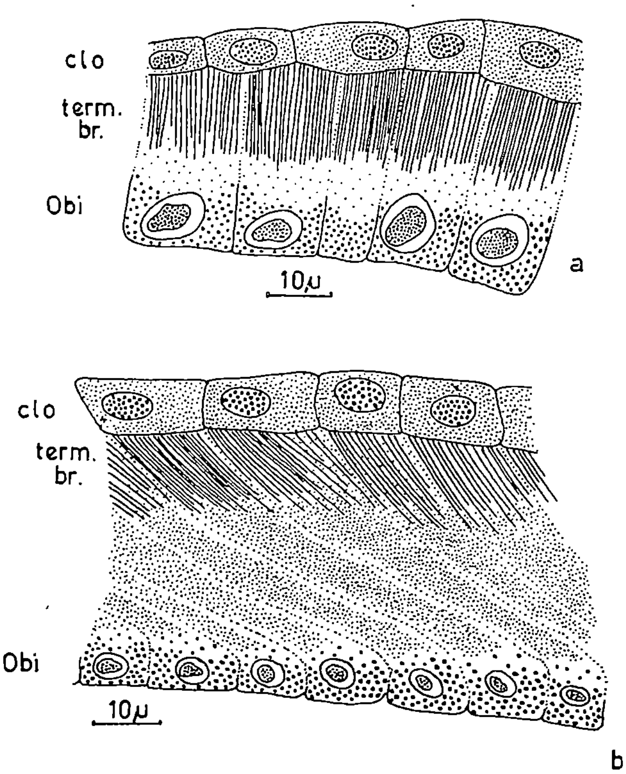

Fig. 2*a*, Diagrammatic drawing of an early stage in the formation of new bristles from the Oberhautchen of the inner epidermal generation; *b*, diagrammatic drawing of a later stage in the formation of new bristles from the Oberhautchen of the inner epidermal generation; *clo*, clear layer of outer epidermal generation; *term. br.*, terminal sub-divisions of major bristles; *Obi*, Oberhautchen of inner epidermal generation

smaller bristles on its outer surface, between the bases of the major formations (Fig. 1). The completion of keratinization of the inner epidermal generation results in the formation of a new β-layer bearing the adhesive bristles on its outer surface. These are revealed when the next slough occurs[5].

These observations are important in assessing the validity of the explanation at present accepted of the adhesive mechanism. Mahendra[2] and Altevogt[3] have put forward a theory which suggests that a gecko uses its adhesive pads in essentially the same way as a climber uses spiked boots. They believed that in the first instance adhesion is achieved by the digital claws clinging to the major irregularities in the substrate, and, secondly, that the bristles, as well as the sub-divisions thereof, reinforce the action of the claws by entering the smaller irregularities. In climbing windows the claws play no part and the adhesion results from the insertion of the terminal sub-divisions into the microscopic irregularities in the glass.

This hypothesis presents a number of difficulties in the light of the observations presented here. Neither Mahendra[2] nor Altevogt[3] referred to the length of the major bristles, nor to the relatively flexible nature of the keratin of which they are composed. The extra adhesion afforded by the spikes on a climber's boot depends not only on the individual spike's ability to engage small crevices, but also on its immobility relative to the sole of the boot. The length of the bristles affords a flexibility which would seem to preclude an analogous function. In the absence of any mechanism which would enable the bristles to actively engage the irregularities in the substrate, it is doubtful whether a direct insertion of the bristles into small crevices, as illustrated by Mahendra's[2] model, could withstand gravitational forces, particularly when the animal is walking upside-down on the ceiling. Furthermore, the effects of abrasion when the animal runs over rough surfaces, for example, stone walls, suggests that the minimum effective diameter of the terminal sub-divisions would be in the order of 0·5–0·1μ, and there is no evidence to suggest that surface depressions of this magnitude are normally to be found in window-glass. However, the presence of a bed of smaller bristles around the bases of the major structures, and the terminal sub-divisions of the latter, suggest that the adhesive mechanism is dependent on the existence of a very large surface area. In spite of the objections of Mahendra[2], the possibility of the existence of a mechanism dependent on electrostatic charges cannot be ruled out, although there is no experimental evidence to support or reject such a theory. It is difficult to see, however, what would be the significance of the terminal sub-divisions if an electrostatic charge was responsible for the adhesion. It is suggested that the present adhesion theory is unsatisfactory and that further attention should be directed to the possibility of a mechanism dependent on physical properties.

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Effect of Low-salinity Water on the Vertical Migration of Estuarine Plankton

Plankton in estuaries is frequently exposed to water of different salinities in layers at different levels, with less dense low-salinity water at the surface[1]. The effects of such salinity layers on the behaviour and distribution of zooplankton have been considered by a number of workers, including in recent years Harder[2], Banse[3] and Lance[4].

Plankton samples pumped from a series of depths in the estuary of the River Test at Southampton during the day and the night indicated that the presence of low-salinity water at the surface inhibited the vertical migration of most species of estuarine zooplankton. A concentration of planktonic animals during the night appeared just below the low-salinity surface water, whereas when there was no such salinity gradient the plankton rose to the surface.

Most workers have found that estuarine plankton will not perform natural diurnal migrations in the laboratory so it is difficult to investigate this behaviour experimentally. In the laboratory most species are attracted to a light[5,6], whereas in the field they may only migrate upwards during darkness.

Lance[4] has shown in an elegant series of laboratory experiments that the strong positive phototactic responses of various estuarine copepods are reduced or inhibited by a layer of low-salinity water. The effect of salinity discontinuities on several estuarine species was reported. However, it seemed desirable to find whether diurnal vertical migrations in the field occurring in darkness were affected in the same way as these responses to a directional light stimulus in the laboratory.

For field experiments an apparatus was required, similar in principle to that designed by Sir Alister Hardy (Hardy and Paton[7]), but in which the enclosed water could come into equilibrium with the environment, and take up any outside salinity layering. Such an apparatus (Fig. 1) was constructed in the Zoology Department of the University of Southampton with the technical assistance of Mr. R. Odell. A transparent tube of square section, 1 m. long, with one side covered solely by 200 mesh/in. nylon netting was separated into three compartments by two sliding trap-doors. Mechanisms were devised for injecting a previously caught sample of living zooplankton and for closing the trap-doors while the apparatus was submerged. The apparatus was designed so that it could be raised without losing the enclosed water through the panels of

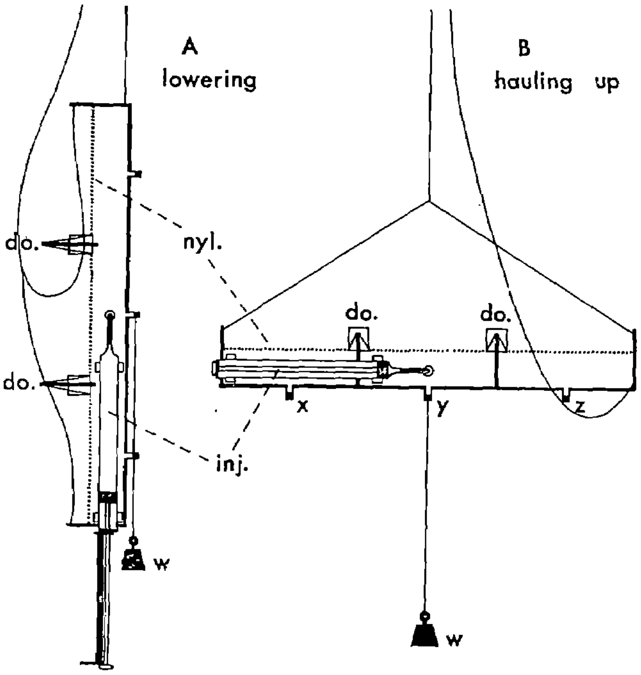

Fig. 1. Apparatus for studying the vertical migration of plankton. *A* shows the position in which it is lowered into the water with the trapdoors (*do.*) open, and a previously caught sample of plankton in the injection mechanism (*inj.*). Water is taken up freely through the panels of nylon netting (*nyl.*). The apparatus is left down for 30 min after injecting the plankton into the main tube before closing the trapdoors (*do.*). *B* shows the apparatus being hauled up horizontally so that the enclosed water is not lost through the panels of nylon netting (*nyl.*), but can be drained separately from the outlets (*x*, *y* and *z*). The weight (*w*) holds the apparatus steady in either position

netting. The water can then be drained from the three compartments separately and the percentages of each species in the upper, middle and lower compartments counted. Sharp salinity discontinuities were produced by surrounding the upper chamber only, by a metal cylinder made from a sawn-off drum, and running fresh water into this, where it was held as a floating layer. The cylinder was large enough in diameter to avoid shading the tube. Slight salinity gradients were also obtained in experiments under completely natural conditions. Several estuarine species appeared to exhibit natural vertical migration patterns in this apparatus and the effects of low-salinity water on their migrations could be determined.

Experiments were carried out using this apparatus in estuaries at Hermanus and Milmerton near Cape Town, with the estuarine copepod *Pseudodiaptomus hessei* (Mrázek)[8]. Groups of experiments in sunlight, shaded daylight, darkness and just before dawn were performed to determine the normal behaviour of the animals in salinities of 24·0, 35·0 parts per thousand. Two further groups of experiments in sunlight and darkness were then done with the top chamber of the apparatus surrounded by water of salinity of 8·5–19·0 parts per thousand. 1,000 and 3,000 *Pseudodiaptomus* were used in each of the six groups of experiments.

In the absence of a low-salinity layer the mean percentage of *Pseudodiaptomus* in darkness and before dawn was greatest in the top chamber, less in the middle and least in the bottom chamber. (Mean in the top chamber, 53·8 per cent; range 41·2–73·6 per cent.) In sunlight and shaded daylight the mean percentage was least in the top chamber, greater in the middle and greatest in the bottom. (Mean in top chamber, 23·9 per cent; range 10·1–36·8 per cent.)

In the presence of the low-salinity layer described here the percentage of *Pseudodiaptomus* in the top chamber, which was above the salinity discontinuity, was less than 1·0 per cent in all cases both in sunlight and in darkness. There was thus an almost complete inhibition of migration into the water of lower salinity, despite the fact that *Pseudodiaptomus hessei* may at times be found living naturally in water of equally low salinity[8], where there is no sharp salinity discontinuity. No consistent differences in the behaviour of the sexes or developmental stages of *Pseudodiaptomus* were apparent in these experiments.

Diurnal vertical migration must be of profound significance to have evolved in almost all planktonic animals. Its survival value to estuarine planktonic animals is probably in helping to maintain their position in the estuary. Complex current patterns occur in estuaries, where there are usually tidal oscillations and a net surface outflow of low-salinity water, and a compensating influx of saline water along the bottom which maintains the salinity gradient[9]. By performing vertical migrations, the planktonic animals drift alternately upstream and downstream, tending to maintain their position in the estuary[10]. In the case of river floods, however, the plankton would be in danger of being swept out to sea if it rose to the surface. The inhibition of migration produced by a strong salinity discontinuity would, however, prevent this occurring. This may well be the reason for the evolution of this particular behaviour pattern. This behavioural mechanism would also reduce the coefficient of reproduction[11], required to maintain a zooplankton population in an estuary, for a given exchange ratio of water escaping seaward at each tidal cycle.

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Relation of Electrical Resistance to Vigour of Apple Scions

THE size attained by a scion apple tree grafted to a clonal rootstock may be modified by interaction between them; but this size is primarily an expression of rootstock effect, and it is usually similar to that of the unworked stock. The vigour rating[1] assigned to the latter is stated in terms of its effect on the ultimate size of scions.

Two electrolytic methods, namely, one involving extraction by either ethanol and/or water and the specific conductance of the extract, and a needle electrode probe method which is applied to living shoots, have identified the accepted vigour of a number of apple rootstocks. Dwarfing and vigorous stocks had high and low specific conductance[2,3], and low and high electrical resistance[4], respectively. This communication examines resistance data from living shoots of 3 scion varieties of apple grafted to 5 apple rootstock varieties.

The accepted vigour ratings 2, 4, of the rootstock clones in the experiment were as follows : *M* (Malling) IX, very dwarfing ; *M* VII, semi-dwarfing; *M* I, vigorous ; and *M* XII, and the Ottawa stock, *Malus robusta* No. 5 (hereafter referred to as *R* 5), very vigorous. The preceding were grafted to each of 3 scion varieties classified in respect of self-vigour as follows: 'Toshkee', semi-dwarf ; 'McIntosh', semi-vigorous ; and an 'Ottawa' selection, 0-2016, very vigorous. Growth and resistance data were obtained when the scions had attained the age of 5 years.

Table 1 gives averaged growth results obtained in October for each of the three varieties of scion trees on the various rootstocks. The heights of 15 trees in each rootstock/scion combination were measured in feet from ground-level, and their trunk circumference in inches at 1-ft. above the ground. The results for each scion variety reveal a direct relationship between height and rootstock vigour, and a similar relationship between circumference and rootstock vigour excepting in the case of the variety 'Toshkee'. On a particular rootstock both the height and circumference of the very vigorous scion 0-2016 were invariably greater than those of either 'McIntosh' or 'Toshkee'. The height of 'McIntosh' exceeded that of 'Toshkee' excepting in the case of the combination 'Toshkee'/*M* XII. The circumference of 'Toshkee' was slightly greater on both rootstocks *M* IX and *M* XII. Scion means for both height and circumference were in the order of accepted scion vigour. Similarly rootstock means identified in the true order of rootstock vigour.

The electrical resistances of living twigs of the previous year's growth of the scion varieties, 8 mm in diameter, were measured in the field on October 5 and November 5 by means of a portable, battery-powered Bouyoucos bridge and steel needle electrode probe assemblage according to procedures[4] previously described. The electrodes, 0·7 mm in diameter, had a tip separation of 7·0 mm. Their penetration of 2·5 mm ensured complete passage of the bark, which becomes progressively thicker with age of shoot. Air temperature ranged from 20° to 28° C; but it must be pointed out that air temperature is one of the least influential factors affecting resistance readings made on living shoots[4]. The averaged results given in Table 2 represent results from 2 twigs on each of 4 trees in each combination of rootstock and scion varieties.

Inspection of the results in Table 2 generally, and of rootstock means in particular, reveals no consistent pattern of electrical resistance attributable to rootstock effect. That the rootstock *per se* had no significant effect on the resistance of the scion grafted to it is further indicated by an analysis of variance and application of the Duncan multiple range test[5] to the data. However, the interaction between the rootstock and the scion was statistically significant at the 5 per cent level.

Table 1. Heights and Circumferences of Trees of Three Apple Scion Varieties grafted to Five Apple Rootstocks

Rootstock		Scion		Rootstock mean
	'Toshkee'	'McIntosh'	0–2016	
		Height (ft.)		
M IX	4·5	5·0	6·1	5·2
M VII	5·0	6·1	6·3	5·8
M I	5·9	6·5	7·1	6·5
M XII	7·8	7·4	9·8	8·3
R 5	7·6	7·9	9·7	8·4
Scion mean	6·2	6·6	7·8	
		Circumference (in.)		
M IX	4·1	4·0	4·4	4·2
M VII	3·9	4·4	4·6	4·3
M I	4·0	4·9	5·8	4·9
M XII	5·8	5·7	6·3	5·9
R 5	5·8	5·9	6·8	6·2
Scion mean	4·7	5·0	5·6	

Table 2. Electrical Resistances (each × 100 ohms) of Living Shoots of Three Apple Scion Varieties grafted to Five Apple Rootstocks

Rootstock		Scion		Rootstock mean
	'Toshkee'	'McIntosh'	0–2016	
		Oct. 5		
M IX	267·0	242·0	270·0	259·7
M VII	212·0	232·0	246·0	230·0
M I	213·0	246·0	258·0	239·0
M XII	230·0	243·0	280·0	251·0
R 5	266·0	254·0	277·0	265·7
Scion mean	237·6	243·4	266·2	
		Nov. 5		
M IX	264·0	259·0	266·0	263·0
M VII	283·0	330·0	332·0	315·0
M I	312·0	292·0	359·0	321·0
M XII	224·0	248·0	271·0	247·7
R 5	253·0	261·0	282·0	267·0
Scion mean	267·2	278·0	302·0	

L.S.D., between scion means: Oct. 5, 5 per cent level, 19·30; 1 per cent, 28·07. Nov. 5, 5 per cent, 24·83; 1 per cent, 36·12.

There were no significant differences attributable to date of sampling. On each of the two dates there existed a direct relationship between self-vigour of scions and their mean resistances. Low resistance was associated with semi-dwarfness and semi-vigour, and high resistance with extreme vigour. The mean scion resistance of 0-2016 differed significantly from those of 'McIntosh' and 'Toshkee', but there was no significant difference between the latter two. The latter result appears attributable to the proximity of 'Toshkee' and 'McIntosh' in the classification scale.

From this experiment it appears that, whereas the height and circumference of a scion apple tree tend to identify the vigour of both the scion and its rootstock, the electrical resistance of living scion shoots is markedly affected by the scion itself, and tends to identify only the accepted self-vigour of the scion variety.

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Feeding of Vitamin C to Tomato Stems and Leaves

Branches of tomato plants ('King Humbert' variety) can absorb, without any apparent ill-effects, large quantities of ascorbic acid (0·1 per cent in tap water). In this solution, oxidation does not proceed very quickly; moreover, the vitamin C content remains constant. In July, in two days, 205* mg were absorbed per 100 g fresh weight; in October, 332 mg per 100 g fresh weight[1–3].

Vitamin C, thus absorbed in the form of ascorbic acid, partly disappears during the absorption itself: in July, the immediate determination found in the plant an excess over the controls of 115 mg vitamin C per 100 g fresh weight, that is, 56 per cent of the quantity absorbed; in October, of 129 mg vitamin C per cent fresh weight (39 per cent of the absorbed vitamin).

For the larger part at least, vitamin C is absorbed as ascorbic acid; it can be found as such in the plant, or, in various percentages, as the non-reducing form. In July, the excess of 115 mg vitamin C contains 95 mg (that is, 83 per cent) ascorbic acid. In October the excess of 129 mg vitamin C per cent g fresh weight was 97 per cent ascorbic acid.

If after two days' absorption of the ascorbic acid solution this solution is replaced by tap water, the excess of vitamin C over the controls does not exceed 29 mg per cent g fresh weight. As 158 mg (per cent g fresh weight) were absorbed, only 18 per cent of the absorbed quantity is found in the plant. These 29 mg vitamin C contain 23 mg ascorbic acid (that is, 79 per cent).

In July, after two days' absorption of the solution the vitamin C content in the stem and in the stalk of the leaves is 84 mg per cent g fresh weight: in the control at the same time, it is 27 mg. Thus the content in the experiment is about three times the content in the control. In the flat parts of the blades, the figures are much higher —experiment, 292 mg per cent g fresh weight; control. 84 mg. The content in the blade is about 3·5 times the content in the control.

* All figures given here are means.

If after two days absorption the solution is replaced by tap water for three days, a large part of the absorbed vitamin C disappears; the content in stem and stalk falls to 45 mg (control, 30 mg) per cent g fresh weight: in the flat part of the blades, to 147 mg (control, 91 mg) per cent g fresh weight: the ratio to the control is thus about 1·5 for both. The distribution of the excess of vitamin C in the experiment is fairly uniform with the distribution of the vitamin in the control, in both cases.

In October, concentration in the stem after absorption for three days rose to 75 mg per cent g fresh weight (control, 26 mg) and in the whole leaves to 223 mg per cent g fresh weight (control, 44 mg). At the end of the experiment the concentration is 3 times the control in the stem, 5 times the control, and more than twice the concentration in the solution, in the whole leaves. Water is always a little more abundant in the experimental plants than in the controls, but this is of little consequence.

At the end of the last experiment, after absorption of tap water for three days, the concentration of vitamin C in the stem is still higher than in the control: this fact points to the incorporation of the excess of vitamin C in the tissues, the increase of concentration in the stem being not merely an appearance resulting from the presence of the solution moving in the vessels.

There is a very sharp difference between the composition of vitamin C in the two kinds of organs: in the leaves, specially in the leaf blades, all the vitamin C absorbed can be in the form of ascorbic acid; in mg per cent g fresh weight 273/292 (control, 68/87) and 217/223 (control, 31/44). In the stem the proportion of ascorbic acid in vitamin C is always much lower: 42/84 (control, 14/27) and 46/75 (control, 7/26). It seems that oxidation in the stem is followed by reduction in the leaves, most probably in the blades.

The absorption of a solution of ascorbic acid (0·5 per cent in tap water) can also be brought about by means of a wick set as a seton in a slot cut in the stem of a normally rooted tomato plant. The results are less regular; but sometimes there is an important increase of the vitamin C content in the stem and the concentration may be lower above the seton than below it.

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VETERINARY SCIENCE

Comparative Digestive Capacities of *Bos taurus* and *Bos indicus* Cattle as affected by Intake of Feed

A comparison of the productivity and efficiency of *Bos taurus* and *Bos indicus* cattle in an environment intermediate to those in which the two species developed was carried out over a 3-year period and reported in 1963 by Howes *et al.*[1]. Using these data it is possible to correlate and regress the apparent digestive coefficients with nutrient intakes (Table 1) for the two bovine species.

All correlation coefficients are highly significant and indicate that the coefficients of apparent digestibility are significantly correlated with the intake of dry matter, protein and gross energy for both species. However, the individual correlation coefficients for the 'Herefords' were consistently larger, although not significantly greater than those for the 'Brahmans', which may be interpreted to indicate that the digestibility of dry matter, protein and energy are more closely related to intake in 'Herefords' than 'Brahmans'. Compared with the 'Brahmans', 'Herefords' over the past century have been subjected to much selection for growth and feed efficiency. This may be the reason for the closer association between intake and digestion for the 'Herefords'. If the correlation between intake and digestion for the 'Herefords' was truly superior, it would indicate a greater efficiency in beef production.

Table 1. Correlation Coefficients and Regression Equations for Intake and Digestion of Dry Matter, Crude Protein and Gross Energy

	Correlation coefficients 'Hereford'	'Brahman'
Dry matter	0·67*	0·62*
Crude protein	0·91*	0·89*
Gross energy	0·76*	0·59*

	Regression equations (*S.E.* in brackets)
Dry matter:	
'Hereford'	$X_1 = 46\cdot2490 + 0\cdot0028\ X_2$ (0·0007)
'Brahman'	$X_1 = 45\cdot9667 + 0\cdot0030\ X_2$ (0·0009)
Crude protein:	
'Hereford'	$X_1 = 19\cdot4490 + 0\cdot0498\ X_2$ (0·0053)
'Brahman'	$X_1 = 29\cdot0271 + 0\cdot0391\ X_2$ (0·0047)
Gross energy:	
'Hereford'	$X_1 = 40\cdot3030 + 0\cdot7805\ X_2$ (0·1577)
'Brahman'	$X_1 = 43\cdot1136 + 0\cdot6094\ X_2$ (0·2175)

* $P < 0\cdot01$. X_1, coefficient of apparent digestibility. X_2, total daily intake.

Based on these investigations the regression equations reveal that the interrelationships for the dry matter and energy measures are similar for both species. However, the relationship for protein is markedly different for the two species ($P < 0\cdot05$). The superior ability of 'Brahman' females to digest crude protein has been discussed in detail elsewhere[1]. Ashton[2] has recently indicated that 'Brahman' steers have a superior coefficient of apparent digestibility for nitrogen. It is appreciated that the fæcal nitrogen involved in these investigations included undigested feed residues, unabsorbed but digested nitrogen, nitrogen secreted from various sources into the alimentary tract, nitrogen from cellular debris from the tract wall and nitrogen from undigested bacteria. No satisfactory system for separating these components has been developed.

Attempts to classify the origins of fæcal nitrogen usually involve more assumptions and errors than they eliminate.

Bos indicus cattle developed in the tropics appeared to possess fundamental digestive and other advantages for tropical beef production[3]. Since crude protein is comparatively low in tropical forages, this may be the result of adaptation. With suitable management and selection, it is possible that tropical cattle production would eventually benefit more from the selection of indigenous types rather than attempts to adapt temperate species to environments for which they are not well suited.

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ENTOMOLOGY

Accelerating and Inhibiting Effects of Crowding on Sexual Maturation in Two Species of Locusts

Isolated pairs of *Locusta migratoria* (R. and F.) kept in optimum laboratory conditions may begin to copulate seven days from their emergence as adults, and the majority of females lay their first egg-pods before the 14th day of adult life[1]. Isolated pairs of *Schistocerca gregaria*

Forsk.) kept in the same conditions seldom copulate or viposit before the fourth week of adult life, and maturaion is frequently delayed for long periods[2]. Crowding ccelerates maturation in *Schistocerca* but retards it in *ocusta*. This difference between two acridids of similar regarious habit seemed anomalous. However, the bservation that continuous crowding of *Schistocerca* vith very young individuals, or fledglings, less than 8 lays old retards maturation suggested that the difference etween the two species might be mainly one of timing[3]. his suggestion is confirmed by the results of the following xperiment which show that in *Locusta*, as in *Schistocerca*[3], nature males exert an accelerating influence on the naturation or readiness to copulate of young males.

Six groups of 10 newly emerged pairs of *Locusta* were et up and three of them were seeded each with two mature nales. The times from emergence until the first copulation of ach experimental male was noted and any male copulating n the non-seeded groups was at once removed and replaced by a female of the same age as the others in the group. The copulation times of the males were as follows:

Groups with mature males	Groups without mature males
13·5 ± 1·7 days	19·3 ± 6·5 days
14·1 ± 1·4 ,,	17·5 ± 2·7 ,,
13·6 ± 1·6 ,,	25·2 ± 3·5 ,,

The average of 13·7 ± 1·5 for all males in the seeded groups differed significantly from that of 20·7 ± 5·5 for the non-seeded ones ($t = 6·566$, $P < 0·001$). Also the average for the most rapidly maturing non-seeded group differed significantly from that of the most slowly maturing seeded group ($t = 3·377$, $P < 0·01$). The synchronizing effect of the mature males on the rates of maturation was, as in *Schistocerca*, very well marked.

In both species, therefore, the young locusts have an inhibiting effect on male maturation and the mature ones have an accelerating effect. The retarding effects of crowding are counteracted by the stimulating effect of the first males which become mature, and maturation is thus synchronized. When young individuals are crowded together there comes a point where the balance is switched over from inhibition to stimulation. In *Schistocerca* this point is reached during the second week of adult life, probably before the crowded males are fully mature, and before any but the most precocious isolated individuals are mature. In *Locusta*, on the other hand, the inhibitory period lasts beyond the second week while most isolated individuals become mature early in the second week. The net result of these opposing differences in the duration of the inhibitory period and in the characteristic maturation time of isolated individuals is that crowding retards in *Locusta* and accelerates in *Schistocerca*.

In *Schistocerca* the accelerating influence of mature males is due to a chemical substance or pheromone secreted by the epidermal cells of the male at maturity[4]. The stimulating agent in mature *Locusta* males has not been isolated but it may safely be assumed to be a pheromone as in *Schistocerca*. There is as yet no direct evidence as to the nature of the juvenile inhibitor in either species but there are strong indications that it is also a pheromone. In general, crowding stimulates activity. Yet older males of *Schistocerca*, whether mature or immature, show depressed activity when permanently crowded with fledglings and become more active when temporarily isolated or crowded with each other. If permanently isolated they show depressed activity when temporarily crowded with fledglings and increased activity when crowded with each other[5]. Since the fledglings are themselves very active, locusts crowded with them are subjected to far more tactile and visual stimulation of a type which normally induces activity than they are when isolated. The inference follows that depression of activity is a response to some non-mechanical, non-visual and therefore presumably chemical stimulus. High activity is associated with rapid maturation in males[5] and enforced activity stimulates ovarial development in females[6]. Although depression of activity in older males may be a response to the same stimulus as that which inhibits maturation it is improbable that delayed maturation is the direct result of depressed activity. During the first two weeks of adult life, by the end of which time ordinary crowded *Schistocerca* are normally maturing, males crowded with fledglings are as active as those crowded with older individuals and more active than isolated ones[5].

In *Schistocerca* the pheromone also has a stimulating effect on ovarial development and on readiness to copulate in the female[3,7]. (As is usually the case in insects spermatogenesis in the male is under independent control and is dissociated from readiness to copulate; spermatozoa appear in the seminal vesicles within 10 days from emergence.) Oviposition in virgin females is stimulated by pheromone action but still more so by copulation[3], and where both sexes are present the effect of the pheromone on oviposition is mainly indirect through acceleration of copulation. The effect on the female was not tested in the present experiment with *Locusta*. The females in the seeded groups certainly copulated with the mature males well before they did so with the young males in any of the groups, but it is probable that this was forced copulation rather than the result of pheromone action on the female.

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Role of DDT-hydroxylation in Resistance

To prove the rate of enzymatic detoxication in resistance, a direct correlation between the degree of insecticide resistance with the enzyme system concentration within the insect must be shown. Thus, in the housefly, DDT * is readily converted to DDE by cell-free preparations and the resistance appears to be proportional to the DDT-dehydrochlorinase activity[1]. DDT is also metabolized to a 'Kelthane'-like material by a microsomal enzyme system, in several insect species, including *Triatoma infestans*[2,3]. However, no significant data are available on the possible relation of DDT-hydroxylation to resistance. Correlation between the level of enzymatic detoxication and resistance can be shown by *in vivo* specific inhibition or stimulation of the enzymatic process, resulting in decreased or increased resistance, respectively. In this respect, it is known that SKF 525–A and other compounds, such as iproniazid, block microsomal hydroxylation reactions[4], while 3-methylcholanthrene acts like an activator[5]. By using this last approach, it has been possible to show that the degree of tolerance to DDT by nymph *T. infestans* can be modified by the foregoing inhibitors and activators, suggesting that DDT-hydroxylation plays indeed a part in resistance.

T. infestans third instar larvæ, which are fairly tolerant to DDT[6], kept under the conditions of Dinamarca *et al.*[3] were used throughout. To groups of 15 insects, 10 μl. of an acetone solution containing 30 μg SKF 525–A or 3-methylcholanthrene were topically applied to the ventral abdominal region of the insects by means of a micropipette. Iproniazid phosphate in aqueous solution (75 μg/3·3 μl.) was intracœlomatically injected at the dose of 75 μg per specimen. After treatment for 24 h, the insects were topically intoxicated with 200 μg DDT in acetone solution per specimen[3], and the survival determined thereafter for 72 h. In order to follow the production of internal polar DDT-metabolites[3], ring-labelled DDT-^{14}C (5,000 c.p.m./μg) was used. The latter was applied at the dose of 4·0 μg per specimen in 10 μl. acetone[3]. After 24, 48 and 72 h of the application of the DDT-^{14}C, the insects were rinsed three times in acetone to eliminate any external insecticide, homogenized in chloroform and extracted according to Dinamarca *et al.*[3]. The chloroform extracts were fractionated in an activated alumina column by the procedure of Sternburg *et al.*[7]. The carbon tetrachloride eluate, containing unchanged DDT and DDE[3], and the combined benzene and acetone eluates which correspond to the polar metabolites, were concentrated to a known volume and an aliquot taken for measuring the radioactivity. Proper functioning of the alumina columns was checked by paper chromatography of the carbon tetrachloride and benzene–acetone eluates[3].

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* Abbreviations: DDT, 2,2,-*bis*-(*p*-chlorophenyl)-1,1,1-trichloroethane; DDE, 2,2-*bis*-(*p*-chlorophenyl)-1,1-dichloroethylene; 'Kelthane', 2,2-*bis*-(*p*-chlorophenyl)-1,1 1-trichloroethanol; SKF 525–A, β-diethyl-aminoethyl-diphenylpropyl acetate; iproniazid; 2-isopropyl-1-isonicotinoyl hydrazine.

Control groups were treated only with either SKF 525–A, iproniazid, 3-methylcholanthrene, acetone, or injected with water. At the doses used, SKF 525–A and 3-methylcholanthrene proved to be non-toxic, while iproniazid alone produced a small mortality of 8 per cent 96 h after injection. Topically applied acetone or injected water was without effect on the insects during the entire experimental period. Ring-labelled DDT-^{14}C was synthesized in the laboratory[3]. SKF 525–A was a gift from Dr. James R. Gillette. The rest of the reagents used were of commercial sources. Radioactivity was measured as described elsewhere[3].

Fig. 1 shows the effect of SKF 525–A, iproniazid, and 3-methylcholanthrene on the toxicity of DDT to *T. infestans* nymphs. It is evident that SKF 525–A and iproniazid potentiate the effects of DDT. At 72 h, DDT alone produced a 58 per cent mortality, while with SKF 525–A and iproniazid, the mortalities were 81 and 89 per cent, respectively. On the other hand, the mortality of DDT-intoxicated insects decreased to 46 per cent by the action of 3-methylcholanthrene, indicating a certain degree of antagonism between DDT- and 3-methylcholanthrene.

When experiments were conducted with DDT-^{14}C, it was found that iproniazid and SKF 525–A substantially decreased the rate of production of polar metabolites, while 3-methylcholanthrene increased their production (Table 1). The latter effect, although fairly small, was consistently found in several experiments.

Our results indicate a negative correlation between the mortality and the rate of production of DDT–polar metabolites. When the latter were decreased, for example, by the effect of SKF 525–A at 72 h, to about 52 per cent, as compared with control insects, mortality increased about 23 per cent. On the other hand, when DDT–polar metabolites were increased to 11 per cent of the controls by the action of 3-methylcholanthrene at 72 h, mortality decreased in 12 per cent. The reported observations would apparently contradict those of Hewlett *et al.*, showing no effect of SKF 525–A on the toxicity of DDT to *Aliphitobius laevigatus*[8]. However, there is no evidence that DDT is metabolized to hydroxylated compounds by this insect, as occurs in *T. infestans*[3]. Further supporting our work, Terriere[9] has recently observed that DDT-resistant houseflies contain microsomal tissue of greater hydroxylating capacity than those isolated from normal insects. Whether the effect of SKF 525–A, iproniazid and 3-methylcholanthrene reflect a depression or an acceleration, respectively, of the rate of specific protein biosynthesis at the microsomal level, or simply an inhibition or activation of microsomal enzymes, is now under investigation. On the basis of this work, it is concluded that DDT-metabolization via hydroxylation plays a part in resistance.

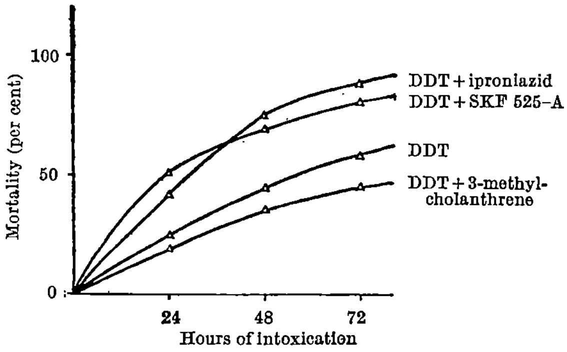

Fig. 1. Effect of iproniazid, SKF 525–A and 3-methylcholanthrene on the mortality of DDT-intoxicated nymph *T. infestans*. The values are the average results of 15 experiments

Table 1. Effect of Iproniazid, SKF 525–A, and 3-Methylcholanthrene on the *in vivo* Production of DDT-^{14}C Internal Polar Metabolites by Nymph *T. infestans*

The values correspond to the average of 5 experiments

Compound	Metabolites production (% of DDT controls		
	24 h	48 h	72 h
Iproniazid	60	78	75
SKF 525–A	52	41	52
3-Methylcholanthrene	124	115	111

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Sex Attractant of Male Scorpion Flies

It is well known that in mecopteran flies of the family Panorpidae courtship and mating behaviour fall into a distinctive pattern[1]. In the case of the Bittacidae, the recorded observations[2–4], although suggestive, have been insufficient to reveal clear-cut feeding and mating patterns

Harpobittacus nigriceps (Selys) is a very important predator of the cinnabar moth (*Callimorpha* (*Tyria*) *jacobaeae* L.) which was introduced into Australia for the control of ragwort (*Senecio jacobaea* L.). Observations carried out in the Gippsland area of Victoria, where *Callimorpha* was released, and later in the Australian Capital Territory on the closely similar *Harpobittacus australis* (Klug), brought to light an extraordinarily interesting pattern of courtship behaviour in which the emission of a sex attractant by the male is combined with the presentation to the female of a nuptial meal. Part of the interest of these observations stems from the fact that the production of a 'female lure' has been very seldom reported in the Insecta.

Both species of *Harpobittacus* search for the soft-bodied insects on which they prey by flying slowly upward along the stems of herbage with their powerful raptorial hind legs outstretched. The long prehensile tarsi vibrate rapidly while they systematically explore the surfaces of the plants. Anything soft or moving is captured instantly by the hind tarsi, which coil around the prey. Once the victim is held securely, the predator seeks out a suitable plant on which to settle and feed. As soon as the biting mouthparts penetrate the integument of the prey, an apparent paralysis sets in, suggesting the presence of some toxic constituent in the saliva. Feeding is usually completed in 1–2 min.

It is significant that the females of *H. nigriceps* and *H. australis* were never observed hunting, capturing o

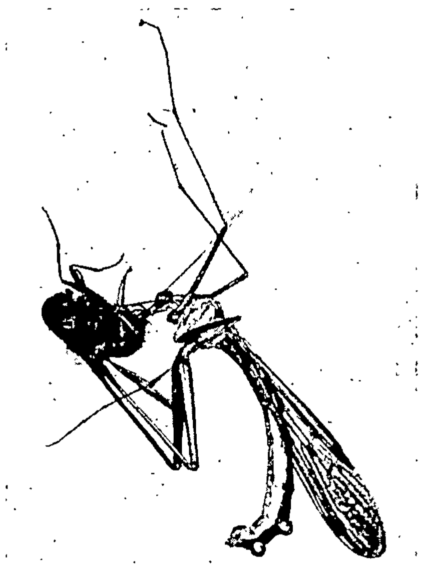

Fig. 1. A male *H. australis* in its female-luring position with vesicles everted (× *c.* 1·5)

killing prey in the field. When the male *Harpobittacus* is holding its prey and starts feeding, two reddish-brown vesicles are everted on the abdomen between tergites 6–7 and 7–8 (Fig. 1); and these can be observed to expand and contract in a slow rhythmic motion. These abdominal vesicles discharge a scent, best described as musty, which is perceivable by the human nose. In the field it acts as a sex pheromone[5] which attracts the females to the vicinity of the male. Females down-wind of a male with prey in its clutches have been repeatedly observed to move unerringly in his direction.

As soon as the female is within reach of the male, the latter retracts its vesicles (presumably ceasing immediately to emit scent) and brings the prey to its mouthparts. The female makes efforts to get hold of the prey, but is prevented in this by the hind legs of the male. During the somewhat agitated proceedings, the abdomen of the male seeks out the tip of that of the female and manipulates it until impregnation commences. Once in copula, the male voluntarily passes the prey with its hind legs over to the female, which eagerly accepts this nuptial food offering.

This courtship and mating pattern, which is virtually identical in *H. nigriceps* and *H. australis*, will be described in greater detail elsewhere. The composition of the *Harpobittacus* sex pheromone is being investigated by colleagues in this Division. A preliminary examination has been carried out of the scent-producing vesicles. They do not appear to have any associated scent reservoirs; and they seem to consist of a specialized intertergal membrane, the cells of which presumably discharge their secretion directly on to the membrane surface (Dr. D. F. Waterhouse, personal communication).

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MICROBIOLOGY

Immuno-clinical Classification of *Staphylococcus pyogenes aureus*

Experimental pyelonephritis in animals and clinical pyelonephritis in man have been under serious investigation at Columbia Presbyterian Medical Center for the past decade. We have been publishing and presenting scientific exhibits at various time-intervals. In 1958, an immunological approach for the possible diagnosis of pyelonephritis in man was undertaken because, bacteriuria being intermittent, the urine culture in such patients was often negative, and no abnormal urinary and radiological changes were demonstrable, particularly early during the course of this disease.

Pyelonephritis is a very common condition. Among the autopsy cases at Columbia–Presbyterian Medical Center in 1940, 11·1 per cent of the kidneys microscopically showed evidence of pyelonephritis, while in 1960 it rose to 26·1 per cent[1]. The results of agar-gel immunological investigations were first reported from our laboratories in the form of a scientific exhibit at the annual meeting of the American Medical Association and American Urological Association in 1960, and a brief reference was made to this technique by us in 1962[2].

While evaluating the results of the agar-gel precipitation bands in patients suffering from chronic pyelonephritis, we observed a very significant fact concerning antibodies to *Staphylococcus pyogenes aureus*. A tabulation showed that some strains of *Staphylococci* reacted uniformly with all patients' sera, while a second group very rarely reacted with such sera, and an intermediate or third group reacted with 5–30 per cent of sera. Having observed this immunological phenomenon, we undertook a survey in 1960 to test as many clinical strains of *Staphylococcus* as possible against the sera we obtained from patients suspected of clinical pyelonephritis.

Every patient suspected of pyelonephritis is routinely examined by the house physician, a urine specimen is collected for culture and 10 ml. blood obtained for serological investigations.

The following eight cultures are being routinely used as antigens in these investigations because they are commonly involved in upper urinary tract infections: *Proteus columbiensis* (*P. rettgeri*, *P. mirabilis*, *P. vulgaris*), *P. morganii*, *K. pneumoniae*, *A. aerogenes*, *E. coli*, *P. aeruginosa*, *enterococcus* and *Staphylococcus pyogenes aureus*. These pathogens are grown for 18 h on Difco brain heart agar in Roux bottles; they are then lysed by sonic oscillation in a Raytheon oscillator *DF* 101, 250 W, 10 kc/s, 115 V and 60 c/s, for 30 min.

One per cent 'Ion agar' (Oxoid) in normal saline is heated to dissolve, and 1 : 10,000 merthiolate is added. Four and a half ml. of melted agar is put on a standard (25 × 75 mm) microscope slide. After the agar has solidified, eight peripheral wells are bored with core borer No. 2, and two centre wells with No. 3. The serum of the patient is put in the centre well, while eight antigens are put in the peripheral wells. The slides are kept at room temperature in a closed plastic freezer box containing moist cotton balls or beakers of water to prevent drying. The readings are taken at the end of 24 and 48 h. The same procedure is carried on using antigens obtained from various *Staphylococcus pyogenes aureus* obtained from surgical and urological patients.

So far 840 sera from patients suffering from chronic pyelonephritis have been tested since 1958, using our eight standard antigens. This observation in pyelonephritis will be reported separately. During this period 221 clinical strains of *Staphylococcus pyogenes aureus* have been tested against 288 sera obtained from cases of chronic pyelonephritis.

A tabulation of these *Staphylococci* showed that 81 *Staphylococcus* or 36·7 per cent gave agar-gel reactions with more than 50 per cent of sera tested. These have been named Group I. Twenty-six of this group are tentatively placed in Sub-group I*a*, and they account for 11·1 per cent of *Staphylococcus*. Sub-group I*a* reacts with almost 100 per cent of sera tested. Forty-four or 19·9 per cent of *Staphylococcus* reacted with less than 5 per cent of these sera, some showing no reaction whatever. These have been named Group III. In the intermediate

Table 1. IMMUNO-CLINICAL CLASSIFICATION OF *Staphylococcus* OBTAINED FROM 221 PATIENTS

Group	No. of *Staphylococcus*	Per cent
I (I*a* included)	81	36·7
I*a*—sub-group	26	11·1
II	96	43·4
III	44	19·9

Group II there were 96 *Staphylococcus* which reacted with 5–30 per cent of sera tested, and they accounted for 43·4 per cent of the pathogens.

We do not know the significance of this observation. We are now preparing typing sera in rabbits with these four types of *Staphylococcus*, so that we shall be able to type all clinical and laboratory strains of this pathogen. Moreover, we shall try to correlate these types with phage 80/81, mutation in relation to chemotherapeutic agents, urease production, coagulase reaction, toxin production, virulence, epidemiology etc. . . . and the possible relationship to superinfection under the impact of chemotherapy with antibiotics.

A brief review of the literature shows that some interesting observations have been reported in this field. Staphylococcal typing by agglutination and agglutinin absorption methods has been in use for some time since first reported by Blair in 1939 (ref. 3). In 1960, Haukenes and Oeding[4] described agglutinins *m* and *n*, and attempted to correlate agar-gel bands with agglutination reaction. Jensen[5] typed 161 *Staphylococci* against sera from 951 donors by agar gel method, and described eight antigens, namely, *A*, *B*, *C*, *D*, *E*, *F*, *G* and *H*; up to six were present in a single strain. Antigen *A* was found in all pathogenic *Staphylococci*, and corresponding antibody found in all normal sera. The sera from infected patients contained more antibodies of the other types than sera from healthy patients. Haukenes, Losnegard and Oeding[6] tested acid and alcohol extracts of *Staphylococci* and found Jensen antigen *A* to be chemically and immunologically distinct from carbohydrate *A* which was identified in all pathogenic *Staphylococci* by Julianelle and Wieghard[7]. Lofkquist and Sjoquist[8] proved the identity of Jensen's *A* and Verwey's type specific protein. Oeding and Haukenes[9] compared agar-gel precipitin bands and agglutinin. Of 9 specific factors used in agglutinin typing, three formed bands in agar gel, namely, *n*, *h* and *i*. Polysaccharide *A* of pathogenic *Staphylococcus* is known to be ribitol terchoic acid mucopeptide.

These observations lead us to believe that there are certain patterns or trends in the nature of the highly mutagenic pathogen, *Staphylococcus*, which have become a great nuisance in the two decades of antibiotic therapy. Apparently, within the species of *Staphylococcus pyogenes aureus*, strain or race variation may be responsible for the erratic behaviour of this organism.

Investigations in Europe and ours in the United States have been carried out independently. Complete and adequate knowledge of these three or four types of *Staphylococcus pyogenes aureus* may prove to be of clinical and epidemiological importance, similar to Lancefield's contribution to the classification of *Streptococcus*.

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CYTOLOGY

Effects of Tannic Acid and Mercury on Huma Erythrocytes

INVESTIGATIONS have been carried out in this laborator on the effect of various substances on the permeability erythrocytes to non-electrolytes in an attempt to elucida the problem of the relationship between the molecul anatomy of the cell membrane and the movement molecules across it. The effect of these same substanc on other characteristics of erythrocytes, including hæm lytic volume, initial volume and fragility, may also gi information which will contribute to a possible solutic of the basic problem. The present report includes da obtained from an investigation of the effect of tannic ac and mercury on these parameters.

Human blood was obtained by venipuncture an citrated. It was refrigerated no more than 24 h befo being washed three times in solution *A* (phosphat buffered, *p*H 7·1, isotonic sodium chloride). For examin tion of initial volumes, 1 ml. of packed cells obtaine following the third washing was added to 10 ml. solution *A* and another 1 ml. of packed cells was added t 10 ml. of solution *A* containing mercuric chloride tannic acid. After 10 min, both solutions were centr fuged at approximately 2,000*g* for 10 min. The supe natant fluid was discarded and the packed cells we used for cell counts[2] and hæmatocrit measurements From these results the initial volume of the cells in th presence and absence of the two substances could b calculated. The highest concentration of mercur chloride used in these experiments was 8×10^{-5} M an of tannic acid $3 \cdot 5 \times 10^{-4}$ per cent.

The method of Hoffman *et al.*[4] was used to measure th hæmolytic volume ($\bar{V}_h$). Stock suspensions of contr and experimental cells were prepared as above. Aliquo of the packed cells were added to different volumes water as described by these authors. The experiment cells were added to water containing mercury or tann acid. In some experiments the value for 100 per cen hæmolysis in these solutions was slightly different fro the control reading. Calculations using both dat however, led to the same conclusions. The highe concentrations used in the cell suspensions in the experiments were $2 \cdot 7 \times 10^{-6}$ M mercuric chloride an $3 \cdot 5 \times 10^{-4}$ per cent tannic acid. The highest concentr tions used in the water were $2 \cdot 0 \times 10^{-6}$ M mercur chloride and $4 \cdot 6 \times 10^{-4}$ per cent tannic acid.

Since previous experiments with 'Triton *X*-100' (ref. had demonstrated that with fragility measurements th concentration of the substance in the salt solutions wa effective while the concentration in the stock solutio made no difference, aliquots of packed cells were adde directly to solutions of sodium chloride of differen osmotic pressures. The highest concentrations used i these experiments were $5 \cdot 9 \times 10^{-6}$ M mercuric chlorid and $2 \cdot 9 \times 10^{-3}$ per cent tannic acid.

A stock solution of tannic acid was prepared freshl each day for all types of experiments. A stock solution o 10^{-3} M mercuric chloride was kept in a 'Desicote'-covere flask. Dilutions of this solution were prepared freshl each day in a desicote-covered flask. All measurement were made at room temperature (20° ± 2° C).

Table 1. EFFECT OF TANNIC ACID AND MERCURIC CHLORIDE ON $\bar{V}_h$ O HUMAN ERYTHROCYTES

Substance	Concentration	No. of observations	$\bar{V}_h$	
Control		127	1·50	
Tannic acid	$3 \cdot 5 \times 10^{-4}$ %	127	1·23	8
Control		27	1·52	
$HgCl_2$	$1 \cdot 0 \times 10^{-6}$ M	24	1·45	1·
Control		70	1·41	
$HgCl_2$	$2 \cdot 0 \times 10^{-6}$ M	69	1·40	0·

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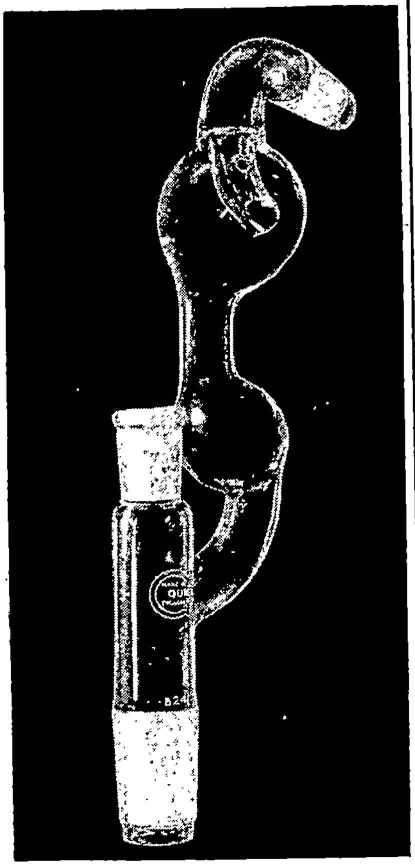

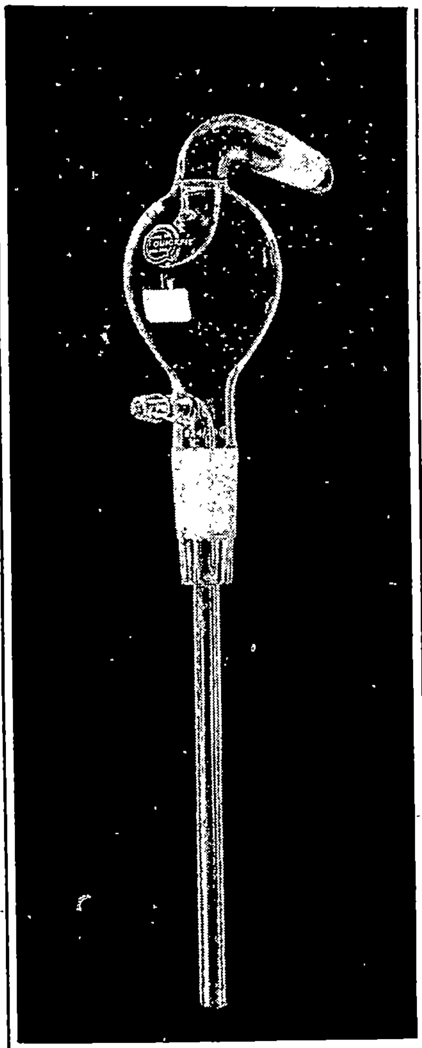

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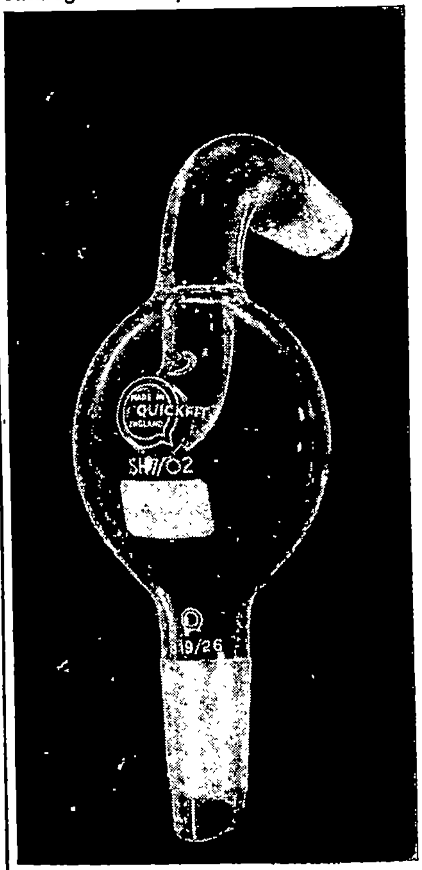

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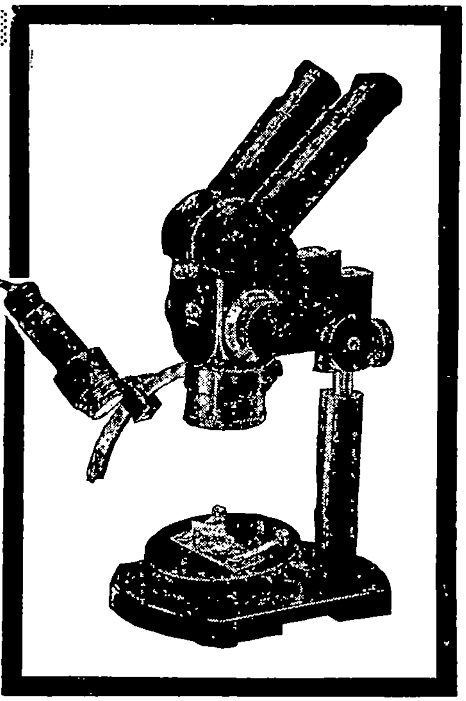

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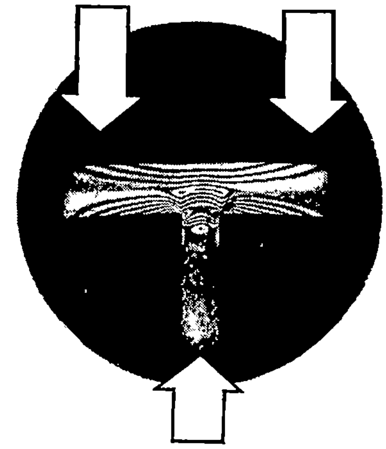

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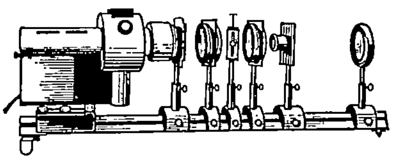

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Both tannic acid and mercuric chloride increased the fragility of these cells slightly. To determine whether or not any of the differences are statistically significant an analysis was made of 44 control values and 65 observations in each of the most concentrated solutions of tannic acid and mercuric chloride. The values in 0·45 per cent sodium chloride were used since the greatest differences were observed in this solution. Using the Student *t*-test, the difference between the cells in the presence of mercury and the controls is significant at the 1 per cent level, and between tannic acid and the controls at the 0·1 per cent level. Neither of these substances produced a significantly different value for the initial volume.

The results of the investigation of the effect of these two substances on $\bar{V}_h$ are summarized in Table 1. It can be seen that high concentrations of tannic acid decrease the hæmolytic volume significantly. Mercury, however, in the concentrations used had no statistically significant effect on this parameter.

Comparing the present results with those previously reported on the effect of 'Triton' (ref. 5), it can be seen that none of these three substances has a marked effect on the initial volume. 'Triton' decreases, while mercury and tannic acid increase, the fragility of these cells. Mercury has no effect, tannic acid decreases and 'Triton' increases the hæmolytic volume. These observations can readily be reconciled with the suggestions previously made that each of these substances is acting on the membrane in a different manner.

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Laser in Cytology

The article by Lang *et al.*[1] suggests that the ruby crystal laser, without mode control, is incapable of producing small areas of tissue destruction, and also that the triocular microscope is not usable. In this laboratory we have been using a ruby crystal laser and a triocular microscope as a routine instrument in biological investigation. The laser, originally designed for ophthalmological coagulation[2], has been modified to be easily connected to the microscope. In numerous experiments we have achieved areas of tissue destruction of $< 2\mu$ diameter, and Fig. 1 shows the effect of the pulsed ruby crystal laser beam on a gametophyte cell of the fern *Osmunda cinnamomea* L. It is worth remarking that we have investigated, theoretically, the temperature distribution in material irradiated by a focused laser beam in the system presented by the retina–choroid interface[3]. We are at present conducting a series of experiments on the volume/temperature relationships of laser irradiation in the case of homogeneous media and have shown, conclusively, the possibility of producing tissue destruction confined to the nucleus of a single cell.

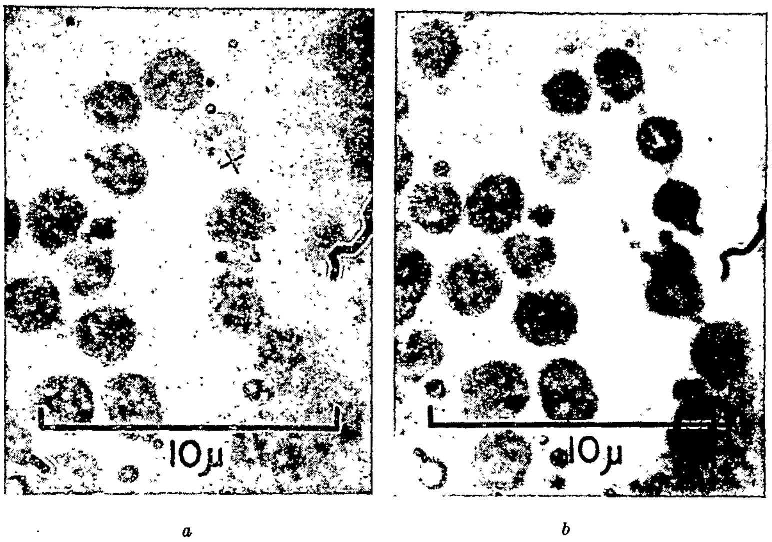

Fig. 1. Effect of a ruby laser beam burst on a living gametophyte cell of *Osmunda cinnamomea* L. (*a*), Before laser beam: the cross marks the point on the chloroplast at which the beam was aimed; (*b*), after a single laser beam burst of approximately 10 m joule into the triocular microscope

This work, which was supported by the National Research Council grant *A*–1356 and the Medical Research Council grant *MBA*–1149, will be reported in full elsewhere.

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VIROLOGY

Effect of Urea on A_2 Influenza Virus

Although many investigations have been reported concerning the action of concentrated urea solutions on plant viruses, relatively few papers discussing the reaction of this reagent with animal viruses have been published.

Cooper[1] has recently described, in some detail, the action of urea solutions on poliovirus. He found that 7·2 M urea initially induced slight surface changes in poliovirus particles and these changes were followed by rupture of the particle. Loss of infectivity and release of RNA were associated with this rupture.

The effect of urea on the hæmagglutinin of influenza virus has been reported by Scott and Lauffer[2], who investigated the action of urea on the thermal stability of hæmagglutinin. Buckland and Tyrrell[3] have recently briefly recorded the response of influenza A_2 virus hæmagglutinin to urea solutions of varying concentration.

In the present work, the effect of concentrated urea solutions on a number of properties of influenza A_2 virus has been investigated. Purified preparations of *A*/Sing./1/57 were prepared by adsorption of virus from infected

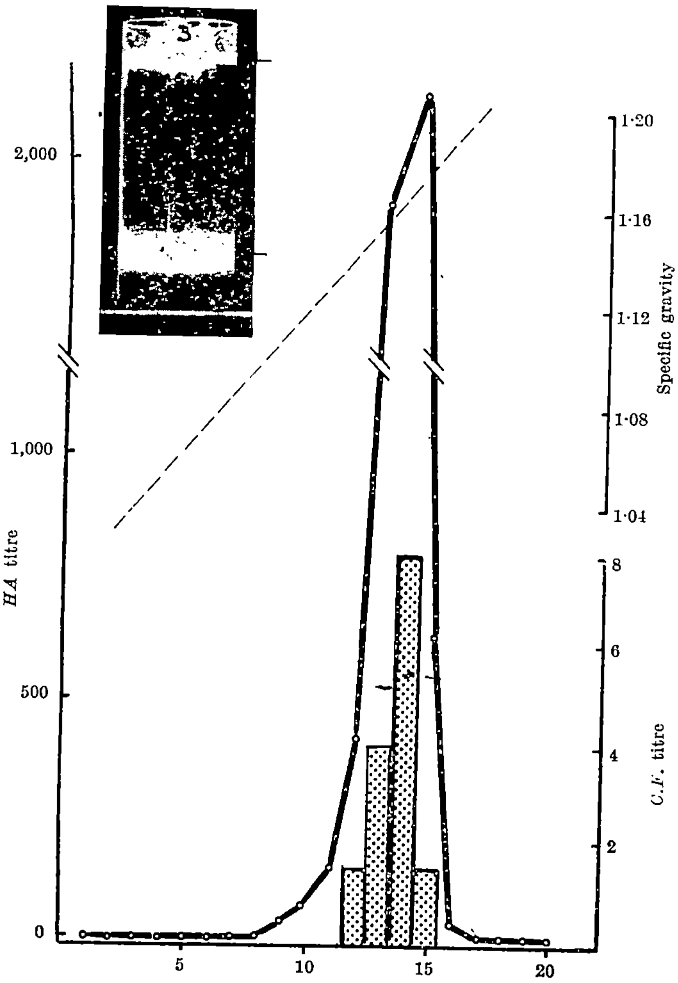

Fig. 1. Control virus. Distribution of light scattering material, hæmagglutinin (*HA*) and complement-fixing antigen (*C.F.*) after centrifugation on a potassium tartrate density gradient. ○, *HA* titre; – – –, specific gravity; stippling, *V* complement-fixing antigen

allantoic fluid on to human red blood cells followed by elution and passage of the eluate through a calcium phosphate column[4]. The virus was further concentrated by centrifugation yielding virus concentrates with hæmagglutinin titres of 1·5–2·0 × 10^5. Urea solutions were freshly prepared by dissolving urea, without heating, in 0·15 M sodium chloride containing 0·01 M phosphate buffer *p*H 7·0. Such solutions were free of cyanate[5,6]. Virus concentrates were treated with urea in the following manner: 0·1 ml. of concentrate and 0·9 ml. of 8 M urea, at the appropriate temperature, were mixed and the reaction allowed to proceed for the selected time. 6·0 ml. of chilled phosphate buffered saline was then added and the mixture transferred to a refrigerator. Control preparations were made by substituting phosphate buffered saline for the urea solution in the above procedure.

Table 1 shows the effect on the hæmagglutinin and the complement fixing antigens of exposure to 7·2 M urea, for 1 min at 0° C. The hæmagglutinin titre is unchanged when measured with guinea pig red blood cells but i reduced when measured with fowl red blood cells. Th *V* complement-fixing antigen is unaffected by the treatment, but in contrast there has been a marked increase in the titre of *S* complement-fixing antigen. Other experiments have shown that no change occurs in the neuraminidase activity of the virus following urea treatment under these conditions.

Table 1. TREATMENT OF A_2 INFLUENZA VIRUS WITH 7·2 M UREA FOR 1 MIN AT 0° C

	Hæmagglutinin* titre		Complement fixing† antigen titre	
	Fowl cells	Guinea pig cells	*S*	*V*
Control	2,560	2,560	<1	24
Urea treated	960	2,560	12	24

* 0·5 ml. volumes of serial, double dilutions were mixed with an equal volume of 0·5 per cent red blood cells and results were read after settling for 1 h at room temperature.

† Determined by the method of Belyavin (ref. 7) but using a single serum dilution.

To explore the foregoing changes in more detail, 4·0 ml aliquots of the control and urea-treated preparations were placed on potassium tartrato density gradient columns contained in 3 in. × 1 in. lusteroid tubes[8,9]. The tubes were then centrifuged in an *SW* 25.1 rotor (Spinco model *L* ultracentrifuge) for 90 min at 20,000 r.p.m. After centrifugation, the distribution of light-scattering material was recorded photographically. The contents of the tube were then displaced by the introduction of 45 per cent potassium tartrate solution into the bottom of the tube and 1·4 ml. fractions were collected from the top through a modified cap. Fractions were tested for hæmagglutinin using fowl red blood cells and, after overnight dialysis against phosphate buffered saline, for *V* and *S* complement-fixing antigen.

The results of the foregoing experiment are shown in Fig. 1, representing the control material, and Fig. 2, representing the urea-treated virus. A comparison of these figures reveals that there has been little change in the sedimentation behaviour of light-scattering material following treatment with urea under the conditions described. Furthermore, the distribution of hæmagglutinin

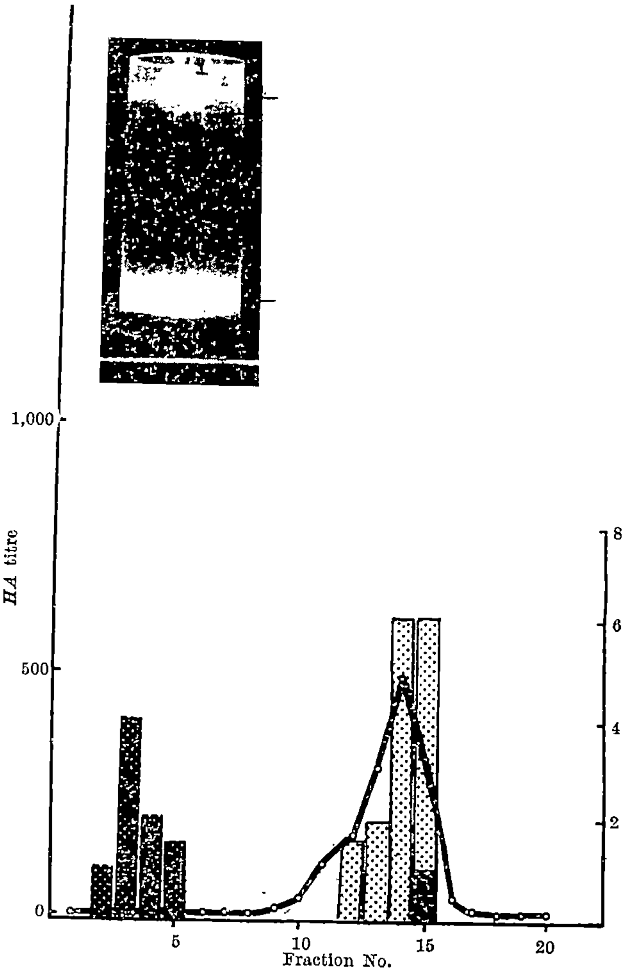

Fig. 2. Urea-treated virus (7·2 M urea for 1 min at 0° C). Distribution of light scattering material, hæmagglutinin (*HA*) and complement fixing antigen (*C.F.*) after centrifugation on a potassium tartrate density gradient. ○, *HA* titre; light stippling, *V* complement-fixing antigen; dark stippling, *S* complement-fixing antigen

s remained unchanged while the change in the dis-bution of *V* complement-fixing antigen is slight. wever, Fig. 2 shows quite clearly that the bulk of the complement-fixing antigen revealed by urea treatment liments much more slowly than either hæmagglutinin *V* antigen.

Brief treatment of A_2 influenza virus with urea thus pears to release *S* complement fixing antigen from the rus particle and in this respect resembles the action of ler on influenza virus described by Hoyle[10].

Extension of this work to the examination of the effect influenza virus of prolonged exposure to urea at higher mperatures has confirmed the rapid release of *S* com-ement-fixing antigen but has revealed marked additional anges in the hæmagglutinin and *V* complement fixing tigen of A_2 influenza virus[11].

I thank Prof. G. Belyavin for his advice. I also thank e Wellcome Trust and the Fleming Memorial Fund for e purchase of equipment used in this investigation. his work was carried out while I was on leave from the stitute of Pathology, Royal Alexandra Hospital for ildren, Camperdown, New South Wales.

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GENETICS

Palatinose Element of the Receptor of the Melezitose Locus in *Saccharomyces*

THE receptor[1,2] of the melezitose (MZ) gene-system omprises at least five 'elements' corresponding to five -glucosidic inductors of the enzyme melezitase, an -glucosidase capable of splitting the five different -glucosidic inductors of MZ, namely, turanose, maltose, ucrose, α-methyl-glucoside and melezitose[3,4]. The otipotent allele, which responds adaptively to all five ubstrates, is designated MZ^{TMSGZ} (abbreviated to TMSGZ). artially recessive alleles, for example, TMSGz, TMSgz, Msgz, Tmsgz, and the totally recessive allele, tmsgz, ere obtained by ultra-violet irradiation[5]. (The small etters indicate specific inabilities of cultures to respond daptively to the corresponding substrates.) The multiple lleles are inferred, on the receptor-hypothesis[1,2], to eflect mutations (heritable changes in folding) of the roteinaceous receptor, a component of the gene con-rolling the capacity to respond to different inductors. t is of special interest to know if the synthetic α-glucoside, alatinose (α-glucosido-6-fructofuranose), can act as a ubstrate and an inductor of this particular enzyme. Emeis[6] has already shown that *Saccharomyces* cultures arry genes controlling the fermentation of palatinose, ut since there are other genes which split α-glucosides and fructofuranosides), and since he did not have genetic-lly identified MZ cultures with which to make specific ests, further analysis was necessary to implicate MZ. The totipotent TMSGZ cultures of the Lindegren Breeding Stock are able to ferment palatinose after 48 h, but specific artial mutants, for example, TMsgz and recessive tmsgz ultures, do not ferment palatinose when tested on EMB-gar and by the Durham tube technique (remained negative for five days)[7]. The fact that the TMSGZ cultures fermented palatinose suggested that palatinose might be one of the substrates (and possibly an inductor) by which the different alleles of the MZ locus may be distinguished from each other.

Melezitase induced in the palatinose-negative, partial mutant TMSgz, *B*4799 (the glucose-grown cells which ferment turanose, maltose, and sucrose after a lag period but which are unable to utilize palatinose, α-methyl-glucoside, melezitose or raffinose), hydrolysed palatinose. The culture was grown on glucose, yeast-extract, peptone nutrient broth for 24 h under aerobic conditions. The cells were adapted to maltose, collected, washed and tested for fermentation[8] of turanose, maltose, sucrose, α-methyl-glucoside, melezitose, palatinose, raffinose and glucose. In control experiments with unadapted cells in which melezitase had not been induced the same

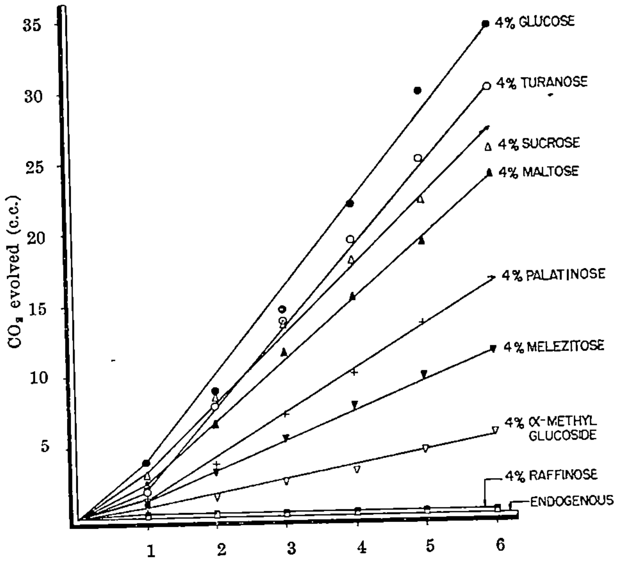

Fig. 1. Culture *B*4799 (TMSgz '*PA*') was grown in 2 per cent glucose nutrient broth for 24 h, adapted to 1 per cent maltose nutrient broth for 12 h and tested for CO_2 production on glucose, palatinose, turanose, maltose, sucrose, α-methyl-glucoside, melezitose and raffinose by the syringe gasometer (1·4 × 10^9 cells in 4 ml. of *p*H 4·5 phosphate buffer at 30° C)

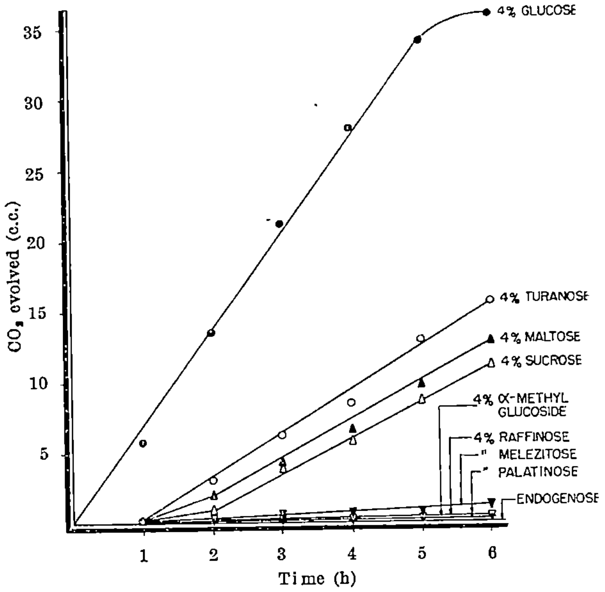

Fig. 2. Culture *B*4799 (TMSgz '*PA*') was grown in 2 per cent glucose nutrient broth for 24 h, then in 1 per cent glucose nutrient broth for 12 h and tested for CO_2 production on glucose, palatinose, turanose, maltose, sucrose, α-methyl-glucoside, melezitose and raffinose by the syringe gasometer (1·4 × 10^9 cells in 4 ml. of *p*H 4·5 phosphate buffer at 30° C)

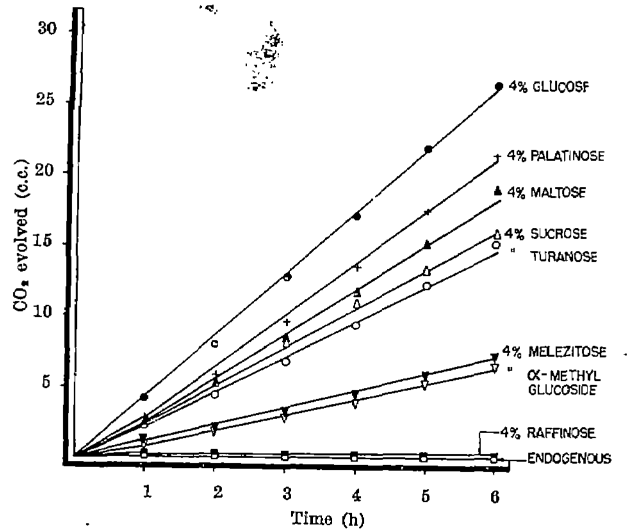

Fig. 3. Culture No. *D*1008Z (TMSGZ '*PA*') was grown in 2 per cent glucose nutrient broth for 24 h, adapted in 1 per cent palatinose nutrient broth for 12 h and tested for CO₂ production on glucose, palatinose, raffinose, turanose, maltose, sucrose, α-methyl-glucoside and melezitose by the syringe gasometer (1·0 × 10⁹ in 4 ml. of *p*H 4·5 phosphate buffer at 30° C)

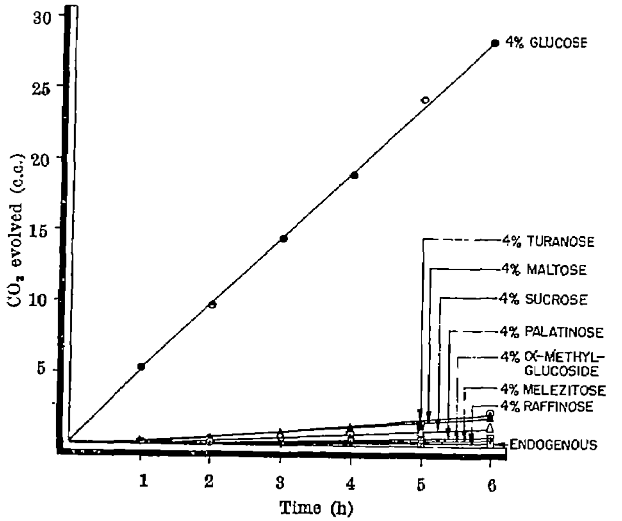

Fig. 4. Culture No. *D*1008Z (TMSGZ '*PA*') was grown in 2 per cent glucose nutrient broth for 24 h, then in 1 per cent glucose nutrient broth for 12 h and tested for CO₂ production on glucose, palatinose, turanose, maltose, sucrose, α-methyl-glucoside, melezitose and raffinose by the syringe gasometer (1·0 × 10⁹ cells in 4 ml. of *p*H 4·5 phosphate buffer at 30° C)

procedure was used (Figs. 1 and 2). The maltose-adapted cells of the TMSgz mutant immediately fermented palatinose and the other five substrates, although the different sugars were fermented at different rates. The unadapted cells were completely unable to utilize raffinose, indicating the absence of β-fructo-furanosidase[9]. These results indicate that the enzyme, melezitase, induced by maltose (in the palatinose-negative culture) is capable of hydrolysing palatinose and the other five substrates.

Similar experiments were performed using the palatinose-fermenting totipotent culture No. *D*1008Z (TMSGZ) (Figs. 3 and 4). Palatinose-adapted cells of TMSGZ fermented turanose, maltose, sucrose, α-methyl-glucoside, melezitose and palatinose, but glucose-grown cells did not ferment any of the six substrates without a lag period. These results indicate that palatinose can induce a melezitase which hydrolyses palatinose as well as the other five α-glucosides, and that another element (the palatinose element, *P*) is presumably a component of the MZ receptor.

Two palatinose-fermenting, totipotent, TMSGZ, single ascospore cultures, derived from the same ascus, were each mated to a recessive tmsgz culture which was capable of fermenting palatinose, and no recombinat were observed between the capacity to ferment palati and the capacity to ferment the other five substr among 23 tetrads from these hybrids. The close lin supports the view that the palatinose element is a c ponent of MZ.

A partial mutant (TMsgz) No. 14 *uv* (which had obtained by ultra-violet irradiation of a palatin fermenting, totipotent, TMSGZ culture, No. *D*10 fermented turanose and maltose but not palatin sucrose, α-methyl-glucoside or melezitose. The re suggests that the putative '*P*' element (along with elements '*S*', '*G*' and '*Z*') had been inactivated by ul violet irradiation. It is unlikely that ultra-violet irr ation would have simultaneously injured two sepa genes ('*MZ*' and '*PA*') in a single cell if one were to ass that '*PA*' was an independent gene rather than 'element' of the *MZ* receptor. Furthermore, when injury of the ultra-violet mutant was back-muta by exposure to melezitose, totipotent back mutants w obtained which had regained the ability to ferm palatinose along with recovery of the ability to ferm sucrose, α-methyl-glucoside and melezitose. Th abilities (once regained) remained tightly linked in s sequent genetical analysis.

We thank Dr. H. Hirschmüller, director of the Be Institute for Sugar Technology, for a supply of palatin We also thank Messrs. J. K. Bhattacharjee and Tom M. Yau for their assistance.

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PSYCHOLOGY

Apparent Size of Holes felt with the Tongue

It is often said that holes, such as dental cavities, fe much larger to the tongue than they appear to the oth senses. This was tested by comparing the apparent si of holes when felt with the tongue and with the finge using the psychophysical method of constant stimuli.

Six research students in psychology, aged 22–30 yea acted as subjects. The subject closed his eyes and pa pated with his tongue a hole accurately reamed in t centre of a 2-in. square of 1/8-in. thick 'Paxolin', whi he held to his lips with his left hand. At the same tin he ran his right index finger round a circular row graduated holes and selected that which felt the san apparent size as the tongue hole. These graduated hol were arranged in steps of 1/64 in., from 1/16 in. up 1/2 in., and were bored in a metal annulus: they we the drill-holding holes in a Dormer drill holder.

Four standard holes were presented to the tongue, diameters 1/8, 1/4, 3/8 and 7/16 in. Each hole was pr sented eight times, in randomized order, and the dri stand felt by the finger was rotated through a rando angle after each trial, to counteract position habits. T

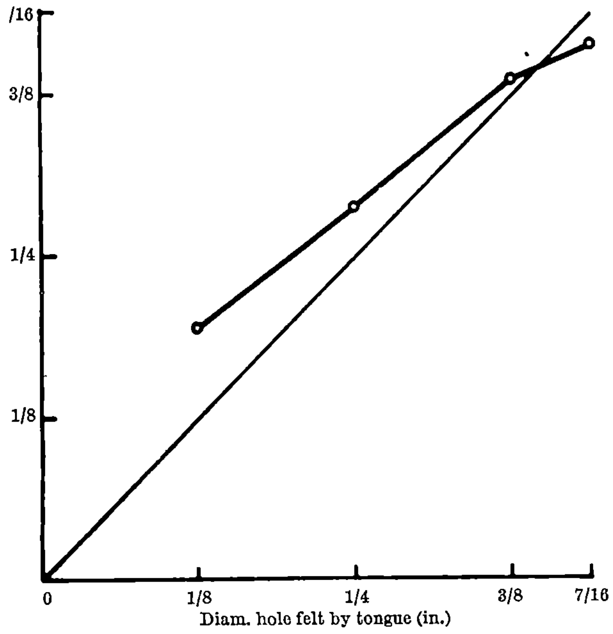

Fig. 1

.bject was allowed to see the finger holes on the drill and before the experiment, but not during it. No .bject saw the tongue holes at any time.

Results are shown in Fig. 1. Each point represents the ean of six subjects, with eight trials per condition. The e of unit slope (45°) indicates no illusion, where the ctual space of the tongue would be congruent with the ctual space of the finger. It will be seen that there is large illusion of just more than 50 per cent for the 8-in. hole, a smaller illusion (15 per cent) for the 1/4-in. le, but no appreciable illusion on the 3/8- and 7/16-in. les. The line which best fits these points by the method least squares is: $y = 0{\cdot}718x + 0{\cdot}106$.

The equations for the individual subjects are: $y =$ 616x + 0·097; $y = 0{\cdot}067x + 0{\cdot}152$; $y = 0{\cdot}768x +$ 089; $y = 0{\cdot}828x + 0{\cdot}117$; $y = 0{\cdot}450x + 0{\cdot}151$; $y =$ 005x + 0·034. A t-test shows that the slope of the dividual subjects' lines is significantly less than unity $< 0{\cdot}05$), and that they intercept the y axis at a value nificantly above zero ($P < 0{\cdot}01$).

So a size illusion is found when the tongue feels 1/8- d 1/4-in. holes, but not when it feels 3/8- and 7/16-in. les.

This work was supported by a research grant from the .S. Air Force (*AF EOARDC* 63–93).

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University of Cambridge.

Alcohol Dehydrogenase Activity and Previous Ethanol Consumption in Mice

PREVIOUS investigations[1,2] have shown marked strain fferences in ethanol preference of mice, and a correlation tween strain means for alcohol preference and for liver cohol dehydrogenase (ADH) activity has been described[3]. urther investigation of this association between ADH d preference is needed to clarify the causal relationships, owever.

Available techniques require that alcohol preference sting precede ADH assay. Thus, if any increase in easured ADH occurs as a result of substrate ingestion, urious correlations would automatically arise from dividual differences in ethanol intake during the eference testing period. Dajani, Danielski and Orten[4] have described such enzyme alteration subsequent to ethanol ingestion in Sprague–Dawley rats, and Figueroa and Klotz[5] have shown an increase with later decrease in ADH activity in Sprague–Dawley rats forced to consume ethanol. Studies on the relationship of ethanol ingestion to ADH in mice should provide data of comparative interest as well as providing information of methodological importance for a planned series of researches on gene-enzyme relationships in ethanol preference of mice. The present investigations are concerned with two strains that differ extensively in the traits of interest: *C57BL/Crgl* mice with high alcohol preference and high ADH activity, and *DBA/2Crgl* mice with strong alcohol avoidance and low ADH activity.

ADH activity was measured in liver homogenates by the rate of reduction of diphosphopyridine nucleotide (DPN) under standard conditions. The procedure used has been described in detail previously[3].

The condition of 'forced' alcohol consumption was imposed simply by providing the animal with only a 10 per cent ethanol solution as a liquid source. Variations of three basic conditions were employed in different studies. The basic conditions were: (*a*) a period of forced alcohol consumption terminating at the time of enzyme assay; (*b*) a period of forced alcohol consumption with a period of forced water consumption intervening before enzyme assay; (*c*) a control condition in which the animal never was provided with alcohol.

Male animals from the *C57BL* and *DBA/2* inbred mouse strains were used. Descriptions of the origin and some of the characteristics of these strains have been given by Jay[6].

Investigations 1 and 2 were designed to determine if the amount of alcohol ingested in the two-week test routinely used for measuring preference could increase measured ADH activity. Forcing animals to consume alcohol solution exclusively for this period of time should provide an estimate of the maximum increase that could be expected in a choice situation.

The effect of forced alcohol consumption, of course, might reasonably be expected to differ in strains which differ markedly in baseline ADH activity and in alcohol preference. In investigation 1, ADH activity was measured in control and experimental (forced alcohol consumption) animals from both the *C57BL* and *DBA/2* strains. All animals were 57 ± 3 days old when killed for ADH assay. The experimental animals had been forced to drink alcohol for either 15 or 16 days prior to killing. Investigation 2 was a partial replication of investigation 1, differing from it in these respects: (*a*) Animals were 77–84 days old when killed for ADH assay; (*b*) the experimental animals had been on a forced alcohol condition for 14 days prior to killing for ADH assay.

Investigations 3 and 4 were undertaken to determine if the alteration in ADH activity observed in investigations 1 and 2 would persist in the absence of ethanol. In *C57BL* and *DBA/2* mice, animals forced to consume alcohol for 3 weeks (3*FAC* group) and control animals were compared to animals which had been forced to consume alcohol for 2 weeks and had then been returned to control conditions (water only available) for 1 week immediately preceding death (2–1 group). Age at time of death was 90 ± 3 days for all groups. The 2–1 group and the 3*FAC* group were thus introduced to the forced alcohol consumption condition at the same age.

Investigation 4 compared 2*FAC* and control animals to animals forced to consume alcohol for 2 weeks and then returned to control conditions for 3 weeks prior to death (2–3 group). Age at time of death was 90 ± 3 days. The 2–3 group was thus introduced to the forced alcohol consumption condition three weeks earlier than the 2*FAC* group.

The results are summarized in Table 1 and Fig. 1. Three measures used to express ADH activity present the same general picture of an increase in liver ADH activity with

Table 1. Means of Measures of Investigations 1–4

Strain	Condition	Study	*N*	Weights Body (g)	Liver (g)	Liver ÷ body	Micromoles ethanol oxidized/min per g liver	Entire liver	Entire liver per g body-wt.
C57BL	3*FAC*	3	4	23·9	1·26	0·052	6·88	8·68	0·361
	2*FAC*	1*	5	25·7	1·38	0·063	6·29	8·63	0·399
		2	7	24·0	1·32	0·055	7·24	9·67	0·395
		4	6	25·1	1·41	0·057	6·01	8·52	0·340
	2–1	3	4	23·0	1·34	0·058	6·13	8·20	0·356
	2–3	4	6	26·2	1·45	0·055	5·49	7·94	0·303
	Control	1	4	23·0	1·23	0·053	5·42	6·69	0·290
		2	7	23·5	1·33	0·056	5·93	7·89	0·336
		3	4	23·4	1·23	0·053	5·86	7·19	0·308
		4	6	25·7	1·36	0·053	5·29	7·19	0·280
DBA/2	3*FAC*	3	4	20·8	0·94	0·052	4·66	4·32	0·236
	2*FAC*	1*	5	18·8	1·01	0·054	4·93	4·95	0·263
		2	7	20·5	1·09	0·054	4·87	5·28	0·259
		4	6	23·7	1·24	0·052	4·10	5·06	0·213
	2–1	3	4	18·7	1·13	0·060	4·01	4·55	0·240
	2–3	4	6	23·5	1·31	0·054	3·56	4·64	0·198
	Control	1	6	18·5	0·94	0·051	4·36	4·07	0·221
		2	7	19·1	1·03	0·054	4·16	4·26	0·222
		3	4	19·9	1·06	0·053	3·84	4·04	0·204
		4	5	21·1	1·19	0·056	3·52	4·17	0·196

* The experimental animals of investigation 1 are approximately 2*FAC*, and are shown as such in this Table and in Fig. 1.

forced substrate ingestion and a reduction of ADH activity with abstinence. The relation is most regular and consistent when ADH activity is expressed in terms of liver weight as in Fig. 1.

These results confirm earlier reports[3,7] that liver ADH activity in *C57BL* mice exceeds that of the *DBA/2* mice.

The increment in ADH activity for the experimental (forced alcohol consumption) conditions appears to be of approximately the same magnitude in the two strains. This, of course, results in a greater percentage increase for the *DBA/2* animals. Because of the aversion of the *DBA/2* animals to alcohol, the total ingested by the forced alcohol consumption groups is less than that of the corresponding *C57BL* groups. For example, in investigation 1 the mean fluid consumption per gram body-weight for the control groups was 0·176 ml. and 0·184 ml. for the *C57BL* and *DBA/2* strains, respectively. The mean fluid intake/g body-wt. for the forced alcohol consumption groups was 0·196 ml. and 0·128 ml. for the *C57BL* and *DBA/2* strains respectively. Under ostensibly the same conditions, then, the *C57BL* animals drank more ethanol although water requirements, as measured by intake in the control condition, are similar in the two strains. Therefore, a detailed analysis of the relative ADH activity increase in the two strains would require controlled substrate administration or correction for consumption differential.

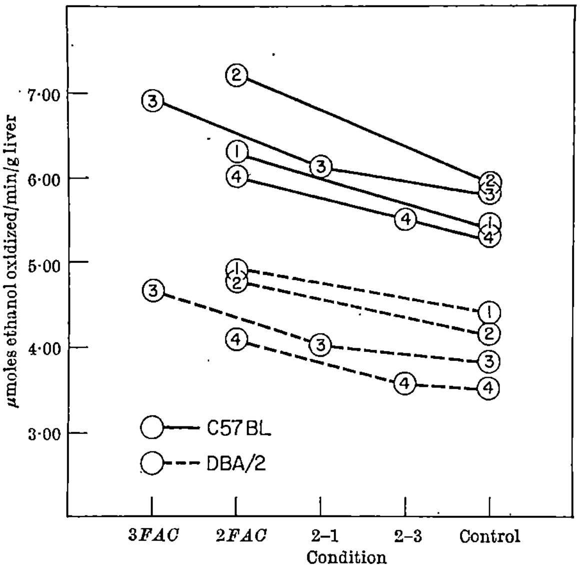

Fig. 1. Alcohol dehydrogenase activity under different conditions. Numbers in circles designate different studies

The results demonstrate that measured ADH activit increases as a function of ingestion of the substrate, an that activity declines toward control values when th substrate is withdrawn. It appears that the absence o ethanol ingestion for three weeks permits almost complet return to baseline values, and, by extrapolation, a period o four weeks or more should constitute an adequate interva in further research on the relationship of ADH to alcoho preference. It is not known, of course, whether th increase in ADH activity represents release of inhibition actual *de novo* synthesis of the enzyme, or some othe process. Nor has it been demonstrated that an increas of this relatively small percentage magnitude has detectable effect upon ethanol metabolism.

The liver weight results, taken primarily for purpose of correcting the ADH activity data for differences i organ size, are of interest *per se*. The larger livers of th *C57BL* animals may simply reflect their greater bod size. The ratio of liver weight to body-weight does no differ between the strains (Table 1). In both investigations (3 and 4) which include a group forced to consum and then to abstain, that group has liver weight in exces of either their control group or the forced alcohol consump tion group. This is true for both strains. Whatever th process responsible for this phenomenon, it appears to b either accelerated by, or initiated by, the cessation c ethanol consumption. Further investigation of thi phenomenon might be of value to the examination of live pathologies associated with alcohol consumption b human beings.

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† Laboratory of Chemical Biodynamics.

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FORTHCOMING EVENTS

Tuesday, August 18—Friday, August 21

ity of Cambridge, School of Clinical Research and Post- Medical Teaching (in the Lecture Theatre of the Department omy, Tennis Court Road, Cambridge), at 9.15 a.m. daily—Symposium rent Research in Leukaemia".

APPOINTMENTS VACANT

pplications are invited for the following appointments on or before dates mentioned:

xperimental Officer in Electrical Engineering in the Faculty of nce, to be responsible for the development and operation of high vacuum electronic equipment—The Registrar, The University, Manchester, 13 gust 22).

esearch Assistant (with a degree) in the Department of Geology, arry out cartographical work and evaluate echo traces for an investigation he submarine topography of the northern North Sea—The Registrar, University, Liverpool 3, quoting Ref. CV/260 (August 22).

esearch Assistants (2) (science graduates) in the Laboratories of Rheumatism Research Centre, to take part in studies of the experi- ntal immuno-pathology and ultrastructure of synovial and other connec- tissues—The Registrar, The University, Manchester, 13 (August 22).

esearch Associates in the following subjects: biology, organic chemis- , mechanical engineering, civil engineering and physics—The Principal, tingham and District Technical College, Burton Street, Nottingham gust 22).

emonstrator (graduate) in Agriculture (Animal Production) in the artment of Agriculture, to assist with experiments and for general duties he University farms—The Registrar, The University, Leeds, 2 (August 24).

ibrarian (with a scientific background, preferably with a pass degree or ivalent, and proficient in German)—The Secretary, John Innes Institute, fordbury, Hertford (August 24).

ssistant Lecturer in Pure Mathematics—The Registrar, The Univer- , Sheffield (August 26).

ssistant Lecturer (with special interests in stratigraphy and sedi- ntation) in the Department of Geology—The Registrar, The Univer- , Manchester 13 (August 27).

xperimental Officer (with a university degree or Higher National tificate, and preferably some knowledge of digital electronics and data dling systems) in the Department of Computer Science, to have ponsibilities for special purpose systems connected to the Atlas computing chine and to assist with the development of new equipment—The Regis- r, University of Manchester, Manchester, 13 (August 27).

raduate Assistant (Administrative) in the Department of Educa- n—The Registrar, The University, Manchester, 13 (August 28).

ecturer (graduate in veterinary science or honours graduate within the ulty of Science, and preferably with postgraduate qualifications and a earch background) in Animal Husbandry at the University of Queens- d, Australia—The Secretary, Association of Commonwealth Universities anch Office), Marlborough House, Pall Mall, London, S.W.1 (London and isbane, August 28).

enior Lecturer (veterinary graduate with postgraduate qualifications honours graduate with a science degree majoring in anatomy and physiol- or biochemistry) in Animal Husbandry (Meat Production) at the iversity of Queensland—The Secretary, Association of Commonwealth iversities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 ndon and Brisbane, August 28).

enior Lecturer (with a higher degree in biochemistry and considerable ching and/or research experience in this field) in Biochemistry at the iversity of Queensland, Australia—The Secretary, Association of Common- alth Universities (Branch Office), Marlborough House, Pall Mall, London, V.1 (London and Brisbane, August 28).

echnician (with Ordinary National Certificate in Chemistry or equivalent alification) to assist with research in the Botany Department—Prof. J. Carr, Department of Botany, The Queen's University, Belfast, Northern land (August 28).

Assistant Lecturer (with medical qualifications registrable in the ited Kingdom) in Anatomy—The Registrar, The University, Manchester, (August 31).

Biochemist or Chemist (Scientific Officer grade) to work in a team studying ion of organo-phosphorus insecticides—The Secretary, Rothamsted perimental Station, Harpenden, Hertfordshire (August 31).

hair of Chemical Engineering at Newcastle University College, The iversity of New South Wales—The Warden, Newcastle University College, ghe's Hill, N.S.W., Australia; and The Agent General for New South ales, 56–57 Strand, London, W.C.2 (August 31).

Chair of Psychology at Newcastle University College, The University New South Wales—The Warden, Newcastle University College, Tighe's ll, N.S.W., Australia; and The Agent General for New South Wales, –57 Strand, London, W.C.2 (August 31).

Lecturer in Theoretical Physics in St. Salvator's College—The Secre- ry, University of St. Andrews, College Gate, St. Andrews, Scotland ugust 31).

Post-Doctoral Research Fellow (with experience in adsorption studies in mass spectrometry) for the spectroscopic investigation of molecules sorbed on surfaces—Prof. N. Sheppard, School of Chemical Sciences, iversity of East Anglia, Wilberforce Road, Norwich, NOR 77H (August 31).

Research Assistant (with a degree in zoology and an interest in fresh- ter biology or ecology) for work on the profundal fauna of lakes—The gistrar, The University, Liverpool 3, quoting Ref. CV/269 (August 31).

Senior Assistant Librarian or Librarian (chartered librarian, and for e higher grade an appropriate university degree) to be employed principally cataloguing and classification duties—The Academic Registrar, Lough- rough College of Technology, Loughborough, Leicestershire, quoting ef. 21/G (August 31).

Research Assistant (with a pass degree in one of the natural sciences) assist in an investigation of unidentified phenolic constituents of plants— e Secretary, Low Temperature Research Station, Downing Street, Cam- idge, quoting Vacancy 64/23 (September 1).

Statistician (Assistant Lecturer or Lecturer) to assist and initiate in- stigations in the Department of Social Medicine and consult with her members of the School and Hospital—Dr. H. W. Holland, Department Social Medicine, St. Thomas's Hospital, London, S.E.1 (September 1).

Post-Doctoral Research Assistant (preferably with an interest in the plication of physico-chemical methods to biochemical problems) in the epartment of Biochemistry, to investigate interaction phenomena in hæmoglobin reactions—Dr. S. Ainsworth, Department of Biochemistry, The University, Sheffield, 10 (September 2).

Administrative Assistant (graduate of a British university and preferably experience of university administration) in the Institute of Education—The Registrar, The University, Liverpool 3, quoting Ref. CV/268 (September 5).

Senior Lecturer (medically qualified man or woman) in the Department of Public Health and Social Medicine—The Secretary, The University, Edinburgh (September 5).

Research Fellow (with a Ph.D. or equivalent postgraduate experience in experimental geophysics, and preferably experience in the maintenance or development of electronic equipment) in Seismology in the Department of Astronomy—The Secretary, The University, Edinburgh (September 7).

Principal Scientific Officer (Senior Zoologist, Research) (with a good honours degree in zoology, considerable postgraduate ecological research experience, and preferably a knowledge of Scottish fauna) at the Edinburgh Headquarters of the Nature Conservancy—The Nature Conservancy (E), 19 Belgrave Square, London, S.W.1 (September 10).

Senior Lecturers; Lecturers; and Assistant Lecturers in Geography, in the following fields of specialization: cartography, climatology, geomorphology, and economic geography, at University College of Cape Coast, Ghana—The Assistant Registrar (Overseas), University College of Cape Coast, 15 Gordon Square, London, W.C.1 (September 11).

Research Assistant (with an honours degree in botany or agricultural botany) to investigate the effects of storage conditions on the life span of crop seeds—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (September 12).

Lecturer in Physiology in the Institute of Obstetrics and Gynæcology at Queen Charlotte's and Chelsea Hospitals and at the Institute of Basic Medical Sciences—The Secretary, Institute of Obstetrics and Gynæcology, Chelsea Hospital for Women, Dovehouse Street, London, S.W.3 (September 14).

Fellow, Senior Research Fellow or Research Fellow in a Unit of Cell Biology and Immunology within the Department of Experimental Pathology, The John Curton School of Medical Research, Institute of Advanced Studies, Australian National University, for studies on acquired immunity and on mechanisms involved in the responses and interactions of cells—Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

Assistant Director of Research in the Mathematical Laboratory—The Secretary of the Appointments Committee of the Faculty of Mathematics, Arts School, The University, Bene't Street, Cambridge (September 16).

Lecturer (preferably with special competence in X-ray diffraction and crystallography and/or electron diffraction) in Physics at the University of Cape Town—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (September 25).

Senior Lecturer or Lecturer in the Department of Mathematics, University of Cape Town—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (September 25).

Reader in Microbial Genetics—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, 2 (September 26).

Chair of Botany at the University of Hong Kong—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, September 30).

Chair of Operational Research in the Department of Engineering Production—The Registrar, University of Birmingham, Birmingham 15 (September 30).

Lecturer or Assistant Lecturer in Food Chemistry in the Department of Nutrition in the School of Home Science of the University of Otago, Dunedin, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

Senior Lecturer or Lecturer (with experience in teaching and research) in Physical Chemistry at the Australian National University—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

Abstractor (science graduate interested in plant pathology and mycology and information handling, and preferably a reading knowledge of one or more foreign languages)—The Director, Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey.

Agricultural Chemist (with a degree in chemistry or agricultural chemistry, and preferably postgraduate experience in agricultural chemistry with specific reference to soil and plant analysis and the design and evaluation of field trials) in Northern Rhodesia, to supervise routine chemical analysis for agricultural purposes, and to undertake development or modification of analytical methods, as governed by crop response to suit the needs of agriculture in Northern Rhodesia—Appointments Officer, Department of Technical Co-operation, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC/213/132/010.

Alfred Capper Pass Chair of Organic Chemistry—The Registrar, The University, Senate House, Bristol 2.

C.E.G.B. Research Fellow in High Temperature Electrochemistry—The Secretary and Registrar, The University, Southampton.

C.E.G.B. Research Student in High Temperature Electrochemistry—Prof. G. J. Hills, The University, Southampton.

Chair of Zoology—The Registrar, The University, Senate House, Bristol 2.

Laboratory Technician in the Department of Veterinary Medicine, to work on research and diagnosis in connexion with animal diseases—The Professor of Veterinary Medicine, University of Bristol, School of Veterinary Science, Langford, near Bristol.

Physicist (with a good honours degree) to initiate and undertake basic research in machining processes—The Secretary, Production Engineering Research Association, Melton Mowbray, Leicestershire.

Research Assistant (honours graduate in zoology, biochemistry, or genetics) in Genetics, to participate in investigations into the physiological genetics of melanotic tumours in the fruit-fly *Drosophila*—The Registrar, The University, Sheffield.

Research Assistant (with an honours degree in physics, biology, medicine or a related subject, and preferably with a broad range of scientific interests) in the Electron Microscopy Unit—Dr. G. Eaves, Department of Experimental Pathology and Cancer Research, School of Medicine, The University, Leeds, 2.

Research Fellow (either medically qualified person with some interest in computers, or a graduate with a knowledge of computer methods wishing to work on medical problems) in Electronic Digital Computer Methods, to undertake studies on the development and application of automatic data processing of medical information—Dr. W. W. Holland, Department of Social Medicine, St. Thomas's Hospital, London, S.E.1.

RESEARCH FELLOW (preferably with a Ph.D. degree and research experience in a subject related to this work) IN THE INSTITUTE OF SOUND AND VIBRATION RESEARCH, for work on the application of high damping alloys in engineering to alleviate noise and vibration—The Secretary and Registrar, The University, Southampton.

VIROLOGIST (preferably with previous experience in microbiology) for tissue culture work in a diagnostic virus laboratory—Consultant in Laboratory Medicine, Belvedere Hospital, Glasgow, E.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Administrators: The Reform of the Civil Service. (Fabian Tract No. 355.) Pp. 45. (London: The Fabian Society, 1964.) 4*s*. [176

British Museum (Natural History). Economic Leaflet No. 7: The Common Earwig. Pp. 4. 6*d*. The Identification of British Mammals. By G. B. Corbet. Pp. iii+46. 4*s*. (London: British Museum (Natural History), 1964.) [176

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 737, Vol. 247 (11 June 1964): Mandibular Mechanisms and the Evolution of Arthropods. By Dr. S. M. Manton (Mrs. J. P. Harding). Pp. 1–183+plate 1, 80*s*.; 12 dollars. No. 738, Vol. 247 (11 June, 1964): Interglacial Deposits at Ilford, Essex. By R. G. West, Camilla A. Lambert and B. W. Sparks. With an Appendix on the Bryophyta by J. H. Dickson. Pp. 185–212. 10*s*. 6*d*.; 1.55 dollars. (London: The Royal Society, 1964.) [176

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The Animal Virus Research Institute, Pirbright. Report for the years 1961–1963. Pp. 34. (Pirbright: The Animal Virus Research Institute, 1964.) 2*s*. 6*d*. [256

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REAPPRAISAL OF TREASURY CONTROL

ONE of the recommendations of the Plowden Committee on Representational Services Overseas (*Nature*, 202, 1039; 1964. 203, 439; 1964) arose out of that Committee's concern that the unified Service proposed in its Report should be in a position to respond quickly and in time to changing requirements. For this the Plowden Committee held that flexibility in the control and deployment of man-power was essential. This is generally true also of scientific and technical man-power, but the Plowden Committee was more particularly concerned that there should be a sufficient margin of man-power to cover the time necessarily spent in travel, training, home leave and sick leave. The Committee received convincing evidence that lack of such an adequate reserve of man-power had seriously impeded the effective deployment of the present Foreign and Commonwealth Services and had led to overstrain.

It was considered vital that this situation should be remedied and the Committee was satisfied that a margin of the order of 10 per cent was reasonable. It recommended accordingly that the authorized man-power of the new Diplomatic Service should be calculated to include such a reserve, though it recognized that it would have to be built up gradually: emphasis was placed on the fact that such a reserve should not be squandered by the creation of new operational posts. Firm management would be required to ensure that this reserve was held and used for the purposes intended, and such management would also ensure that, as requirements change and new commitments are accepted, old commitments which have ceased to be worth while should be dropped. Further, it believed that a reasonable man-power ceiling such as is proposed accentuates the departmental sense of responsibility and encourages both real economy and morale.

The cost of providing and maintaining such a reserve of man-power was put at £750,000 a year, but the Government was not prepared to accept the recommendation in full. Instead, it was agreed that the position should be reviewed again when a margin of 7·5 per cent had been achieved, probably in about two years time. There would then be a careful reappraisal as a basis for deciding whether or not it was necessary to go so far as the 10 per cent recommended. The main basis of the Committee's argument was unchallenged, and the decision taken accords with what the Plowden Committee itself recognized as being practical.

In spite of this, however, this recommendation of the Plowden Committee has now been rejected outright by the Estimates Committee, without seriously examining the evidence presented to the Plowden Committee and questioning the fact that the Diplomatic Service might, under the present arrangements, be understaffed. In its fifth report for the Session 1963–64, *Treasury Control of Establishments**, the Estimates Committee, refusing to discuss any such considerations of overstrain and possible undermanning, refuses peremptorily to consider the proposal of the Plowden Committee on the ground that it endangers the present system of Treasury control of the Civil Service. The purpose which that control is intended to preserve is ignored, and accordingly the vital question whether such control effectively serves the national interests is unasked. Control for control's sake is advocated nakedly and unashamed, and the present system of determining reserves for the Foreign Service should be retained, regardless of its proved defects.

There are two major reasons why this attitude of the Estimates Committee should not be allowed to pass unchallenged. First, such an attitude has the most serious implications for the association of scientists and technologists in the task of government, whether in the strictly scientific and technical sphere, or in that of administration. Inevitably, it must hinder scientific and technical work, leading to frustration and inefficiency and waste. It is also likely to discourage scientists and technologists from taking their fitting share in administration—a point on which considerable emphasis was placed in a recent Fabian Group pamphlet, *The Administrators: the Reform of the Civil Service* (*Nature*, 203, 555; 1964), as well as in the earlier Plowden Report, *Control of Public Expenditure*, issed in July 1961 (*Nature*, 192, 485; 1961).

The second reason is that the unenlightened and restrictive attitude which marks this Report from the Estimates Committee is detrimental to the valuable work which that Committee has done in the past and also to the public esteem of Parliament. It is no service to the effective control of public expenditure to refuse to consider the purposes which such expenditure is intended to service and subordinate the end to the means. There is already a large volume of criticism of the effectiveness of Parliament and suggestions for reform are being freely, if seldom convincingly, advanced. This is a vital element in arriving at an appropriate system for the public discussion of policy and for integrating advice and judgment in ministerial decisions. Solution will not be easier if the standing or prestige of Parliament is diminished by recommendations as ill-founded as that of the Estimates Committee in this matter. The report could well be the subject of some consideration by the Parliamentary and Scientific Committee, all the more in that there are other recommendations which are constructive and enlightened.

It should be noted that the Plowden Report on *Control of Public Expenditure* arose essentially out of a recommendation in an earlier report from the Select Committee on Estimates in 1958 on *Treasury Control of Expenditure* and the comment of the Treasury in a subsequent special report in the Session 1958–59. The recommendations of the Plowden Report led to certain changes in the internal organization of the Treasury in November 1962, designed to contribute more positively to good management throughout the Civil Service, and the present arrangements for Treasury Control are outlined in this latest Report from the Estimates Committee. The Report, however, is not limited to the methods by which the numbers and grades of personnel in departments are kept under scrutiny by the Treasury, but considers also the management functions of the Treasury, including recruitment and the introduction of computers. Moreover, the Report includes among the minutes of evidence a memorandum submitted by the Treasury on Treasury control of departmental establishments and the minutes of an interview with Sir Ronald Holroyd, which covers the experience of Imperial Chemical Industries, Ltd., in the recruitment and management of staff.

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* Fifth Report from the Estimates Committee together with the minutes of the Evidence taken before Sub-Committee C and Appendixes, Session 1963–64—*Treasury Control of Establishments*. Pp. xxxvi+172. (London: H.M.S.O., 1964.) 12*s*. 6*d*. net.

Where the Estimates Committee has gone astray is clear early in the Report. The present system for controlling numbers and grading in the Civil Service is basically that established in 1949. The primary method is a twice-yearly review of man-power, at which the total ceiling of a department's non-industrial staff is fixed. Below this figure, departments can vary the numbers and grades of their staff in particular fields up to the level of their delegated authority, but the creation of senior posts and staff expenditure on any new function or any considerable expansion of an existing function requires Treasury approval. To ensure that complementing and grading standards are properly applied in departments, test checks are made by a team of staff inspectors from the Treasury.

The Estimates Committee agrees with the principles underlying the present system, though it makes certain recommendations. The April review of the man-power of Civil departments should be abolished and the Treasury should require departments to submit their October returns in regular sequence. The Treasury should establish an interdepartmental committee to review the existing arrangements for delegated authority with the view of raising the level throughout the Civil Service for the professional, scientific and technical classes, and for the general service classes also in departments where the establishment officer is of a rank lower than under-secretary. Establishments (Complementing) Division should make regular and systematic examinations of the staff of all Civil departments over a period of 3–5 years.

The Committee tried hard to induce Sir Laurence Helsby, Joint Permanent Secretary to the Treasury and head of the Home Civil Service, to commit himself to the Committee's view that the recommendation of the Plowden Report regarding the staffing of the Diplomatic Service would make it impossible for the Treasury to perform its primary duty, "to ensure that no more staff are employed than are needed; that posts are correctly graded by reference to duties; that suitable staff are assigned to the posts; and that consistent standards are maintained throughout the Service". However, it is obvious that Sir Laurence Helsby has an open mind on this matter. He pointed out, in fact, that the 10 per cent margin recommended by the Plowden Committee was not a full 10 per cent increase, though the existing margin which the Plowden Committee thought inadequate was not disclosed. Sir Laurence thought that it might not be necessary to exceed the 7·5 per cent margin adopted for the present, but preferred to reserve judgment until we had seen over the next two years what a modest increase in the existing reserve did to make for a greater flexibility. He gave no support to the idea that removal of Treasury scrutiny from this reserve violated the whole principle of Treasury control.

On the evidence of this report, the Estimates Committee's concern that one of the most important functions of the Treasury is to be the agent of the House of Commons and its committees in determining the amount of public expenditure and the means by which the House controls the size and pattern of the public service has blinded it to the real issues. It is irrelevant that other departments are, in general, satisfied with the present system. We are faced with a proved defect which the Estimates Committee will not really face, least of all the fact that the size of the Civil Service is ultimately determined by Government policy which increases or curtails the work to be done. So naïve a recommendation could scarcely have come from the Committee if its members had read Lord Bridges's recent book, *The Treasury* (*Nature*, 203, 555; 1964), particularly the chapter, "Treasury Staff Control: How it Works".

Even so, it is difficult to account for so futile a recommendation in a report characterized otherwise by much sound sense and insight, particularly when, as already noted, the recommendation could hinder the recruitment of scientific and technical staff with which the Estimates Committee was also concerned. The interdepartmental committee it recommends is intended partly as a contribution to the more effective use of such staff. However, the Committee's major recommendation in this field is that the Treasury should take positive steps to stimulate the recruitment of scientists by producing a better career structure within the Scientific Class and should encourage the transfer of members of the Scientific Class to the Administrative Class. Sir Laurence Helsby, in evidence, admitted that there was a considerable shortage of scientists in the Civil Service, while the Treasury memorandum referred to the difficulty of discerning and applying standards or objective criteria in the work of professional and scientific staff. This is a prime reason for seeking to increase the delegated authority in such classes.

Sir Laurence pointed out that in the recruitment of scientists the Treasury and the Civil Service Commission were already co-operating to the maximum extent. However, much of this recruitment was on a temporary basis and the research fellowships offered by the Commission had attracted some exceptional scientists who did not respond to establishment on a career basis. He agreed that the dividing line between the administrative and the scientific and professional classes might have been too rigid and hindered recruitment. Nevertheless, he did not think that the scientist was at a grave disadvantage. While they had been disappointed about the willingness of potential administrators to move from the scientific field into the administrative class, within the past three months a competition to that end had been more successful than any for some years and fifteen really promising administrators had been recruited from the scientific class.

It was at this point that Sir Ronald Holroyd's evidence was relevant. However, while the Estimates Committee does not consider that there should virtually be no boundary between administrative and specialist tasks, it wishes to encourage to the full the incorporation, so far as possible, of scientists into the management team of a department, provided recruitment for the purely scientific posts was fully maintained. The Estimates Committee considers that, in any event until recently, there has been too rigid a barrier between the administrative and scientific classes and that this should be relaxed. This is not of itself likely to contribute greatly towards making the Civil Service more attractive as a career to the younger scientists: other factors are probably much more important, and the interchange between the Civil Service and industry, with which the Estimates Committee was next concerned, could contribute something to the establishment of the atmosphere which is most likely to overcome the reluctance of a scientist to adopt the Civil Service as a career.

In spite of recommendations in the earlier Plowden Report and elsewhere, such interchange does not occur on any appreciable scale—though the Treasury was considering whether it would increase its initiative here. Sir Ronald Holroyd in his evidence referred to the need for people in the Civil Service who can speak the language of industry. Even though there was good agreement on this point, it is an open question whether this broadening of experience is best achieved by physical interchange of staff or by the exchange of experience and ideas. Sir Laurence had reservations as to how far the former had really benefited the Civil Servants who had enlarged their experience in this way, and very clearly there are considerable practical difficulties in arranging such interchange which do not apply, for example, to interchange with universities. Nevertheless, the Estimates Committee is satisfied that the more informed discussion and exchanges of ideas can occur the better for all concerned. The barrier between the Civil Service and industry is, indeed, gradually being broken down, but the Estimates Committee urges that the process should be accelerated as much as possible. The new syllabus at the Centre for Administrative Studies, which provides for visits to industrial concerns, is one example of increasing Treasury initiative in this field, and the Committee recommends that the Treasury should make every effort to increase the scope for interchange of knowledge and experience between the Civil Service and industry both at home and abroad.

For the rest, the Estimates Committee in this report considers at some length the management services of the Treasury, now organized under an Under-Secretary, responsible to the Third Secretary (Management). While it has no doubts as to the skill and efficiency of the Organization and Methods (General) Division, it questions whether this skill is fully utilized, partly through lack of control over departments which have their own organization and methods branch and partly through the limitations imposed by the Division's advisory role. The Committee thinks that the Treasury should require such departmental branches to submit a forward programme of their assignments, in which the Treasury should participate if it thought fit. It also recommends that the Division should carry out planned reviews of all the departments for which it had responsibility.

The role of the Organization and Methods (Machine) Division is also advisory, but it participates in any major mechanization scheme in the Civil Service, and in this sphere of machines the Treasury plays a much more central part. The Division has already, rightly, in the Committee's opinion, taken on itself immense responsibilities which it is believed to be fulfilling with care and skill. Contrary to some rather vocal criticism elsewhere, the Estimates Committee found the Civil Service at least as far advanced in this field as a commercial concern such as Imperial Chemical Industries, Ltd., without plunging recklessly into excessive expenditure without proper consideration. For this position the credit is given to the Treasury, and steps are also being taken to make all departments fully aware of the great potentials of mechanization. Welcoming the Treasury's initiative, the Committee also expresses the hope that still further steps will be taken to instil into all departments a proper appreciation of the immense potentialities of computers as a means of speedier and more efficient management.

The third of the management divisions, Management Services (General Division), has only operated since November 1962, and is responsible for investigating problems outside the strict field of organization and methods, and acting as an organ of the Treasury, to compare the practices of several departments. The Estimates Committee welcomes this wider opportunity but does not consider that the opportunity has yet been used. It recommends accordingly that the organization and functions of the Division should be reviewed to enable it to carry out a wider range of investigations which the Committee believes are of the highest importance.

Generally, the Committee holds the opinion that the greatly increased emphasis in the Treasury on management since November 1962 is to be welcomed and it hopes that the initiative will be maintained and strengthened. To a large extent, the impulse here comes from the Plowden Report on *Control of Public Expenditure*, but the Estimates Committee does not accept all the implications of that Report. It challenges, for example, the view which, it suggests, is implicit in that Report that the Treasury and the other departments are more or less equal partners, and that the work done by the complementing divisions is far less important than the political work to be done by the management divisions. Unhesitatingly, the Committee affirms the supremacy of the Treasury, at least to the extent of the right and duty to institute regular and thorough inspections of the departments.

The Plowden Committee maintained that there should be no interference with a department's responsibilities, and it can certainly be argued that regular inspection by the Treasury tends to undermine departmental responsibility. The Estimates Committee's anxiety to restore the supremacy of the Treasury, however, really stems from its desire to reassert the authority of the House of Commons. That much is plain from any reading of A. Hill and A. Whichelow's *What's Wrong with Parliament?* They point out that to-day control by the House of Commons of Government spending is now largely non-existent and only the Treasury can help. While Members are aware of this and are now more wary about the full financial implications of the policies they approve, they still cling to old procedural forms which no longer grip the matters they were designed to control.

Mr. Hill and Mr. Whichelow argue firmly that the influence and prestige of Parliament would be restored by use of a series of parliamentary special committees—as opposed to the Party committees which have developed since the Second World War—and they cite as an example the Select Committee on the Nationalized Industries. In this system, they suggest, the Estimates Committee would itself occupy an important place: its inhibition in respect of policy would be removed and its six sub-committees might form the basis of the new committees. The Public Accounts Committee would probably continue to function as at present, but if the Estimates Committee were no longer debarred from criticizing Government policy, the futilities in its present report would be unlikely to recur.

While there is some substance in the view that the weaknesses in the present Report arise out of out-dated procedures, more is involved than procedures. There is also the question of the function of the Treasury itself, and it is obvious that the Estimates Committee is poles asunder from the view put forward by the Fabian Group in the pamphlet mentioned earlier. The Fabian Group sees the functions of the Treasury as very different and does not regard it as good for the Civil Service that the department responsible for financial control should also be responsible for policy and management of personnel, since it is judge in its own cause: considerations of finance may take precedence over ideas of enlightened management. Whether in fact that happens seems a little doubtful on the evidence of the Estimates Committee's report, but the function of the Treasury as viewed by the Fabian Group should be considered in the light of its proposal to transfer to the Civil Service Commission the responsibility for personnel management and the structure of the Civil Service. For that, however, the pamphlet must be considered in greater detail, and all that can be added here is that, like the Estimates Committee itself and Mr. Hill and Mr. Whichelow in discussing the reform of Parliament, the Fabian Group is intensely concerned with the recruitment and use of scientists on an adequate scale and up to the highest levels.

THE INTERNATIONALE OF SCIENTISTS

The Dawn of a New Age

Reflections on Science and Human Affairs. By Eugene Rabinowitch. Pp. viii + 332. (Chicago and London: University of Chicago Press, 1963.) 6.95 dollars; 52*s*.

THIS book by the internationally known, respected and loved editor of the *Bulletin of the Atomic Scientists* contains his reflexions on human affairs extending over almost twenty-five years. He first presents his credentials as a prophet in two astonishingly accurate forecasts of the Second World War, written on September 1, 1939, and on May 5, 1941. After this it comes as less of a surprise that in his later essays, written at the time of the Korean war, of the Vietnam crisis, of the first *Sputnik* and of the Cuban crisis, he has displayed the same sound judgment; always confirmed, never contradicted by what has happened since then.

Eugene Rabinowitch was born in the U.S.S.R. and is now professor of biophysics at the University of Illinois. He has maintained friendly relations with a great number of Soviet scientists, without ever ceasing to be critical of their system. His essay on "Russian and Soviet Science" is a masterpiece of balanced judgment. In this, and in all his other essays collected in this volume, he pleads a realistic gospel of understanding and of patience, which is probably equally uncongenial to the average unsophisticated American with a somewhat fundamentalistic cast of mind, who can see nothing but diabolic wickedness in Communism, and to the fundamentalists among his fellow-scientists, who recommend conscientious objection, and who can see no hope short of "Banning the Bomb".

Rabinowitch is a Fabian of disarmament, who keeps his eye steadfastly on what is possible, without ever forgetting the final goal. The report to the Secretary of War, 1945, of which he was one of the chief authors, and which has become known as the Franck Report, and his speech for the President in 1954, are fine examples of his method of approaching influential non-scientists. If not even words of such wise moderation so closely argued from incontrovertible facts have an effect on politicians, then what use are words? Rabinowitch must have often asked himself this question, yet he has never despaired, and never given up his patient pleading. If, as is very likely, he and people like him have made even a small contribution to the tremendous fact that fifteen years after both sides came into possession of the atomic weapon not a single nuclear bomb was exploded in anger, this ought to be considered a rich reward for their patience.

The book contains thirty essays, grouped under the headings "Looking Ahead", "Taking Stock", "Talking with World Scientists", and "Heretical Thoughts". If anybody looks for really heretical thoughts, he will be disappointed. Rabinowitch offers plain common sense, and he scorns Bernard Shaw's trick of dishing up commonsense as a shocking paradox. It is somewhat regrettable, but in the nature of things, that out of his many pages of carefully balanced arguments, it will be scarcely possible to lift out even one epigram, which might take root in the common man's mind. He is a fluent and polished writer, eminently readable, yet sometimes I wish he had taken advice from 'Mad. Ad.'. But any serious reader, scientist or humanist, will find great enjoyment in reading his book.

D. GABOR

PHYSICIAN AND PHILOSOPHER

John Locke (1632–1704): Physician and Philosopher

A Medical Biography, with an Edition of the Medical Notes in his Journals. By Kenneth Dewhurst. Pp. xii + 331 + 11 plates. (London: The Wellcome Historical Medical Library, 1963.) 42*s*.

JOHN LOCKE'S abiding fame rests on his philosophy. He may be regarded as the founder of empiricism, which is the doctrine that all our knowledge (with the possible exception of logic and mathematics) is founded on experience. He was born in 1632, but did not publish the *Essay Concerning Human Understanding* until 1687. Six years (1687–1693) sufficed for this and his further works on *Toleration*, *Government* and *Education* to make him the most influential and perhaps the greatest of British philosophers. His teaching in his lifetime and after his death exerted a far-reaching political influence. The British Constitution was based on Locke's doctrines until some sixty years ago; with Montesquieu's additions they are part of the American Constitution; they had a determining influence for the French philosophers and the moderate reformers in the French Revolution and inspired the French Constitution in 1871[1].

So great is Locke's stature as a philosopher that it is often forgotten that he was also a scientist, and throughout the greater part of his life a practising physician, a writer of medical essays and of clinical reports and papers. The view may well be accepted that it was his interest in science and prolonged observation of disease that led him to reflect on human knowledge and to enunciate his views that this equally was based on experience.

When Locke died, his large store of manuscripts, notebooks and journals went to his cousin, Peter King, later Lord Chancellor. The seventh Lord King, in 1829, published an indifferent biography, entitled *Life and Letters of John Locke*, with a few extracts from the journals. The medical aspect of Locke's life was brought forward in Dr. John Brown's discursive essay, "Locke and Sydenham" (*Horae Subsecivae*, 1866). In 1876, H. R. Fox Bourne's reliable biography, the *Life of John Locke*, appeared in two volumes, which examined new material including some of Locke's medical writings. This led to the brief but admirable studies of two medical historians, Dr. E. T. Withington[2] and Sir William Osler[3], who both concluded that Locke was an experienced and skilful physician of sound judgment and advanced views. They surmised that he gave more time to his chosen profession than the manuscripts then disclosed. These beliefs were confirmed in 1948, when the Bodleian Library, with the aid of the Clarendon Press, purchased from the Earl of Lovelace (a descendant of the first Lord King) a large collection of Locke's unpublished papers including much material of medical interest.

Dr. K. Dewhurst, Wellcome Research Fellow in medicine, Corpus Christi College, Oxford, has sedulously studied and deciphered the medical papers in this new source of material and has written this scholarly medical biography of John Locke, which reveals the philosopher's attainments as scientist and physician. Included are a complete edition in English of the medical notes in Locke's journals and a biographical survey of his other medical writings.

John Locke, the son of a Somersetshire lawyer, in 1646 entered Westminster School under Dr. Busby. Among his school-fellows were Richard Lower, Walter Needham and John Mapletoft, future physicians. In 1652 he proceeded to Christ Church, Oxford, graduating B.A. in 1655 and M.A. in 1658. In 1659 he was elected to a senior studentship at Christ Church; in 1660 he became lecturer in Greek and in 1662 lecturer in rhetoric. Dr. Dewhurst notes that Locke kept a medical note-book and studied science and medicine from the beginning of his university career; and also that through his friend, Lower, he was early in touch with the precursors of the Royal Society, that group of experimental philosophers, including William Petty, Thomas Goddard, Ralph Bathurst, Christopher Wren, Thomas Willis, and Robert Hooke, who met in the rooms of John Wilkins, Warden of Wadham College. In 1660 Locke's friendship with Robert Boyle augmented his interest in medical chemistry or pharmacology; and he studied botany in the Oxford Botanic Garden and attended Willis's lectures on therapeutics. In 1665 he served as secretary to Sir Walter Vane's diplomatic mission to the Elector of Brandenburg. Returning to Oxford, he worked with his friend, Dr. David Thomas, at practical chemistry and in 1666 joined him in medical practice. Lord Clarendon, Chancellor of the University, requested that Locke might now accumulate the M.B. and M.D. degrees. Fearing opposition and rejection, Locke did not submit the request but ensured continuation of his studentship by a royal dispensation.

In 1667 Locke went to London as personal physician to Lord Ashley. This led to his association in clinical work with Thomas Sydenham, and he forsook the laboratory to study disease at the bedside. An early success was his operation on Lord Ashley for a suppurating hydatid cyst of the liver. In 1668 he was elected a Fellow of the Royal Society of London and served on a committee of the Society. In 1670 Lord Ashley nominated Locke for the degree of M.D. Oxon., but Locke asked his patron to withdraw the request on hearing that John Fell, Dean of Christ Church, and the Provost of Eton both opposed the award. When Ashley became Earl of Shaftesbury, Locke was appointed Secretary of Presentations. In 1674 he became M.B. Oxon., a medical graduate at last, but he never proceeded to the M.D. He was appointed to a medical studentship at Christ Church.

In the smoky atmosphere of London, Locke appears to have contracted asthma and chronic bronchitis. After Shaftesbury's fall from power in 1673 he lost his post, and in 1675 he went to France for the sake of his health. He stayed fifteen months at Montpellier studying medicine and science and, afterwards, went to Paris for the same purpose. His medical services were constantly in request by British residents. He revisited Montpellier, then Paris again, and returned to London in 1679. From then until 1682 he was again with Shaftesbury in public affairs and renewed his friendship with Sydenham and other physicians. After Shaftesbury's death he fell under suspicion for his political views and from 1683 until 1689 sought refuge in Holland. Here he wrote his celebrated *Essay*, but also studied Dutch medicine and met scientists and physicians. In 1685, on the order of James II, he was expelled from his studentship.

After the Revolution, he returned to England and accepted the office of Commissioner of Appeals. For his health he spent the winter months with Lady Masham's family at Oates in Essex. In 1696 he was appointed a Commissioner for Trade and Plantations. In 1700 he resigned his appointments, but as late as 1701 he was giving medical advice to some of his poorer neighbours. He died probably from heart failure supervening on bronchitis and emphysema in 1704.

Such in brief outline was the career of this great genius whose medical biography Dr. Dewhurst has so fully and ably put on record.

ARTHUR S. MACNALTY

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[1] *Vide* Bertrand Russell, *History of Western Philosophy* (G. Allen and Unwin, London, 1946). Book 3, Chaps. 13, 14 and 15, describe Locke's philosophy and his influence.

[2] Withington, E. T., "John Locke as a Medical Practitioner", *Med. Magazine*, 7, 47, 375, 573 (1898); *Janus*, 4, 393, 457, 527, 579 (1899).

[3] Osler, Sir William, *An Alabama Student and Other Biographical Essays*. "John Locke as a Physician" (Clarendon Press, Oxford, 1908).

LAW AND TECHNOLOGISTS

Law and Technologists

By Dr. Roy Douglas. Pp. 158. (London: Gee and Company (Publishers), Ltd., 1964.) 30*s*. net.

LAW and science are rightfully regarded as significant tool subjects for developing the logical mind. It has frequently been argued that a scientific background can be as valuable as a humanistic one for the study of the law. Many scientists and engineers at some stage in their career divert towards the legal aspects of industry and technology. In the world of to-day the applied scientist finds the law increasingly impinging on his activities—employment, contracts, patents, company affairs. A book such as *Law for Technologists* is assuredly, therefore, in principle to be welcomed, though it was written primarily for those studying for the Diploma in Technology and other students who require a measure of legal knowledge as an adjunct to a course which is primarily technological or scientific.

This publication is not submitted as "every technologist his own lawyer", though it provides a brief, lucid insight into the structure and operation of the English legal system and an analysis of certain key topics directly relevant to the application of science or engineering to industry. Anyone so interested reading these chapters will not only be better informed but will also become conscious of the wisdom of the author's injunction "this book may perhaps help people to understand what the law and lawyers are saying, and therefore it may also help them to avoid unnecessary legal trouble—but is most certainly not designed to extricate people from trouble once they have got into it!".

The author has at the outset been faced with the difficult problem of deciding to what extent he is to concern himself with formal aspects, such as the evolution and procedure of the law, in order to help pave the way to an adequate understanding of the substantive portions of his subject. He has attempted to solve this by treating first of all with legal sources and machinery, leaving such matters as proof and international aspects to the last; sandwiched between are chapters on contracts, torts, crimes, master and servant, trade secrets, patents and other monopoly rights and restrictive practices.

It may be commented that the section on private and public companies, which is to be found within the chapter on "Machinery of the Law", would well merit a little expansion into a chapter in its own right, with some reference to the duties of directors; and that perhaps the section on restrictive covenants as regards employees, sound as it is, should fall under the heading "Master and Servant" rather than "Trade Secrets". A list of key reference books on the various legal subjects discussed would be of value.

A word about the selection of cases: this is always a pitfall for the legal writer, but the author has struck a fair balance between ancient historical cases which still to-day often constitute important landmarks in the evolution of the law, and the modern ones which may reflect more directly on present-day industrial conditions. This is particularly noteworthy in the short sections on nuisance and restrictive covenants as between master and servant. It is to be noted that the treatment of the subject of resale price maintenance may shortly be out of date by reason of the new legislation at present before Parliament.

Although limitations of space and the nature of the intended readership have necessitated not only careful selection but also a certain amount of superficial treatment of some subject matter, the style is always readable and intelligible. This book should be of real assistance to those for whom it is primarily intended, even though inevitably they cannot end up with much more than a general appreciation of this complex subject, or rather interrelation of subjects. B. J. A. BARD

A GREAT CIVILIZATION OF ANTIQUITY

Ancient Iraq

By Georges Roux. Pp. 431 + 33 plates + 5 maps. (London: George Allen and Unwin, Ltd., 1964.) 50*s*. net.

THE three great civilizations of early antiquity with the longest past history were those of Iraq, Egypt and China. For Iraq, Dr. Georges Roux has contributed a history from the Palæolithic and Mesolithic periods until about the seventh century A.D., and those who have worked in a part of Mesopotamia will be grateful for his outline history of other parts and periods. The book will also be useful to students who wish to get a general view of their position in any special subject they choose to make their own.

From 1928 until 1934, I was closely associated with the University of Oxford and Field Museum Expedition to Mesopotamia working at Kish (the Harsagkalamma end) under the late Prof. Langdon and the late M. Louis C. Watelin, and was fortunate to be in the field in the year when we at Kish, Sir Leonard Woolley at Ur, and Dr. Jordan at Uruk (the Erech of *Genesis*, now Warka), all reached virgin soil, ourselves after digging through 60 ft. of buried civilizations and passing downward through more than 5,000 years of history. Thus, for the first time, we were able to establish the main succession of events including the position of the three pre-dynastic periods: Al 'Ubaid, with its fine pottery painted with one-colour designs, and copper (including *cire perdue* casting), its affinities in Iran; Uruk, with the origin of writing in pictographic script, its affinities with the north shown by its polished red, polished black and polished grey pottery, and its open-spouted jars; and Jemdet Nasr, with its polychrome red, buff and black pottery, again with affinities with Iran and farther east.

Here, I think, Dr. Roux is rather severe in restricting the Jemdet Nasr period to pottery. I remember well the work of Prof. Langdon in saving at Jemdet Nasr a large number of semi-pictographic unbaked clay tablets and baking and publishing them in the *Oxford Edition of Cuneiform Texts* (7, 1928), each sign with a series of the forms taken by this through all periods to the Assyrian.

More too might have been made of the slow progress in copper and bronze, and the development of iron, both through a long experimental period, and of their diffusion to the rest of the civilized world, and their work on copper and alloy which saved bronze users of other areas from a long experimental chalcolithic period.

In connexion with irrigation, something might be made of the wealth of tablets dealing with water and its use between individuals and between cities. Here perhaps was an early start of the principles of both individual and of international law.

In dealing with chronology, it is somewhat surprising to find no mention of the pioneer work of Langdon and Fotheringham on *The Venus Tablets of Ammizaduga* (found at Kish) with its first establishment of the date of the beginning of the first Babylonian dynasty, later corrected to the next cycle of Venus 275 years later by Sidney Smith and Brigadier-General Sewell in *Alalakh and Chronology*.

Dr. Roux is quite right in his statement that Sumerian and Semite (including Akkadian) are linguistic terms, and not racial, and that there is no racial difference. Both had the Armenoid and Mediterranean mixture, the latter predominating. In my report on the early dynastic skeletal material in *Kish IV*, in agreement with Dr. Dudley Buxton's earlier report in the *Journal of the Royal Anthropological Institute* on a longer series, I mentioned three racial groups, Mediterranean, Eurafrican (a sturdier form of Mediterranean) and Armenoid, and said that throughout Mesopotamian history there was a mixture of the same three races which helped us in excavations to-day.

My few observations are not made in a carping spirit, and I fully appreciate the chapters and sections on law, religion, social and economic life, science, and military and political organization.

To have sorted out the rise and fall of so many cities and dynasties, and to have placed in order so long a period from the Palæolithic to the Parthian, is a considerable achievement, let alone the large amount of varied bibliographical material required to support it, all listed with notes at the end. The book is illustrated, and has four maps of different periods. T. K. PENNIMAN

AGRICULTURAL RESEARCH IN NEW ZEALAND

Thomas Cawthron and the Cawthron Institute

By David Miller. Pp. vi + 248. (Nelson, New Zealand: The Cawthron Institute Trust Board, 1963.) 30*s*. New Zealand currency.

MANY agricultural research institutes were started by benefactions from rich men; the Cawthron Institute at Nelson in the South Island of New Zealand began in this way. The Cawthron family went to New Zealand in 1849 when Thomas was 15; they were poor. Nelson was founded only a few years before the Cawthrons arrived and there were large rewards for hard work and initiative in the rapidly-growing colony. Thomas made a start in business by working as a contractor, first in the Australian gold fields, and afterwards at Nelson. From the late 1850's he had "a thriving business at Port Nelson as a shipping agent, merchant and prudent investor leading to the accumulation of his substantial wealth"—ultimately amounting to £264,000. He had plans to found a Solar Physics Laboratory but died before the arrangements were completed. By his will the bulk of his fortune was left to start "an Industrial and Technical School, Institute and Museum to be called the Cawthron Institute". The Trustees decided that the new Institute should work mainly in agriculture—the main industry of New Zealand—and particularly in fruit growing, which was important around Nelson.

In 1917, just before the Institute was formed, only £10,000 a year was available to Government Departments and the Dominion Museum for scientific research. The Cawthron Institute endowment of £231,000 made much more research possible. Work began in 1920 and for the next forty years was mainly in 'biology' (entomology and plant diseases) and 'agriculture' (soils, plant nutrition and agricultural chemistry). The Institute had many early successes, particularly in the biological control of insect pests, such as woolly aphis, and of weeds, through the introduction of predators. Later, grass grub was controlled by insecticides; this latter work is said to have saved New Zealand farmers £15 million a year. Other pioneer work was in soil surveying, fertilizer use, the control of deficiency diseases in plants and animals, and forestry. By 1959, 760 papers had been published by the staff.

Many agricultural research institutes have expanded greatly in attempts to cover their subjects as more money has become available from Governments. The Cawthron Institute has been an exception; it has started many branches of agricultural research and developed them until they could stand alone. Recent expansions in research in New Zealand have generally involved the formation of new institutes, mostly under the Department of Scientific and Industrial Research. The Institute was reorganized between 1956 and 1959; it still has the autonomy of a privately-endowed institution and works mainly on problems of plant and animal nutrition of the Nelson district.

The first third of the book describes the origins of the Cawthron family and the life of Thomas as colonist, business man, and public benefactor. The next third summarizes the development of the Institute. The remainder of the book lists fully the papers published by Institute staff up to 1959 and gives short biographies of the directors and many senior staff. (David Miller, the author, worked at the Institute for more than 30 years and was director from 1956 until 1959.)

The book is of general interest for its accounts of the career of a distinguished citizen of Nelson (which also produced Lord Rutherford—in fact the Cawthron family arrived there the same year as he was born) and of the development of this part of New Zealand. The excellent history of the first forty years of the Cawthron Institute, and the summaries of investigations that have been done, inevitably make it an essential source-book for all interested in the development and history of agricultural science in the Dominions. There is a good index, and sources of information on the life of Cawthron and the development of Nelson are all listed. G. W. Cooke

FUNGAL AND YEAST CHEMISTRY

The Chemistry and Biochemistry of Fungi and Yeasts

Proceedings of the Symposium on the Chemistry and Biochemistry of Fungi and Yeasts, Dublin, 18–20 July 1963. (International Union of Pure and Applied Chemistry, Royal Irish Academy and Irish National Committee for Chemistry.) Pp. v + 525–705. (London: Butterworth and Co. (Publishers), Ltd., 1963.) 45*s*.

THESE *Proceedings* fall into three unequal parts. Section 1, on "Fungal Metabolites", contains four papers. Under the title "Biosynthesis of Antibiotics", A. J. Birch (United Kingdom) examines the question whether antibiotic production can be manipulated by the chemist to make it more efficient, and whether an individual antibiotic can be endowed with more useful properties by effecting minor alterations in its molecule. Penicillin, tetracyclines, novobiocin, fumagillin and one non-antibiotic substance, gibberellic acid, are used to illustrate how intimate knowledge of details of the metabolic routes involved in antibiotic biosynthesis might be usefully applied for these ends. The remaining papers in Section 1 are excellent examples of the contributions which organic chemists make to biological sciences. D. Dolphin, A. W. Johnson, R. Rodrigo and N. Shaw (United Kingdom) describe, in their paper on "The Vitamin B_{12} Coenzyme", the chemical synthesis from reduced vitamin B_{12} of vitamin B_{12} coenzyme (5′-deoxyadenosylcobalamine) and a range of its alkyl coenzyme analogues. Their properties and reactions seem particularly interesting in relation to possible mechanisms of the biochemical role of vitamin B_{12}. In "Constitution of Rifamycins", V. Prelog (Switzerland) unfolds how the structure of the complex rifamycin molecule has yielded to the formidable weapons of modern organic chemistry. The similar success attending the attack on the sclerotiorin group is described by W. H. Whalley (United Kingdom) in "The Sclerotiorin Group of Fungal Metabolites: Their Structure and Biosynthesis".

Section 2 comprises three papers on "Biochemistry of Fungi": "Fungal Metabolism of Certain Aromatic Compounds Related to Lignin" by M. E. K. Henderson (United Kingdom); "Carbohydrate Metabolism by Fungi" by D. Gottlieb (United States); and "Influence of Peat Substances on the Metabolism of Fungi" by E. Küster (Ireland). The first and final papers of this group contrast sharply with those of Section 1, and show how remote is the stage when the organic chemist will find lignin and peat rewarding subjects for research. Yet lignin, through its role in the maintenance of soil fertility, and peat, because of its economic importance, demand investigation and, as these contributions show, the research prospects in the chemistry and biology of lignin and peat are rich. Dr. Gottlieb presents a general review, surveying the operation in hyphal fungi of the major pathways of carbohydrate metabolism.

Section 3, devoted to "Chemistry and Biochemistry of Yeasts", consists of seven papers. D. J. D. Hockenhull (United Kingdom), in "Changing Approaches to Antibiotic Production", reviews in general terms the historical development of the nutritional and cultural conditions needed for antibiotic production, and develops the thesis that "the concept of antibiotic production as a steady state system based on an understanding of rate processes concerned with the assimilation of simple nutrients is here to stay". The complex subject, "Protein Synthesis in Yeast", is viewed by A. H. Cook (United Kingdom) from four angles: age aspects of yeast cultures, the occurrence and nature of yeast 'peptide-nucleotides', synchronous yeast cultures and enzyme development in yeast. Despite its seemingly academic nature, Dr. Cook aptly indicates possible technical advances resulting from the pursuance of such research as he describes. This point also emerges in the contribution by H. Suomalainen (Finland), "Changes in the Cell Constitution of Baker's Yeast in Changing Growth Conditions", in which are described some changes in the chemical and biochemical make-up of baker's yeast taking place in response to the sequence of environmental changes involved in the stages of its industrial propagation. In "Sulphur Utilization by Yeast", G. A. Maw (Ireland) deals with the distribution of sulphur in *Saccharomyces cerevisiae*, its ability to utilize various sulphur compounds for growth and sulphur transport and uptake in this species. Interest in the glycogen, glucan and mannan of yeast is heightened by recent investigations described by D. H. Northcote (United Kingdom) in "The Structure and Organization of the Polysaccharides of Yeast", in which their chemistry and properties are related to their function and synthesis in the total organization of the cell. G. Ehrensvärd (Sweden), in "Biochemistry of Yeasts—an Analysis of Some Conditions for Growth", reports experiments designed to throw light on the metabolism of acetate and formate during the lag and log phases of growth of the fodder yeast, *Candida utilis*. The final contribution, by S. R. Mardashev (U.S.S.R.), provides "Some Data on the Metabolism of Dicarboxylic Amino Acids and Their Amides in Yeast". In particular, the biosynthesis and breakdown of aspartate, glutamate, β-methylaspartate, asparagine and glutamine by extracts of *Saccharomyces* strains are considered.

This review of its contents indicates that the volume is one for specialists, of whom the yeast specialist is likely to derive the greatest benefit from its perusal. Not only is the largest individual section devoted to yeast (except for Dr. Hockenhull's paper, which is surely more appropriate to Section 1), but also it achieves a better focus than the other sections can hope to achieve, with so few papers in so vast a field, despite the individual excellence of the contributions. The book is well produced and contains few typographical errors, although there are some remarkable Latin plurals on p. 677. C. Rainbow

Progress in Ceramic Science

Vol. 3. Edited by Dr. J. E. Burke. Pp. vii + 262. (London and New York: Pergamon Press, 1963.) 80*s*. net.

THE second volume in this series was reviewed on p. 932 of the March 9, 1963, issue of *Nature*. The present volume contains four essays, the first by Prof. G. W. Brindley (Pennsylvania State University), "Crystallographic Aspects of Some Decomposition and Recrystallization Reactions", in which orientation relationships between parent and product phases are discussed and illustrated, including the aluminium hydrous silicates—a field which Prof. Brindley has made especially his own. The second essay, by F. M. Ernsberger (Pittsburgh Plate Glass Co.), deals with the current status of the Griffith crack theory of glass strength. He surveys techniques (ion-exchange, pulsed-stress, bifluoride etch and sodium-vapour treatment) for the direct detection of Griffith cracks. It is suggested that mechanical-damage cracks form a distinct class of strength-impairing structures, and that the description 'Griffith crack' should be reserved for such defects, distinguishing them from hypothetical intrinsic flaws which are presumably responsible for the failure of glass with damage-free surfaces. There appears to be a good deal of information on this aspect of the problem which so far does not present a coherent picture. A. E. Owen (Westinghouse Research Laboratories) contributes a report on electric conduction and dielectric relaxation in glass, in which, after a brief review of the Zachariasen–Warren work, he outlines the present-day theories of d.c. conductivity and relaxation, directing particular attention to the role of the alkali ion. He feels that, in spite of the present-day work, a quantitative understanding of even the simpler electrical phenomena in glass has still not been achieved. The final essay is by R. L. Coble (Massachusetts Institute of Technology) and J. E. Burke (General Electric Research Laboratory) on sintering. This covers a good deal of fairly well-established work, but is particularly interesting in its comments on the inhibition of discontinuous grain growth and on pore trapping.

The book in some curious way lacks impact, and one must hope that future volumes in this series will be more exciting. However, it is a useful addition to the library and contains extensive bibliographies. N. F. ASTBURY

Encyclopedia of Chemical Technology

By Kirk-Othmer. Executive Editor: Anthony Standen. Vol. 2: Aluminium Compounds to Azo Dyes. Second completely revised edition. Pp. xv + 910. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1963.) 338*s*.

THE publication of the revised second volume of 'Kirk-Othmer' within a year of the publication of the first volume is a notable achievement, and full credit must be given to the editorial board on fulfilling their promise to publish two to three revised volumes per year of this excellent *Encyclopedia of Chemical Technology*.

This volume contains thirty-nine articles ranging from "Aluminium Compounds" to "Azo Dyes", the majority of which appeared in Volumes 1 and 2 of the first edition. Each article, written by a specialist, has been completely revised and extended. For example, "Amines", included in the first edition, has been replaced by three complete articles covering, "Amines aliphatic", "Amines fatty" and "Amines chloroalkyl". New leading articles covering material only briefly mentioned in the first edition include the following subjects. "Antistatic Agents", an article which reviews the theory and use of these agents in fabric and polymer technology. "Aquametry", a review of analytical methods for the determination of water, with special reference to instrumental techniques, such as nuclear magnetic resonance, infra-red and ultra-violet spectroscopy, etc. "Automobile exhaust control", a subject of increasing importance, dealing with the health hazards and methods of control of the exhaust gases from automobile engines.

The preface to the first edition stated that contributions from other countries would be included in an attempt to widen the scope of the *Encyclopedia* beyond the limits of technology in the United States. In this edition attempts to do this have been made by including leading articles written by Swiss, French and Japanese authors. The lists of trade names, although still predominantly of American origin, have all been revised and extended.

It is disappointing that so many of the cross-references that were presented and found so useful in the first edition have been omitted—a loss that will no doubt be overcome when the index volume is published. The omission of the review on "Analytical Chemistry" will, we hope, be replaced by the inclusion, in later volumes, of separate articles on individual analytical procedures. K. FIELD

A Manual of Experiments in Reactor Physics

Edited by Prof. Frank A. Valentine. Pp. xv + 335. (New York: The Macmillan Company; London: Collier-Macmillan Ltd., 1963.) 55*s*.

IT is inevitable that any account of specialized experiments using equipment available in modern laboratories, such as those catering for reactor physics students, must contain a certain amount of detailed description of items of equipment which are special to the country of origin. This means that accounts of experiments and details of how they are to be performed must inevitably lose some of their value in a country other than that which has the equipment readily available.

For non-American users, therefore, this is a fault from which *A Manual of Experiments in Reactor Physics* suffers. However, despite this, it is generally so good an account as largely to make up for this defect. There is an excellent opening chapter on the elementary statistics of experiments, a subject which is so often neglected altogether or dealt with inadequately. This is followed by a short discussion of radioactivity, which includes a description of the method of analysis of decay curves. The standard methods of detection of particles are then described, together with a very short account of some electronic techniques. Also included in this chapter is an account of neutron sources. As a number of the experiments which are described later in the book require a pulsed-neutron source, there is a short discussion of the theory and methods of production of pulsed-neutron sources, and the final chapter in the introductory section (rather less than half the book) deals with radiological considerations relating to safety requirements in a reactor physics laboratory.

The remainder of the book describes actual experiments which fall into three broad categories; those requiring only a steady source of neutrons (ten experiments); those requiring a subscribed assembly and a steady source of neutrons (six experiments); and those requiring a sigma-pile or a subcritical assembly and a pulsed source of neutrons (seven experiments). All are well described according to a standard pattern of introduction, object, theory and method, materials and apparatus, procedure, results and discussion. It should, however, be noted that the theory given generally amounts only to a discussion of the necessary and relevant formulæ required and not to the derivation of them. However, very adequate references are given for the experimenter to be able to read these up for himself.

For those who wish to have ready-made descriptions of many important, yet relatively simple, experiments in reactor physics this is an excellent book, with the proviso that some of the particular pieces of equipment described will not be available outside American laboratories. J. F. HILL

EMERGENCE OF POTATO BLIGHT, 1843–46

By P. M. AUSTIN BOURKE
Irish Meteorological Service, Dublin

NO outbreak of a plant disease, before or since, has received the public attention given to the epidemic of potato blight (*Phytophthora infestans* (Mont.) de Bary) which commenced in Europe in 1845. The detailed consideration of the disease in agricultural journals like the *Gardeners' Chronicle* is not surprising; it is more unexpected to find no fewer than sixty-six entries on the subject in the official Belgian gazette, *le Moniteur Belge*, during the five-month period, August–December 1845, and a number of articles in the French *Journal des Débats*.

The first appearances of blight were mostly recorded in local newspapers, which sometimes were led by political or sectional allegiances to minimize or exaggerate its intensity and extent. In the professional Press, there was quite an amount of sharp jostling as to which group was most competent to deal with the outbreak. The medical men felt that all disease fell within their ambit, so the *Lancet*, in common with the corresponding continental journals, gave considerable space to the epidemic. In Ireland, the *Dublin Medical Press*, feeling the first chill breath of socialized medicine, suspected that Governmental concern about food shortage, famine and fever was merely a stratagem to ease the passage of a "medical charities bill", and for a long time denied the gravity of the situation. The chemists argued that the potato rot was primarily a chemical change and thus came within their field of competence; for this they earned the scorn of the London *Medical Times*, which dismissed them as "the Giessen-bitten boys of the present day", mesmerized by "the tasmalic name of Liebig". Commercial concern, and, in particular, possible repercussions on the grain market, explain the continued interest of journals like the *Mark Lane Express*; but, in fact, every periodical from the *Bankers' Circular* to *Punch* featured blight to such an extent that the American *Monthly Journal of Agriculture* was moved to complain that the British papers of the time spoke of nothing except the potato disease and the railway mania. The botanists and mycologists were quite outnumbered in this rush into print; the plant pathologists had not yet been invented.

The epidemic broke out at an awkward time for the learned societies. Some, like the Société Royale et Centrale d'Agriculture of Paris, were summoned back by ministerial demand from their summer holidays to urgent special sessions; the remainder made up for lost time when they reassembled in the autumn. Many set up special committees to examine and to experiment on the disease, and, in due course, to add to the voluminous and perplexed reports which were emanating from officially inspired commissions in Belgium, Holland, France and Ireland. But it was the pamphleteers who had the real field day. An eruption of brochures broke out all over Western Europe, written by physicians, surgeons, botanists, noblemen, pharmaceutical and other chemists, geologists, excisemen, gardeners and accountants; even a plain housewife added her meed of wisdom. Many of these pamphlets put forward highly eccentric views, but few were without useful factual information.

Although the attack took Europe completely by surprise, blight had already been ravaging the potato crops of North America in the previous two seasons. Stevens[1] has charted the annual progress of the disease, from its beginnings in 1843 in the five States closest to the great ports of the east coast of the United States, to an expanded area in 1845 which closely approximates to the limits within which blight is a serious problem to-day. Fig. 1 is based primarily on Stevens's work, with the addition of a limited amount of extra information on the impact of the disease in Canada. The diagram bears quite a resemblance to charts of the ten-yearly progress of the Japanese beetle from its introduction near Philadelphia in 1916.

From what has been said, it will be clear that there is no lack of material on which to base a similar chart of the geographical progress of potato blight in Europe in 1845; indeed, the major problem is the sheer volume of the relevant data. Eye-witness accounts of the earlier stages in Belgium and neighbouring countries are given by Dumortier[2] and by Martens[3]. Bonjean[4] adds details of the onset of the disease in southern France, Switzerland and north Italy. Münter[5] and others describe its progress in Germany. Decaisne[6] is one of the few to attempt a comprehensive verbal picture of the spread of the disease. In France[7] and in Scotland[8], reports were collected and co-ordinated by a central committee. In Ireland, the constabulary were instructed by an order dated September 16, 1845, to submit periodic confidential reports on the progress of the disease; the original returns from more than 250 police stations are filed in the Public Records Office, Dublin. Data from all these sources and from very many others, including British, Irish, French and Belgian newspapers, are summarized in Fig. 2.

Onset of Potato Blight

The disease was first noticed in the Courtrai area of Belgium towards the end of June 1845. By mid-July it extended all over Flanders, and had appeared in neigh-

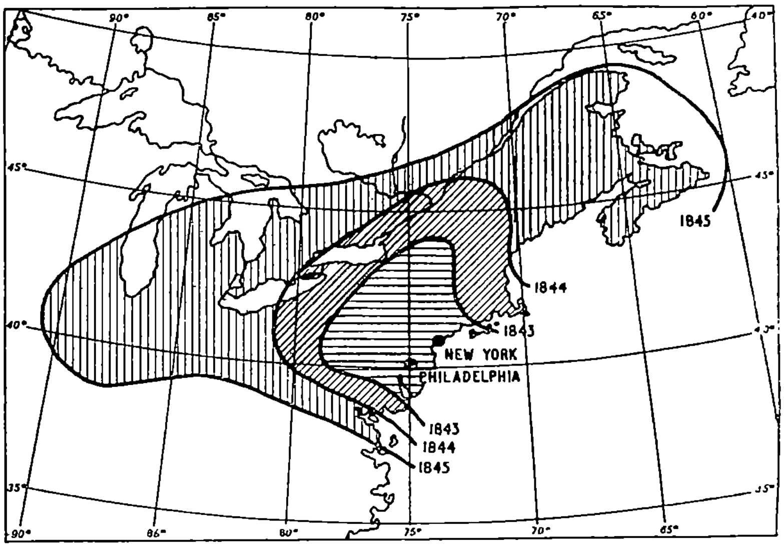

Fig. 1. Approximate extent of potato blight attacks in the United States and Canada during 1843–45 (after Stevens)

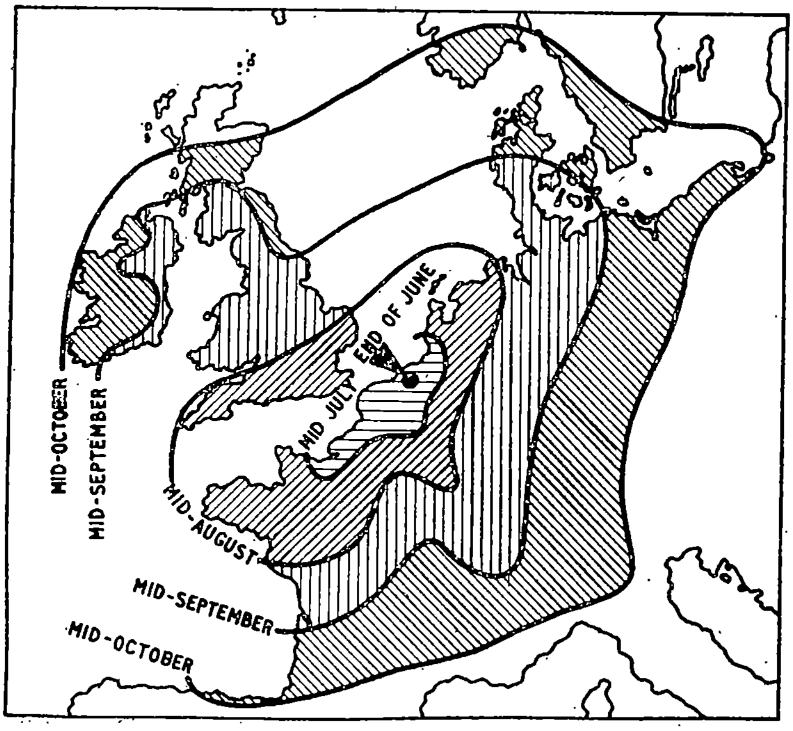

Fig. 2. Approximate dates of first report of potato blight in Europe in 1845

bouring parts of The Netherlands and France. The first newspaper references to the strange and alarming disease occurred in the second half of July. As early as July 31, 1845, *l'Organe des Flandres*, a newspaper published at Gand, printed an article by the Abbé Édouard van den Hecke, in which he suggested that the disease was due to a fungus which he described briefly, with particular reference to the remarkable mechanism by which it launched its spores. A previous suggestion as to the fungal nature of potato blight had been made in the United States by Mr. J. E. Teschemacher of Boston, but had caused no reaction there. Van den Hecke's independent conclusion proved to be the first innocent shot in what soon developed into a violent controversy between those who considered the fungus to be the unique cause and those who dismissed it as a consequence of the disease. There were authentic grounds at the time for scientific doubt, but the issue was embittered by personal jealousy, international rivalry and even religious conviction. The standard bearer of the fungal theory was Prof. Charles Morren of Liège, who launched, in the Brussels newspaper *l'Indépendence belge* of August 20, 1845, a vigorous manifesto which was reproduced all over Europe and in the United States. He was supported by Rev. M. J. Berkeley in England and, more cautiously, by A. Payen in France; but the opponents of the theory were far more numerous and influential, and carried the waverers with them. The fungal theory quickly fell into disfavour with all except the faithful few, and did not emerge from the shadows until de Bary's time, some fifteen years later.

Spread of the Disease

To return to Fig. 2, the extension of the disease from Belgium to Luxembourg seems at first to have been blocked by the Ardennes; it did not appear there until it had flanked the mountains from north and south. At a later stage, the entry of the disease into Switzerland appears similarly to have been made, virtually simultaneously in September, along the valleys of the Rhône and the Rhine.

The disease was found in the Paris area about mid-August. On August 20, M. Élisée Lefebvre showed examples of blighted potato foliage and tubers to a session of the Société Royale et Centrale d'Agriculture. In Paris also, Dr. C. Montagne hastened to comply with the botanical canons by describing the fungus in proper detail to a meeting of the Société Philomathique on August 30, and thus gained priority over a number of Belgian writers—van den Hecke, Anne Marie Libert, René van Oye and Morren—who had already given less formal accounts of the fungus in popular newspapers.

By mid-August the disease had also extended to the lower Rhineland, to north-west France, to the Channel Islands and to southern England. In England, the alarm was first sounded by Dr. Bell Salter of the Isle of Wight in a letter which appeared in the *Gardeners' Chronicle* of August 16; the following week's issue confirmed the presence of the disease over much of southern England. It had not yet appeared in the Midlands, for when Rev. M. J. Berkeley of King's Cliffe, Northamptonshire, received on August 26 some infected foliage from his friend Dr. Montagne in Paris, he had not succeeded, despite careful search, in finding any traces of the disease in his area. A few days later it made its appearance, and he followed its progress in the field and checked its characteristics against Dr. Montagne's specimen and description.

The advent of potato blight in Ireland was announced simultaneously, on September 6, in brief paragraphs in the *Waterford Freeman* and the *Dublin Evening Post*. On September 13, Dr. John Lindley, impressed by the advanced stage of destruction of the potato crops in the London area, stopped the press to announce the grim news from Ireland in the *Gardeners' Chronicle*. In Ireland itself, however, the newspapers and journals, irrespective of their political allegiances, tended in the earlier weeks to play down the disease, and it was not until considerable tuber rot was noted at harvest time in October that the popular panic spread to the Irish newspapers.

In the later stages of a potato-growing season, it is difficult to distinguish the effects of blight on haulm which is ripening to decay. The outer boundary line in Fig. 2 thus encloses, in large part, areas in which tuber blight was discovered at or soon after harvesting. It is difficult to place with absolute certainty in Scandinavia and central Europe. There is little doubt that the disease in Scotland was virtually limited to the area south of the Grampians and, in particular, did not extend to the Highlands in 1845. Neither did it appear in the Shetland, Orkney or Scilly Islands, and it made little or no impact in Achill Island on the Irish west coast. The southern limit of the disease did not quite reach the Mediterranean, with the possible exception of the Genoa area about which there were conflicting reports.

Extent of Crop Losses in 1845

Losses in the potato crop in 1845 were most severe in the area of earliest attack. In Belgium, Holland and north-east France, the foliage of the main crop was destroyed in most cases while the tubers were no bigger than marbles. In the regions of late-season attack where the potatoes were almost fully developed, the extent of loss by tuber rot depended on the wetness or otherwise of the season before and at harvest time. In rainy areas, losses were quite serious, particularly where panic digging took place while the fungus was active. Appreciable damage was suffered in parts of Switzerland, and the ffect on the lives of the poor is vividly described in the well-known novel, *Käthi, die Grossmutter*, by Jeremias Gotthelf (Albert Bitzius), published in 1847. In Ireland, he effects of the disease were very variable; but the mean loss by tuber rot was about 40 per cent in a crop which had yielded remarkably well, so that the production of sound potatoes was about a quarter or a third below normal. In Bavaria and Prussia, where the autumn was dry, crop losses were slight. A contrary mpression was created abroad by the decision of the Prussian Government, by a decree dated September 18, 1845, to prohibit the export of potatoes, but this was merely a temporary safeguard directed against heavy buying by Belgian and Dutch importers.

Pattern of Blight Progress in 1845 and 1846

Outside Belgium, the pattern of disease progress in 1845 (Fig. 2) passed virtually unnoticed, and in each area which was attacked later in the season there was a tendency to interpret the data as showing a linear progression. Thus, in Britain, the apparent path from Belgium through England to Ireland was extrapolated backwards to include Germany and even Poland; the outbreak of blight was thus seen as "coming from the east", and, with the path of the cholera pandemic of 1826–37 still fresh in the public mind, the disease was often described as the "potato cholera". In the south of France, potato blight appeared to travel from north to south, and its late season appearance was interpreted as support for the theory that the potato was suffering from a kind of cool weather degeneration. Writing about forty years later, Jensen of Copenhagen[9] recalled the sequence of successive blight appearances in 1845 from the Isle of Wight through England to Scotland, and from The Netherlands through Holstein to Denmark, and claimed that it was identical with the apparent progress of the disease in normal later years, as controlled by climatic factors.

In fact, however, the cycle of events as shown in Fig. 2 is completely untypical of the annual course of potato blight in Europe, nor is it explicable in terms of abnormal weather conditions. The summer of 1845 in north-west Europe was everywhere cold, sunless and wetter than normal, but, while it was a depressing season, it was by no means an unusual one. Growing seasons more favourable to blight development had occurred in every part of the region within the past 3–6 years. The only adequate explanation of the pattern of development of blight in 1845 is that the fungus had recently been introduced into Europe, and that the main concentration of inoculum in that year lay within the region in which the disease was obvious by mid-August.

This interpretation is reinforced by the sequence of events in 1846 when the apparent geographical march of the disease followed what was afterwards to be recognized as its characteristic climatic pattern[7,10]. It first appeared in the west, striking Ireland fully two months before it was noted in the previous year, and its apparent progress was eastwards or north-eastwards "at about 50 miles per week". In Ireland the crop was destroyed in most cases in early August at a time when tuber formation in the main crop, then sown later than nowadays, had barely begun. The sole food of a great part of the population was thus wiped out, and the year which followed marked the most intense stage of the great hunger so eloquently described in Mrs. Woodham-Smith's book[11]. The Scottish Highlands, which had emerged unscathed in 1845, suffered an early and severe attack of blight in 1846, and distress in this area was second only to that in Ireland[12]. In south-east England and on the neighbouring European mainland the disease not only appeared later, but also, since the summer there was dry and warm, such damage as the potato crop suffered was due more to drought than to blight.

In 1846, the disease made an appearance in new areas on the European mainland, in the Orkney and Shetland Islands, and even in Algeria.

A Political Consequence

The different courses of the disease in 1845 and 1846 had political repercussions which are of some historical interest. In 1845, the London area was attacked sooner and more spectacularly than was Ireland. We have seen that Dr. Lindley, in his *Gardeners' Chronicle*, was already sounding the alarm for Ireland a month before the Irish journals commenced to treat the matter seriously. As a result, Peel's Government in London took prompt action, required the constabulary to survey the Irish crops, and set up a scientific commission of three, including Lindley, to go to Ireland and report on the situation. Impressed by Lindley's estimate that five-sixths of the Irish potato crop would be lost, Peel placed an early order for maize in North America. The provision made was parsimonious by modern relief standards; but, since the actual loss was only about half the original estimate, the emergency measures blunted the sharper edge of starvation in the spring and early summer of 1846.

By the time the disease reappeared in Ireland in 1846, the parliamentary opposition which had berated Peel for exaggeration and unjustified panic was itself in power. Further, the disease was rampant in Ireland at a time when south-east England was virtually free. Russell's Government, treating the earlier appeals from Ireland as cries of 'Wolf!', moved with tragic slowness and inadequacy. Meanwhile, in Ireland, Lindley's forecast was being more than justified, with a time-lag of a year. The result is a matter of history.

A New Disease?

Although most observers in 1845, and particularly practical farmers, insisted that blight was a disease new to Europe, there was by no means complete agreement on the point. A new appearance was difficult to explain, irrespective of the theory advanced, and there was a rich source of error in the confusion of blight with earlier potato diseases, which was mined with particular industry by the academic commentators who lacked direct experience of the disease in the field. There were also those who felt that the first need was to dispel public panic and that this consideration justified the mild deception of identifying the disease with earlier outbreaks which had died down after a short period.

In the past, the potato had suffered from drought in dry seasons, from water-logging and consequent rot in wet years, and from frost damage in growth and storage when the weather was unusually cold. It was also subject to a number of minor ailments, like scab, which were more a nuisance than a matter of concern. Two diseases only had claimed major attention. The first was the 'curl', which we can now recognize as a virus attack. This was prominent from the last quarter of the eighteenth century and gave rise to a voluminous literature which has been fairly exhaustively reviewed by Atanasoff[13]. The development of new varieties and the regular replacement of seed potatoes from areas free of the complaint proved to be effective devices for the restraint of 'curl'. It led, however, to a continuous and uncontrolled exchange of potatoes which guaranteed the rapid extension and development of any new plant diseases.

The 'curl' differed so radically from blight that it is difficult to credit that anyone could confuse them; yet there was a small group who wielded Occam's razor with fanatical zeal and argued that 'curl', blight and other maladies of the potato were merely different aspects of a single disease.

The 'taint' or 'dry rot', now recognizable as a fungal infection caused by ***Fusarium*** spp., appeared to a troublesome degree about 1830 and caused serious concern in Germany towards the end of the same decade. Again there was a voluminous output of articles, prize essays and pamphlets, culminating in the famous study by von Martius in 1842 (ref. 14).

Again, the course of the disease was quite distinctive, and only a small group of contemporary observers took the line that dry rot and blight were two aspects of a single malady, the respective characteristics developing according as the season was dry or wet. They were sufficiently vocal, however, to create a confusion regarding the time when blight first appeared in Germany and elsewhere in Europe. This confusion has persisted to the present day and has led to the supposition, which is in reality an extension of the cholera fallacy, that the disease might have travelled from Europe to North America.

The fact is that if blight existed in Europe prior to the 1840's, it passed completely unnoticed either by von Martius, who carefully recorded even the minor ailments of the potato, or by the hundreds of other commentators who wrote on the potato. All the evidence points to the conclusion that blight had arrived in Europe only shortly before the epidemic of 1845.

Date of Introduction of Potato Blight into Europe

Accepting that the fungus had been recently introduced, the question arises as to whether the spread of blight in 1845 to a large part of Europe could have occurred in the course of a single season. The possibility cannot be entirely ruled out. It was a summer in which shallow thundery depressions penetrated into the continent and gave rise to a recurrent wind pattern which favoured the transport of spores eastwards as well as westwards in moist sunless weather.

A further favourable factor was the extreme susceptibility to blight of most of the potato crop of the time. No variety proved immune, but a few showed a fair resistance to the disease; it was from a build-up of these partially resistant potatoes that the crop continued to be grown successfully up to the time, towards the end of the century, when treatment with fungicides came into use. But the varieties then most widely grown, such as the 'Lumper' in Ireland, proved highly susceptible and went out of general use after 1846. No doubt there was much exaggeration in the frequent reports of fields which were blackened overnight or even in a matter of hours. But there is solid evidence that the disease worked its destruction with terrifying rapidity. This has recently been confirmed in a remarkable way by Prof. van der Plank[15]. Using potatoes from a stock introduced by missionaries into Basutoland in 1833, which have since then survived in an ambient unfavourable to blight, he has tested the old varieties and found them to be intensely susceptible to the disease.

There is, however, some evidence that blight was already present in parts of Europe in 1844, when the summer was mainly dry. The areas for which a prior appearance are claimed with some plausibility are Belgium, the Lille district in France, the south-east corner of England (Kent and Sussex) and Dumfriesshire in Scotland. There are some reports in 1845 of appearances of the disease in Cheshire and in Dumfriesshire some weeks earlier than the orderly advance depicted in Fig. 2 would suggest. Claims from South Africa, Poland and Portugal that the only crops in which blight appeared were grown from British seed potatoes of the 1844 crop would be decisive, if one could be sure that the disease in question was, in fact, blight. However, the accumulation of evidence does suggest strongly that the disease had already gained a foothold in 1844. There are isolated grains of evidence that the disease may have dated back locally to 1843 or even 1842, but they are far from conclusive. For 1841 and earlier, there is little risk in a dogmatic claim that potato blight did not then exist in Europe.

Source of Blight

It may be deduced with some confidence that blight was not introduced from Europe into the United States; it may have come from the latter, where it was already widespread in 1843, to Europe or it may have arrived independently in the two areas from a common third source. Where this source was located remains a debatable point, on which further detective work is required. Modern commentators have suggested Mexico[16]. Recent work by Dr. Niederhauser *et al.*[17] has shown that the blight fungus there comprises a highly specialized population of races and that it retains the power to mate and form oöspores freely. These characteristics give strong support to the claims of Mexico as the primary site of origin of potato blight; they are less convincing as evidence that Mexico was the immediate source of the less versatile fungus which invaded the United States and Europe.

The nineteenth-century theory was that blight came from the northern Andes region in South America. The direct evidence that the disease existed there prior to 1845 is not very satisfactory; but the region has recently proved to be one in which genes of hypersensitivity to blight occur in wild potatoes[18]. Certainly, the timing and method of disease entry are easier to demonstrate for the Andes area. The newly independent South American States opened up trade with Europe and North America, and this was greatly expanded with the guano boom which commenced in 1840. The problem of the dry rot may have induced the European growers to experiment with fresh stocks from South America, "the home of the potato". Certainly the guano ships brought South American potatoes to Europe, and among the newly introduced varieties which went down in the first attack in Belgium in 1845 were three which bore the significant names, 'Lima', 'Péruviennes' and 'Cordillières'[6]. The available evidence suggests that the disease may well have come from the Andes area; but there are several outstanding problems to be solved before a dogmatic answer can be given.

Acknowledgments

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SPACE AND TIME IN CHEMISTRY*

By Prof. ALLEN MACCOLL
Department of Chemistry, University College, London

THERE seems good reason for a practising scientist occasionally to spend a little time in reviewing the wider area of science into which his interests fit and in assessing their relationship to the subject as a whole. I therefore propose to examine the structure of chemistry and the way in which my own research interests fit into the general pattern. Traditionally, chemistry is divided into three main areas, inorganic, organic and physical. As chemistry occupies a place between physics on one side and biology on the other, further subdivisions have occurred, leading, for example, to chemical physics and biochemistry. This immediately poses the question, from the point of view of a physical chemist, as to whether the traditional subdivision is necessarily the most natural or convenient one. Especially from the point of view of a physical chemist, since his subject has to do more with methods of investigation and with theories, than with synthesis and analysis, which are the stuff of organic and inorganic chemistry. The physical chemist must provide the basic ideas that underpin progress in the other two main areas of chemistry. In the analysis of chemistry which I am going to develop I claim no originality. What I am in fact doing is borrowing as my theme part of the title of Ingold's book, *Structure and Mechanism*. Since I am going to use these terms quite extensively let me first explain what I mean by them.

The term 'structure' is used at three different levels in chemistry. In the first place the organic and inorganic chemists use the term in an almost topological sense in saying that the structure of a molecule is known when the spatial arrangement of the atoms forming it is known, but not necessarily the distances between them. Thus at this level (Fig. 1) the cobaltinitrite anion is described as six nitro groups surrounding a central cobalt ion octahedrally, and the ketene molecule as one carbon atom to which two hydrogen atoms are attached, joined to a second carbon atom, which in turn is attached to an oxygen atom. The second sense in which the term 'structure' is used is essentially a geometrical one; the distances between the atoms in a molecule are specified as are the angles that a pair of atoms subtend at a given atom. By various physical techniques the structures in this sense of the two compounds mentioned earlier have been determined leading to the distances and angles shown in Fig. 1. The third sense in which the term 'structure' is used relates to the way in which the electrons which bind together the nuclei of molecules are organized. This description is essentially a mathematical one.

The three levels discussed here may be differentiated by the terms 'chemical', 'physical' and 'mathematical'. In the chemical method of approach to questions of structure, the behaviour of a substance in a series of chemical reactions is observed and deductions made as to the structure of its molecules. Thus the tetrahedral structure of methane, the planar structure of benzene and the octahedral structure of the cobaltamines were all inferred by this method and represent some of the highlights of deductive reasoning in chemistry. This approach was equally successful in the investigation of the structure of complex, naturally occurring, organic molecules. In the physical method of approach, not only the topology but also the metrical properties of molecules are established. The most powerful technique is that of X-ray diffraction, in that it can fix to a very high degree of precision the positions of all the atoms in a molecule. Spectroscopy, for small and intermediate-sized molecules, can also yield precise values of the structural parameters of molecules. Again there is a recently developed technique which is closely related to the chemical technique discussed here, namely, mass spectrometry. In this a beam of electrons is used to fragment a molecule and structural deductions can be drawn from the nature of the fragments. I shall have more to say about this method later. Finally, there is the third, or mathematical, approach which, by using the techniques of quantum mechanics calculates the energy of the molecule in terms of structural parameters, and thus is able to predict the stable structure. Since problems of structure are of importance for the excited states of molecules as well as for the ground-states, problems of the energy-levels of atoms and molecules can rightly be included within the general term structure as developed here; and so can energy relationships between atoms and molecules.

Mechanism is concerned with the intimate details of the way in which molecules interact to give new molecules. It too can be subdivided in the same way as structure. Thus there are again three levels at which mechanism can be investigated, namely, the chemical, the physical and the mathematical. In the first the method is to take a parent molecule and to substitute various groups at different positions in order to find the effect on the rate of reaction. In the physical method, since it is known that two molecules can only react when they possess excess energy, the question arises as to how they gain or lose this energy. In the mathematical approach an attempt is made first to calculate how the energy of a molecular system varies with various displacements of the constituent atoms and, secondly, to calculate the rate at which the displacement leading to reaction will occur. The chemical method has been widely used in investigations both of reactions in solution and of reactions in the gas-phase. The physical method, based as it is on examinations of intra- and inter-molecular energy transfer, is developing very rapidly at present. The mathematical method, because of the inherent difficulty of the problem, has not as yet been widely applied, although the general principles underlying such a treatment are becoming clearer.

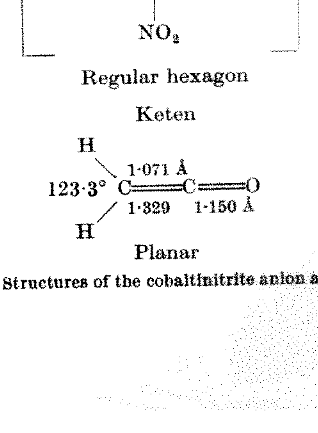

Fig. 1. Structures of the cobaltinitrite anion and of keten

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* Substance of the Inaugural Lecture delivered on April 28.

Chemistry is essentially the study of interactions, for it is only by allowing the substance that we wish to investigate to interact with something else that we can learn anything about it. Thus we can examine the relation between the pressure and volume of a gas by investigating its interaction with the walls of the containing vessel. By a refinement, the interactions between the molecules themselves can also be investigated by the same technique. So it should not seem surprising if the term interaction looms large in a classification of chemistry. One possible classification is shown in Table 1. This is based on the three methods, namely, mathematical, physical and chemical, by which chemical phenomena can be investigated. No mention is made of the classical subdivisions, namely, inorganic, organic and physical. This is the logical outcome of the physical–chemical approach.

Table 1. A Subdivision of Chemistry

STRUCTURE	MECHANISM
Mathematical Quantum mechanics	
Interaction of electrons and nuclei in atoms and molecules. Bonds and intermolecular forces.	Interactions of atoms and molecules with fields (for example, radiation), with particles (for example, electrons) and with each other (chemical reactions).
Statistical mechanics	
Systems in equilibrium	Systems not in equilibrium
Physical	
The interaction of atoms and molecules with fields and particles. Non-reacting interactions of atoms and molecules with each other. Reacting interactions of atoms and molecules with each other.	Rates of intra- and inter-molecular energy transfer. Rates of reaction from the properties of atoms and molecules.
Chemical	
The stoichiometry of chemical reactions. Structure by the study of the interactions of chemical substances. Substituent effects on equilibria.	The effect of substituents on the kinetics of reacting systems.

Such a scheme is based on a vertical division according to whether or not time is one of the variables investigated, and a horizontal division according to the method of investigation used.

This subdivision allows us to see that what is normally called 'physical chemistry' provides the scaffolding inside which chemistry can be built. The correctness of this view was by no means so apparent a few decades ago when inorganic and organic chemists used the simplest of physical techniques in their problems. This raises one of the most important pedagogical difficulties for the teachers of physical chemistry. Some of the more complex concepts and methods of physical chemistry are required in the early stages in the teaching of inorganic and organic chemistry; long before they can be rigorously presented. Two examples come to mind—the use of equilibrium theory in a discussion of the properties of inorganic systems and the use of spectroscopic data in the determination of organic structures. In both cases, the best solution seems to be to give a short phenomenological discussion of the topic, in order to enable the method to be applied, and postpone the more rigorous discussion until later in the physical chemistry course.

Physical chemistry has two major contributions to make in the development of a chemist. The first of these is the imparting of a sense of rigour, so well exemplified in the study of thermodynamics. This subject plays much the same part in the development of the young chemical mind as does geometry in the development of the young mathematical mind. It is a discipline in the real sense of the term; if one proceeds logically within its framework, one cannot be led to the wrong conclusion. The second contribution lies in the area of numerical analysis. An ability on the part of a student to produce formulæ relating chemically significant quantities counts for little if he or she is unable to apply these formulæ to specific examples and to get chemically sensible answers. By a judicious selection of numerical problems the development of a feeling for the quantitative aspects of chemical phenomena can be developed in the student, a feeling that will be of the utmost importance in his future scientific life, whether in pure or applied research. Much more serious than the lack of ability of a student to get the correct answer to a problem is his lack of realization that the answer he has produced may make chemical nonsense. This feeling for the quantitative aspects of chemistry will also facilitate the making of rapid order-of-magnitude calculations, which are an important guide in accepting or rejecting a possible interpretation of experimental results.

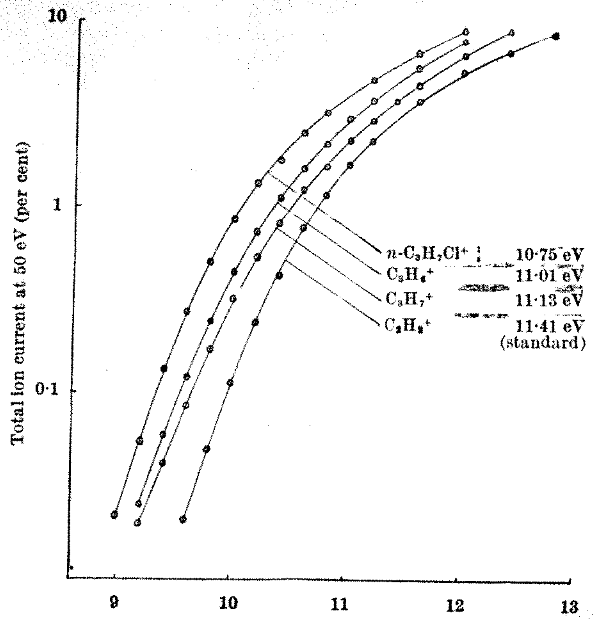


Fig. 2. Ionization efficiency curves for species derived from *n*-propyl chloride

I would like now to turn to the areas of chemistry in which my collaborators and I are most actively working. We are interested both in structure and mechanism. It is only very recently, thanks to the Department of Scientific and Industrial Research, that we have been able to enter the field of structure by the acquisition of a high-resolution mass spectrometer. Work in mechanism through the kinetics of gas-phase reactions has been proceeding for more years than I care to remember.

A mass spectrometer is an instrument which takes a beam of atoms or molecules and sorts them according to their mass, just as an optical spectrometer takes a beam of light and resolves it into its component wavelengths. In its simplest form (Fig. 2) the mass spectrometer uses a beam of electrons to knock off an electron from a molecule, forming a beam of charged species or ions:

$$M + e \rightarrow M^+ + 2e$$

for example:

$$C_3H_7Cl + e \rightarrow \{C_3H_7Cl\}^+ + 2e$$

The ions are then accelerated by an electric field along the line of motion, and mass analysis is effected by a transverse magnetic field, the ions of a given mass being brought to a focus and finally collected. For a given velocity of the ions, and a given magnetic field, the heavier ions are deviated from a straight-line course less than the lighter ones. This property provides a means of weighing atoms and molecules. Such an instrument is known as a single-focusing mass spectrometer. In addition to the magnetic analyser there is included an electrostatic analyser, and the geometry is such that the ion beams may be brought to a very sharp focus. This is known as a double-focusing mass spectrometer, and it can be used for very accurate mass measurements, as we shall see later.

Besides being used for determining the masses of charged particles, the mass spectrometer may also be used for an investigation of the energetics of ionization. For if we vary the energy of the ionizing electron beam we can determine the minimum energy required to ionize a given molecule, namely, the ionization potential. Fig. 2, which gives a plot of the logarithm of the ion current against the energy of the electron beam, shows the way in which the former increases as the latter decreases. In an actual determination of an ionization potential, a standard substance (acetylene in this case) is added along with the unknown (n-propyl chloride) and the efficiency curves for these two species are the inner and outer ones. The horizontal distance between the curves then gives the difference in ionization potential between the unknown and the known species. In this way, the ionization potential of n-propyl chloride has been determined as 10·75 eV.

Besides merely ionizing a molecule, the electron beam may fragment it:

$$M + e \rightarrow P^+ + Q + 2e$$

for example:

$$C_3H_7Cl + e \rightarrow \{C_3H_7\}^+ + Cl + 2e$$

and the minimum energy required to produce a fragment P^+ is known as the appearance potential of P^+ or $A(P^+)$. This quantity is of importance, since it leads to chemically important information regarding the chemical bond. For consider the fate of a species RX. This can fragment in three different ways to give $R + X$, $R^+ + X^-$ or $R^+ + X$. The energy required to bring about the first process is known as the homolytic bond dissociation energy $D(R—X)$, that for the second is the heterolytic bond dissociation energy $D(R^+X^-)$, while that for the third, as we have already seen, is the appearance potential of R^+ from RX. There is one further quantity required, namely, the ionization potential of the radical R, namely $I(R^+)$. The relationship between these quantities is shown in Fig. 3, $I(X^-)$ being the electron affinity of X. It is immediately apparent that:

$$D(R—X) = A(R^+) - I(R^+)$$
$$D(R^+X^-) = A(R^+) - I(X^-)$$

and that these two quantities may be obtained if one can determine $A(R^+)$, $I(R^+)$ and $I(X^-)$. Returning to Fig. 2, it is seen that the appearance potential of the n-propyl ion $(C_3H_7^+)$ is 11·13 eV. This gives for the heterolytic bond dissociation energy of n-propyl chloride the value of:

$$11{\cdot}13 - 3{\cdot}80 = 7{\cdot}33 \text{ eV} = 169 \text{ kcal mole}^{-1}$$

3·80 eV being the electron affinity of chlorine. The relevance of such an investigation of energetics will be seen when we come to discuss our interests in mechanism.

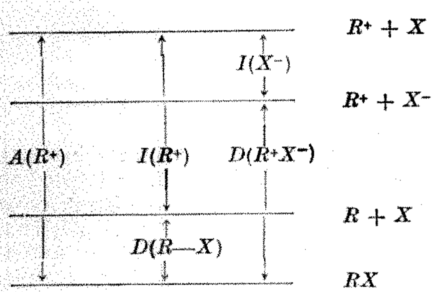


Fig. 3. Energetics of ionization and dissociation

Leaving now for a while the energetics of ionization, let us examine some other implications of fragmentation. The first arises from the fact that the mass spectrum of a molecule is normally uniquely characteristic of that molecule; it can be regarded as a molecular fingerprint, and used for identification. But one can go further than this; the mass spectrum of a mixture of substances can be analysed for the relative abundance of the components of the mixture, that is quantitative analysis of mixtures can be carried out. A simple example is shown in Fig. 4, where the mass spectra of methane and of ethane are shown, together with the mass spectrum of an equimolar mixture of the two. In a diagram such as this the heights of the lines at different mass numbers represent the ease of production of the given species. The most abundant peaks are at 16 in methane (top spectrum), namely, the parent peak, and 28 in ethane (middle spectrum), namely, a fragment peak. The results for the mixture (bottom spectrum) would enable us to conclude that it contained

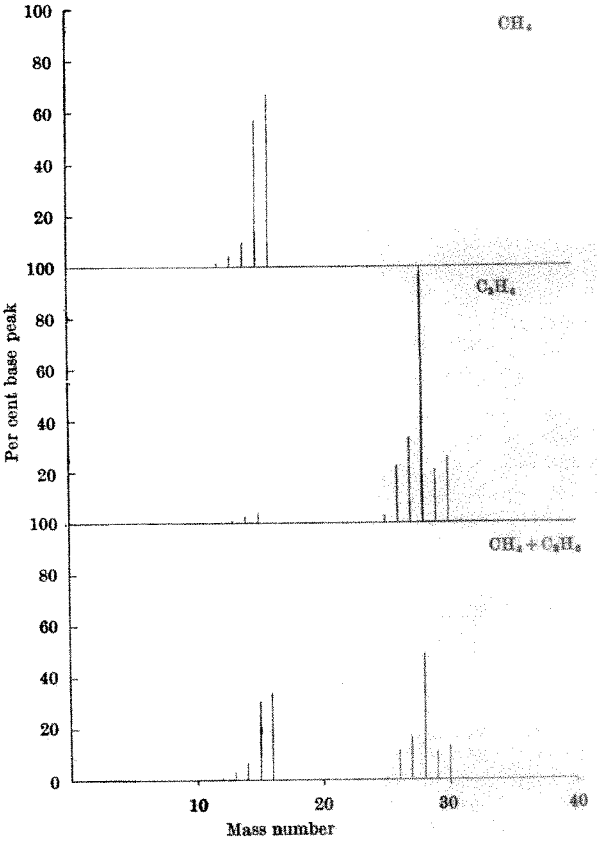


Fig. 4. The mass spectra of methane, ethane and an equimolar mixture of the two substances

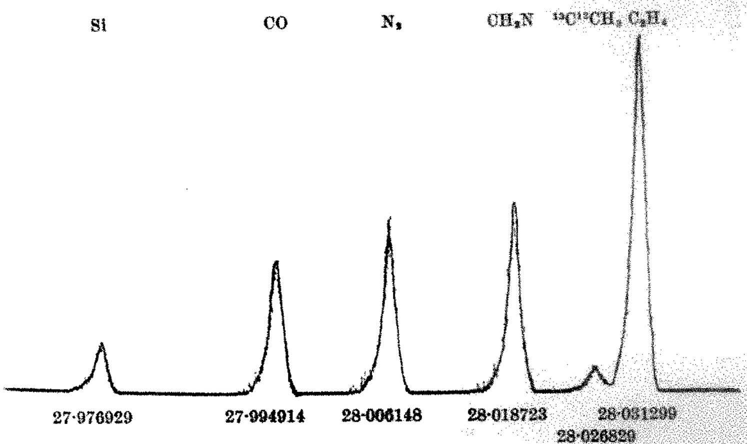


Fig. 5. A sextuplet at mass number 28 resolved on the A.E.I. *MS*9 mass spectrometer

equal amounts of methane and ethane. Problems of this sort, which were particularly relevant to the oil industry, resulted in the first commercially produced mass spectrometers. This aspect of mass spectrometry we hope to utilize in our investigations of reaction mechanisms, in particular in the identification of the products of reaction.

The mass number of an ion or a fragment is not sufficient to identify the atomic composition of the fragment. For consider the case of mass number 28. This could arise from Si, CO, N_2, CH_2N, $^{13}C^{12}CH_3$ or C_2H_4, the mass numbers of hydrogen, carbon, nitrogen, oxygen and silicon being 1, 12 (or 13), 14, 16 and 28 respectively. However, the atomic weights vary slightly from whole numbers as shown in Table 2.

Table 2. Some Atomic Weights

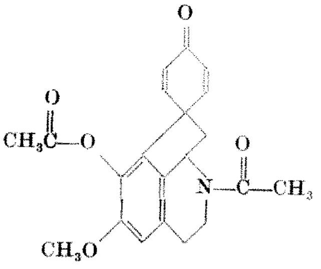
Element	Atomic wt.
H	1·007825
C	12·000000
	13·003355
N	14·003074
O	15·994914
Si	27·976929

Such a sextuplet resolved on the *MS*9 mass spectrometer is shown in Fig. 5. If mass measurements can be made to a sufficiently high degree of accuracy, the atomic composition of the ion or fragment may be determined. This aspect has immediate application to the investigation of molecular structure, since in one step the molecular formulæ of a given compound can be unambiguously determined. Thus in our examination of a compound isolated from an East African plant and containing only carbon, hydrogen and oxygen, we found the molecular weight to be 412·224 ± 0·002. Examination of possible formulæ (about thirty in all, some of which are shown in Table 3) showed it to have the composition $C_{25}H_{32}O_5$.

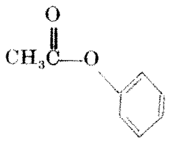
Table 3. Some Possible Formulæ of a Compound of Mass No. 412

$C_{24}H_{28}O_6$	412·189
$C_{25}H_{32}O_5$	412·225
$C_{26}H_{36}O_4$	412·261
$C_{27}H_{40}O_3$	412·283
$C_{27}H_{56}O_2$	412·428
observed	412·224

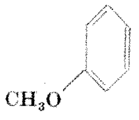
The next aspect of mass spectrometry I wish to discuss is the determination of the structure of a given molecule from its fragmentation pattern. This is, of course, a problem of much greater difficulty, due to the mass of information afforded by the many lines in the mass spectrum. However, as an example, let us look at the inverse problem, namely, the interpretation of the mass spectrum on the basis of an established formula. Thus diacetyl crotonosine, a substance isolated from a West Indian plant, was shown to have the structure *A* by Profs. Barton, Hayes *et al.* In order to simplify the problem, spectra of the model compounds related to *A*, namely, phenyl acetate (*B*), anisole (*C*) and *N*-acetyl piperidine (*D*), were run, and are shown in Fig. 6.



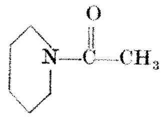
A, $C_{21}H_{21}O_5N$, 367



B



C



D

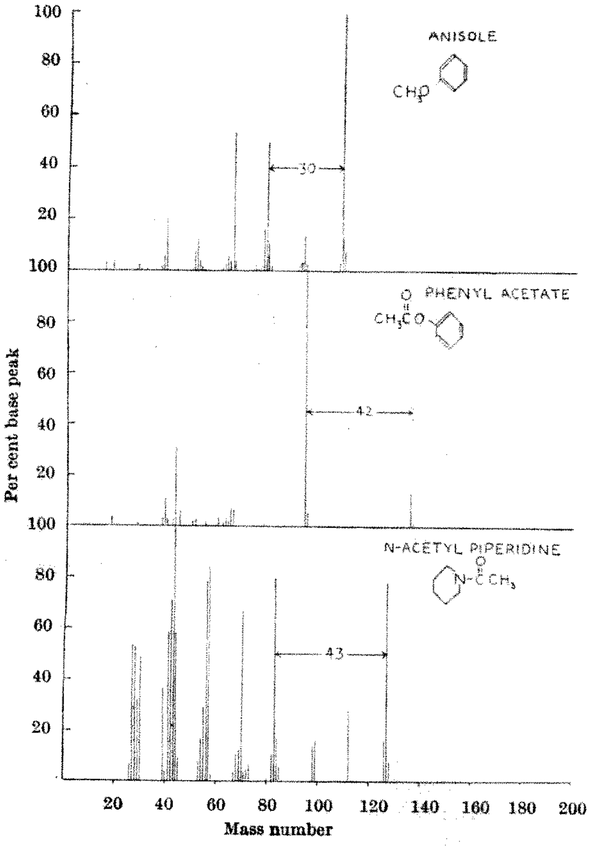


Fig 6. The mass spectra of anisole, phenyl acetate and *N*-acetyl piperidine

Large peaks occur in these compounds due to the loss of ketene (mass 42), formaldehyde (mass 30) and the acetyl radical (mass 43) respectively. In diacetyl crotonosine, peaks at 337 ($C_2H_{19}O_4N$), 325 ($C_{19}H_{17}O_4N$) and 324 ($C_{19}H_{16}O_4N$) can be interpreted as due to losses of formaldehyde, ketene and the acetyl radical respectively. The interpretation of the many remaining lines will have to await another occasion.

There is one further aspect of mass spectrometry that we are investigating, namely, the phenomenon of metastable peaks. These arise from processes of the type:

$$M_1^+ \rightarrow M_2^+ + M_3$$

for example:

$$\{C_2H_5Cl\}^+ \rightarrow \{C_2H_4\}^+ + HCl$$
$$\{C_6H_5OCH_3\}^+ \rightarrow \{C_6H_6\}^+ + CH_2O$$

which occur after the ion has been accelerated but before it enters the magnetic analyser. Now the mass spectrometer only sees those metastable reactions that occur in the region of the entrance to the magnetic analyser. A simple relation exists between the masses of the original and of the offspring ions, and the non-integral mass at which the metastable peaks occur. It is:

$$m^* = \frac{m_2^2}{m_1}$$

where m_1 and m_2 are the masses of the original and offspring ions, and m^* is the mass at which the metastable peak occurs. Metastable peaks may be used to confirm a postulated fragmentation. In this way the production of olefines from alkyl halides and of formaldehyde from anisole can be shown to have arisen, at least in part, by electron bombardment and not by thermal decomposition. The metastable peak in anisole due to the elimination of formaldehyde is shown in Fig. 7. The characteristic

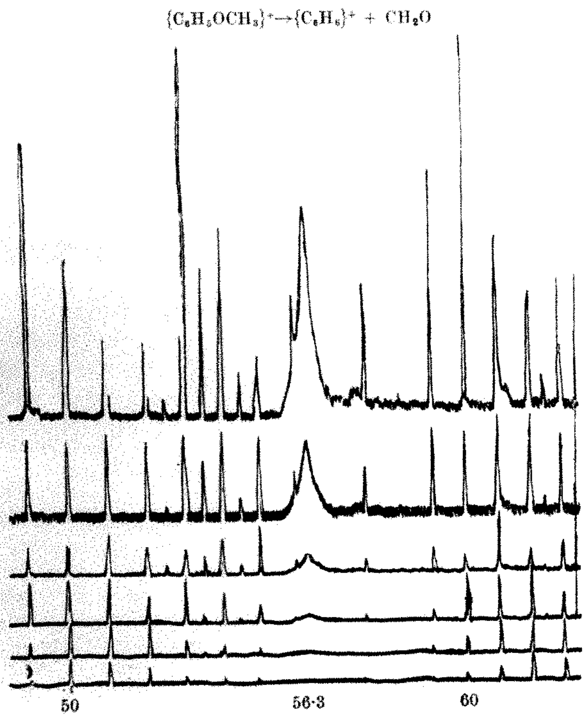
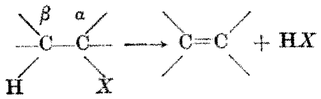


Fig. 7. A metastable peak in anisole

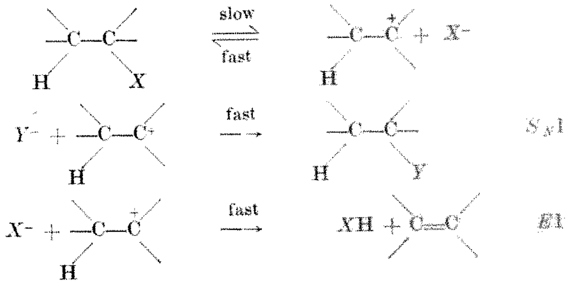
width of such peaks compared with the normal peaks is apparent, the width in this case extending over more than one mass number. Further, by observing the variation of the intensity of the metastable peak under various conditions, an estimate may be made of the rate of the unimolecular reaction giving rise to it. The half-lives of such processes are of the order of a microsecond.

This leads me back to the other area of our researches, namely, the kinetics of gas-phase reactions. The essential problem here is the prediction of the rate at which a reaction occurs in terms of some previously known properties of the atoms or molecules of the reactants. As we have already seen, the problem may be tackled at the three different levels, which we have called the mathematical, the physical and the chemical. We have applied the chemical method to homogeneous unimolecular gas-phase elimination reactions of alkyl halides:



yielding an olefine and a hydrogen halide (X being Cl, Br or I). The method is to take a parent molecule, namely, the ethyl halide, and determine the effect of substitutions at the α- and β-carbon atoms on the rate of elimination. The basic method has been used to great effect by organic chemists and in particular by Prof. Ingold and the late Prof. Hughes, in their investigation of reactions in solution. It was perhaps understandable, although still surprising, that Dr. Thomas and I were led to postulate an analogy between the gas-phase elimination reaction and reactions occurring in solution in a polar solvent by the unimolecular mechanism. Understandable because we were working in a department which had devoted so much of its energies towards an interpretation of reactions in solution. Surprising because, while the solution reactions occur at temperatures between 0° and 100° C, the gas-phase reactions take place between 200° and 450° C.

The solution reactions have been established as proceeding through a slow, rate-controlling ionization. Thus the following scheme holds for either substitution (S_N1) or elimination ($E1$). On the basis of these mechanisms, it follows that the rate of the unimolecular reaction will be increased by the attachment of electron-repelling groups at the α-carbon atom (and to a lesser extent at the β-carbon atoms) and decreased by the corresponding attachment of electron-attracting groups. Or, put in another way, factors which tend to stabilize the carbonium ion will also tend to speed up reactions by the unimolecular mechanism.



The evidence on which the analogy was based, namely, the effect of substitution on rates of reaction, is shown in Table 4. In the case of the chlorides the data are in the main taken from the work of Prof. Barton, Dr. Howlett *et al.*, while that for the bromides and iodides has mainly been obtained in this Department. It might be said in passing that, although only rate sequences are used in Table 4, the investigations have usually led to the complete temperature-dependence of the rate constant, through the Arrhenius equation:

Table 4. EVIDENCE FOR THE ANALOGY BETWEEN *Eg* AND *El*

1 $(CH_3)_3CX \gg (CH_3)_2CHX \gg CH_3CH_2X$ $X = Cl, Br, I$
2 $(CH_3)_3CCX(CH_3)_2 > (CH_3)_2CHCX(CH_3)_2 > CH_3CH_2CHX(CH_3)_2$
$CH_3CX(CH_3)_2$ $X = Cl$
$(CH_3)_2CHCH_2X > CH_3CH_2CH_2X > CH_3CH_2X$ $X = Cl, Br$
3 $CH_3CCl_3 > CH_3CHCl_2 > CH_3CH_2Cl > CH_2ClCH_2Cl$
$CH_3CHBr_2 > CH_3CH_2Br > CH_2BrCH_2Br$
4 $C_6H_5CH_2CH_2X \sim CH_3CH_2X$; $C_6H_5CHXCH_3 \sim (CH_3)_3CX$ $X = Cl, Br$
5 p-F–$C_6H_4CHClCH_3 > C_6H_5CHClCH_3 > p$-Cl–$C_6H_4CHClCH_3$
6 $CH_2=CHCHClCH_3 \sim CH_3CHClCH_3$
$CH_2=CHCH_2CHBrCH_3 \sim CH_3CH_2CHBrCH_3$
7 $CH_3OCHClCH_3 >>> CH_3CH_2X$
8 Wagner–Meerwein rearrangements

$$k = A \exp(-E/RT)$$

both A and E being determined for the reactions. It is found that the log A values are distributed about a value of 13·5 in an approximately Gaussian fashion. Thus the variations in activation energy are the major cause of variations in rate of elimination throughout a series of related compounds. This justifies the use of either relative rates or of activation energies in an attempt to correlate the effect of substitution on the rates of reaction. The striking feature of Table 4 is that the rate sequences shown are just those which have long been established as characteristic of reactions proceeding by the unimolecular mechanism in a polar solvent. This suggests that the activation energies for gas-phase elimination should be related to the heterolytic bond dissociation energies $D(R^+X^-)$ of the R–X bonds: and this provides a link between our two major areas of interest, gas-phase kinetics and mass spectrometry. For we have already seen how heterolytic bond dissociation energies can be obtained using mass-spectrometric techniques. On the basis of values of appearance potentials obtained from the literature, the plot of the energy of activation for elimination against the heterolytic bond dissociation energies is shown in Fig. 8. It will be seen that a nearly linear relationship exists between the two quantities. We are at present engaged in extending the range of appearance potentials (and hence heterolytic bond dissociation energies) so that the correlation with activation energies may be further tested.

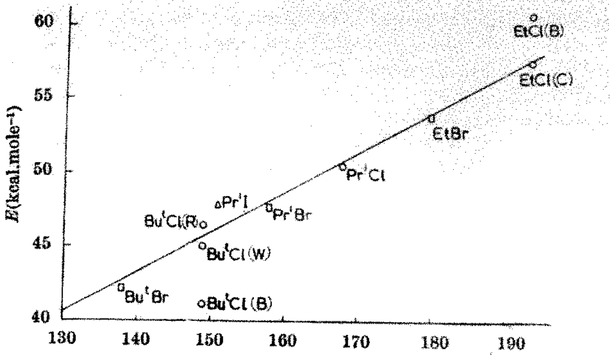
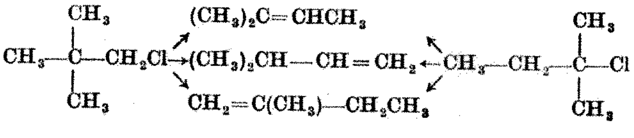


Fig. 8. Relation between the activation energies for gas-phase elimination and the heterolytic bond dissociation energies

There is one further aspect of Table 4 that I would like to mention in greater detail, namely, observation of Wagner–Meerwein rearrangements in the gas-phase. The first case reported was that of neopentyl chloride, in which it was shown that the major route of pyrolysis of this compound was a homogeneous, unimolecular one, the products being those that would have been expected from *t*-pentyl chloride. This again is analogous to the results:

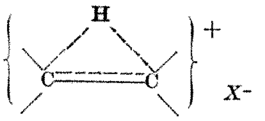


neopentyl chloride

t-pentyl chloride

observed in the examination of the solvolysis of similar compounds. During the reaction, the carbon skeleton has changed, which implies the migration of a methyl group from the β- to the α-carbon atom. Corresponding rearrangements have been observed in the gas-phase pyrolyses of bornyl and iso-bornyl chlorides, which again confirm the proposed analogy.

This then leads us to the main object of any kinetic study, namely, the molecular description of the process of reaction, or, more precisely, the assignment of a mechanism to the reaction or alternatively a physical model of the transition state. We have seen that those factors which lead to the stabilization of the carbonium ion also lead to an enhancement of the rate of gas-phase elimination. This, in turn, provides the essential clue to the mechanism. For it clearly suggests a carbonium ion character for the transition state. The essential experimental facts are in accord with the model of the transition state as an elongation, with polarization, of the $C^{\delta+}—X^{\delta-}$ bond in the sense indicated. This in turn may be represented by the formula:



on the understanding that the charge separation has not been carried to completion, and further, that the system is stabilized by polarization forces. A somewhat better than order-of-magnitude calculation shows that such a formulation cannot be ruled out on energetic grounds.

This then is the position at which we stand at present. Gas-phase elimination reactions of the type I have discussed can be understood on the basis of the postulated transition state. In addition the correlation of the solvolysis reaction in polar solvents with the gas-phase elimination reactions of the same compound can be rationalized on the basis of the gas-phase transition state presented here. What still remains is a more detailed model for the transition state which will enable more precise estimates of rates of reaction to be made. This problem is a challenging one on which we hope eventually to throw more light.

What I have tried to do is to define what I understand to be physical chemistry, to outline the areas of mass spectrometry and gas-phase kinetics which we are investigating, and to indicate the progress which has been made up to the present time. In conclusion I should like to thank those people who have helped me at various stages. First, Prof. Fawsitt and Dr. Iredale of the University of Sydney for encouraging my interest in chemistry and, secondly, the University of London for the award of an Imperial Chemical Industries fellowship. Thirdly, all my research associates, without whose help the work described here could not have been carried out. Finally, I would like to express my appreciation of the technical assistance I have at all times received, of the support I have had from this College and of the advice I have been given by colleagues past and present.

OBITUARY

Prof. J. Bartels

Prof. J. Bartels, professor of geophysics in the University of Göttingen, died on March 6.

Though deeply interested in all aspects of the physics of the Earth, and indeed much involved from time to time in seismology (results from Heligoland explosions) and meteorology (atmospheric tides and solar radiation), Bartels's life-long interest was in geomagnetism. In this discipline his early training at Potsdam Observatory under Dr. Adolph Schmidt ensured that he had the soundest possible basis on both observational practice and classical theory. One of his lasting contributions to the subject dates from that early period. He deduced from Prof. S. Chapman's analysis of the quiet day solar and lunar magnetic variations the pattern of the electric current system in the atmosphere which could produce these variations. He also became much interested in the slow secular changes in the Earth's main magnetic field and brought out clearly the regional rather than planetary nature of this phenomenon. The recognition of this later led to the cause of the isoporic foci being attributed to fluid eddies in the Earth's core.

After Potsdam, Bartels spent several periods in the Department of Terrestrial Magnetism of the Carnegie Institution of Washington. There he had direct contact with the records from various observatories which the Institution had set up in specially selected parts of the World. The abnormally large daytime amplitude of the diurnal variation at Huancayo, Peru, attracted his attention and led him with colleagues in the Institution to make a series of detailed studies. These resulted in the discovery of what has become known as the equatorial electojet, a narrow band of intensified electric current in the high atmosphere over the magnetic equator, now explicable in terms of a steep increase in conductivity where the angle of inclination of the field is small.

To follow up his long-standing interest in relationships between geomagnetic, solar and other related phenomena, Bartels needed an index of magnetic activity covering shorter intervals than the old international character figures for whole days; and, more particularly, he wanted the index to distinguish the effects of solar corpuscular radiation as shown by short-period irregular disturbance from the effects of electromagnetic wave radiation on the quiet day variation. The outcome of his work on this theme, which covered the period from before the Second World War to his later years, was the 3-h K_p index, and, more experimentally, the 15-min index Q. Thanks to its sound physical basis and the many careful investigations Bartels made to evaluate its validity and usefulness, K_p is now internationally accepted in many geophysical disciplines as an authoritative index not just for geomagnetic activity but for comparison with all effects associated with the solar corpuscular wind.

In addition to his comprehensive grasp of the physics and mathematics of geomagnetism, Bartels had an exceptional flair for handling statistics and succinctly displaying their main import. His personal contributions to the subject in a series of papers on the harmonic dial, stereograms, harmonic analysis of a single day's data, and random fluctuations and persistence in quasi-periodicities have been adopted as present-day practice in many fields. He had a special and justifiable pride in the amount of concentrated information displayed in the 'musical diagrams' which he issued regularly from Göttingen to show the sequence, every three hours, of the planetary indices, K_p, over successive rotation intervals of the Sun.

In addition to his personal research work, the results of which appeared in a long series of papers, Bartels wrote several texts on magnetic observatory practice and the theory behind it: in 1940 with S. Chapman he produced the two great volumes on geomagnetism which immediately became and still remain the definitive comprehensive text on the subject.

Since 1946 Bartels had held the joint posts of director of the Geophysical Observatory and professor of geophysics in the University of Göttingen. He took an active part in the international organizations related to his subjects, being president of the International Association of Geomagnetism and Aeronomy (1954–57), and vice-president of International Union of Geodesy and Geophysics (1957–63), and chairman of various committees and commissions related to International Geophysical Year, International Year of the Quiet Sun and, more recently, the Committee on Space Research (COSPAR). In 1953 he was awarded the Charles Chree Medal by the London Physical Society. He was awarded posthumously the Bowie Medal by the American Geophysical Union. In recent years (since the death of Prof. Regener) he had been director of the Max Planck Gesellschaft Institute of Aeronomy at Lindau.

Bartels was a kindly, friendly man with a great fund of quiet humour and with catholic tastes in music and the arts.

S. K. RUNCORN

NEWS and VIEWS

Director of the Post Office Radio Services: Mr. H. G. Lillicrap

MR. H. G. LILLICRAP has been appointed director of the Radio Services Department of the Post Office in succession to Mr. Alan Wolstencroft, who became deputy director general on August 15. Mr. Lillicrap is at present a deputy director of finance in the Accountant General's Department at the Post Office. He started work in the Research Laboratories of the General Electric Co., Ltd., at Wembley, and joined the Post Office as a probationary assistant engineer in 1936. Apart from an early spell at Exeter, he spent most of the next ten years or so at the Post Office Research Station at Dollis Hill, London, working on the design of radio transmitters and receivers. In 1947 he moved to the Radio Branch of the Engineer-in-Chief's Department, where he was concerned with frequency allocation and the technical side of broadcasting. In 1951 he became a principal in what was then the Overseas Telecommunications Department (now the External Telecommunications Executive), of which he became a deputy director in 1958. While in this work he attended numerous international conferences at Buenos Aires, in the United States, Canada, Japan and various European countries. In 1960 he became a deputy director of finance.

School of Applied Sciences in the University of Sussex: Prof. J. C. West

PROF. J. C. WEST, at present professor of electrical engineering in Queen's University of Belfast, has been appointed professor of engineering and dean of the School of Applied Sciences in the University of Sussex. Prof. West graduated at the University of Manchester in 1943 and then joined the Royal Navy Volunteer Reserve, being stationed in Northern Ireland for a period, engaged in anti-submarine warfare. He returned to the Electrical Engineering Department at Manchester in 1946, where he was appointed successively assistant lecturer, lecturer and senior lecturer. In 1958 he was appointed professor and head of the Department of Electrical Engineering in Queen's University of Belfast (*Nature*, 180, 1168; 1957), where he has been responsible for the rapid expansion of the Department. Prof. West is well known for his contributions to the analysis of non-linear servomechanisms, and for his more recent work on unconventional electrical machines. He is the author of two text-books on automatic control. As dean of the School of Applied Sciences in the new University of Sussex, Prof. West is eager to introduce undergraduate courses differing significantly from the traditional specialized subjects, and one of his first tasks is the specification of new buildings for engineers, the third time he has performed this operation.

Electrical Power Engineering in the University of Southampton: Prof. P. Hammond

MR. P. HAMMOND, Fellow and director of studies in electrical sciences in Pembroke College, Cambridge, has been appointed to the chair of electrical power engineering at the University of Southampton. Mr. Hammond was educated at Steyning Grammar School and Emmanuel College, Cambridge. He served a graduate apprenticeship in electrical and mechanical engineering with the English Electric Co., Ltd., and afterwards joined the staff of this Company as a designer of synchronous machines. He was appointed to a lectureship at Cambridge in 1949 and was later elected to a fellowship at Pembroke College. He has twice been invited to serve as visiting professor overseas: at Stanford in 1955–56 and at Kuala Lumpur in 1962–63. Mr. Hammond is the author of a number of papers and one book on electromagnetism. His research work has been largely concerned with eddy current losses in rotating machines and he has written a number of articles on the early history of electromagnetism.

Fibre Science in the University of Leeds: Prof. A. Robson

DR. A. ROBSON has been appointed professor of fibre science in the Department of Textile Industries in the University of Leeds as from September 1. After graduating from the University of Leeds in 1947, Dr. Robson worked under the late Prof. F. A. Paneth at the Londonderry Laboratories for Radiochemistry in the University of Durham, on the microanalysis of uranium. He was awarded a Ph.D. degree in 1949, and in the autumn of that year he joined the staff of the Wool Industries Research Association at Torridon, Leeds, as a senior scientific officer; latterly he has been head of the Fibre Chemistry Department. Dr. Robson's main interests have been in the application of radioisotope techniques to problems in wool chemistry. With his colleagues he has developed methods for the analysis of amino-acids and amino-acid derivatives, determined the amino-acid composition of wool and of proteins isolated from wool, and examined the distribution of sulphur in wool. The last-mentioned of these topics was supported by a grant from the United States Department of Agriculture, and led to the discovery of two new sulphur-containing amino-acids. Dr. Robson will retain responsibility for the direction of a new research project on which he and his colleagues at the Wool Industries Research Association are at present engaged, and which is also sponsored by the United States Department of Agriculture.

Medical Biochemistry in the University of Manchester : Prof. D. S. Jackson

DR. D. S. JACKSON has been appointed to be the first holder of the chair of medical biochemistry in the University of Manchester. Dr. Jackson, now aged forty-three, was educated at Farnworth Grammar School, and served from 1940 until 1946 in the Royal Air Force before entering the University of Manchester. He graduated B.Sc., with honours in chemistry, in 1949, and Ph.D. in 1954. He has been interested throughout his career in the medical applications of chemistry and biochemistry. As a research assistant in the Rheumatism Research Centre, he became concerned with the nature of collagen and reticulin, and with the response of tissues to the irritant carrageenin. In 1954–55 he was Visiting Fellow at the National Institute for Medical Research, with Prof. A. Neuberger. From 1957 until 1959 he was a research associate in biochemistry at the Harvard Medical School, and in 1959 he went to the medical school of the University of Oregon as assistant, and later associate professor. In 1962 he was appointed professor of biochemistry, and also head of the Surgical Research Laboratories in Oregon. Dr. Jackson's published work has been concerned with the proteins of connective tissue, and more recently with wound healing and tissue reaction to foreign materials and to tumours. He has also developed new approaches in the teaching of biochemistry to medical and biological students. His return to Britain, and to his own University, is welcome.

Experimental Psychology in the University of Sussex : Prof. N. S. Sutherland

THE progress of the behavioural sciences in Britain will be notably furthered by the establishment of a chair of experimental psychology at the University of Sussex and the appointment of Dr. N. S. Sutherland to it. A recruit to psychology from 'Greats', Dr. Sutherland followed this with the Oxford Final Honour School of Psychology, Philosophy and Physiology, and was afterwards elected to a fellowship by examination at Magdalen College. Encouraged by Prof. J. Z. Young in his interest in the visual discrimination mechanisms in *Octopus*, he and his colleagues have, in the past ten years, made a massive and penetrating investigation of stimulus-analysing mechanisms in both vertebrates and invertebrates. Dr. Sutherland is also well known for his contributions to human psychology, especially in the field of learning and memory. Appointed University lecturer in experimental psychology at Oxford in 1960, he was elected to an official fellowship at Merton College in 1962. He has played a large part in the development of the subject at Oxford, through a period of rapid and difficult evolution. His many friends and colleagues, not only in Britain, but also in the United States, will look forward with great interest to the development of the new department at Sussex.

1964 Guggenheim International Astronautics Award : Prof. W. O. Fenn

THE Daniel and Florence Guggenheim International Astronautics Award for 1964 has been conferred on Prof. Wallace O. Fenn, of the Department of Physiology in the University of Rochester School of Medicine and Dentistry. Dr. Fenn is well known for his work in many of the biological sciences, including respiratory problems, the mechanics of running and bodily movements, heat production of muscle, potassium and other electrolytes in muscle, and oxygen requirements of stimulated nerves. Since 1959 he has been secretary-general of the International Union of Physiological Sciences. He has been an active member on numerous committees of leading American scientific institutions, such as the National Research Council, the Research and Development Board, the National Science Foundation, and he has served at times as chairman of the Physiological Training Grant Committee and of the Physiology Study Section of the National Institutes of Health. His achievements have been widely recognized and earlier this year he received the Antonio Feltrinelli Prize for experimental medicine. The presentation of the Guggenheim International Astronautics Award will be made during the fifteenth Congress of the International Astronautical Federation, which will be held in Warsaw during September 7–12. The Award, which carries a prize of 1,000 dollars, has been made annually since 1961, but this is the first time that it has been awarded to a scientist working in the field of physiological research.

British Aviation Research

REPLYING to a question in the House of Commons on July 8, the Minister of Aviation, Mr. J. Amery, said that the Ministry's research and development establishments, in co-operation with industry, were devoting considerable effort to problems of approach and landing guidance, long-range navigation, communications, ground data handling and processing systems, the application of automation to future air traffic control systems and the use of micro-electronics techniques for civil aviation equipment. His department would also finance most of the British share in the development of the *Concord* supersonic transport aircraft, which entails the development of advanced electronic systems. Links with industry had recently been strengthened and they were very much aware of the need for the fullest dissemination of information.

Research and Development for Power

IN a written answer in the House of Commons on July 29, the Parliamentary Secretary to the Ministry of Power, Mr. J. Peyton, stated that the combined expenditure on research and development by the nationalized coal, gas and electricity industries had increased from about £6 million in 1958–59 to about £12 million in 1963–64. In conjunction with manufacturers, the National Coal Board had made significant progress in developing equipment for the widespread mechanization of coal winning, and more recently for applying remote control techniques to mining. Pioneer research by the Gas Council had led to the development of new processes which were bringing about a technological revolution in the production of gas. The Central Electricity Generating Board had expanded its central research laboratories and built new laboratories for nuclear and engineering research.

Solway Firth Barrage

IN reply to questions in the House of Commons on July 22, Mr. G. Campbell, Under-Secretary of State for Scotland, said that the proposal for a barrage in the Solway Firth was being examined jointly by the Scottish Development Group in its investigation of South-west Scotland and by the North-west England Regional Development Group. The Secretary of State for Scotland was aware of the interest of the University of Strathclyde in this problem and he was sure that it was being examined urgently. The Scottish Office was considering all representations which had been made on this subject, including the memoranda submitted by Dr. R. Drew.

The Price of Education

In reply to questions in the House of Lords on July 7, the Earl of Bessborough said that, for the present year, capital and recurrent Exchequer grants to the universities were estimated at £133 million compared with £58 million in 1959, when there were 24 university institutions, compared with the present 33. Over the same period the number of full-time students had risen from 104,000 to 125,000, and of full-time teachers from 12,000 to 15,000. These figures did not include the colleges of advanced technology and certain Scottish institutions which were achieving university status. The proportion of women students had risen from about 24 to 27 per cent. In the House of Commons on July 6, in reply to a question, the Secretary of State for Education and Science, Mr. Q. Hogg, stated that the total educational expenditure of public authorities in Great Britain, including expenditure on school meals and milk, rose from £479 million in 1952–53 to £1,277 million in 1962–63, or from 3·4 per cent to 5·1 per cent of the gross national product. In terms of constant prices the increase was estimated at about 75 per cent over the period, about half being attributable to an increase in the number of pupils and students.

British Aid to Development

In a written answer in the House of Commons on July 30, the Chief Secretary to the Treasury, Mr. J. Boyd-Carpenter, stated that the Government had announced to the United Nations Conference on Trade and Development Britain's intention of increasing her contribution to the United Nations Special Fund and to the Expanded Programme of Technical Assistance, to contribute to the African Development Bank, and to examine sympathetically the possibility of assisting other regional development banks. Britain was contributing in one form or another £7·1 million to the resources of the Inter-American Development Bank and her annual contribution to the International Development Association had been increased. At the recent meeting of Commonwealth Prime Ministers proposals were announced for a scheme of Commonwealth Development Projects, increased capital aid for higher education and an expansion of training facilities for senior administrators from developing countries in the Commonwealth. On this the Prime Minister had said in the House of Commons on July 23 that the proposals for a Commonwealth Foundation to encourage professional exchanges had been remitted to Commonwealth officials for study. He stated that he would not exclude the idea of a Foundation extending its activities to include training and exchanges of technical personnel. Much would depend on the funds available and the Foundation was devised essentially to promote professional links. Mr. Boyd-Carpenter continued that Britain would, at the third Commonwealth Education Conference at Ottawa in August, make proposals to expand and improve the Commonwealth Educational Co-operation Scheme. Recent specific bilateral commitments included provision of £52 million of economic aid for Kenya over the next three years, £51 million for Malta over the next ten years subject to satisfactory continuation of the defence arrangements, and increased aid for the Sudan. Expenditure on non-military aid in 1963–64 was £175 million and on military aid £30 million.

Japanese Aid

In the past decade Japan's gross national product has risen by about 10 per cent per annum, and in the non-Communist world only the United States, West Germany, the United Kingdom and France have a higher gross national product. With a programme of aid to developing countries of £60 million a year, she is also the fifth largest of the non-Communist donors, but a survey, *Japanese Aid*, by J. White, issued by the Overseas Development Institute, concludes that most of this is designed to help Japan's economic expansion rather than developing countries (Pp. 78. London: Overseas Development Institute, Ltd., 1964. 6*s*.). The survey reviews first the motives and objectives of Japanese aid, and then the mechanisms used and the flow of resources. It is pointed out that Japanese aid should not be judged by Western standards and attention is directed to some defects in the present system. This appears to be based on principles of selection which have little to do with the requirements of the recipients and neither enhances the programmes of other donors nor is enhanced by them. The booklet should contribute to a better understanding between Japan and the West, and Mr. White suggests that in practice Japan should be encouraged to participate to a greater extent in programmes which require detailed consultation and co-operation with other donors, and to administer a higher proportion of its aid on the basis of Government-to-Government agreements.

Infant and Neo-natal Mortality

The Office of Health Economics set up in 1962 by the Association of the British Pharmaceutical Industry has recently published a pamphlet entitled *Infants at Risk*, which gives an account of infant and neo-natal mortality, and makes a number of historical comparisons (Pp. 36. London: Office of Health Economics, 1964. 2*s*.). As is well known, infant mortality in Britain has been declining since the beginning of the twentieth century, but the rate of decline has slackened since the mid-nineteen-fifties. However, the social pattern of infant mortality has persisted almost unchanged since Edwardian days: in other words, different social groups in the community have benefited uniformly. The recent slowing down in the rate of decline means that Britain now lags behind countries such as Sweden, and a reduction of the British infant mortality rate to the Swedish level would mean a reduction of 4,000 infant deaths per annum. The report suggests that a more extensive use of ante-natal services on the part of expectant mothers might be instrumental in highlighting their present inadequacies and help in reducing the wastage immediately after birth.

Scientific Research in British Universities and Colleges, 1963–64

The 1964 edition of *Scientific Research in British Universities and Colleges* contains brief notes on scientific research in progress in British universities and colleges (including colleges of technology), and describes the nature of the projects in sufficient detail to indicate the scope of the research carried out by the various science departments and individual teams of investigators (Pp. xxv + 727. London: H.M.S.O., 1964. 35*s*. net). The social sciences and industrial administration are not included except where work in these fields normally occurs within the science faculty or technology department. Universities and colleges are listed alphabetically and under each are entered departments, and in many cases sub-departments and affiliated institutions, etc. The staff of each department are listed along with their qualifications and present fields of research. Indication is made where members of staff are affiliates of the Agricultural Research Council or Medical Research Council. The three indexes, "List of Subject Headings", "Name Index" and "Subject Index", make this publication one of the most useful guides to scientific research in British universities and colleges. The publication is issued jointly by the Department of Scientific and Industrial Research and the British Council.

Soviet Technical Literature

Tematicheskiy Plan—1964 is a complete listing in English by subject-matter of all books scheduled for publication by the Soviet Academy of Sciences in 1964 (Pp. iv + 132. London: Scientific Information Consultants, Ltd., 1964. 70*s*.; 10 dollars). Each entry gives author, title, sponsoring agency, size (in terms of printing sheets), size of edition and scheduled time of publication. Except where otherwise stated, all books are published by the Academy of Sciences. Synopses can be supplied on request at a cost of 7*s*. 6*d*. each, including postage. Some 1,255 titles are listed.

The Directorate for Scientific Affairs of the Organization for Economic Co-operation and Development has issued an index to issues 1 to 12 of the bulletin *Russian Technical Literature*, covering the period February 1960–October 1963 (Pp. 58. Paris: The Directorate for Scientific Affairs of the Organization for Economic Co-operation and Development, 1963). Entries are arranged alphabetically under author and subject, and entries relating to original contributions are differentiated. Titles of translated books are entered in the language into which they have been translated.

Bulletin of the International Computation Centre

The April issue of the *Bulletin of the International Computation Centre* in Rome contains three interesting papers concerned with the use of integrated computer systems in research and education—the latter both from the point of view of administration and of instruction. A paper by Tadatoshi Sakai, who is head of the Data Processing Section of the Bureau of Statistics, attached to the Prime Minister's Office in Japan, is of particular interest in showing the way in which the Japanese have adapted some notions on automatic programming to their IBM 705 data-processing system. In addition to the scientific papers there are a number of book reviews and notes of forthcoming conferences in the field of computation.

A Television Station in the Desert

According to a recent article in *The Lamp* (45, No. 4; Winter 1963), quarterly publication of the Standard Oil Company (New Jersey), Baghdad was the first television transmitting station to be established in the Middle East. In 1957 the Arabian American Oil Company ('Aramco'), an affiliate of Standard Oil, sponsored the setting up of the second station at Dhahran in the desert region of eastern Saudi Arabia. This was later followed by the establishment of television stations in Beirut, Cairo, Damascus, Kuwait, Abadan and Tehran. 'Channel 2 Dhahran', as it is called, can claim certain distinctions. In the first place 'Aramco Television' is probably the only social and cultural facility of its kind operated by an industrial firm. Secondly, it is the only station broadcasting entirely in Arabic. When it first came on the air the intention of the Company was to provide entertainment, information and education for 11,000 local employees and their families. "This audience is vastly outnumbered to-day by an estimated 100,000 or more viewers who can be reached by the Dhahran transmitter and a newly opened relay station sixty miles away. The number of receivers in the region has grown to about 16,000, and selling and servicing sets has provided new jobs and a brisk business for Saudi Arab merchants." The Dhahran station operates from a building situated not far from the original discovery oil-well in this desert region, the antenna being sited on the top of an old drilling rig. The station includes studio and control room for live programmes, film-processing laboratory, offices and a 'dubbing' booth; this last facility is of great importance because by means of 'dubbed-in' dialogue rendered by people who speak fluent Arabic, the characters and themes of many well-known American television films and productions are thus made available to these desert audiences. As regards programme policy, Aramco television devotes about one-third of its air-time (32 hours per week) to information and education; local events are covered by the station's own camera and sound-truck; Arabic-speaking doctors discuss hygiene, and pre- and post-natal care, among other subjects concerned with the health of the community. Although provision is made for non-Arabic viewers to enjoy imported films, by tuning their sets to a wave-length broadcasting the original soundtrack, one of the perhaps unexpected results of this enterprise has been to encourage many to learn Arabic.

New Barley Varieties in Europe

The European Brewery Convention *Report of the Barley Committee: Trials, 1962* (Pp. 138. 1964), presents the results of comparative trials of new barley varieties in twelve European countries both from the agricultural and malting points of view. From the first point of view, the trials consisted of (*a*) comparisons of the variety 'Kenia', which was regarded as the standard, with three obligatory varieties, and (*b*) local trials of some thirty additional varieties. Perhaps the major importance of these results in scientific literature is not the numerical values and assessment of specific qualities; rather is it to emphasize the complex organization required to give an overall picture of the acceptability of a new barley variety and perhaps of any agricultural crop when account has to be taken of climatic differences from country to country, climatic variations from year to year and differences in, for example, agricultural methods and susceptibility to disease in varying localities. Nevertheless, the summarized results and the country-by-country reports contain a wealth of tabulated information of a kind which would be very difficult to locate elsewhere. In addition, the *Report* includes summaries of papers presented at a joint meeting at which wider topics such as quality in malting barley, the development of barley breeding programmes and specific problems such as premature sprouting were considered. Radical new information is necessarily limited, but it will probably be helpful in similar fields to see how appreciation has grown of the value of regional agricultural experience and of objectively assessing a crop in terms which go far beyond the first consideration of yield per acre.

British Palæozoic Fossils

A genuine welcome is accorded the appearance of the Natural History Museum's publication *British Palæozoic Fossils* which completes the set of three very reasonably priced handbooks illustrating a representative selection of British fossils from the beginning of Cambrian times onwards (Pp. vi + 208 (69 plates). London: British Museum (Natural History), 1964. 12*s*. 6*d*.). Though intended primarily as an aid to the interested layman, this series also provides a wealth of readily accessible information for the specialist, and one may cite, by way of example, the synonyms given, where necessary, for the species selected for illustration. In this third handbook, an introductory chapter outlines the geological history of the British Isles during the Palæozoic Era, while subsequent sections include stratigraphical tables for each of the six systems involved, the geological distribution of the species illustrated, and brief notes on the intricacies of species nomenclature. The greater part of the book (pp. 54–191), however, is devoted to annotated illustrations of the 443 species selected to represent the Palæozoic. Having in mind the problems which must have confronted the authors, one is reluctant to criticize, but it is felt that certain improvements could have been implemented and at no extra cost. Thus, the illustrations are frequently too heavily shaded, giving insufficient contrast and consequent loss of detail, and it is unfortunate that less than 50 per cent of the species depicted are on display in the Museum. But such drawbacks are slight in comparison with the services rendered by this handbook.

Canadian Anthropology

A valuable collection of a pre-First World War field survey with excavations appears in the November 1963 issue of the *National Museum of Canada Anthropology Papers* (No. 4. *The W. B. Nickerson Survey and Excava-*

tions, 1912–15, of the Southern Manitoba Mounds Region. By Katherine H. Capes. Pp. iii + 178 (19 plates). Ottawa: Queen's Printer, 1963). This investigation utilizes the extensive field notes of Nickerson to produce a full report of his work and to show the relationship of the burial mounds of the area to those of other regions. On the basis of form, burial site and grave goods, and on distribution, the author suggests that the mounds form a north-western periphery to a great burial mound complex on the Northern Plains, evolving perhaps from Middle Woodland antecedents, and introduced into southern Manitoba by the Assiniboine.

A recent number of *Études Anthropologiques, Musée National du Canada* (No. 6, March 1964), describes the excavation of an Early Woodland site near the north bank of the St. Lawrence River (*Le Gisement de Batiscan. Notes sur des vestiges laissés par une peuplade de culture Sylvicole inférieure dans la valle du Saint-Laurient.* Par L'Abbe Rene Levesque, F. Fitz Osborne et J. V. Wright. Pp. 49 (5 plates). Ottawa: Queen's Printer, 1964). Although little organic material survived at this eroded site, diagnostic pottery and stone tools were recovered from a protected and homogeneous occupation layer. This provided one of the few culturally pure sites of Early Woodland nature in the north-east. While some quantitative analysis of the material has been attempted here, the materials thereby available would have been of greater value had the analysis been more comprehensive and the illustrations of artefacts more legible.

Index to Publications of the Bureau of American Ethnology

The Bureau of American Ethnology has published an index to the first 100 numbers of its *Bulletin*, which is likely to be of immense value, even though the *Bulletins* are now well advanced in their second century (*Bulletin* No. 178: *Index to Bulletins 1–100 of the Bureau of American Ethnology*, with *Index to Contributions to N. American Ethnology, Introductions and Miscellaneous Publications.* By Biren Bonnerjea. Pp. vi + 726. Washington, D.C.: Government Printing Office, 1963. 3.50 dollars). In addition to the subject index, there are separate indexes of authors and titles, and of illustrations. For good measure, indexes of the ethnological material in three other series which are largely out of print are added, namely, *Contributions to North American Ethnology, Introductions,* and *Miscellaneous Publications.*

University News :

Leeds

Dr. G. W. Johnson has been appointed lecturer in the Department of Metallurgy. Mrs. J. Tough has been appointed lecturer in the Institute of Education.

London

The following titles have been conferred: professor of metallurgical chemistry, on Dr. C. B. Alcock, in respect of his post at the Imperial College of Science and Technology; professor of rural economy, on Dr. G. P. Wibberley, in respect of his post at Wye College; professor of histology, on Dr. F. R. Johnson, in respect of his post at the London Hospital Medical College; professor of embryology, on Dr. P. H. Spencer-Silver, in respect of his post at the Middlesex Hospital Medical School; reader in biophysics, on Mr. D. K. Hill, in respect of his post at the Postgraduate Medical School of London; reader in hæmatology, on Mr. J. G. Humble, in respect of his post at Westminster Medical School; reader in psychology, on Dr. R. J. Audley, in respect of his post at University College.

Sheffield

Prof. R. S. Waters, professor of geography in the University of Canterbury, New Zealand, has been appointed to a chair of geology.

Battersea College of Technology

Battersea College of Technology, in anticipation of the granting of university status, has conferred the title of professor on the following heads of departments: Dr. F. M. Arscott (mathematics); Dr. L. R. B. Elton (physics); Dr. V. S. Griffiths (spectroscopy and chemical physics); Dr. Z. S. Makowski (civil engineering); Dr. J. E. Salmon (chemistry); Dr. S. R. Tailby (chemical engineering). A petition is in preparation for presentation to H.M. The Queen in Council for a royal charter incorporating the University, which it is hoped may be entitled the University of Surrey.

The International Institute for Conservation of Historic and Artistic Works

At the annual general meeting of the International Institute for Conservation of Historic and Artistic Works the following officers were elected: *President*, Dr. A. van Schendel; *Vice-President*, Mr. N. Brommelle; *Secretary-General*, Mr. N. Reid. Mr. F. I. G. Rawlins, the first secretary-general of the Institute and vice-president, was elected an Honorary Fellow of the Institute.

Announcements

The Elwell Award papers for 1964 will be presented at an ordinary meeting of the Midlands Section of the Society for Analytical Chemistry, which will be held in the University of Birmingham on September 16.

A half-day meeting of the Thin-Layer Chromatography Group of the Society for Analytical Chemistry will be held in Nottingham on September 23. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

An international conference on "The Earth Sciences" will be held at the Massachusetts Institute of Technology during September 30–October 2. Further information can be obtained from Dr. H. G. Houghton, Department of Meteorology, Massachusetts Institute of Technology, Cambridge 39, Massachusetts.

A joint meeting of the Microchemical Methods Group and the Scottish Section of the Society for Analytical Chemistry on "Automatic Methods of Analysis" will be held in the University of Aberdeen during September 25–26. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

The *Australian Science Index*, April 1964 (8, No. 4), contains a further 300 entries of articles published in Australian scientific and technical periodicals. The entries are classified under general; physical sciences, engineering, technology and biological sciences, agriculture; and there are subdivisions of sciences, etc. There is also an author index and a subject index.

A course of instruction in "Powder Technology", leading to a College postgraduate diploma, will be held in King's College, University of London, commencing in October, under the direction of Prof. H. E. Rose. The course, which will extend over one academic year, is designed for students having a first degree in engineering, physics, chemistry or certain other approved branches of applied science. Further information can be obtained from A. Davies, King's College, Strand, London, W.C.2. The fee for the course will be £87.

Erratum. In the communication entitled "Ammonium Production in Soil under Waterlogged Conditions as an Index of Nitrogen Availability", by S. A. Waring and Prof. J. M. Bremner, which appeared on p. 951 of the February 29 issue of *Nature*, it is stated in the twenty-first line of the second paragraph that the standard sulphuric acid used was 0·0005 N, whereas the correct figure is 0·005 N.

SCIENCE INFORMATION

A SUCCESSFUL first conference was held by the Institute of Information Scientists at Merton College, Oxford, during July 17–19, attended by 64 persons. The conference dinner on the Saturday evening was also attended by Prof. J. W. S. Pringle, representing the College, and by Mr. L. Wilson, director of Aslib (Association of Special Libraries and Information Bureaux), who proposed the toast of the Institute and spoke of the successful growth of the Institute since its inauguration in 1958, and of the courses in information science started at Northampton College of Advanced Technology, London, on the basis of the Institute's syllabus. Of these courses, the full-time, one-year postgraduate course, leading to the Diploma of the College, was the first full course in information science to be given in any country; the two-year part-time evening courses, which were started in 1961, lead to the Certificate of the Institute. The Institute now has nearly 400 members, of whom some sixty are in various countries overseas.

Of the papers presented, two concerned information services direct to management. Miss P. H. Cutting outlined the work at Messrs. Urwick, Orr and Partners, where she is information officer, and Mr. S. J. Batchelder described the work of the information department of the Leeds Incorporated Chamber of Commerce, of which he is the secretary. The wide scope and extent of the services provided at Leeds were of great interest, and indicated that many firms were finding that the Chamber of Commerce could provide answers to technical as well as economic questions. Mr. Batchelder remarked, however, that there were still many small firms which had not yet realized the value of obtaining information to improve their productivity. This is a problem which the Department of Scientific and Industrial Research and other bodies have been attacking for many years.

An interesting point of view, provoking much discussion, was put forward by Dr. F. J. Wallace, senior research engineer at the Pressed Steel Co., Ltd., Cowley; he suggested that a research worker, especially an engineer, would prefer not to be given too much information early in the start of a new project. He would want to work with a general picture of the problem, and would welcome some basic data, but then needed a period in which to develop his own ideas before again seeking the services of an information department for the provision of detailed literature on specific points which he could then select. In the discussion, it was suggested that the engineer's outlook was, perhaps, always of a more practical and less theoretical nature and, therefore, different from that of, say, a chemist who generally required the full literature in advance of a project. The differences in the information requirements of workers in different subject fields would appear to deserve further investigation.

A paper entitled "Information work in A.D. 2000" was given by Mr. J. Farradane, the honorary secretary of the Institute and Senior Research Fellow (also course organizer) at Northampton College. This was a necessarily fanciful presentation of serious considerations of possible developments of present trends in information science within the next fifty years. He considered in particular the necessity for control of scientific publications by channelling better research work into a limited number of 'official' organs which would be reviewed, abstracted and indexed in large regional information centres; all other literature, especially trade literature, would receive secondary consideration or be ignored for the record. Abstracting would have to give place to reviews of groups of papers at a time. (In discussion, Miss I. M. Slade suggested that special regional applied science and technology centres would also be needed, and could deal with their appropriate literature.) He also suggested that mechanized storage and retrieval would never be satisfactorily performed by present types of computers, which were appropriate for handling data, but not linguistic information, and that machines based on a closer approximation to the working of the human brain would have to be developed. Close liaison between such centres and industrial and other information departments (the latter feeding back information) was also considered important.

Mr. J. S. Rippon (information officer, Metal Box Co., Ltd.) gave a detailed survey of the extent to which information services could relieve research workers of the need to undertake their own literature searches. Although the research worker must clearly undertake adequate personal reading, and make his own decisions, the information department provided an essential service in view of the complexities of the literature problem and could do useful pre-evaluation of the material found. It was important that management, and research and development personnel, should appreciate the full scope of possible services which could be provided by an efficient service.

The problems of foreign literature, and especially those of technical translation and its cost, were considered by Mr. F. Liebesny (intelligence officer, British Aluminium Co., Ltd.). Since literature written in English is now only little more than half the total, with Russian now in second place, the need for skills in reading languages and for accurate translation was rapidly increasing; the exacting qualities required for good work were not common. He suggested that the only satisfactory course, in the long run, was for children to be taught foreign languages as a regular cultural necessity from an early age. The technical complexities were such that it was doubtful whether mechanical translation, even with pre- and post-editing, would ever be an economic possibility, or even feasible.

Finally, Mr. J. Blackwell (B. H. Blackwell Ltd.) and Mr. C. W. Hanson (head of research, Aslib) discussed the need for a new effort in publicizing the value of information work (and the work of the Institute). Mr. Blackwell suggested that the matter should now be put in the hands of professional publicity experts so that the subject could, in particular, be brought convincingly to the attention of management. Mr. Hanson pointed out that, as with research, it was difficult to give evidence of the direct economic value of information services; cases which might show convincing evidence were often related to confidential matters and could thus not be published. Adequate information was necessary in order to keep up to date and abreast of competition. Researches carried out at Aslib had indicated the probable extent of wasted effort due to lack of adequate information in support of research, and this waste was considerable. He therefore supported the policy of increased publicity.

The conference provided a demonstration of the growing importance of information work since the Second World War and the consolidation of the profession of information scientist. It is expected that the full *Proceedings* will be published in the autumn.

J. FARRADANE

NOMENCLATURE OF MULTIPLE ENZYME FORMS

SINCE the original demonstration that some enzymes may exist in a number of different forms in the same species or the same tissue, a considerable literature has accumulated on the topic. There has been no unanimity in these papers on the method of identifying a particular form, as Wieme[1] has pointed out. In a later note, King and Thompson[2] stated that the specific question of the numbering of isoenzymes which have been separated by electrophoresis had been referred to the Standing Committee on Enzymes and the International Commission of Editors of Biochemical Journals. The Enzyme Commission in its report[3] had made no recommendation about multiple enzyme forms, and the International Union of Biochemistry, when it dissolved the Enzyme Commission and set up the Standing Committee on Enzymes[4], also set up a Sub-committee on Isoenzymes composed of the late E. J. King, C. L. Markert, R. J. Wieme, F. Wroblewski and E. C. Webb. After the death of E. J. King, N. O. Kaplan was appointed to the Sub-committee by the Bureau of International Union of Biochemistry. This Sub-committee reached certain decisions which have been approved by the Standing Committee on Enzymes and are set out here.

Multiple enzyme forms may be distinguished from one another by any of several means, for example, electrophoresis, chromatography, salt fractionation, ultracentrifugation, immunochemistry and reaction kinetics. The electrophoretic method has been most commonly used, particularly in clinical laboratories, and numbering systems which have been utilized have usually related to electrophoretic separation. Unfortunately, two quite different systems have been used; it is now recommended that:

"When multiple forms of an enzyme are identified by electrophoretic separation, they should be given consecutive numbers, the form having the highest mobility towards the anode being numbered one".

This system is in conformity with that universally used for the fractions obtained by electrophoresis of serum proteins.

Such numbering systems are probably to be regarded as temporary expedients until information is available about the chemical differences between the various forms. If the molecules of the different forms vary in the nature and arrangements of protein sub-units, a nomenclature should be used analogous to that which has been successfully used in the field of hæmoglobin chemistry.

The Sub-committee also considered the question of a suitable word to be used to describe multiple enzyme forms. Markert and Møller[5] originally proposed "the term *isozyme* to describe the different molecular forms in which proteins may exist with the same enzymatic specificity". Since then the forms *iso-enzyme* or *isoenzyme* have also been widely used, and the term has been limited to multiple forms in a single species. The majority of the Sub-committee felt that the latter forms were preferable as being more logical and in line with such terms as *isotope*.

It is, therefore, recommended that:

"Multiple enzyme forms in a single species should be known as *isoenzymes*, although since either form is readily intelligible this recommendation is not to be interpreted as excluding the use of 'isozyme' if any individual author prefers it".

EDWIN C. WEBB

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[1] Wieme, R. J., *Lancet*, i, 270 (1962).

[2] King, E. J., and Thompson, R. H. S., *Lancet*, i, 589 (1962).

[3] *Rep. Commission on Enzymes of the International Union of Biochemistry* (Pergamon Press, Oxford, 1961).

[4] Thompson, R. H. S., *Nature*, **193**, 1227 (1962).

[5] Markert, C. L., and Møller, F., *Proc. U.S. Nat. Acad. Sci.*, **45**, 753 (1959).

THE MEDICAL RESEARCH COUNCIL

THE latest report of the Medical Research Council* again impresses us, as previous reports have done, by the immense range and outstandingly practical value of the research work supported by the Council and carried out in numerous research centres in Britain and abroad. Established in 1913 as the Medical Research Committee, the Council was created and was granted its Royal Charter in 1920, and in November 1963 it celebrated its fiftieth anniversary at a dinner at the Goldsmiths Hall, at which the guest of honour was the Minister for Science. The Council re-formulated in its report for 1960–61 its concept of the part it should play, that is, "to watch over the whole field of medical and related biological research, so as to foresee the needs and opportunities: to give support to any promising research in these fields irrespective of the agent concerned; to work in partnership with the universities and professions on the one hand, and the various departments of Government on the other, so that new knowledge may be made available as the need arises". Following this aim, the Council has supported research in two main ways: by employing its own staff in its own institutes and research units; and by helping the work of the staffs of other agencies such as universities or hospitals by means of temporary or block research grants or by setting up research groups. Thus, apart from the National Institute for Medical Research at Hampstead, the Council has established 72 research units, of which four are abroad (one each in Gambia and Uganda and two in Jamaica) (see also *Nature*, **200**, 1039; 1963). During 1962–63 the Council set up three new research units: one, now in Cambridge, is investigating abnormal hæmoglobins: another, at Oxford, is investigating the psychological aspects of speech and language; while another, at the Imperial College of Science and Technology, is investigating metabolic reactions in the tissues of higher animals and in micro-organisms. Five other new research groups are devoted to biomechanics, megaloblastic anæmia, respiration and energy metabolism in the new-born, the immunology of certain skin diseases, and genetic problems in orthopædic conditions such as club foot and congenital dislocation of the hip. During the year 1962–63, research workers at the Medical Research Council published 4,730 papers in various scientific journals.

After paying a well-deserved tribute to the Rockefeller Foundation and its work, this report records the value the Council places on its happy relationships with this Foundation and its gratitude for the help given by the Foundation to medical research in the United Kingdom, especially by its establishment of the Rockefeller travelling fellowships between the years 1923 and 1963. Since their foundation, 203 fellowships have been awarded and many of the Fellows have become university officers of high rank. In 1964 the fellowships will be discontinued, but the Council will now award travelling fellowships of its own to replace the Rockefeller awards.

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* Committee of Privy Council for Medical Research, Report of the Medical Research Council for the year 1962–1963. Pp. vii + 280 + 3 plates. (Cmnd. 2382.) (London: H.M.S.O., 1964.) 16*s*. 6*d*. net.

The report refers to the much-publicized subject of "Investigations on Human Subjects", and in this report 4½ pages are devoted to an important statement of the Council's views as to the considerations which should govern medical men who may propose for one reason or another to carry out experiments on human volunteers in order to advance beneficent knowledge. This statement will be heartily welcomed by all medical men and research workers. Important also and of great interest to all medical men and biologists is the section of the report entitled "Some Aspects of Medical Research", which will be published separately together with the Council's statement on responsibility in investigations on human subjects, under the title *Current Medical Research* (H.M.S.O. 4*s*. 6*d*.). As most readers will know, this section has been a feature of previous reports and has been published separately under the same title. This year it discusses the theoretical basis of organ transplantation; chromosome damage in man following exposure to ionizing radiations; clinical genetics and its applications (illustrated by 3 plates); recent studies on the control of ovarian function; microbial drug resistance; the chemotherapy of cancer; protein turnover in the central nervous system; and epidemiological research in psychiatry. All these articles have valuable bibliographies.

The rest of the report is devoted to an extensive summary of all the research work being done under the auspices of the Council, with details of the personnel employed in various capacities and indexes of their names and of the subjects they are working on and of the establishments in which they are working.

Taken as a whole, the report provides a valuable epitome of the main trends of the medical and biological research now being done not only in Britain but also in the tropics and elsewhere. The Council's work is unique. It deserves the widest publicity, for a nobler example could scarcely be found of the devoted co-operation of men and women of all grades of skill and experience for the benefit of their fellows all over the world.

G. LAPAGE

A HALF-CENTURY OF FRUIT RESEARCH

SINCE there have been less than forty half-centuries since the beginning of our present dating era, a jubilee provides a sufficient lapse of time over which to review progress in any field of sustained human effort. That East Malling Research Station came into being before the First World War was a major achievement, for money and effort were then but sparsely invested in the pursuit of new knowledge. The Station's first contribution to the fruit-growing industry was spectacular—the classification of rootstocks to give, for the first time, reliable, regulated production in orchards—and the name of its early director, R. G. (later Sir Ronald) Hatton, is inseparably linked with this. It was almost inevitable that subsequent contributions had to become more detailed—more intensive investigations of smaller parts of the whole problem of fruit production. Papers delivered during the three-day jubilee celebrations therefore give a unique opportunity for reviewing the work at East Malling as a practical whole*.

A true perspective was introduced at the outset in a paper by W. S. Rogers and D. W. Way, who, speaking of strawberry culture, stated bluntly "the need is for cheaper production and greater economy of labour, and the most likely contribution to this, on the cultural side, is in the use of herbicides". This conclusion followed a gratifyingly long-term review of all aspects of culture, for W. S. Rogers has been concerned with the trials for thirty-six years. R. I. C. Holliday spoke of the herbicides, of which simazine has proved the outstanding material so far for soft fruits. The work on rootstocks still continues, and W. S. Rogers discussed the features of Malling-Merton 104, 106, 111 and Malling XXV among others. Trees are also being worked 12 in. above the ground to give a 'built-in' insurance against the soil-borne fungus causing collar rot. Sufficient water is a basic need for any crop, and J. E. Goode demonstrated average increases per acre of 4 cwt. of black currants per in. of water applied as irrigation. D. W. P. Greenham, investigating problems of manuring and soil management, has also linked them with water needs. Dessert apples have not responded greatly to applications of phosphate or potassium, and after being planted for 32 years have only responded to light dressings of nitrogen during the past ten. This work seems to indicate the need for careful timing of the applications of water and nitrogen to give the maximum desired effect. The long-term nature of this work also is particularly valuable. Still on the subject of management, J. F. Wilson showed that proper composting of hop bines and trash after picking by stationary machine destroys the *Verticillium* fungus causing wilt. This is a most welcome adaptation to a modern economic tendency, and provides better facilities for checking the spread of *Verticillium* than those previously possible.

It takes a great deal of detailed research to simplify and improve measures of direct control of fungal and bacterial diseases, but there are three most welcome instances of this. *Botrytis* fruit rot of strawberries is a tantalizing disease of greatly varying severity between localities in the same season, and between seasons in the same locality. M. H. Moore has shown that much infection arises from earlier attack of the fungus during flowering, so a spray of thiram or captan then is far more effective than later. Apple scab and apple mildew are now being controlled by sprays designed to reduce infection in the spring, and *Gloeosporium* diseases in store by reducing infection in the orchard between July and September (J. E. Crosse). Spores of the *Verticillium* wilt fungus of hops are relatively short-lived in the soil (G. W. F. Sewell and J. F. Wilson). They do not attack grasses, thus suggesting measures of control by grassing down for short periods and linking with the control by composting mentioned here. Life-cycle investigations of codling moth and tortrix have also led to more effective timing of sprays against these insects, as described by G. H. L. Dicker. There are now highly efficient insecticides, but their very efficiency brought an increase of fruit-tree red spider mite which requires separate control.

Virus diseases limit fruit production greatly, and A. F. Posnette reviewed the methods of virus control—relative isolation of stocks, the planting of healthy material, the control of aphids and other vectors and the thrilling development of heat therapy. The recognition of leaf-hopper and soil nematode vectors has brought new methods of control, but the older methods of planting only virus-free stocks are still highly effective, for "trees (now ten years old) of our cherry and plum mother tree clones have yielded three times the crop produced by virus-infected trees often used for planting orchards in the past". J. T. Legg showed that degeneration of the hop can be explained by the joint attack of 'nettlehead' and 'line pattern' viruses, but here again, vigour can be maintained by planting only virus-tested stocks supplied by the Station through the Ministry of Agriculture's A-plus scheme.

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* The Kent Incorporated Society for Promoting Experiments in Horticulture. Jubilee Annual Report of the East Malling Research Station, Maidstone, Kent, 1st October, 1962, to 30th September, 1963. Pp. xxx + 208. (Fifty-first year.) (East Malling, Maidstone: East Malling Research Station, 1964.) 17*s*. 6*d*.: 2.50 dollars.

R. V. Harris, in an introductory paper on hop problems, outlined, *inter alia*, the part that could be played in disease control by the breeding of resistant or tolerant varieties. There are always variations within such varieties, but P. W. Talboys concluded that "variation in response to local environmental factors will always tend to be large in varieties of intermediate resistance, but small in very sensitive or very resistant varieties". He also showed that resistance to *Verticillium* wilt depends on the more rapid completion of endodermal suberization, and the production of highly tolerant varieties is thus placed on a sure basis. East Malling has produced new varieties of black currant and a whole range of raspberries; R. L. Knight and Elizabeth Keep discussed patterns for remodelling varieties for resistance to aphids and *Botrytis* fungi, black currant varieties for new strig type and resistance to gall mite, gooseberry for spinelessness and resistance to American gooseberry mildew, and all for high yield and quality. There are also some interesting interspecific crosses.

One might perhaps predict for the future that, as the great practical work of the Station is now to reduce costs for the industry, more assessment will become necessary of the relative expenditure required for the various new operations introduced by research. There is nothing mercenary about this, for as the World wants more food, it wants it without the expenditure of undue human energy, and the scientific measure of such energy is ultimately based on its cash value. In reviewing the sustained work of the Station as a whole I have, unfortunately, omitted mention of twenty-five detailed papers, a general review and four bulletins for fruit growers which indeed occupy the bulk of the report. Apologies to the authors of these, but warmest congratulations on the Jubilee, thus fortified by an acute appreciation of the Station's work. J. GRAINGER

SCIENCE IN THE JUNIOR SCHOOL

IN the initial competition for the Guinness Awards for Science Teachers in Training, held in 1963, the first prize was gained for their joint work by two students, Sheila Stafford and David Horswill, of the City of Leeds Training College. A slightly edited version of their entry has now been published as a special Supplement to *The Science Teacher* (7, No. 7; July 1964). It needs to be appreciated that, at the time when they were producing their essay, the classroom experience of these two students was limited mainly to their periods of school practice and observation of teachers in action, so that they were drawing on their own academic studies, which included among others the writings of Susan Isaacs, J. Piaget, and Cyril Burt.

Following a trend which is gathering strength, they have broken away from the tradition that for young children nature study provides the best introduction to science. Instead, they consider that the aim should be to help boys and girls to gain confidence in scientific methods of enquiry and experiment, and train them to think methodically and logically, so that, within the scope of their abilities, they may gain an understanding of selected aspects of the world around them, in accordance with the needs of society. There should be no fixed syllabus, although, while allowing for flexibility, the course should not lack cohesion; and the driving-power should come from the children's own interests, the teacher sensing "when to follow, when to discuss, explain, teach or guide". In this way they believe the teacher can provide opportunities for progressive observation and experiment which will lead to deeper understanding and the formulation of simple, general laws—as, for example, those relating to elementary magnetism and the reflexion of light.

The essay includes sections on such topics as the help teachers can give in building up concepts, project work, and the value of class discussion; and it ends with an outline syllabus for children of seven to eleven years old. Although somewhat discursive and repetitive, it is a commendable piece of work, well in line with modern developments in the junior school; and it indicates that the scheme of Guinness Awards for Science Teachers in Training is serving a useful cause. It also highlights the need for more attention to be directed, at full research level, to a still greater cause, that of gaining a better understanding of individual attitudes, interests and abilities, in relation to science, throughout the whole of the educational period. R. WEATHERALL

INDUSTRIAL DESIGN

IN a debate in the House of Lords on June 17 Lord Peddie directed attention to the annual report of the Council of Industrial Design and referred to the recent Fielden Report on *Engineering Design*. The latter had expressed the view that Britain's share of international trade in engineering goods had been declining because, in spite of many notable successes, many British products were being outclassed in performance, reliability and sales appeal. Lord Peddie referred to the importance of raising the standards of design. He thought that design in industry was never more important than it is to-day, and he also thought it unfortunate that industrial design was mentally closely identified with art. During the past twenty years the Council of Industrial Design had played a significant part in increasing the awareness that good design was an expression of creative genius and of the importance of the whole subject. But he thought that it was necessary to increase substantially the work of the Council and to extend its activities into designs not at present covered by the Design Centre, capital goods and engineering industries. He thought a closer association would be justified between the Council and the Department of Scientific and Industrial Research. It was encouraging to see that the Royal College of Arts was now recognizing the importance of greater attention to the problem of industrial design, and Lord Peddie also referred to the proposal for a university on design and technology in northern Middlesex.

The Minister of State (Board of Trade), Lord Drumalbyn, referred briefly to the development of industrial design to put the question of design into perspective. He pointed out that the new profession of industrial designing had formally been recognized less than thirty years ago (1936) when the Royal Society of Arts founded the distinction of Royal Designer for Industry, of which there were now 48 in addition to 13 honorary foreign holders of the distinction. The Council of Industrial Design and its Scottish Committee had been formed in 1944 to promote by all practical means the improvement of design in the products of British industry, and in 1956 the Design Centre had been established at 28 Haymarket, London, S.W.1: the

Design Centre in Glasgow was opened in the following year. Small Design Centres with duplicate copies of the *Design Index* had recently been set up in Bristol, Nottingham and Manchester with financial support from local interests. The Council was trying to convince firms that it was in their interest to employ qualified designers and to give them their proper place in the managerial structure. It also sought to encourage a progressive attitude towards design among retailers and their customers and the recognition that the pressure for good design must come largely from the users and from the trade. At the present time the Council was concentrating on seeking to persuade buyers in the services, Government departments, local authorities, hotels, hospitals, offices, etc., to give serious thought to the design of what they bought. Lord Drumalbyn pointed out that a critical and well-informed public taste tone was important if Britain's manufacturers were to have well-designed goods to offer in competitive export markets. The grant to the Council in 1963 was £250,000 and this had been increased to £325,000 in the current year. In its new development of thematic exhibitions to show manufacturers how the industrial designer could be of service in designing such products as aircraft, vehicles, machine tools, scientific instruments, etc., the Council worked in close co-operation with the Federation of British Industries and the Department of Scientific and Industrial Research as well as the British Productivity Council.

Other speakers in the debate mentioned the importance of the education of the designer, particularly those working in the field of industrial design, and Viscount Eccles pointed out that scientific and technical education were proving a most powerful stimulant to better industrial design. Baroness Burton of Coventry thought that industrial design was now being given more careful consideration by many sections of the economy, but suggested that research into consumer needs was much more constructive than collecting complaints about the unsatisfactory products. Baroness Elliot of Harwood asked for some help and encouragement of craftsmen.

In replying on the debate, Lord Drumalbyn said that the point about postgraduate schools was very much in the mind of the Secretary for Education and Science, and suggestions from the Coldstream Council were being considered. He agreed that there was need for greater encouragement and improvement in the status of designers, but thought that more attention was being given to the display of craft goods than Baroness Elliot of Harwood had suggested.

VOLCANIC DUST COLLECTED AT AN ALTITUDE OF 20 KM

By DR. S. C. MOSSOP

C.S.I.R.O. Division of Radiophysics, University Grounds, Sydney

THE eruption of Mt. Agung, Bali (8° 25′ S., 115° 30′ E.), on March 17, 1963, caused the ejection of large quantities of volcanic dust to great heights in the stratosphere. Optical effects have been reported from both the southern hemisphere[1] and the northern[2]. This article deals with the concentration and size distribution of volcanic dust at an altitude of 20 km over a period of a year subsequent to the eruption. These measurements were made using techniques developed for the detection of extra-terrestrial particles, a purpose which the incursion of volcanic dust into the stratosphere effectively frustrated from April 1963 onwards.

Particles were collected at an altitude of 20 km by the use of a 'dust probe' carried by a *U*-2 aircraft of the U.S. Air Force. The collecting surfaces were electron microscope grids covered with carbon-collodion films and mounted on a 3-mm wide metal strip. This was housed in a dust-tight cover from which it could be caused to protrude for the desired time by the aircraft pilot. In this way the electron microscope grids could be exposed normal to the airstream, to collect airborne dust by impaction. Following Ranz and Wong[3], it can be shown that this system collects virtually all particles larger than $0{\cdot}2\mu$ in diameter of density 2 g/cm^3 under the exposure conditions.

Collections were made with varying durations of exposure while the aircraft was on a north-south track at longitude 145° E. The flights fall into two groups which we will designate northerly, between latitudes 15° and 35° S. and southerly, between 40° and 45° S.

The nature of particles collected by this technique prior to the eruption of Mt. Agung has been described elsewhere[4]. Typically they consisted of flattened 'rosettes' of water-soluble material of about 1μ diameter, within which were found denser water-insoluble particles of about one-tenth this diameter. The latter are thought to be mainly of extra-terrestrial origin, and to form nuclei on which the soluble material, ammonium sulphate and persulphate[5], is deposited when produced by gaseous reactions at these altitudes. The flattened, rather than shattered, nature of the sulphate particles indicates that they are not completely dry when collected. Their typical indented appearance in the electron microscope is illustrated in Fig. 1.

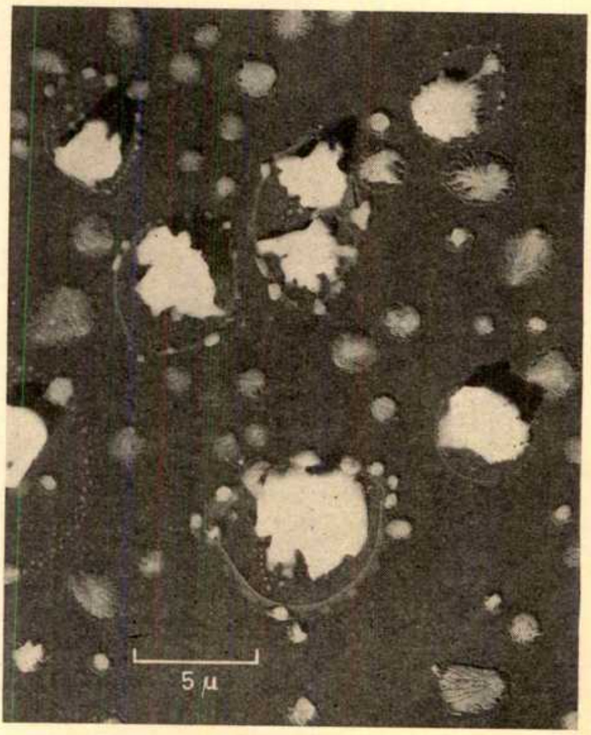

Fig. 1. Particles collected at 20 km between latitudes 44° and 40° S. on May 21, 1963, with metal shadowing. The large particles which cast an appreciable shadow are volcanic particles encased in soluble material, while the smaller flat 'rosettes' are typical of particles present at this level before the incursion of volcanic dust

Table 1. SIZE AND CONCENTRATION OF VOLCANIC PARTICLES AT 20 KM

Date	Elapsed time since eruption (days)	Latitude limits of collection (degrees south latitude)	Concentration (particles per l.)	Sampled volume (l.)	Particle dimensions (μ)		
					Maximum	Minimum	Median
11.4.63	25	15–35	8	17	3·6	0·4	1·1
14.5.63	58	19–32	41	7	3·4	0·2	0·9
28.5.63	72	18–25	160	2·6	2·5	0·2	0·85
11.6.63	86	21–28	145	2·6	2·3	0·1	0·6
9.7.63	114	15–21	150	2·6	4·8	0·1	0·55
6.8.63	142	15–21	140	1·3	2·6	0·1	0·55
12.12.63	270	15–19	107	1·3	1·1	0·1	0·3
2.4.64	382	15–18	32	2·0	0·9	0·1	0·3
23.4.63	37	42–39	0·7	83	6	0·6	2·0
7.5.63	51	44–41	30	7	8	0·2	1·0
21.5.63	65	44–40	7	13	2·5	0·2	1·0
4.6.63	79	45–41	59	2·6	2·8	0·2	0·75
18.6.63	93	45–40	84	2·6	2·4	0·2	0·65
3.7.63	108	40–44	89	2·1	1·7	0·1	0·55
30.7.63	135	41–44	102	2·6	2·0	0·1	0·45
3.12.63	261	43–44	38	1·3	0·8	0·1	0·25
7.4.64	387	41–45	35	2·0	1·0	0·1	0·2

This description applies to collections of particles made during the period January 29–March 14, 1963. Particle concentrations averaged about 30 per litre. Larger concentrations have been reported at this altitude at other times and places[5,6].

Particles of an entirely different appearance were found in a collection made on the following sampling mission on April 11, 1963, between latitudes 15° and 35° S., and in all subsequent collections at 20 km. These particles were insoluble in water, electron-dense, angular, and much larger than the non-soluble particles observed before this date. Their appearance and size, coupled with the fact that their first detection coincided with reports of vivid sunset and sunrise displays moving southward from Mt. Agung, left little doubt that the new particles were in fact volcanic dust.

As in the case of the stratospheric particles previously examined, the volcanic dust particles were covered in water-soluble material as illustrated in Fig. 1. By floating the electron microscope grids on water, specimen side uppermost, this soluble material was removed by dialysis. A typical collection of volcanic particles revealed in this way is shown in Fig. 2.

The concentration of volcanic dust in our collections was obtained by examining electron micrographs at a magnification of 7,000. The diameter of each particle was taken as the average of two dimensions at right angles to one another. In this way the size distributions shown in Figs. 3 and 4 were derived. The swept volumes corresponding to the examined area are shown in Table 1 and range from 1·3 to 83 l. depending on particle concentration. It is of interest to note that 8-μ particles were carried as far south as 41° S., and that 1-μ particles were still present at this level a year after the eruption.

At first there was no difficulty in distinguishing the volcanic dust from other insoluble stratospheric particles. From December 1963 onwards, however, many volcanic dust particles as small as 0·1μ in diameter were present, and at this size the distinguishing criterion of angularity, as against the rounded, nodular shape of non-volcanic particles, was difficult to apply with certainty. These most recent measurements may therefore underestimate

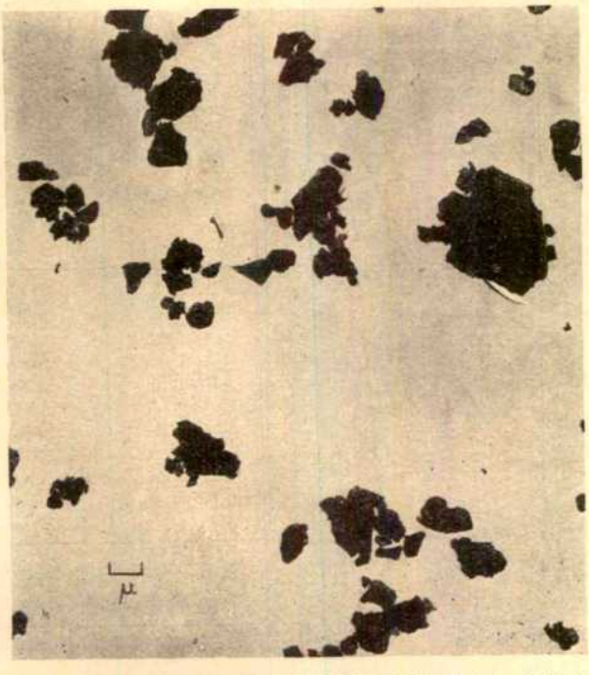

Fig. 2. Volcanic particles collected at 20 km altitude, between latitudes 15° and 21° S. on July 9, 1963. Water-soluble material has been removed by dialysis

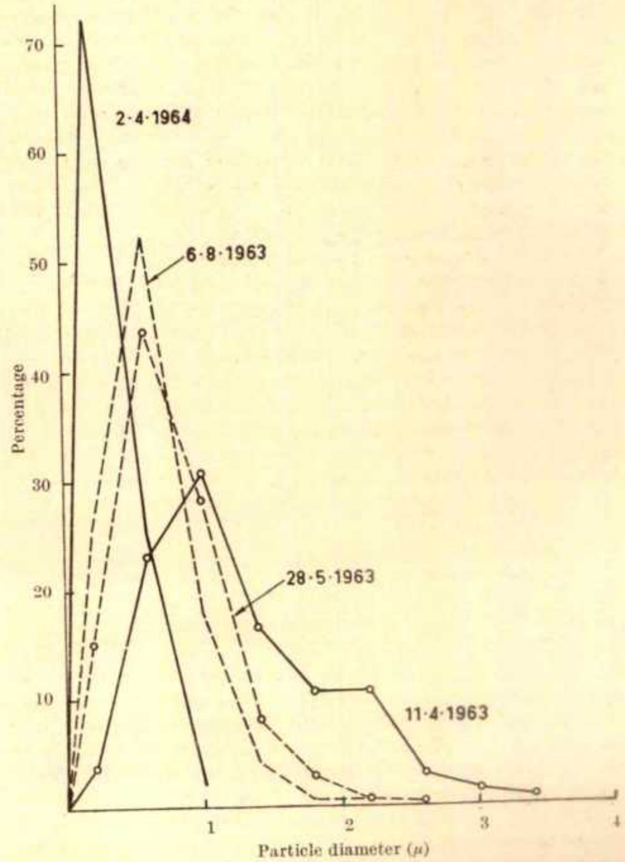

Fig. 3. Size distribution of volcanic dust particles collected at an altitude of 20 km on northerly flights (between 15° and 35° S. latitude), plotted at diameter intervals of 0·4μ

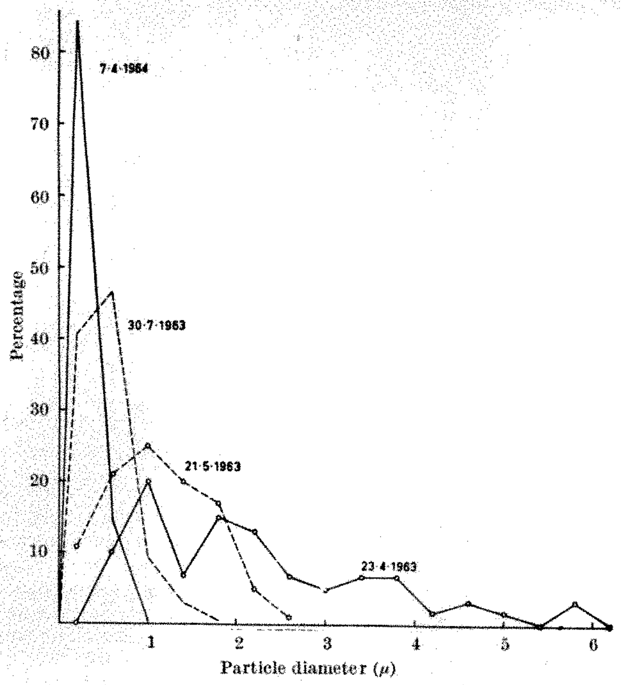

Fig. 4. Size distribution of volcanic dust particles collected at an altitude of 20 km on southerly flights (between 40° and 45° S. latitude), plotted at diameter intervals of 0·4μ

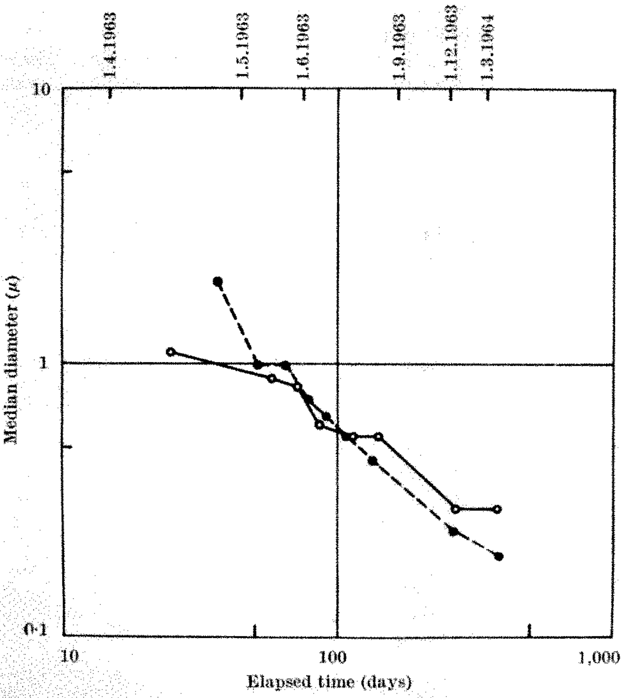

Fig. 5. Change of median diameter of volcanic dust particles with time. Full line indicates data from northerly sampling flights, broken line southerly

the concentration of the smallest volcanic particles and cause corresponding errors in the estimate of median size.

As shown in Fig. 5, the median sizes at the two sampling latitudes were very similar apart from the first southerly collection when only the largest of the southward-diffusing particles had descended to the 20 km level.

Fig. 6 shows that for a period of 80–150 days after the eruption a concentration of about 100 particles per litre prevailed at this altitude between latitudes 20° and 40° S. The mean diameter of the volcanic particles ranged from 0·8 to 0·5μ and these sizes were increased to an unknown extent by the coating of soluble material. The arrival of these high concentrations of dust was marked at Sydney (latitude 34° S.) by the disappearance of the vivid sunset and sunrise colours that had been apparent from April 14, 1963, onwards, presumably owing to the heavy attenuation of all twilight wave-lengths. These phenomena afterwards reappeared and have persisted at Sydney until June 1964 at least.

A careful search was made for spherical particles in 15 of our collections dated April 11–December 3, 1963. The total number of particles examined was of the order of 1,000 in each case. Only on one occasion, May 7, 1963, did the collected particles contain spheres and spherules.

It therefore appears that in the size range below 10μ that we are concerned with, spheres usually constituted less than 0·1 per cent of the total number of volcanic particles. This particular eruption therefore lends no support to the suggestion of Fredricksson and Martin[7] that volcanic sources provide an appreciable proportion of the black spheres and spherules found in polar ice, and in deep-sea sediments.

In the collection of May 7, 1963, there were 82 spheres and spherules, ranging from 0·5 to 5μ diameter, median value 1·7, among the total of 1,050 particles examined. As compared with this, Hodge and Wright[8] find on average that only 1·4 per cent of particles are spherules in the magnetic components of various volcanic dusts. There is therefore strong reason to believe that the spheres collected on May 7 were of extra-terrestrial origin. Their concentration at the sampled level was about two per litre.

It has previously been noted that dust particles in the stratosphere become coated with soluble material. After the incursion of dust from Mt. Agung, the quantity of this coating material seems to have increased and its

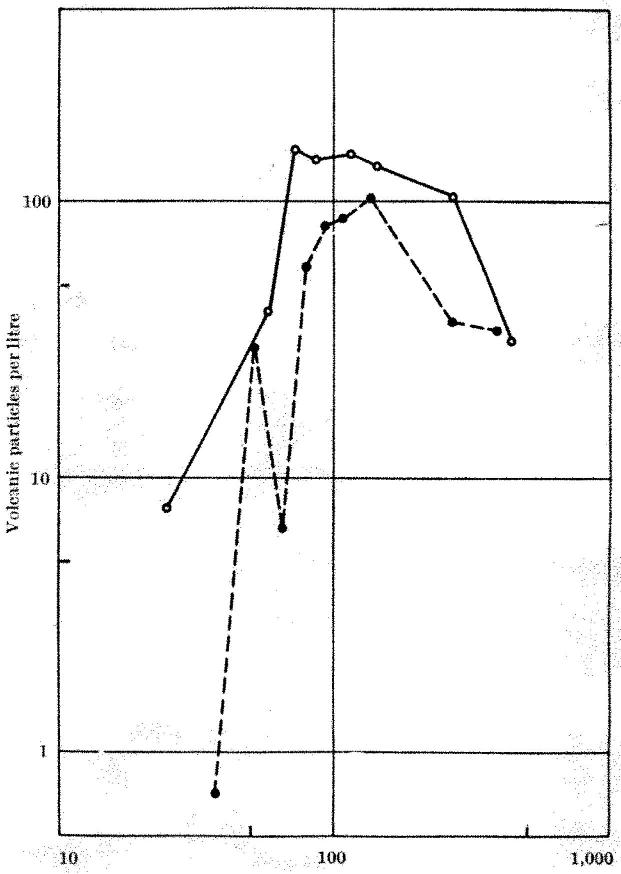

Fig. 6. Change of concentration of volcanic dust particles with time. Full line indicates data from northerly sampling flights, broken line southerly

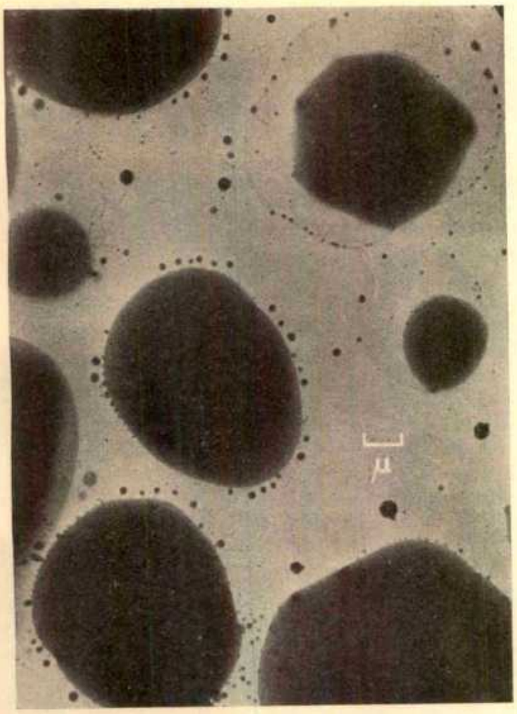

Fig. 7. Fluid particles collected at 20 km on April 7, 1964, between latitudes 41° and 45° S.

nature to have changed. This change was immediately apparent in the northerly samples. The water-soluble material is much more fluid and spreads over the collecting surface, sometimes in a continuous layer. In the case of the southerly collections this change did not become marked until June, and the 'rosette' structure of the dried sulphate particles can still be seen in the collection of May 21 (Fig. 1). In subsequent collections the coating material was much more fluid, as illustrated in Fig. 7.

This fluid material did not evaporate to any marked degree in the electron microscope vacuum but could be driven off by an intense electron beam. It caused blackening and corrosion of exposed parts of the copper electron microscope grids. The material collected on the aircraft windshield is acid to litmus paper and painfully acid to the tongue. More sophisticated tests are in preparation.

The most likely composition of this material is thought to be sulphuric acid. Reports of the Mt. Agung eruption mention the sulphurous fumes present[9] and the high sulphur content of the solid products[10]. The entry of large quantities of sulphur-bearing gases into the stratosphere might cause an excess of sulphur as compared with the ammonia present so that the action of ozone would form sulphuric acid in addition to the usual ammonium sulphates.

I thank the personnel of *O.L* 11, 4080 Strategic Reconaissance Wing, U.S. Air Force, for carrying and operating the particle-sampling apparatus on their aircraft. I also thank the Sydney University Electron Microscope Unit for electron microscopy and in particular Mr. C. D. Shorey for his assistance.

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EVIDENCE FOR ASYMMETRY OF JUPITER'S VAN ALLEN BELT

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MOST of the decimetric radio emission from Jupiter is believed to be synchrotron radiation from the electrons of a Van Allen belt surrounding the planet. Using the C.S.I.R.O. 210-ft. telescope at Parkes, New South Wales, we have recently examined the polarization of this radiation at wave-lengths of 6–100 cm and will submit a detailed report of the observations to *Icarus*. This article discusses two interesting features which suggest that the belt is not symmetrically disposed about the planet.

The Van Allen belt radiation is substantially linearly polarized, and Morris and Berge[1] have shown that as the planet rotates the direction of the polarization rocks through about ± 10°. Their suggested explanation of this effect, namely, a tilt of about 10° between the direction of the magnetic dipole axis and the axis of rotation of the planet, appears to be generally accepted. Several other authors have investigated this effect[2–6], but our observations, in which the linear polarization is completely determined with reasonably good signal/noise ratio at intervals of 15–45 min, serve to define the variation much more clearly and show it to possess a characteristic asymmetry. In addition they demonstrate unambiguously another feature which was inferred indirectly by others[1,5,6], namely, the beaming of the radiation, which manifests itself as a variation of the total intensity as the planet rotates.

Fig. 1 shows the variation of the total intensity as a function of the system III longitude of the central meridian. There are seen to be two maxima and minima per revolution, the minimum near longitude 200° being deeper. The curves are reasonably symmetrical about longitudes 18° and 198°.

To a first approximation this observed variation of intensity may be ascribed to the change, as the planet rotates, of the magnetic latitude of the Earth relative to a magnetic dipole centred on Jupiter. Fig. 2*a* shows the predicted variation of this latitude, when the dipole is assumed to lie in the plane of longitude 18° and 198°, and to be tilted at 10° to the rotational axis. The different depths of the two minima result from the tilt of the North rotational pole of the planet towards the Earth. The resemblance of this curve to the observed total intensity curve (Fig. 1*a*) is striking, and when, as in Fig. 2*b*, the intensities are replotted as a function of magnetic latitude it is seen that to a good approximation they are a function of the magnetic latitude alone. This is the result expected for a centred dipole.

In attempting to relate this observed beaming to the distribution of the pitch angle, α, of the electrons in the belt we have used the published computations of Thorne[7] supplemented by further calculations which he communicated to us privately. The observed beaming and the degree of polarization could be accounted for by the

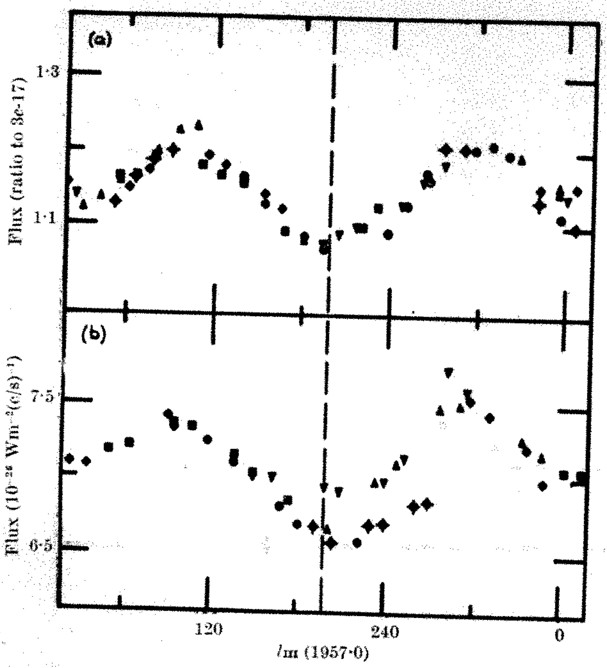

Fig. 1. Variation of total intensity of Jupiter emission with system III longitude. The intensity has been normalized to a polar semi-diameter of 22·75" arc (4.04 A.U.). The significance of the symbols is indicated in the caption to Fig. 3. (*a*) 21 cm, August 1963. (*b*) 11 cm, November 1963

superposition of two distributions of the form $\sin^9 \alpha$, in which one-quarter of the electrons were in very flat helices ($q \approx 25$) while the remainder were nearly isotropically distributed ($q \approx 2$). Such a double-humped distribution might suggest two radiation belts, an idea already proposed for other reasons by Chang and Davis[8] and Warwick[9].

Departures from the centred dipole case are apparent in Fig. 2*b* in the small but systematic displacement of the values for longitudes near 18° (shown by squares) relative to the values for longitudes near 198° (circles). This separation is not removed by changing the assumed tilt of the dipole by a few degrees, and it seems to represent a genuine asymmetry of the source. Clearer evidence for an asymmetry is provided by the variation of the direction of polarization with longitude.

The variation of the direction of polarization with planetary rotation is shown in Fig. 3. It is obvious that the direction does not vary sinusoidally. The curve is distinctly asymmetric: the downward cross-over is steeper than the upward cross-over and crosses the centre line at an earlier phase.

The effect does not appear to be transitory, as the partially incomplete curve for August–September 1962 (Fig. 3*b*) shows the same features as the curve for August 1963 (Fig. 3*a*). This comparison also shows that the rotation period of the magnetic field structure is very close to the system III period determined by the decametric radio bursts. The curves for 1962 and 1963 coincide within the accuracy of the measurements and fix the rotation period as coincident with the I.A.U.[10,11] system III within ±0·5 sec.

Although casual inspection of Fig. 3*c* suggests that the asymmetry is less marked at a wave-length of 11 cm, the differences between the 11 cm and the 21 cm data are, in fact, scarcely significant. Evidently Faraday rotation, which would be four times greater at 21 cm than at 11 cm, is not sensibly affecting the form of Fig. 3*a*.

It is easily shown that if the emitting area were completely symmetrical about an axis through the centre of the planet, but inclined at an angle ~10° to the rotational axis, the resulting variation of the direction of polarization would be a symmetrical curve and very nearly sinusoidal. Hence the observations imply some asymmetry of the belt in longitude. At the upward cross-over at longitude 17° the slope of the curve is much nearer the slope of a sinusoid of amplitude 9° or 10° than at the downward cross-over[12]. This suggests that near longitude 17° the belt may be less affected by the asymmetry. Since the total intensity curves (Fig. 1) show symmetry about a similar longitude (18° and 198°) it seems possible that a basic dipole (inclined at 9° or 10°) intersects the surface at longitude 18° in the southern hemisphere and 198° in the northern hemisphere.

The nature of the asymmetry of the belt is not clear, but it probably reflects some permanent departure of the magnetic field from the centred dipole-configuration. An asymmetrical magnetic field has already been suggested to explain the 360° (rather than 180°) periodicity in the occurrence of the decametric radio bursts[9,13–16]. If Ellis and McCulloch's[16] representation of the field as that of a centred dipole plus a local anomaly is correct, the present observations imply that the effect of the anomaly is still evident at several radii from the planet. On the other hand, Warwick[12] has shown that with a pure dipole model, but with the dipole displaced to the south, shadowing by the planet will produce qualitatively the type of asymmetry observed. Further discussion requires the extension of theoretical developments already begun[7,8,17] to provide two-dimensional distributions of the intensity and polarization for the dipole case (to test the shadowing theory), and alternatively an analysis of the non-dipolar case.

We wish to express our gratitude to the many people who have contributed to the acquisition of the data and their subsequent analysis and discussion. In particular we would like to thank Mr. K. S. Thorne and Dr. J. W. Warwick for making available their unpublished calculations, and Mr. C. S. Higgins and Mr. D. W. Orchiston

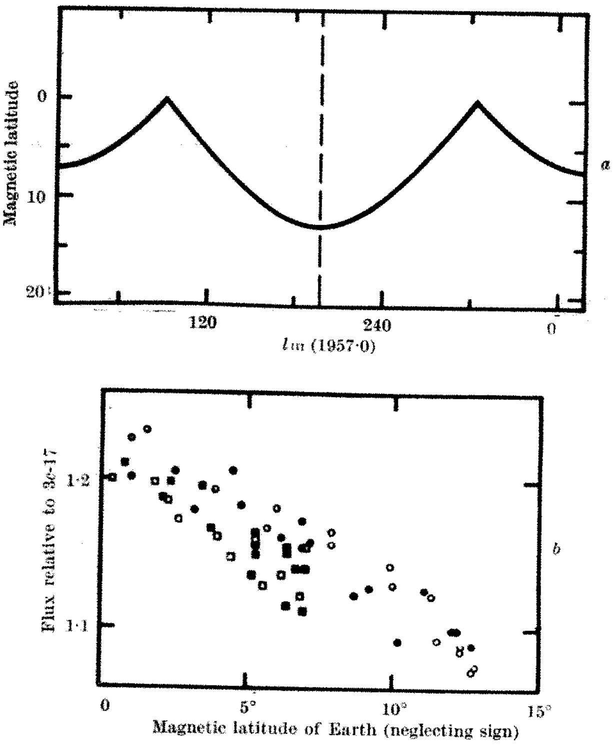

Fig. 2. *a*, The computed magnetic latitude of the Earth with respect to an assumed magnetic dipole inclined at 10° to Jupiter's rotational axis, the pole in the northern hemisphere being at $l_{\text{III}} = 198°$. A 2·9° tilt of the north rotational pole towards the Earth has been included, this being the value appropriate to the 1963 August observations. *b*, Observed total intensity at 21 cm as a function of the magnetic latitude of the Earth. This figure is derived from the data of Figs. 1*a* and 2*a*. Points corresponding to the four longitude ranges in 2*a* are distinguished thus: 18°–92° (□), 92°–198° (○), 198°–304° (●), 304°–18° (■)

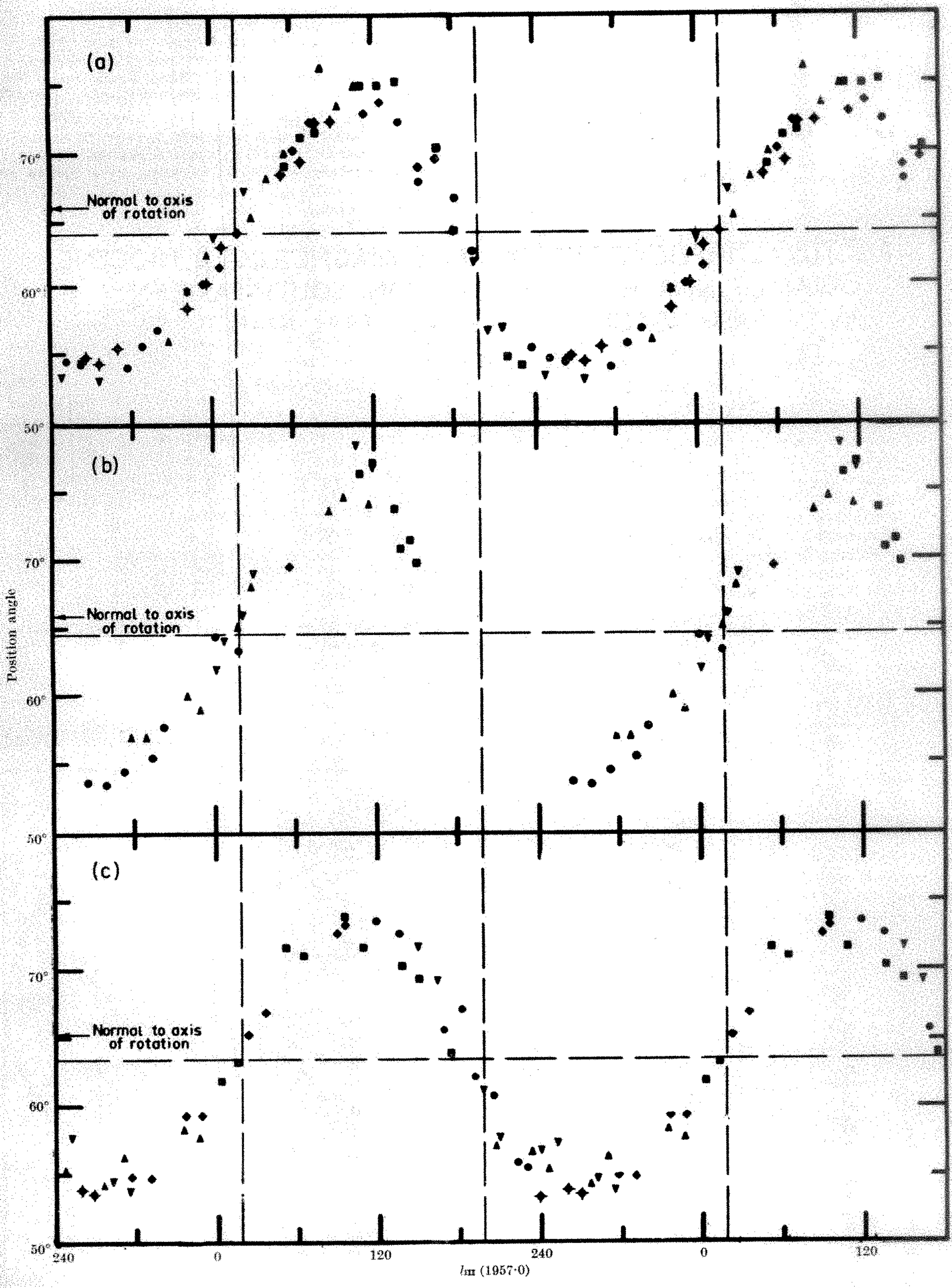

Fig. 3. Position angle of the maximum electric vector of Jupiter radiation as a function of I.A.U. system III longitude. *a*, 21 cm, August 1963; *b*, 21 cm, August/September, 1962; *c*, 11 cm, November, 1963. To assist in the comparison of the shapes and symmetries of these curves and those in Fig. 1, dashed lines are shown (i) at the position angle midway between the maxima and minima of the curves and (ii) at longitudes 18° and 198°, the longitudes of approximate symmetry of the total intensity curves (Fig. 1). The different U.T. observing days are distinguished by different symbols thus: (*a*) 1963 -●- August 2, ■ August 3, ▼ August 4, ◆ August 5, ● August 6, ▲ August 7. (*b*) 1962 ● August 28, ■ August 29, ▼ August 31, ▲ September 24, ◆ September 27. (*c*) 1963 ● November 6, ▲ November 7, ■ November 8, ▼ November 9, ◆ November 10, -●- November 11

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STRUCTURAL IMPLICATIONS OF PALÆOMAGNETISM IN MIOCENE LAVAS OF NORTH-EASTERN OREGON, SOUTH-EASTERN WASHINGTON AND WEST-CENTRAL IDAHO

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A PALÆOMAGNETIC survey of the Miocene lavas of south-eastern Oregon[1] was extended northwards during the summer of 1963. This communication concerns the lavas sampled in seven widely separated sections close to the sharp bend of the suspected gigantic structural feature or orocline, as proposed by Carey[2] (Figs. 1 and 3).

Waters[3] has described the lithologic variation in four of the seven sections, and has divided the Columbia River Basalt into two distinct varieties. In at least one section (Fig. 3, section *G*, Imnaha) both the younger Yakima and older Picture Gorge Basalt outcrop. The type section of the older basalt lies under the Middle to Late Miocene Mascall formation[4], and the Yakima Basalt is intercalated with Middle or late Miocene flora (*ibid.*). A few Early Pliocene lavas[5] may be present at the top of sections *E* and *F*.

More than three hundred accurately orientated cores, representing at least two cores from each lava, have been taken from a total of 154 lavas. It is probable that some of these represent repeated sampling of the same lava in different sections. The sections sampled are the largest local outcrops available, and the nature of the exposures, most of which are along the steep canyons of the Snake River and its tributaries, has in most cases enabled complete sections of successive lavas to be sampled.

Campbell and Runcorn[6] have examined the palæomagnetic properties of the Columbia River lavas, and point out that normally and reversely magnetized lavas occur in approximately equal numbers. Their results showed considerable scatter in direction of natural remanent magnetism. Lack of apparatus for removing unstable components[7] at that time no doubt contributed to this condition. Cox[8] found unusual directions of natural remanent magnetism in Eocene lavas in southern Idaho, and Irving[9] suggested that the anomalous natural remanent magnetism might be a function of tectonic rotation, since the area is still tectonically active.

The magnetic properties of each core have been determined using a spinner magnetometer and a triaxial demagnetizing apparatus[10]. Following experiments in which samples from at least four lavas in each section were subjected to progressive demagnetization in alternating magnetic fields[11], a field of 200 œ was chosen as sufficient to remove unstable components. Application of this field to each specimen resulted in a great reduction of scatter of natural remanent magnetism. Statistical analysis of the mean natural remanent magnetism in each lava has been carried out for all sections, using the methods of Fisher[12], and the results are presented in Table 1.

Table 1. STATISTICAL ANALYSIS OF NATURAL REMANENT MAGNETISM (NRM) IN EACH SECTION

Section	*N*	*D*	*I*	*R*	α95°
A	22	337° 07′	+66° 17′	21·22	6·1
B	10	334° 29′	+66° 20′	9·14	15·7
C	16	339° 08′	+73° 04′	15·08	9·2
D	30	1° 57′	+67° 47′	28·53	6·0
E	18	359° 01′	+54° 04′	17·13	8·0
F	22	0° 05′	+68° 01′	20·92	8·6
G	36	12° 45′	+61° 43′	33·28	6·9

N, No. of lavas in section; *D*, mean declination of NRM; *I*, mean inclination; *R*, resultant vector, assigning unit vector to each lava; α95°, semi-vertical angle of 95 per cent confidence cone. All reversed directions of NRM have been transposed to the northern hemisphere.

The natural remanent magnetism in all cores after removal of viscous components is presented, for two of the seven sections, in Fig. 2. The size of the unstable component in some lavas is large enough to produce pseudo-normally magnetized lavas, which Mumme[13] has described elsewhere. Removal of this unstable component to produce stable reversely magnetized lavas has resulted in the reduction of the number of the successive variations in polarity in most sections. Should

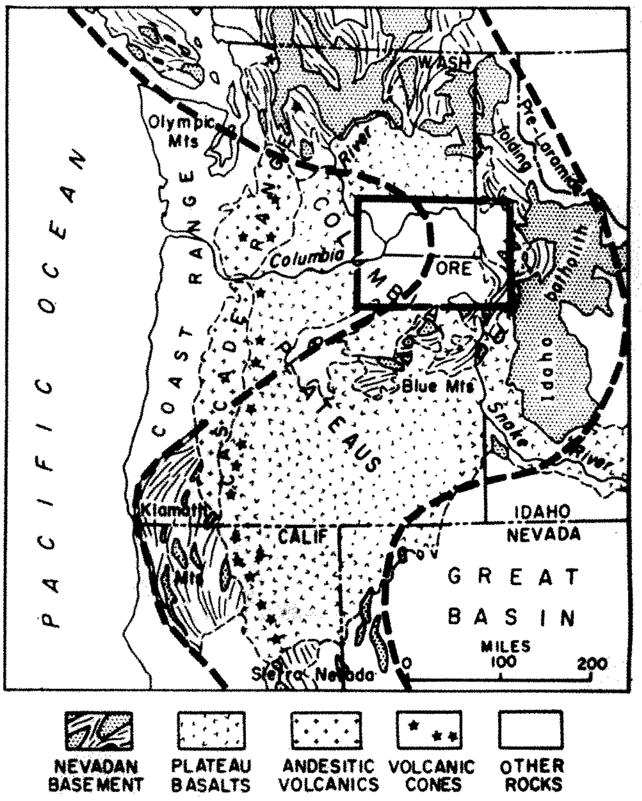

Fig. 1. Map showing relation of area of investigation and the Nevadan Orogenic Belt (or orocline)

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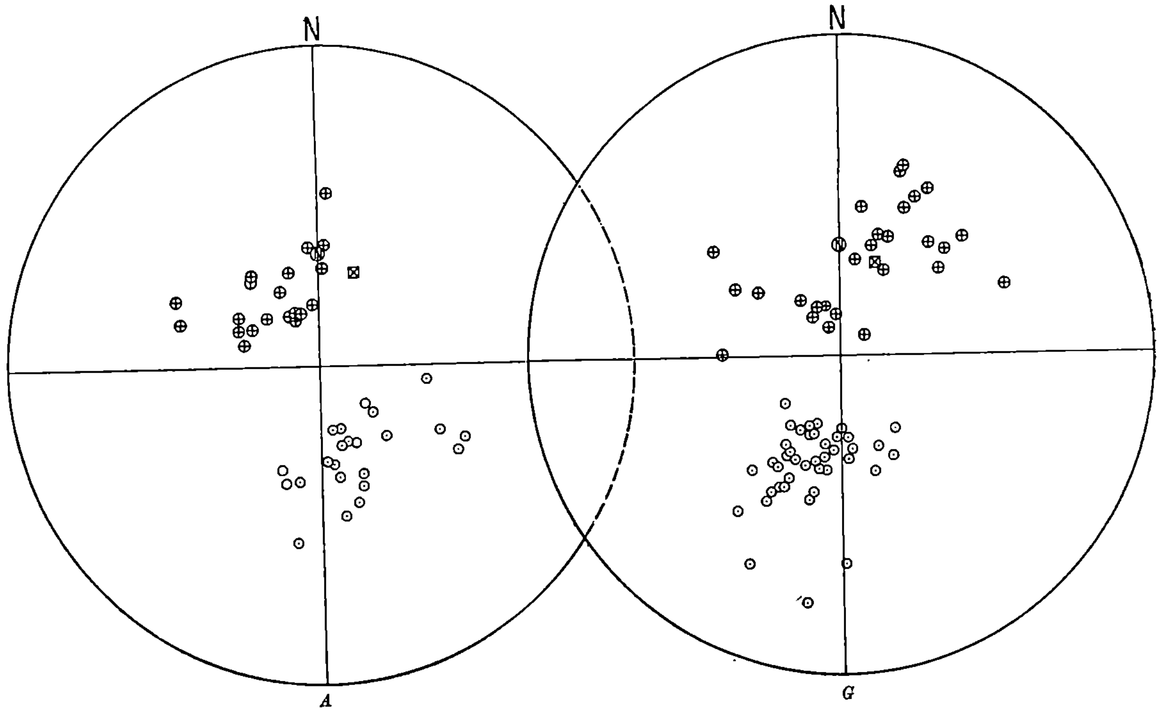

Fig. 2. Remanent magnetism in sections *A* and *G* after treatment in alternating magnetic fields. Left, Snake River; right, Imnaha. ⊕, Lower hemisphere; ○, upper hemisphere; (N), direction of geocentric dipole field; ☒, direction of present geomagnetic field

the surface geologist integrate field measurements of natural remanent magnetism[14] into stratigraphic work in this area, as Einarsson[15] and others have done elsewhere, pseudo-normally magnetized lavas would be encountered. In terms of the implied Miocene geomagnetic field this would be misleading, although there is no reason why such a lava should not be a suitable mapping unit, if the property is homogeneous in the magnetically unstable lava.

All sections feature both polarities. This polarity variation in single sections is one of several properties contrasting with properties revealed in the palæomagnetic survey of Miocene lavas of the Basin and Range area of the Columbia Plateaux to the south[1]. Here eight of ten sections possess only a single polarity in up to forty successive lavas. This difference implies a more rapid rate of extrusion in the southern region, if magnetic polarity epochs encountered are of the same order of duration as those defined in younger rocks[16]. In the present survey, the number of successive polarity variations in each section increases eastward. This may be due to the time-span of sections increasing eastwards.

The most significant result of the survey is illustrated in Fig. 3. The mean palæomagnetic declinations, determined from the mean direction of natural remanent magnetism in all lavas in each section, are plotted at each section location. The sections well to the north of the Oregon–Washington border have declinations differing from those sections to the south. (This fact is very obvious in Fig. 2.) The conditions may be caused by one of the following circumstances :

(1) The sections north and south of the Oregon–Washington border are not contemporaneous. Their different mean palæomagnetic declinations are due to the fact that insufficient time is represented by the sections to average out non-axial dipole components to the spin axis. If the polarity epochs encountered in the sections are of the same order as those defined for more recent rocks by Cox, Doell and Dalrymple[16], then the time represented would be at least five million years. The duration of the persistence of non-axial dipole conditions is fundamental in the palæomagnetic model, particularly in the application of data to inter-continental studies. The time-span required to average non-axial components to the spin axis has previously been suggested to range from ten thousand years[17] up to one million years[18].

(2) The sections north of the Oregon–Washington border are not contemporaneous with those to the south. Sufficient time is represented in the sections to average non-axial dipole components to the spin axis, but the spin axis moved considerably in Miocene items.

(3) The sections involved are roughly contemporaneous. The spin axis did not move considerably during the Miocene period, and the different declinations are the result of post-Miocene differential tectonic rotation, in the same sense as the orocline indicated in Fig. 1. Similar explanations of non-coincident palæomagnetic data are common in the literature. Wise[19] has summarized the diverse geological, geophysical and geochemical data which support a tectonic origin for the broad features of this part of the Cordillera. He does, however, point out factors contradicting the implied sense of rotation. Recently, Bodvarsson and Walker[20] have presented evidence for crustal drift in Iceland, and suggest that consideration be given to similar phenomena in the other great flood basalt areas. The enormous dyke swarms

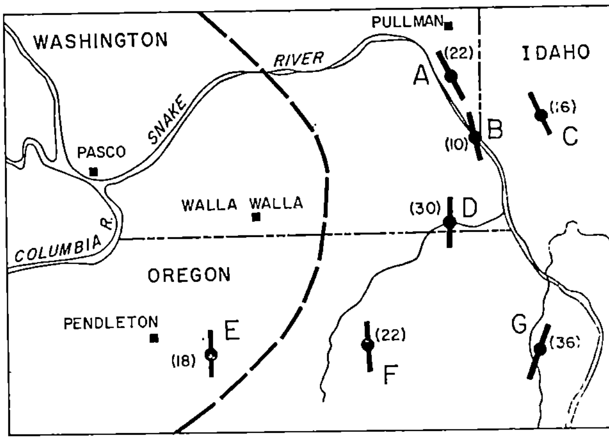

Fig. 3. Mean palæomagnetic declinations in seven sections of Miocene lavas in South-eastern Washington, North-eastern Oregon and West-central Idaho. No. in brackets is No. of lavas in each section

at Grande Ronde (in the area of section *D*) and Cornucopia (south of section *G*), described briefly by Waters[3], complement the ideas of Bodvarsson and Walker[20], who state that large-scale intrusion of dykes is a significant factor in crustal spreading in the great Icelandic lava pile. It can be stated that the palæomagnetic data are consistent with crustal spreading of the part of the Columbia Plateaux under consideration, but by no means are independent proof of the event.

Since it has been shown[1] that the Miocene geomagnetic field may be subject to apparently sudden movement, and due to the uncertainty of the time required to average non-axial dipole components to the spin axis, the possibility that ambient geomagnetic field variation may be the cause of the declination variations described in this communication must be stressed.

The ambiguity in interpretation of the observations should be resolved to a large extent by further collection in north-central Washington, and by dating of the lavas concerned in order to determine the degree of contemporaneity.

I thank Drs. A. Cox and R. Doell of the U.S. Geological Survey for the loan of equipment, and Prof. A. C. Waters of the University of California, Santa Barbara, for guidance in selection of the sections. This work was carried under a National Science Foundation administered U.S.–Japan Co-operation Programme grant to Stanford University.

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RHEID AND RIGID ROTATIONS

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IN rocks deformed by rheid folding, rotational phenomena displayed by componental segregates appear to allow contradictory kinematic interpretations where the accepted sense of individual rotation cannot readily be reconciled with the proposed mechanism of environmental shearing. The subject of rheid deformation by solid laminar flow, as developed by Carey[1], provides idealized concepts that are readily susceptible of analysis through the use of laminar models where planar translations by rotational and irrotational shear are conceived. Such models as have been described[2-7] represent ideal rheid deformations in homogeneous isotropic material in terms of direct shear movement.

To facilitate geological field investigations of flow phenomena, specifically those related to the effects of rotation and stretching of dissimilar bodies embedded in a strained rheid matrix, I have recently used a combination plasti-laminar model in which to observe the behaviour of inhomogeneous material aggregates enclosed in a rheid medium that deforms by plane laminar flow resulting from experimentally controlled progressive shear deformation. These aggregates include the idealized extremes of both rigid and rheid bodies as well as intermediate anisotropic bodies that, by virtue of preferred internal slip surfaces, behave both as rigid and rheid bodies according to variations of internal shear induced in them during the controlled progress of matrix deformation. The examination of these 'rheoplex' models has disclosed some curious and seemingly paradoxical rotational and orientational qualities that are interdependent and consistent with the one superimposed deformation, although their counterparts in the field might otherwise be regarded as cogenetically incompatible.

The essential feature of the models is a rheid matrix of silicone putty (*P*, Fig. 1), which is uniformly rabbeted in the upper edge-surface (*L*, Fig. 1) of a standard laminar model. This attachment provides a direct coupling whereby transcurrent movement of the 'basement' is reproduced directly in the rheid matrix by plane laminar flow without producing visible shear planes. Within this matrix are set small bodies of various shapes and orientations. In the accompanying illustrations, rigid bodies, marked *G*, remain undeformed but free to rotate in the matrix. Rheid bodies, marked *H*, are represented by shapes engraved in the matrix, and these may be regarded as bodies of the same rheidity as the matrix and deforming with it by internal laminar flow parallel to the superimposed shear according to a direct function of the strain ellipse. Anisotropic bodies, marked *N*, are small hand-set laminar prisms, free to deform by internal laminal slip (or induced shear) which may be variously divergent from the superimposed direct shear, depending

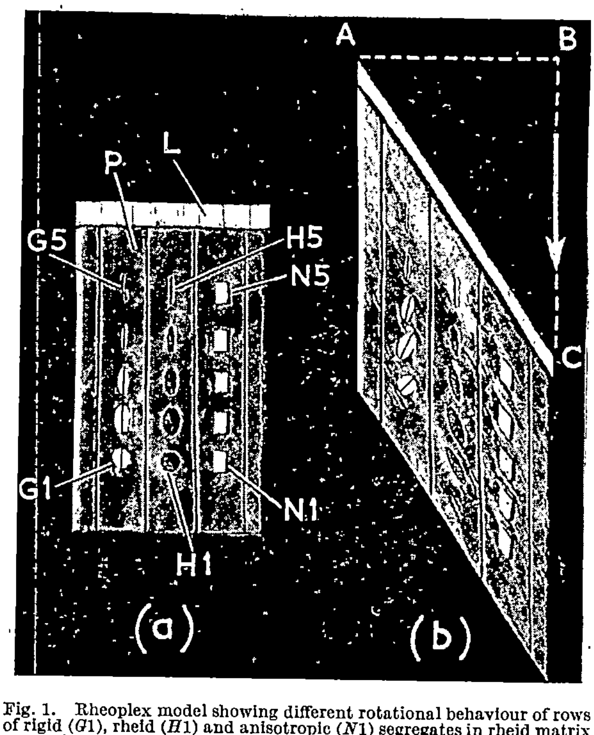

Fig. 1. Rheoplex model showing different rotational behaviour of rows of rigid (*G*1), rheid (*H*1) and anisotropic (*N*1) segregates in rheid matrix deformed by laminar flow imposed by shear parallel to rows

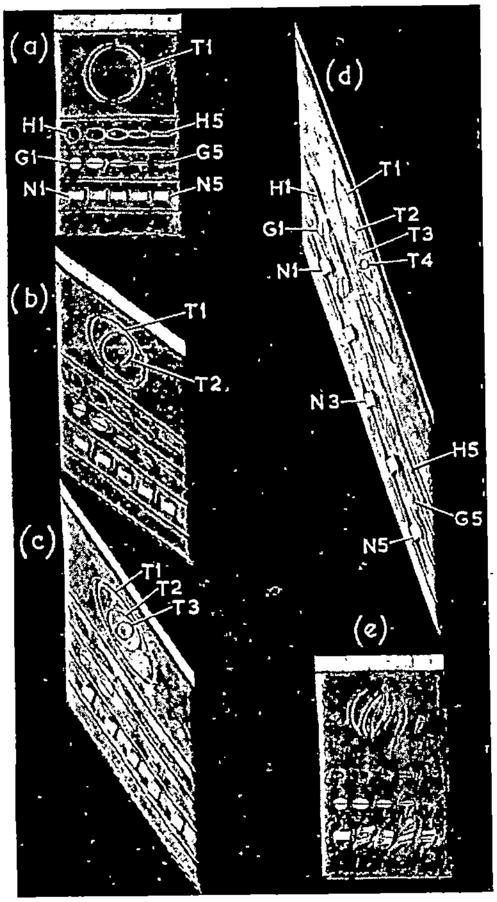

Fig. 2. Similar model to Fig. 1, except that imposed shear is perpendicular to rows of segregates. *e* shows attempted restoration by 'unshearing'

on the initial orientations of the bodies. The anisotropic *N*-bodies may be regarded as combining the behaviour of the rigid *G*-bodies and the rheid *H*-bodies depending on the divergence between the direct and the induced shear planes. By adaptation of the terms described by Wood[8], the *G*-, *H*- and *N*-bodies are called segregates, and the long axis of each segregate is called the segregate axis. Groups of segregates are called aggregates, and the long axis of distribution of such a group is called the aggregate axis.

Fig. 1*a* shows a rheoplex model containing three parallel rows of segregates embedded in a rheid matrix, *P*. The left-hand row of rigid bodies, marked *G*, contains five segregates ranging from circular (*G*1) to linear (*G*5) shape. The segregate axes of these bodies coincide with their aggregate axis, which, in turn, is parallel to the shear planes in the laminar basement, *L*. The centre row of five rheid segregates, *H*1–*H*5, has shapes and alignments identical with those of the corresponding *G*-segregates by which their respective deformational behaviour may be the more easily compared. The right-hand row contains five rectangular laminated anisotropic segregates, *N*1–*N*5, which have their segregate axes, their aggregate axis and their internal laminæ all parallel to the shear direction of the basement.

Fig. 1*b* shows the effect of a uniform superimposed right lateral shear (tan *BAC*) applied to the basement, *L*, and transmitted to the rheid matrix, *P*. It is seen that the rigid *G*-segregates rotate in a right lateral sense in sympathy with the superimposed shear, whereas the rheid *H*-segregates rotate in the opposite sense, the amount of rotation of a particular segregate in each case depending on its formal aspect ratio (length/breadth). The *N*-segregates behave in a composite manner. They deform internally by right lateral induced shear, and also rotate slightly in a right lateral sense so that their internal laminal directions assume a left-hand *en échelon* alignment along the aggregate axis. This pattern is to be compared with the left-hand *en échelon* of the rigid *G*-segregates. However, the formal long axes of the *N*-segregates now assume new diagonal positions in right-hand *en échelon* alignment, which is to be compared with the behaviour of the rheid *H*-segregates.

In the foregoing terms the remaining illustrations are largely self-explanatory. Fig. 2 shows an example where the *G*-, *H*- and *N*-segregates have their aggregate axes initially perpendicular to the superimposed shear direction. At each stage of deformation by right-hand rotational shear, a time-circle, *T*, is added to the matrix to provide a progressive deformation history, as shown by *T*1, *T*2, *T*3 and *T*4 in Fig. 2*d*. Figs. 2*a*, *b*, *c* and *d* may be compared with the right limb of a shear fold anticline during progressive deformation in which the rows of segregates define the strata, and the segregate and aggregate axes coincide initially with the bedding planes. It will be seen that for a considerable part of the deformation, the rigid *G*-segregates rotate in a left-hand sense relative to their bed, that is "in a sense just opposite of that assumed in shear folding"[9]. In the extreme deformation of Fig. 2*d*, this sense is eventually reversed. The rheid *H*-segregates behave in a contrary manner except that their sense of rotation relative to their bed does not suffer a reversal in Fig. 2*d*. Instead, the *H*-segregates are stretched to more than three times their original lengths in the plane of the bedding. It may be noted that the distance between segregates increases progressively, in contrast to Fig. 1 where the initial spacing is largely maintained during 'bedding slip'. In Figs. 2*b* and *c*, the *N*-segregates display a composite behaviour. The shear induced in them initially as left-hand internal slip in Fig. 2*c* has afterwards suffered a reversal in Fig. 2*d* in which the segregates have been restored by internal 'unshearing' to nearly their original shapes. Fig. 2*e* shows the restoration of the entire model matrix to its original shape by complete reversal of the superimposed direct shear. Both the *G*- and *H*-segregates are restored with considerable fidelity, but the *N*-segregates have undergone irreversible changes. It may also be noted in Fig. 2*e* that the matrix surface has developed a set of strain striations which supersede those in Fig. 2*c*. To the extent that these striæ or 'folds' could become fixed during deformation, a cross-fold relationship

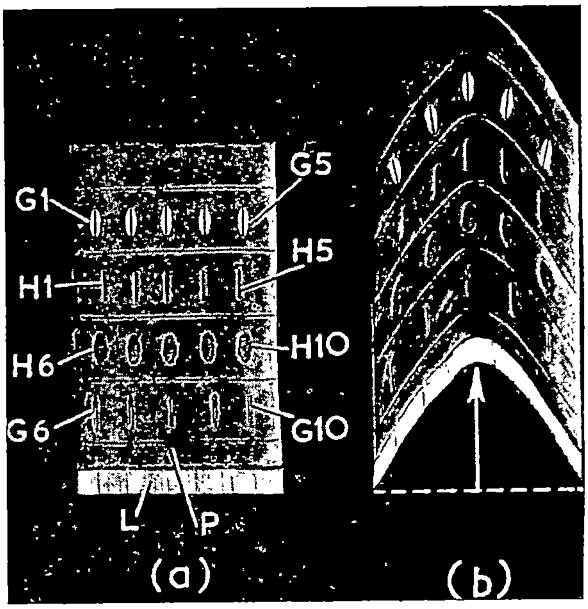

Fig. 3. Rheoplex model of anticline in which shear folding produces respectively divergent, parallel and convergent orientations in successive bands of particular segregates

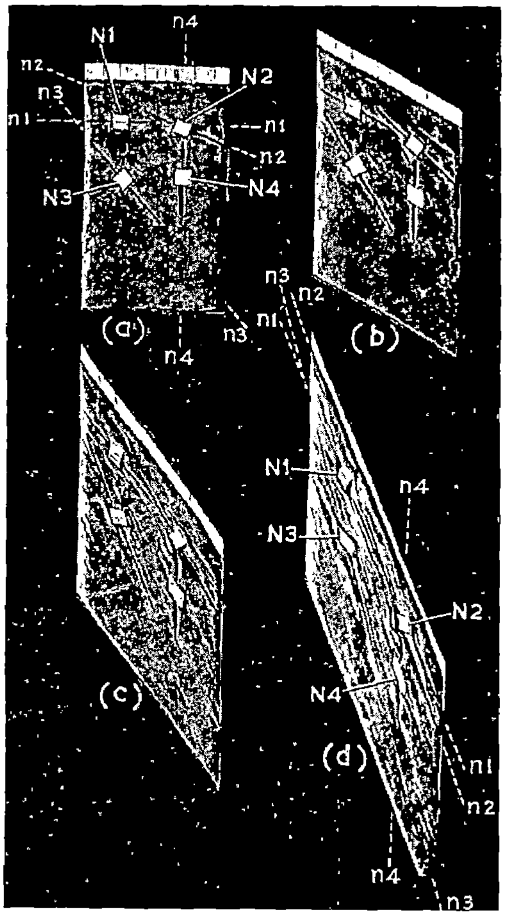

Fig. 4. Four identical anisotropic segregates deform differently according to their initial orientations in rheid matrix deformed by laminar flow

is established by the reversal in sense of the superimposed shear.

Fig. 3 illustrates the case where both the segregate axes and the superimposed shear are perpendicular to the aggregate axes. In Fig. 3*a*, the aggregate axes, *G*1–*G*5 and *G*6–*G*10, which may be equated to stratal bedding, are the locus of rigid segregates respectively of low and high aspect ratio, while *H*6–*H*10 and *H*1–*H*5 represent the corresponding sets of rheid segregates. Fig. 3*b* shows the effect of superimposing an anticlinal fold by axial plane shear. The segregates of high aspect ratio, *H*1–*H*5 and *G*6–*G*10, resist rotation and remain in the axial (shear) plane. Of those of lower aspect ratio, the *G*1–*G*5 segregates assume orientations corresponding to slaty[10] cleavage divergent toward the anticlinal crest, while the *H*6–*H*10 segregates assume orientations convergent toward the anticlinal crest after the manner of fracture[10] cleavage. This model suggests an analogy to the attitude of fold cleavage where it is determined by the orientation of mineral segregates, varying with the strata, which have originated as mimetic growths in early axial plane cleavage, and thereafter responded unequally and differentially to continuing deformation. In this context, three accepted types of cleavage orientation may be causally united under the one shear fold system.

Fig. 4 illustrates the different behaviour of four similar *N*-segregates according to their initial orientation with respect to the superimposed shear. The axes *n*1, *n*2, etc., are specified as the respective aggregate axes of the individual segregates, corresponding to their internal laminal direction before deformation. When right lateral shear is imposed, the subsequent internal deformation of the segregates is alternatively by right-hand or left-hand slip, or, as in *N*2, by both, successively, according to the stage of movement. In the advanced stage of this type of shear deformation, as in Fig. 4*d*, all formal elongations and all aggregate axes except those in the shear plane (for example, *n*4) tend to become parallel to the gross strain elongation.

Fig. 5 illustrates how the one uniform shear may appear to produce contrary rotations in similar *G*-segregates according to the initial angle of shear with the bedding planes (aggregate axes). Thus, *G*3 and *G*4 (Fig. 5*a*) rotate anticlockwise with respect to their aggregate axis (Fig. 5*b*), whereas *G*7 and *G*8 do the opposite. Of particular interest is the 'rigid area' divided in two parts (*G*9 and *G*10) by a line of scission perpendicular to the superimposed shear direction. It may be seen in Figs. 5*a* and *b* that although the 'basement' shearing is in a left lateral sense, not only do the rigid areas, *G*9 and *G*10, move in a right lateral sense with respect to one another, but the 'fault' separating them also rotates relatively in the same sense. In this context, the 'sense of rotation' is a relative matter depending on the interaction of rigid and rheid qualities which vary according to initial orientations as well as to the stage of deformation.

It is of interest to consider a composite segregate that consists of a rigid nucleus with a rheid periphery (as if *G*1 were inside *H*1 in Fig. 2), or one that grows by progressive syntectonic accretion, especially if the accretion is polarized along the infinitesimal strain axis. With appropriate orientation, a superimposed clockwise shear can cause such a segregate to assume a formal '*S*-structure' even though the shear rotates both the segregate and its environment in a clockwise sense with respect to a fixed direction. This is of importance when recalling that an *S*-structure in a mineral segregate is accepted as indicating anticlockwise rotation[11]. On a global scale it is also of interest to extend the implications of Fig. 5 to the conclusion that sub-crustal rheid flow in a given direction may induce fault movements in crustal blocks that may appear to contradict it in both direction and rotational sense.

The role of model presentations must be kept in proper perspective and their limitations appreciated. As Suppes[12] claims, a model drastically simplifies the true physical phenomena and describes only certain aspects of them. In this way, the idealized extremes shown in rheoplex models may be regarded as qualitative components effective in varying degrees in an ultimate deformation which is variously a compromise between rigidity and rheidity. The separate representation of these properties is intended to help the elucidation of what Simpson[13]

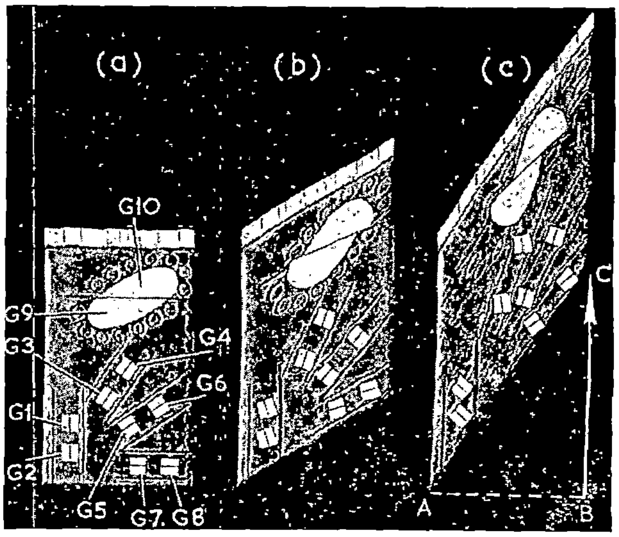

Fig. 5. Left lateral shear of rheid matrix induces contrary rotational effects in various rigid bodies disposed within it

distinguishes as "immanent" qualities, that characterize particular relationships, from "configurational" qualities, that are related to particular materials.

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RECOMBINATION OF CHLORINE ATOMS

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THE recombination of chlorine atoms has been the subject of two recent communications[1,2], Linnett and Booth[1] having obtained a value for the overall termolecular rate constant which is approximately two orders of magnitude less than that reported by Bader and Ogryzlo[2].

Both authors used the isothermal calorimetric technique[3] to measure the decay of the atom concentration. This method is known to give results of dubious reliability in the presence of excited species, and it is sometimes found that only a fraction of the energy of the atom recombination is accommodated on the catalytic wire[4]. We have used an alternative technique to monitor the atom concentration, and have therefore measured our rate constants by an independent method.

The atoms were produced in a flow system by a radio-frequency discharge through pure chlorine gas. The reaction cell was of 2·9 cm internal diameter and the walls were 'poisoned' with sulphuric acid[5].

Downstream from the point of injection of the chlorine atoms a multiperforated jet permitted the addition of nitrosyl chloride gas, which was used to estimate the absolute value of the atom concentrations by means of the fast reaction:

$$\mathrm{Cl} + \mathrm{NOCl} \rightarrow \mathrm{Cl_2} + \mathrm{NO}$$

Chlorine was obtained from Imperial Chemical Industries, Ltd., and quoted as 99·99 per cent pure. The nitrosyl chloride was prepared by the reaction of nitric oxide with molecular chlorine and was purified by fractional vaporization. Pressures were measured on a McLeod gauge filled with sulphuric acid, which was standardized against a conventional mercury McLeod gauge.

For each experiment the absolute value of the chlorine atom concentration was determined at one position in the reaction tube by titration with nitrosyl chloride, and the relative concentrations of the atoms along the remainder of the tube were obtained by measurement of the intensity (I) of the red glow emitted from the recombining gas.

The dependence of I on the chlorine atom concentration was determined[6], and hence absolute values for its concentration obtained at several positions along the tube.

If the recombination mechanism is expressed[1,2]:

$$\mathrm{Cl} + \mathrm{Cl} + \mathrm{Cl_2} \xrightarrow{k_{\mathrm{Cl}_2}} \mathrm{Cl_2} + \mathrm{Cl_2}$$

$$\mathrm{Cl} + \mathrm{wall} \xrightarrow{k_w} \tfrac{1}{2}\,\mathrm{Cl_2}$$

then

$$\frac{-\mathrm{d[Cl]}}{\mathrm{d}t} = 2\,k_{\mathrm{Cl}_2}\,[\mathrm{Cl}]^2\,[\mathrm{Cl_2}] + k_w\,[\mathrm{Cl}] \qquad (1)$$

From low-pressure experiments at low chlorine atom concentration a value for k_w of 0·70 sec^{-1} was obtained corresponding to a wall recombination efficiency (γ) = $3{\cdot}5 \times 10^{-5}$, which is in good agreement with previous work[2].

k_{Cl_2} was determined over a range of pressure and chlorine atom concentrations (Fig. 1); our results are approximately half those obtained by Bader and Ogryzlo, and k_{Cl_2} decreases with increase in chlorine atom concentration. Bader and Ogryzlo's results, which are plotted for comparison on the same graph, show the same general trend.

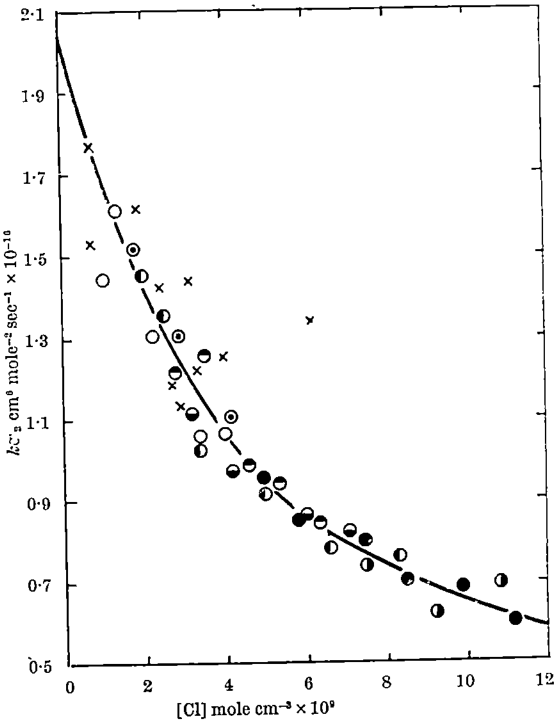

Fig. 1. Experimental values of k_{Cl_2} at the pressures (torr): ⊙, 2·03; ○, 1·65; ◐, 1·21; ⊖, 0·91; ⊖, 0·66; ◑, 0·58; ●, 0·33; ×, values obtained by Bader and Ogryzlo (divided by 2 for comparison)

Such a decrease is not in accordance with equation (1), which must therefore represent an over-simplified presentation of the reaction.

A more detailed mechanism for the removal of the chlorine atoms is:

$$\mathrm{Cl} + \mathrm{Cl_2} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Cl_3}$$

$$\mathrm{Cl_3} + \mathrm{Cl} \xrightarrow{k_2} \mathrm{Cl_2} + \mathrm{Cl_2}$$

$$\mathrm{Cl} + \mathrm{wall} \xrightarrow{k_w} \tfrac{1}{2}\,\mathrm{Cl_2}$$

Hence:

$$\frac{-\mathrm{d[Cl]}}{\mathrm{d}t} = \frac{2\,k_1\,k_2[\mathrm{Cl}]^2\,[\mathrm{Cl_2}]}{k_{-1} + k_2[\mathrm{Cl}]} + k_w[\mathrm{Cl}] \qquad (2)$$

From equations (1) and (2) the measured overall termolecular rate constant (k_{Cl_2}) is given by:

$$k_{\mathrm{Cl}_2} = \frac{k_1\,k_2}{k_{-1} + k_2\,[\mathrm{Cl}]}$$

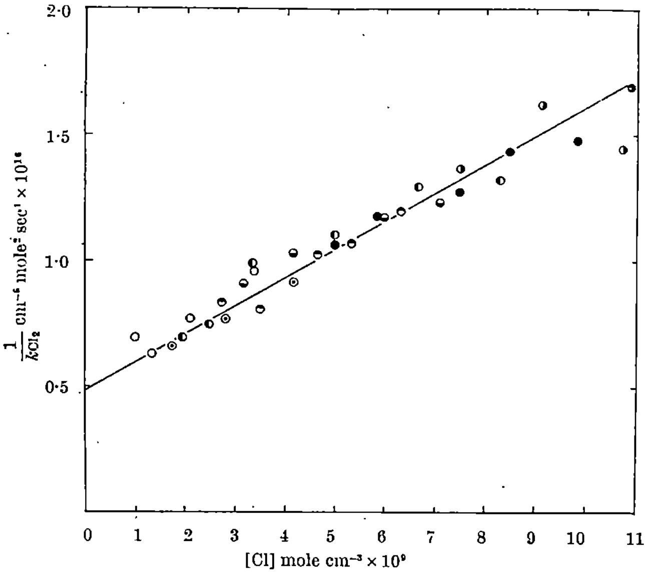

Fig. 2. Dependence of the reciprocal of the termolecular rate 'constant' on the atomic chlorine concentration

or:

$$\frac{1}{k_{Cl_2}} = \frac{[Cl]}{k_1} + \frac{k_{-1}}{k_1 k_2}$$

At very low concentrations where $k_{-1} \gg k_2$ [Cl], then $(k_{Cl_2})_{[Cl]\to 0} = \frac{k_1 k_2}{k_{-1}}$. $\frac{1}{k_{Cl_2}}$ is plotted against [Cl] in Fig. 2. The slope of the line gives $\frac{1}{k_1}$ and from the intercept we obtain $\frac{k_{-1}}{k_1 k_2}$.

From Fig. 2:

$$k_1 = 9{\cdot}09 \times 10^7 \text{ cm}^3 \text{ mole}^{-1} \text{ sec}^{-1}$$

and

$$\frac{k_1 k_2}{k_{-1}} = 2{\cdot}04 \times 10^{16} \text{ cm}^6 \text{ mole}^{-2} \text{ sec}^{-1}$$

hence

$$\frac{k_2}{k_{-1}} = 2{\cdot}24 \times 10^8 \text{ cm}^3 \text{ mole}^{-1}$$

If we assume that the reaction of Cl and Cl_3 takes place with unit collisional efficiency, then we obtain an upper limit for k_2 of $1{\cdot}7 \times 10^{14}$ cm^3 mole^{-1} sec^{-1} and, by substitution, $k_{-1} \leqslant 7{\cdot}6 \times 10^5$ sec^{-1}.

At low atom concentrations:

$$\frac{[Cl_3]}{[Cl]} = \frac{k_1 [Cl_2]}{k_{-1}}$$

and at a pressure of 2 torr:

$$[Cl_3] \geqslant 1{\cdot}2 \times 10^{-5} [Cl]$$

The value of the termolecular rate constant obtained for chlorine atom recombination at very low atom concentrations ($2{\cdot}04 \times 10^{16}$ cm^6 mole^{-2} sec^{-1}) is in fairly good agreement with the value of 4×10^{16} cm^6 mole^{-2} sec^{-1} obtained by extrapolating Bader and Ogryzlo's data from experiments carried out in pure chlorine, and is in good agreement with their data from indirect determinations in the presence of inert gases.

A full account of this work is to be published in detail elsewhere. I thank Mr. H. Williams for helpful discussion and criticism.

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QUANTITATIVE DETERMINATION OF PROGESTERONE IN HUMAN PLASMA BY THIN-LAYER AND GAS-LIQUID RADIOCHROMATOGRAPHY

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IN 1954, progesterone was isolated from human peripheral venous blood and the concentration determined in samples collected during late pregnancy[1]. Later, although several methods were developed for the determination of the hormone in plasma samples of reasonable volume[2-4], it was apparent that a considerable increase in sensitivity would be required for the determination of plasma concentrations in early pregnancy and the menstrual cycle. For this purpose and to achieve a higher degree of specificity, the method developed in this department[2,4] which involved alumina chromatography and the formation of the isonicotinic acid hydrazone, was modified by the introduction of preliminary purification on silica gel, followed by paper chromatography and the formation of the thiosemicarbazone. There was a ten-fold increase in sensitivity and, using an internal standard of progesterone-4-^{14}C, the method was suitable for the assay of 10-ml. samples collected during the luteal phase of the menstrual cycle[5]. Further information on the plasma concentration of the hormone during the cycle has been obtained by a double isotope dilution technique[6] and the application of a fluorimetric method has been reported[7]. However, the former technique is time-consuming and expensive, while the latter is liable to interference by non-specific material.

In general, physicochemical methods for the determination of steroids in blood are inconveniently laborious—specificity being achieved by the use of several chromatographic steps and re-chromatography of a derivative. The time involved in the analysis may be from 3 to 14 days. The introduction of gas-liquid chromatography for steroid analysis offered the hope of a more rapid and, at the same time, highly sensitive and specific method. There were, however, two main problems—to effect adequate preliminary separation without resort to laborious techniques and to ensure that the method is quantitative. The present report describes the manner in which the former has been resolved by the use of two-dimensional thin-layer chromatography; the latter by the simultaneous determination of an isotopically labelled internal standard in terms of integrated radioactivity. The new method has a high degree of specificity and the sensitivity is adequate for the determination of the hormone in 5-ml. samples of pregnancy plasma and 10-ml. samples collected during the menstrual cycle. The time involved is three hours plus the duration of the gas-liquid radiochromatography (40–80 min depending on choice of temperature and stationary phase).

While this work was in progress there were preliminary reports on the application of gas-liquid chromato-

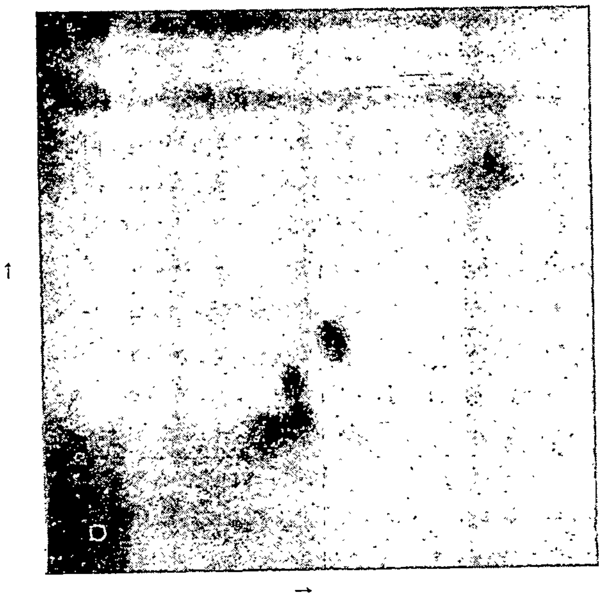

Fig. 1. Two-dimensional thin-layer chromatogram showing 20α-hydroxy-pregn-4-en-3-one (left); Δ4-androstene-3,20-dione (right); 20β-hydroxy-pregn-4-en-3-one (above); progesterone (right)

graphy to the determination of progesterone in extracts of human myometrium[8] and to the identification of the hormone in human pregnancy plasma[9].

All solvents are purified by standard techniques and redistilled prior to use. The stationary phase for thin-layer chromatography is 'Kieselgel' with calcium sulphate binder and fluorescent indicator from Camag, Ltd. (obtained from Griffin and George Ltd., London). Gas-liquid radiochromatography is performed on a Pye panchromatograph with a macro-argon ionization detector; the radioactivity being measured simultaneously by a gas proportional counter coupled to an Ekco ratemeter (type *N701A*) which incorporates an integration unit. The instrument was modified so that all the effluent gas from the column passes through the argon detector before combustion. The columns are 5′ in length, and for the routine method the stationary phase is 1 per cent *cyclo*hexane-dimethanol succinate (CDMS) on 'Gas Chrom. *P*.' 60–80 mesh (W. G. Pye and Co. Ltd., Cambridge). Progesterone-4-^{14}C (21·7 mc./mM) was obtained from the Radiochemical Centre, Amersham, Bucks.

Peripheral venous blood is withdrawn by a non-greased syringe, transferred to a centrifuge tube containing heparin B.P. (10 units/ml. blood), centrifuged and the plasma removed. An internal standard of 0·25 μg progesterone-4-^{14}C is added to each sample. After the addition of an equal volume of N/3 sodium hydroxide, the mixture is shaken for 5 min; extracted with 2 × 100 ml. ether; the extract washed with 20 ml. distilled water and evaporated to dryness *in vacuo*. The extract is then transferred with 3 × 5 ml. methanol/chloroform (1 : 1) to a small conical test tube and evaporated to dryness under nitrogen at 60° C (a small volume of the same mixture is added to wash the extract into the tip of the tube and after re-evaporation the tube may be stored in a desiccator).

Glass plates 20 cm × 20 cm are coated with 'Kieselgel' and stored in a desiccator prior to use. The extracts are applied to separate plates (using a glass capillary and 3 × 0·05 ml. methanol : chloroform, 1 : 1) on a spot 2 cm in from either side of the right-hand corner. The first system used is benzene/ethyl acetate (3 : 2), the solvent front being allowed to travel 15 cm. The plates are then removed, thoroughly dried, turned through 90°, and re-run in ether/dimethylformamide (99 : 1), the solvent front again advancing 15 cm. The plates are then removed and dried. The compounds are located under ultra-violet light (2537 Å) and demarcated with a fine needle. As an additional safeguard, the position of the steroid may be located by extrapolation from two standards applied to the contralateral corners of the plate at the beginning of each chromatogram. The silica gel containing the compound is loosened with a microspatula; aspirated on to a filter disk (1 cm diam., No. 3 grade); eluted with 3 × 0·5 ml. ethanol under positive pressure and the eluate evaporated under nitrogen at 60° C. (The elution apparatus was devised in the department by Dr. M. I. Stern.) Fig. 1 is a photograph of the ultra-violet absorption of four authentic steroid standards, and Fig. 2 shows the result obtained in an extract of pregnancy plasma. In some instances, examination of the plate in ultra-violet light reveals other compounds which are under investigation.

Where gas-liquid chromatography is not available, the progesterone in the eluate can be determined by formation of the thiosemicarbazone and the recovery of the internal standard obtained by liquid scintillation counting.

For gas-liquid radiochromatography, the eluate is transferred with 3 × 20 μl. methanol/chloroform (1 : 1) to the centre of a rectangle of folded stainless-steel gauze (previously flamed and held in a clamp over a warm surface) which is dropped on to the top of a 1 per cent CDMS column after cessation of the gas-flow. All conditions are carefully standardized and the chromatography carried out at 210° C. The retention time of progesterone at this temperature is 72 min and the relative retention time of other steroids can be expressed in terms of cholestane or using progesterone itself as the marker. For routine work it is permissible to shorten the retention time of progesterone by using a higher temperature. The amount of progesterone on the differential trace from the argon detector is calculated from the peak area (mm^2) and reference to a calibration curve. From this, the amount of internal standard recovered (calculated from integrator units on the radiochromatography recorder) is subtracted and the results corrected for loss. Table 1 shows the relationship between μg progesterone, peak height, area and integrator units at one of the concentrations used for the calibration. Peak height is not satisfactory at relevant concentrations whereas satisfactory calibration curves are obtained from peak area and integrator units. The sensitivity of the argon detector

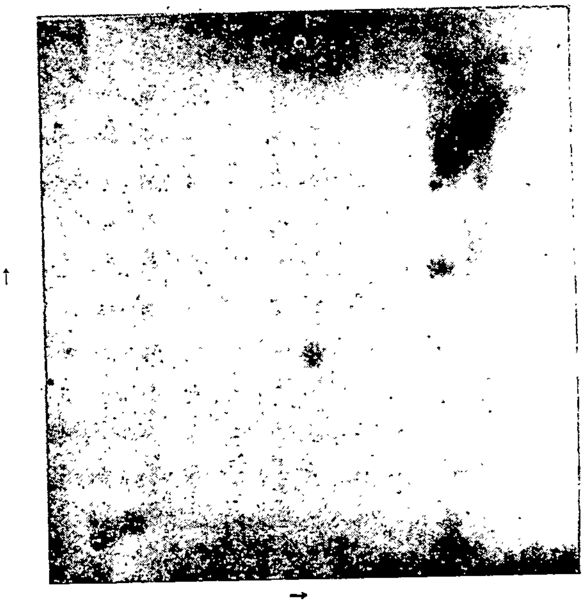

Fig. 2. Two-dimensional thin-layer chromatogram showing progesterone (centre of plate) from extract of 8 ml. pregnancy plasma (spot includes internal standard)

is tested daily by calculating the peak area (and recovery) of a sample of pure progesterone-4-^{14}C. In this connexion, it was found that a small proportion (2·5 per cent) of the standard consists of an isotopically labelled component (possibly the 17α-epimer) which appears immediately before the main peak.

Table 1. Quantitative Gas-liquid Radiochromatography

	0·50 μg progesterone-4-^{14}C		
	Argon (differential trace) 210° C : 10^{-9} amp : 1,000 V : 5″/h		Integrated radioactivity FSD* 1,000 p.p.s. 1/3 recorder sensitivity
	Peak height (mm)	Peak area (mm^2)	Integrator units
	128	694	330
	110	710	320
	125	705	330
	141	709	335
	123	721	330
	147	730	335
Mean ± *S.D.*	129 ± 13·3	711 ± 12·5	330 ± 5·5
Coeff. of variation (%)	10·3	1·8	1·7
	0·10 μg progesterone-4-^{14}C		
	225° C : 10^{-9} amp × 3 : 1,000 V : 10″/h		FSD* 1,000 p.p.s.
	101	1,111	205
	101	1,313	210
	102·5	1,308	202
	99	1,138	218
	92·5	1,156	215
	98·5	1,231	208
Mean ± *S.D.*	99·1 ± 3·5	1,258 ± 80·2	209 ± 6·1
Coeff. of variation (%)	3·6	6·4	2·9

* Full-scale deflexion.

For the determination of progesterone during the luteal phase of the menstrual cycle and pregnancy, adequate sensitivity is obtained on the panchromatograph with a current range of 10^{-9} amp; the ratemeter at minimum sensitivity (full-scale deflexion 1,000 p.p.s. × 1/3 recorder sensitivity) and a chart speed of 5″/h. This permits determination of 0·01 μg. However, as shown in Table 1, higher sensitivity can be obtained by decreasing the attenuation or amplifying the signal from the argon detector through a second integrator and by increasing the chart speed.

Figs. 3 and 4 are photographs of differential traces at 10^{-9} amp. It will be seen that there is adequate separation of a variety of steroids which in the thin-layer system have R_F values close to that of progesterone. Δ4-Androstenedione is not shown in Fig. 3, but it has a retention time between that of 5β-pregnane-3,20-dione and Δ^5-pregnen-3β-ol-20-one. At this sensitivity progesterone is the only steroid present in the eluate from plasma collected during the luteal phase of the cycle or pregnancy. It will be noted that the minimum of non-steroid material is applied to the column and this obviates contamination of the detector.

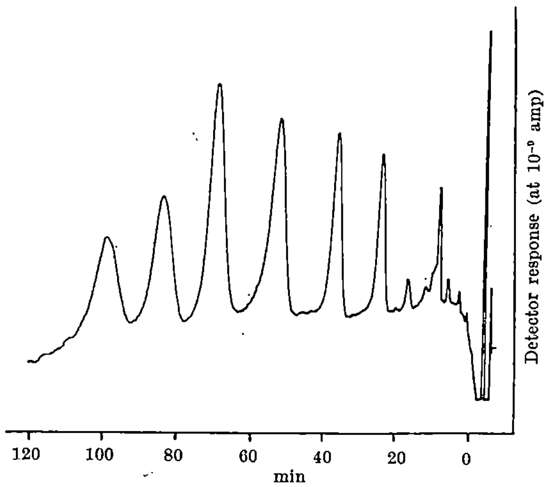

Fig. 3. Differential trace from macro-argon detector (1 per cent CDMS; 210° C, 10^{-9} amp; 1,000 V; chart speed 5″/h). Peaks represent from right to left: solvent; 5β-androstane-3,20-dione; 5β-pregnane-3,20-dione; Δ^5-pregnen-3β-ol-20-one; progesterone, 20β-hydroxy-pregn-4-en-3-one; 20α-hydroxy-pregn-4-en-3-one

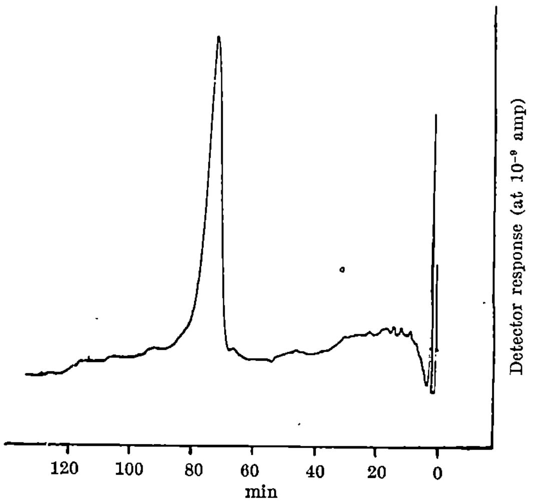

Fig. 4. Same conditions as Fig. 3: progesterone (endogenous plus internal standard) eluted from plate shown in Fig. 2

Table 2 shows examples of the application of the method and includes replicate determinations and the recoveries of the internal standards. The latter are usually higher than 80 per cent, and the loss appears to be mainly due to the technique of transferring the eluate to the metal gauze (since a similar loss occurs in a proportion of standards).

Table 2. Male Plasma

Subject No.	Age (yr)	Internal standard (μg)	Recovery (%)	Progesterone (μg/100 ml.)
1	28	—	—	0·20
2	30	—	—	0·27
3	55	0·5	75·6	0·30
3	55	0·5	81·5	0·29

Female Plasma

(*a*) Early pregnancy (replicates on pool)

Recovery of internal standard (%)	Progesterone μg/5 ml. aliquot
84	0·22
100	0·21
20	0·22
85	0·19

(*b*) Menstrual cycle

Subject No.	Age (yr)	Day of cycle	Duration (days)	Recovery of internal standard (%)	Progesterone (μg/100 ml.)
1	28	4	32	24	0·52
2	23	9	29	95	0·75
3	24	10	30	76	0·97
4	18	13	32	96	0·9
5	17	17	29	12	3·9
6	34	19	28	101	1·4
7	35	19	28	90	2·3
8	38	21	27	99	0·6
8	38	23	27	100	0·53
4	18	24	30	100	1·1
3	24	26	30	94	1·1
9	25	28	35	80	1·1

Very occasionally (for example, three of the recoveries in Table 2), major loss has occurred due to a faulty seal between the base of the column and the molecular entrainer. The analysis may be repeated or the result corrected in terms of the recovery of internal standard. The validity of the correction is indicated by the results of the replicate determinations on pooled pregnancy plasma.

Finally, with regard to specificity, this is achieved by the two-dimensional thin-layer chromatography and by the 1 per cent 'CDMS' column. The simultaneous recording of radioactivity confirms the identity of the progesterone peak at each analysis. If other Δ^4-3-ketones are detected on the thin-layer plate, these can be eluted separately or as a group and then subjected to gas-liquid chromatography. Further evidence on specificity has been obtained by the use of other stationary phases—1 per cent neopentyl glycol adipate (NPGA) and the silicone polymers, 3 per cent '*QF*-1' and 3 per cent '*SE*-30'. In addition, the 1 : 1 dimethylhydrazone and the *bis*ethylene ketal derivatives of endogenous progesterone have been chromatographed on 1 per cent CDMS and 3 per cent '*QF*-1'.

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ENZYMATIC CONVERSION OF ALL-*cis*-POLYUNSATURATED FATTY ACIDS INTO PROSTAGLANDINS

By D. A. VAN DORP, R. K. BEERTHUIS, D. H. NUGTEREN and H. VONKEMAN

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IN view of the results obtained by our group[1] and by S. Bergström *et al.*[2] the relationship between essential fatty acids and prostaglandin seems now established. In both laboratories arachidonic acid could be converted into prostaglandin E_2 (PGE_2) in high yield.

It has further been predicted[1,2] that 'homo-γ-linolenic acid' (all-*cis*-eicosa-8,11,14-trienoic acid) will act as precursor for PGE_1, which has now been proved. Moreover, we were interested to find out whether the enzyme system could also convert other polyunsaturated acids of related structures. This appeared to be the case.

All-*cis*-[1-^{14}C]-eicosa-8,11,14-trienoic acid was obtained by carboxylation of the Grignard derivative of all *cis*-1-bromo-7,10,13-nonadecatriene with $^{14}CO_2$. The product was purified as methyl ester by column and thin-layer chromatography over silver nitrate[3,4] and saponified; the specific activity of the acid was 32 × 10^6 d.p.m./mg, the purity 98 per cent as checked by gas–liquid chromatography, and the *trans* content less than 1 per cent as determined by infra-red spectroscopy.

The vesicular glands of sheep were collected immediately on slaughter and stored up to 2 months on dry ice. After thawing, the glands were stripped of connective tissue and cut finely. This material was homogenized with a turmix in an equal volume of ice-cold 0·15 M phosphate buffer (*p*H 7·5).

1 mg [1-^{14}C]-eicosa-8,11,14-trienoic acid dissolved in 0·1 ml. 96 per cent ethanol was added to a homogenate obtained from 25 g glands, and the mixture incubated aerobically at 37° for 30 min. The incubation was terminated by addition of 160 ml. 96 per cent ethanol containing 2·4 ml. 1 N hydrochloric acid, and the prostaglandins were concentrated according to Samuelsson[5]. As control we used an equal amount of homogenate with the same radioactive substrate to which at the start 160 ml. of the acidified 96 per cent ethanol had been added.

From both concentrates 0·1 part was subjected to thin-layer chromatography on 'Silicagel *G*', using solvent system '*A* I' according to Greén and Samuelsson[6], the reference being PGE_1. The area with R_F equal to PGE_1 was scraped off, and the rest of the spots stained with iodine. In this way it could be checked whether the removal of PGE from the plate was complete; degradation of the PGE by iodine was avoided as much as possible, and other spots were made visible. The various spots were eluted from the 'Silicagel' with methanol, the methanol evaporated and the radioactivity measured in a Packard 'Tricarb' liquid scintillation spectrometer.

As seen in Fig. 1, the bulk of the radioactivity was present in the spot with the same mobility as the reference sample of PGE_1 (R_F 0·60), whereas the control did not show a peak of radioactivity in this region. In several incubation experiments we have observed the appearance of a peak *X* between the PGEs and the solvent front. The nature of peak *X* is not yet clear, but the fact that a similar peak did not appear in the controls indicates that it is also formed from the unsaturated fatty acid by an enzymatic reaction. Unconverted substrate remained for the greater part in the light petroleum used during the concentration procedure.

Thin-layer chromatography was repeated with another 0·1 part of the concentrate and the fraction with R_F 0·60 isolated and re-chromatographed on a thin-layer of 'Silicagel *G*' impregnated with 10 per cent silver nitrate using solvent system '*A* II'[6]. After development the zones were scraped off, eluted from the silica-gel[6], and the radioactivity measured. It was found for the greater part at R_F 0·86, which was equal to that of PGE_1 (R_F PGE_2 0·71) (Fig. 2).

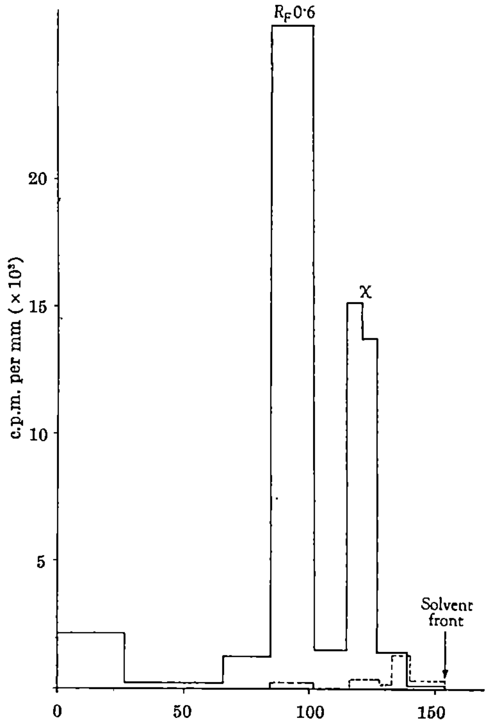


Fig. 1. Distribution of radioactivity (thin-layer chromatography on 'Silicagel *G*') of PG*E* concentrate obtained after incubation of [1-^{14}C]-homo-γ-linolenic acid with homogenate of vesicular glands of sheep (——); (- - - -) is the control

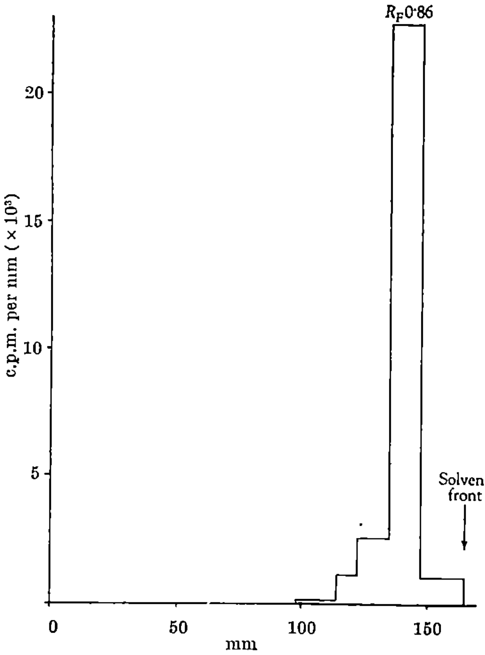
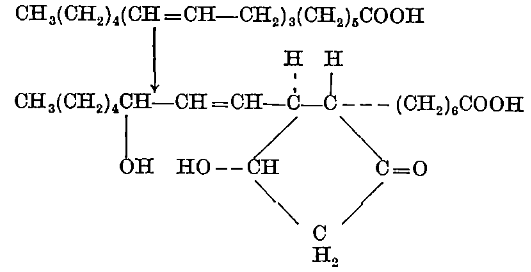


Fig. 2. Distribution of radioactivity (thin-layer chromatography on 'Silicagel *G*'/AgNO₃) of the PG*E* spot isolated on 'Silicagel *G*' (Fig. 1)

Based on the radioactivity found in the PGE_1 thus formed, it is concluded that homo-γ-linolenic acid was converted into PGE_1 to the extent of at least 18 per cent:



Conversions of straight-chain fatty acids with 18 and 19 carbon atoms containing 3 and 4 skipped double bonds* have also been investigated. A homogenate of the vesicular glands of sheep was made in the same way as described here and diluted with three times its volume of phosphate buffer. By centrifuging during 10 min at 4,000*g* the connective tissue was removed from the homogenate. The turbid and light-brown supernatant contained 5–10 mg protein and 2–5 μg PG*E*/ml. and was suitable as such for examining the conversion of non-labelled fatty acids into prostaglandins. To this end, 0·1 mg of a fatty acid in 2 ml. *tris* buffer (*p*H 9) was incubated with an amount of supernatant containing 10 mg protein. The PG*E* content after the incubations could be determined directly in the crude ethereal extract of the acidified incubation mixture by addition of methanolic potassium hydroxide (final concentration 0·5 N) followed by measuring the increase of the extinction at 280 mμ[7]. In this way we could demonstrate the conversion of all-*cis*-eicosa-8,11,14-trienoic acid ($\Delta^{8,11,14}$ $C_{20:3}$) (purity 92 per cent), all-*cis*-nonadeca-7,10,13-trienoic acid ($\Delta^{7,10,13}$ $C_{19:3}$) (purity > 98 per cent), all-*cis*-eicosa-5,8,11,14-tetraenoic acid ($\Delta^{5,8,11,14}$ $C_{20:4}$) (purity > 98 per cent) and all-*cis* - nonadeca - 4,7,10,13 - tetraenoic acid ($\Delta^{4,7,10,13}$ $C_{19:4}$) (purity 95 per cent) into prostaglandins and probably *nor*-prostaglandins.

The corresponding fatty acids with eighteen carbon atoms, all-*cis*-octadeca-6,9,12-trienoic acid (purity > 99 per cent) and all-*cis*-octadeca-3,6,9,12-tetraenoic acid (purity 92 per cent) appeared to be scarcely or not at all converted into prostaglandin-like substances.

Additional proof for the conversion of $\Delta^{4,7,10,13}$ $C_{19:4}$ into '*nor*-prostaglandin E_2' was obtained as follows:

Methyl nonadeca-4,7,10,13-tetraynoate was reduced with tritiated hydrogen gas* over Lindlar's catalyst. The crude methyl ester was purified by thin-layer chromatography on 'Silicagel *G*' impregnated with silver nitrate and afterwards saponified. The specific activity of [³H]-$\Delta^{4,7,10,13}$ $C_{19:4}$ thus obtained was 2×10^9 d.p.m./mg. This acid (0·1 mg) was incubated with a homogenate of 7·5 g vesicular glands of sheep in an equal volume of 0·15 M phosphate buffer (*p*H 7·5) during 30 min at 37°, and the incubation mixture worked up in the usual way. The prostaglandins were isolated from the concentrate by thin-layer chromatography on 'Silicagel *G*' (see aforementioned). To 0·3 part of this purified material non-radioactive PGE_1 (46 μg) and PGE_2 (39 μg) were added. The mixture was afterwards subjected to thin-layer chromatography on 'Silicagel *G*' impregnated with 10 per cent silver nitrate, and the various zones were scraped from the plate and eluted. The radioactivity of each fraction was measured as well as the increase of ultraviolet absorption at 280 mμ after treatment with alkali. Fig. 3 shows that the radioactive prostaglandin formed coincides with PGE_2, from which we conclude that the enzyme system had converted $\Delta^{4,7,10,13}$ $C_{19:4}$ into *nor*-PGE_2.

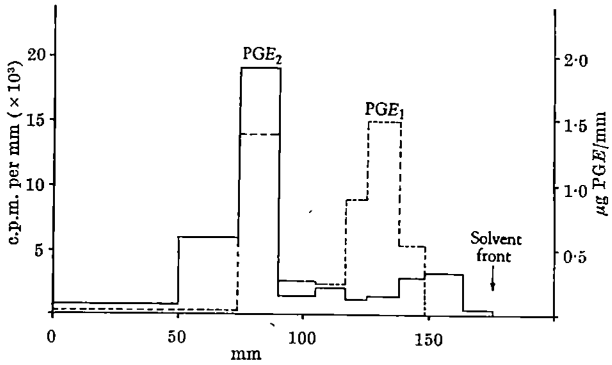


Fig. 3. Distribution of radioactivity (——) (thin-layer chromatography on 'Silicagel *G*'/AgNO₃) and of PG*E* (– – – –) after incubation of [³H]-$\Delta^{4,7,10,13}$ $C_{19:4}$ with a homogenate of vesicular glands of sheep and after addition of PGE_1 and PGE_2

In other experiments 30 mg $\Delta^{8,11,14}$ $C_{20:3}$ and 30 mg $\Delta^{7,10,13}$ $C_{19:3}$ were each dissolved in 60 ml. 0·2 M *tris* buffer (*p*H 9) and incubated with 240 ml. (2·4 g protein) of the aforementioned 4,000*g* supernatant during 2 h at 37°. The reaction mixtures were acidified and extracted with diethyl ether. Based on the molar extinction of 26,800 for PG*E* 278 (ref. 7) the ethereal extract contained 11·4 mg prostaglandin in the experiment with $C_{20:3}$ and 12·1 mg '*nor*-prostaglandin' with $C_{19:3}$. The control (incubation without added substrate) contained only 1·0 mg PG*E*. These experiments indicate that by incubation with a suitable fatty acid the amounts of prostaglandin present in the homogenate can be increased at least by a factor 10.

Part of the PGE_1 and the *nor*-PGE_1, obtained in the experiment described here, was purified by thin-layer chromatography on 'Silicagel *G*' and 'Silicagel *G*' impregnated with silver nitrate successively. The pure PGE_1 thus obtained was tested on guinea pig ileum against a pure preparation of PGE_2 prepared from vesicular glands of sheep. Its activity was about half that of PGE_2. The purified *nor*-PGE_1, containing maximum

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* Of these acids all-*cis*-nonadeca-4,7,10,13-tetraenoic acid and all-*cis*-octadeca-3,6,9,12-tetraenoic acid have not yet been described in the literature. Their preparation will be the subject of a publication by H. J. J. Pabon, D. van der Steen and D. A. van Dorp.

* The reduction was carried out by Mr. J. Parmentier, Central Laboratories T.N.O., Delft, Holland.

5 per cent PGE_1, had scarcely any activity at all when compared with the same standard. It is interesting to note that all-*cis*-nonadeca-7,10,13-trienoic acid scarcely showed any essential fatty acid activity[8]. *Nor*-PGE_2 obtained from an incubation experiment with $\Delta^{4,7,10,13}$ $C_{19:4}$ was purified by the same techniques. After purification the sample still contained about 25 per cent PGE_2 derived from the original homogenate. Owing to this relatively high amount of natural PGE_2 we cannot yet give values for the biological activity of *nor*-PGE_2.

So far as the properties of the enzyme system are concerned, our first impressions can be summarized as follows: By centrifuging the 4,000*g* supernatant during 1 h at 100,000*g* 20 per cent of the protein was precipitated. This protein was able to convert the polyunsaturated fatty acids into prostaglandins while only little activity was shown by the remaining clear supernatant. It should be noted that a supernatant obtained by centrifuging a homogenate in 0·25 M sucrose at 20,000*g* during 1 h was found to be fully active[1].

When the incubations are carried out anaerobically, only small amounts of prostaglandins are formed, so oxygen is essential. Usually the incubations were carried out at *p*H 9, where the yield of PGE is at its maximum.

These investigations have once more shown the relation between essential fatty acids and prostaglandins.

We thank Prof. S. Bergström, of the Karolinska Institutet, Stockholm, for a sample of prostaglandin E_1 and E_2, and Dr. J. de Boer, of our laboratory, for testing the various samples for biological activity.

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PHYTOHÆMAGGLUTININ-INDUCED CYTOTOXIC ACTION OF NORMAL LYMPHOID CELLS ON CELLS IN TISSUE CULTURE

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THERE is some evidence that immunological cell injury can be mediated by lymphoid cells, most probably lymphocytes. When antigen-carrying 'target' cells, usually grown in monolayer culture, are exposed to lymphoid cells from spleen, lymph nodes, thoracic duct or peripheral blood of sensitized animals, an appreciable proportion of the target cells will be lysed within 24–36 h. This cell injury is immunologically specific and no complement needs to be added. Lymphocyte-induced cell damage has been found in autoimmune[1–4], isoimmune and heteroimmune systems[5–9]. It occurs under conditions where sera from the sensitized animals were found to have no effect[1,2,4,6,8]. Homologous lymphoid cells from non-sensitized animals seem to be inactive. However, a certain potentiating effect of serum from sensitized animals has been observed in some cases[1,5,10].

The mechanism of this lymphocyte activity is unknown. One of the main features observed in this reaction is the early aggregation of the lymphocytes round the target cells. This can be seen already 1–3 h after the beginning of the incubation, before any noticeable cell damage appears under the microscope[1]. This step, which obviously is immunologically specific, seems to be important for establishing a necessary close contact between lymphocytes and target cells. The second step, the tissue cell damage, may then represent either a specific or a non-specific activity of the lymphocytes (cf. ref. 10).

In order to examine the significance of lymphocyte-target cell aggregation for the initiation of cell damage, it seemed desirable to induce this phenomenon with lymphoid cells from non-sensitized animals. Phytohæmagglutinin (PHA), a crude extract from the bean *Phaseolus vulgaris*[11], was found suitable for this purpose. PHA is known to agglutinate red as well as white blood cells from many animal species. The addition of PHA to suspensions of lymphocytes stimulates cellular differentiation and proliferation[12]. The induction by PHA of lymphocyte-target cell aggregation and an aggregation-dependent target cell injury will be described in this article.

Primary cultures were set up from fœtal human kidneys (legal abortions) or from kidneys of new-born Sprague–Dawley rats after trypsinization (0·30 per cent trypsin in phosphate buffer, *p*H 7·6) of the minced tissue. The cells were grown in milk-dilution bottles, containing Eagle's medium[13] supplemented with 15 per cent heat-inactivated (30 min 56° C) calf serum and 100 I.U. of penicillin and 100 μg of streptomycin per ml. After 1–2 days, 2 μc. of thymidine labelled with carbon-14 (spec. activity 30·4 mc./mM, Radiochemical Centre, Amersham, England) was added and the incubation was continued for an additional 2–4 days. The cultures were finally washed twice with medium and the cells were afterwards removed from the bottles by treatment with trypsin. The cells were washed three times with cold medium. The number of living cells was determined by counting in a Bürker chamber, after staining with trypan blue. Monolayer cultures of HeLa cells were prepared in the same way.

Lymphoid cells were isolated from heparinized peripheral blood, taken from a cubital vein of normal persons or by heart puncture from normal Sprague–Dawley rats (randomly bred). Human lymphocytes were also isolated from lymph obtained from uræmic patients by drainage of the thoracic duct. An equal volume of 6 per cent dextran (Macrodex, Pharmacia, Sweden) was added to the blood and the red cells were allowed to sediment for 30–60 min at room temperature. After centrifugation at 400 r.p.m. for 5 min, the supernatant, containing white cells, was pipetted off and centrifuged at 1,500 r.p.m. for 5 min. Contaminating red cells were lysed by treatment of the sediment with 0·35 per cent sodium chloride for 1 min[14]. The white cells were suspended in Eagle's medium containing 5 per cent calf serum, and were allowed to stand for 1–2 h at room temperature, during which time most of the remaining granulocytes were adsorbed to the glass. Before use, the cell suspension consisting mainly of monocytes and lymphocytes was washed twice with medium.

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Table 1. Cytotoxic Effect of Normal Lymphoid Cells on ^{14}C-Thymidine-labelled Target Cells after 18 h Incubation in the Presence of PHA

Exp. No.	Target cells	Ratio lymphoid cells: target cells (living cells)	Lymphoid* cells	PHA added: Total radio-act. in DNA (c.p.m.)	PHA added: Per cent released to Sup. II	No PHA: Total radio-act. in DNA (c.p.m.)	No PHA: Per cent released to Sup. II
(1)	Fœtal human kidney	10 : 1	Human	1,182	28·5	1,026	18·1
		,,	Human	929	29·8	1,253	17·4
			—	928	23·1	657	19·2
(2)	Fœtal human kidney	20 : 1	Human	220	37·7	320	22·6
			—	289	21·7	304	26·8
(3)	HeLa	100 : 1	Human	2,031	42·2	2,736	10·5
			—	1,399	12·9	1,770	11·6
(4)	Rat kidney monolayer on coverslips	?	Rat	292	13·5	256	6·8
		,,	Rat	358	14·6	383	2·9
			—	282	16·5	365	16·0

* All 6 samples of lymphoid cells in this column were obtained from different donors.

Varying numbers (1–10 × 10^6) of these white cells were mixed with labelled target cells (10^5) in roller tubes, suspended in Eagle's medium (5 per cent calf serum). The final volume of the incubation mixture was 1·5 ml.; 0·1 ml. of PHA-extract was added to some of the tubes. (The PHA-extract was prepared by extracting 40 g of crushed *Phaseolus vulgaris* beans with 200 ml. of 0·15 M phosphate buffer (*p*H 7·2) at 4° C for 24 h. The suspension was centrifuged at 4,500 r.p.m. for 30 min and the supernatant dialysed in the cold for 24 h against several changes of phosphate buffer.) The tubes were fitted loosely with screw caps, placed at 45° angle, and incubated at 37° C for 18 h in an atmosphere of air containing 5 per cent carbon dioxide.

After incubation, the tubes were centrifuged and the cell-free media were removed (supernatants I). In order to obtain a release of isotope from the damaged cells the sediments were treated with 0·25 per cent trypsin in phosphate buffer (*p*H 7·6) for 30 min at room temperature with occasional shaking[15]. After cooling with ice, the tubes were finally centrifuged at 2,000 r.p.m. for 6 min and the supernatants were withdrawn (supernatants II).

Deoxyribonucleic acid (DNA) was extracted from the supernatants and the cell sediments according to Schmidt–Thannhauser–Schneider[16]. After drying on plastic planchettes, isotope was determined at infinite thinness in a thin-window gas-flow counter (efficiency 10 per cent). The radioactivity (c.p.m.) released into supernatant II after trypsin treatment was expressed as per cent of the total radioactivity recovered in trypsin supernatant plus cells, and used as a measure of cell damage[15].

For morphological observations unlabelled target cells were incubated with lymphoid cells under the same conditions, but in Leighton-tubes containing coverslips. During incubation, the cells settled on the coverslips; these were then removed, washed once with phosphate buffer, fixed in methanol and stained with May–Grünwald and Giemsa.

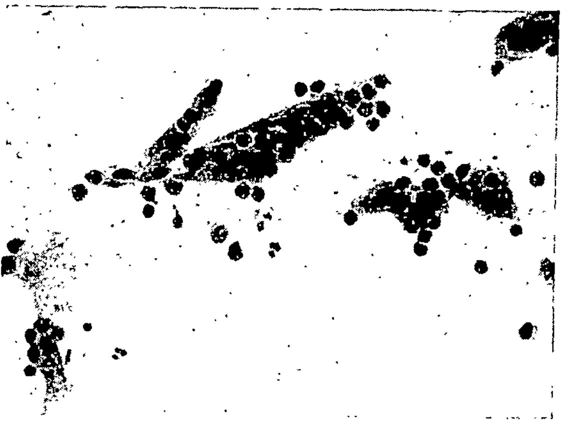

Fig. 1. Normal human lymphoid cells incubated with human fœtal kidney cells for 18 h in the presence of PHA (× 250)

The typical appearance of a culture of human fœtal kidney cells, incubated for 18 h with human lymphoid cells in the presence of PHA, is seen in Fig. 1. The aggregation of lymphocytes to each other and to target cells is striking. No such aggregation was observed in the absence of PHA (Fig. 2). Moreover, under the present conditions, PHA alone did not seem to affect the kidney cells.

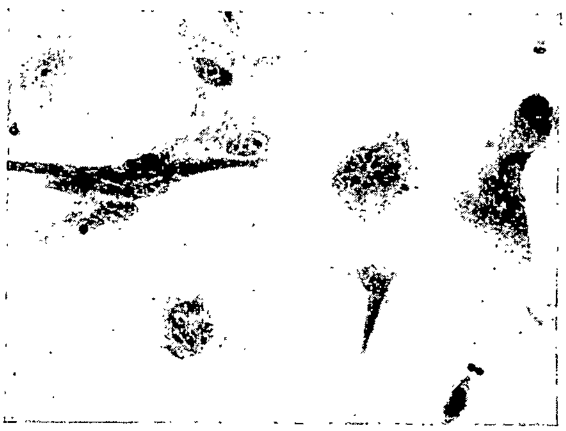

Fig. 2. Control culture from the same experiment as in Fig. 1. No PHA added (× 250)

Table 1 shows the cytotoxic effect of lymphoid cells from various sources, incubated with different target cells and PHA. As can be seen, regardless of the cell combinations used, isotope release was always higher in the presence of PHA than in its absence. However, the magnitude of this effect depended on the ratio of the lymphoid cells to the target cells. As a general rule, only a slight cytotoxicity was obtained with a ratio of 10 : 1, whereas a ratio of 100 : 1 gave a pronounced cytotoxic effect. A low ratio of lymphocytes to target cells may be an explanation of the weak cytotoxic effect seen in experiment 4, where the lymphoid cells were added to an unknown number of rat kidney cells, growing in monolayer culture. It will be noted that an increase of this ratio did not give rise to elevated cell damage in the controls, where PHA was absent. Under the experimental conditions used here, PHA alone caused no, or only negligible, damage of the target cells. (The contents of isotope in the incubation media (supernatants I) were variable, apparently depending on the health of the cells during cultivation[4]. However, since the release of isotope to supernatant I was not significantly elevated in the presence of PHA, it will not be discussed in this article.)

From these experiments it can be concluded that PHA induced a cytotoxic activity of the lymphoid cells.

In order to investigate whether or not these effects were due to the release of cytotoxic agents from the lymphoid cells, experiments were performed with lymphoid cells in 'Millipore' chambers. Normal human lymphoid cells (3×10^6) in 0·3 ml. of medium were placed in 'Millipore' chambers (diam. 14 mm, pore size 0·45μ), which were then incubated in Petri dishes with $1{\cdot}5 \times 10^5$ of human kidney cells in 5 ml. medium. The concentration of PHA was the same as in the experiments already described here. In some controls, PHA was omitted; in others the kidney cells were exposed to free lymphoid cells under similar conditions. Table 2 shows the results of a typical experiment. As can be seen, this arrangement prevented cell damage completely.

Table 2. EFFECT OF 'MILLIPORE' CHAMBERS (MC) ON THE PHA-INDUCED CYTOTOXICITY OF HUMAN LYMPHOID CELLS ON HUMAN FŒTAL KIDNEY CELLS

The lymphoid cell/target cell ratios (living cells) were 10 : 1. Time of incubation 18 h

Lymphoid cells	MC	PHA	Total radio-activity in DNA (c.p.m.)	Per cent released to Sup. II
+	+	+	453	7·8
+	–	+	743	14·5
+	–	–	957	6·2
–		+	1,244	7·9
–		–	928	6·2

In a second series of experiments, 100×10^6 human thoracic duct lymphocytes were grown in 10 ml. of medium for 24 h in the presence or absence of PHA. The media were then centrifuged and 1·5 ml. was added to 10^5 human fœtal kidney cells. No cell damage was observed after 18 h incubation (Table 3). Controls with living lymphocytes from the same donor gave positive results.

Table 3. EFFECT OF SUPERNATANTS FROM PHA-TREATED OR UNTREATED NORMAL HUMAN LYMPHOID CELLS ON HUMAN FŒTAL KIDNEY CELLS

Time of incubation 18 h

Lymphoid cells	Medium supplemented with	Total radioactivity in DNA (c.p.m.)	Per cent released to Sup. II
–	Sup. from PHA-treated cells	336	23·3
–	Sup. from untreated cells	289	20·6
+ *	PHA	181	38·6
+ *	—	358	21·2
–	PHA	329	22·8
–	—	300	25·8

* In these controls the lymphoid cell/target cell ratios (living cells) were 20 : 1.

These negative results are similar to those reported by other authors who used 'Millipore' chambers or various lymphocyte extracts in iso- and hetero-immune systems[7,10]. However, it is realized that cytotoxic agents, released from the lymphoid cells under these conditions, may become considerably diluted in the incubation medium. In contrast, when lymphoid cells are aggregated to target cells, cell damage may be produced, because of high local concentration of such agents in the contact areas between the cells[17].

Although the results presented here underline the importance of close contact between lymphoid cells and target cells in order to produce damage of the latter, they do not disclose how this injury is brought about. The simplest explanation would be to ascribe an entirely passive role to the lymphoid cells. Hence, one could envisage the target cells being damaged by the blocking of their surfaces by the lymphoid cells, thus depriving them of nutrients or inducing various metabolic disturbances. Although unlikely (cf. ref. 8), an explanation of this sort cannot yet be ruled out.

Alternatively, one could expect the lymphoid cells to play an active part in producing cell damage. The observation made in an isoimmune system, that hydrocortisone inhibits the cytotoxicity of immune lymphocytes but not their aggregation to target cells, would perhaps speak in favour of this assumption[18]. If this were the case, one may ask whether or not cytotoxic activity of the lymphoid cells was induced solely by their contact with the target cells, the contact being mediated by PHA in some unknown manner. However, when lymphoid cells from normal mice were non-specifically aggregated to mouse fibroblasts in monolayer by means of polylysine treatment, no obvious cell damage of the target cells was observed under the microscope[10]. If these findings can be confirmed and extended to other experimental systems, they would suggest that the injury of the target cells, in our experiments, could be an expression of a more general stimulation of the lymphoid cells by PHA, related to its mitogenic activity[19].

Obviously, it cannot be decided at present whether or not the target-cell injury observed in these experiments reflects the induction in the lymphoid cells of an immune response[20,21], possibly potentiated by PHA[15]. However, since target cells and lymphoid cells in our experiments were obtained from histoincompatible animals or human beings, experiments with inbred, histocompatible animals should throw some light on this point. Such experiments are in progress.

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RADIATION-INDUCED CHANGES IN METABOLISM OF THYMINE IN RAT

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IN an examination of the effects of X- and γ-irradiation on human beings, it was observed that increased urinary excretion of β-amino-isobutyric acid (BAIBA) occurred in some, but not all, patients[1]. Metabolism of the BAIBA precursor, thymine, is still obscure, but degradation occurs predominantly in the liver. Since human beings possess only a limited ability to destroy BAIBA, Armstrong *et al.*[2] suggest that the excessive excretion of BAIBA in normal people, where there is no evidence of marked tissue destruction, could reflect impairment of liver function. Thus the possibility exists that changes in liver function may have modified the excretion pattern in the patients examined by us. In order to evaluate this point, radioactive thymine was administered to irradiated rats and the excretion of radioactive metabolites was determined.

In an initial experiment, 14 Sprague–Dawley female rats were exposed to varying amounts of X-irradiation (0–803 r., 250 kV, 15 m.amp, 1 mm copper/1 mm aluminium filter) to total body. Urine was collected for 2 days before and 5 days after irradiation, and each sample was analysed for BAIBA by the method of Smith and Dymond[3]. The results in Table 1 show that the mean daily output of BAIBA did not differ between the pre- and post-irradiation periods—furthermore, no correlation existed between BAIBA excretion and irradiation dose.

Table 1. EFFECT OF X-IRRADIATION ON EXCRETION OF BAIBA IN THE RAT

Rat	Dose of X-radiation (r.)	Daily output of BAIBA in μM (pre-irradiation)	Daily output of BAIBA in μM (post-irradiation)
1	0	1·53	—
2	0	1·34	—
3	62	2·31	1·50
4	62	2·05	1·60
5	123	1·89	1·45
6	123	2·64	2·57
7	246	2·38	1·84
8	246	2·64	2·26
9	511	0·53	0·89
10	511	1·75	1·57
11	803	0·61	1·15
12	803	1·07	0·79
13	0	0·92	—
14	0	1·01	—

In man, thymine is first reduced to dihydrothymine. This is hydrolysed in two steps to β-ureido-isobutyric acid and finally BAIBA[4]. Pyrimidine ring cleavage occurs on the formation of β-ureido-isobutyric acid. BAIBA is then probably transaminated to form methyl malonic acid semi-aldehyde which is degraded via pyruvate and the tricarboxylic acid cycle. The pathway is similar in rat although there appears to be a reduced ability to convert thymine to dihydrothymine[5].

By using two differently carbon-14 labelled forms of thymine, it is possible to compare rates related to the formation and to the destruction of BAIBA. Thymine-2-^{14}C is metabolized with the liberation of $^{14}CO_2$ at the pyrimidine ring cleavage, whereas thymine-5-^{14}C does not produce $^{14}CO_2$ until decarboxylation of its metabolites in the tricarboxylic acid cycle. Thymine-2-^{14}C, obtained from the Radiochemical Centre, Amersham, was administered intraperitoneally to two groups of rats. One group served as the control. The other group was irradiated, several hours prior to injection of the thymine, with 2,000 rads to upper abdomen only. Each animal was given 1 μc. in 13 μg thymine and then placed in a metabolic unit which was continuously purged with air free of carbon dioxide and the latter in expired gases was collected by absorption in primene solution. $^{14}CO_2$ was determined by mixing an aliquot of this solution with *NE*213 liquid scintillator and assaying in an Ekco *N* 664*A* counter. The excretion of $^{14}CO_2$ was found to be similar in irradiated and non-irradiated groups, 90 per cent of the injected radiocarbon being recovered in the expired air within 4 h after injection. Thus the degradation of thymine to the point of ring cleavage was not affected by liver damage as a result of irradiation. Thymine-5-^{14}C was synthesized by the method of Henderson, Fink and Fink[6] with certain modifications. Using sodium propionate as starting material, these authors produced thymine labelled in the methyl group. In our synthesis, sodium propionate-2-^{14}C was converted to ethyl propionate. This was condensed with diethyl oxalate in the presence of sodium ethoxide to form the sodium salt of diethyl methyl oxosuccinate. Reduction of this by catalytic hydrogenation gave the ethyl ester of β-methylmalic acid which was saponified and finally condensed with urea in concentrated sulphuric acid to yield thymine. The product was salted out into methyl alcohol from the neutralized reaction mixture. The specific activity was 0·875 μc./mg and 0·07 μc. in 80 μg was administered to each animal of three groups. The first group acted as control,

Table 2 DISTRIBUTION OF CARBON-14 AT 5 DAYS AFTER THYMINE-5-^{14}C ADMINISTRATION

Treatment	Carbon-14 injected (per cent) Combined urines	Liver	Intestine
Control	14·4	2·4	3·8
Total-body irradiation (350 rads)	18·7	1·3	7·7
Upper abdomen (1,500 rads)	34·9	2·9	2·8

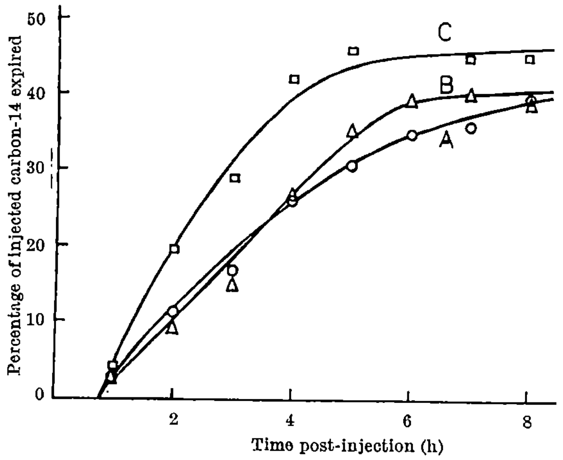

Fig. 1. Expiration rate of $^{14}CO_2$ resulting from the catabolism of thymine-5-^{14}C
A, control; *B*, total-body irradiated (350 rads); *C*, upper abdomen irradiated (1,500 rads)

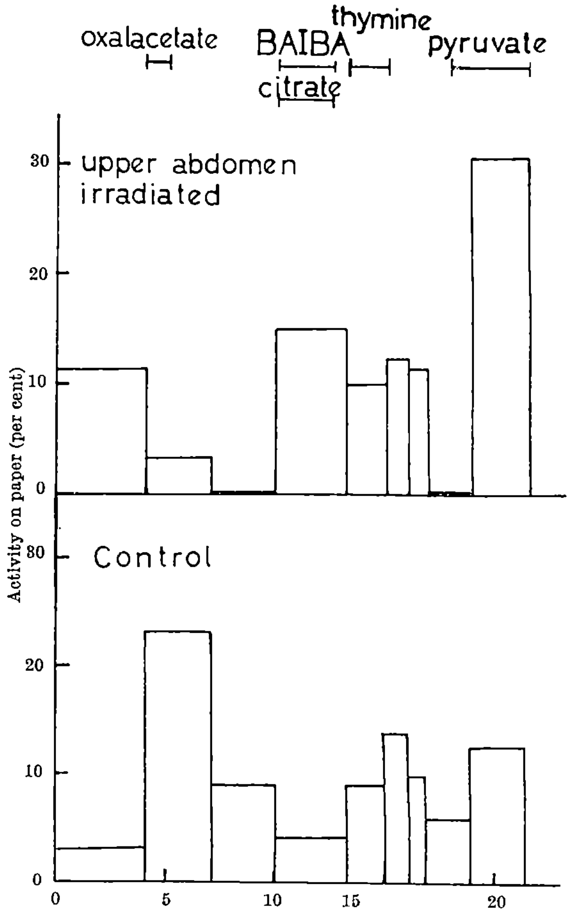

Fig. 2. Effect of irradiation on the distribution of carbon-14 compounds in rat urine
The positions of the migration bands of five substances are indicated. Solvent mixture, butanol : formic acid : water = 77 : 10 : 13

and the other groups were irradiated, 24 h prior to injection, with either 350 rads to total body or 1,500 rads to upper abdomen only. The 24-h delay allowed the development of biochemical injury before the introduction of thymine-^{14}C into metabolic pools. In addition to expired air, all urine was collected for five days after the thymine injection. After this period, the animals were killed and tissues analysed for carbon-14 content by combustion using an oxidation train incorporating a silver/vanadium oxide catalyst; the carbon dioxide was absorbed in primene solution. The results are shown in Table 2 and Fig. 1.

Within the first 8 h 45 per cent of the carbon-14 had been released as $^{14}CO_2$ in expired air and during the next four days 18 per cent was excreted by this route. The amount of $^{14}CO_2$ was similar in control and irradiated groups although the rate of release may have been higher in the irradiated groups. In the five post-injection days more than twice as much carbon-14 activity was found in the urine of the liver-irradiated group than in the controls. Chromatographic analysis (ascending, butanol/formic acid/H_2O, 77 : 10 : 13) followed by radio-assay of the separated bands revealed a significant difference in the distribution of activity on the papers. Identification of the areas of activity is tentatively ascribed as shown in Fig. 2. Analysis of control urine showed that the carbon-14 was associated with urea and metabolites of the Krebs cycle, whereas the activity in the liver-irradiated group was associated predominantly with a fast-moving, ultra-violet absorbing band, believed to be pyruvate. No accumulation of BAIBA occurred after irradiation and there appeared to be a decrease in the amount of radioactive urea formed. This was confirmed by comparing activities of urines before and after incubation with urease. The concomitant accumulation of pyruvate after irradiation is in agreement with the work of Rust[7], who associated the increase in pyruvate with increased glycogenesis after irradiation.

It is suggested in conclusion that irradiation involving moderate liver damage does not alter the rate of catabolism of thymine to BAIBA, but thereafter, accumulation of the metabolites of BAIBA occurs with the formation of carbohydrates as pyruvate builds up. These carbon-14 carbohydrate compounds are gradually metabolized during 4–5 days after the injection with the liberation of carbon-14 compounds predominantly in the urine.

We wish to thank Mr. G. S. Weir for carrying out the synthesis of thymine-5-^{14}C.

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GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE ACTIVITY IN GALACTOSÆMIA

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THE presence of some degree of galactose-1-phosphate uridyl transferase activity in galactosæmia has been suggested by a number of investigators. The work recorded here, based on an examination of intermediates of galactose oxidation, provides further evidence for the incompleteness of the enzymatic block.

Using a manometric procedure, Schwarz *et al.* found no demonstrable erythrocyte transferase activity in 10 of 23 galactosæmic children, but in the remainder an activity averaging 7 per cent of normal value was found[1]. Hsia *et al.*, on the basis of direct measurement of enzyme activity, also concluded that a low degree of activity was present in some patients[2]. Segal *et al.* have examined *in vivo* utilization of injected carbon-labelled galactose[3]. Recovery of carbon label in exhaled carbon dioxide was comparable to normal controls in two of eight galactosæmic individuals. In a separate *in vitro* investigation by Weinberg[4], whole blood and leucocytes from one of these individuals was able to convert galactose-1-^{14}C to labelled carbon dioxide at a substantially greater rate (12–15-fold) than observed with other galactosæmics in his group. However, the amount of galactose oxidized was only 5–8 per cent of normal. Erythrocyte transferase activity could not be demonstrated in any of the galactosæmic subjects, and Weinberg suggested that an alternate pathway might be operative in the unusual case, perhaps the uridine diphosphogalactose pyrophosphorylase system described by Isselbacher[5].

In addition to the patients previously reported, Isselbacher has found substantial *in vitro* production of carbon dioxide from labelled galactose by erythrocytes from two galactosæmic individuals of 20 and 11 years of age, respectively[6].

We have applied a modification of Weinberg's *in vitro* procedure to our own galactosæmia patients, including affected sibling pairs in five families. The results are shown in Table 1. The expected difference in carbon dioxide production between whole blood from galactosæmics and from normals was found, but three points are of particular interest. First, there was variation among galactosæmics in the capability of their blood cells to produce carbon dioxide from galactose. Secondly, three individuals differed markedly from the remaining ten. Thirdly, correspondence of values in sibling pairs suggested a familial influence.

Table 1. *In vitro* OXIDATION OF GALACTOSE-1-^{14}C BY WHOLE BLOOD*

Normal	$^{14}CO_2$, c.p.m.	Galactosæmic	Age	$^{14}CO_2$, c.p.m.
A	41,631	1	13 yr	1,829
B	39,500			
C	44,358	2*a*	3	1,724
D	34,113	2*b*	1	1,306
		3	12	238
		4	5	175
		5*a*	2	48
		5*b*	6 months	0
		6*a*	2 yr	40
		6*b*	4	41
		7*a*	2	21
		7*b*	6	20
		8*a*	12	25
		8*b*	11	26

* 2 μc. of Gal-1-^{14}C (sp. act. 1 mc./mM) was incubated with 2 ml. whole blood for 90 min. $^{14}CO_2$ liberated on acid addition was collected in hyamine and counted in a liquid scintillation system.

Sibling pairs are indicated as 2*a* and 2*b*, 5*a* and 5*b*, etc.

Subjects 1 and 3 are Negroes: all others are Caucasian.

The contribution of leucocytes to the whole blood results was examined in separate experiments on three of the galactosæmic subjects (1, 8*a* and 8*b* in Table 1) and on one of the normal controls. Erythrocytes were sedimented by fibrinogen, and the leucocyte suspensions

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* Predoctoral Fellow: U.S. Public Health Service.

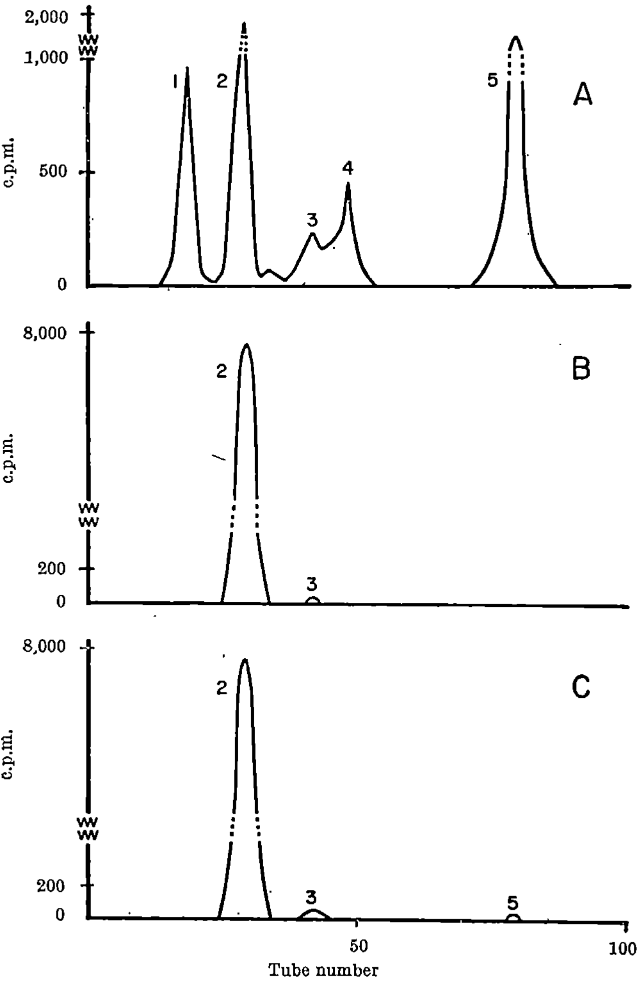

Fig. 1. Column chromatography of galactose intermediates. *A*, pattern for blood from normal person; *B*, from the usual galactosæmic patient; *C*, from one of the unusual galactosæmic individuals. The peaks represent components as follows: 1, lactic acid; 2, galactose-1-phosphate; 3, UDPHexose; 4, triose phosphate (and other compounds as yet unidentified); 5, diphosphoglyceric acid

obtained were incubated in the same manner as whole blood, utilizing the same labelled galactose pool. The relationships among the results were similar to the findings on whole blood. The amounts of labelled carbon dioxide produced by leucocytes from individuals 8*a* and 8*b* were almost negligible. In the case of individual No. 1, one of the unusual cases, the counts were substantially higher, but 20-fold less than produced by leucocytes from a normal control.

To examine for individual differences in more detail, labelled intermediates in the residue after incubation of whole blood with galactose-1-^{14}C were separated by gradient elution of 'Dowex-1-formate' columns[7]. Typical results are shown in Fig. 1. After incubation of blood from normal individuals, the principal labelled peaks, in order of emergence from the column, correspond to lactic acid, galactose-1-phosphate, uridine diphosphohexose (UDPHexose), triose-3-phosphate (and other compounds unidentified as yet) and finally diphosphoglyceric acid (DPGA). In the galactosæmia patients, the anticipated large amount of galactose-1-phosphate was found. Unexpectedly, a peak in the UDPHexose region also was obtained consistently. In the usual type of galactosæmic subject (less than 200 c.p.m. of labelled carbon dioxide), the amount of UDPHexose was very small. However, in the three unusual galactosæmics examined (1,306–1,829 c.p.m.), the UDPHexose peaks were markedly larger, and an additional small peak in the diphosphoglyceric acid area was present.

To determine the identity of the compounds in the UDPHexose region, appropriate eluates were concentrated by lyophilization. After adsorption on charcoal, labelled compounds were eluted with 50 per cent ethanol containing 0·1 per cent ammonia. This procedure was carried out separately for normal controls and for the three unusual galactosæmics. It was necessary to pool column eluates from several experiments for the usual galactosæmics. In all instances, on paper chromatography with ammonium acetate–ethanol at *p*H 7·5 (ref. 8), the radioactive material migrated to the same region as did known UDPGlucose or UDPGalactose. After acid hydrolysis and chromatography with pyridine–butanol–water[9], both labelled galactose and labelled glucose were identified. All galactosæmia bloods showed a ratio of glucose to galactose of 2 to 1: in contrast, the ratio for normal bloods was 1 to 3. These results reflect the relative differences in transferase and epimerase activity in the two groups under the non-equilibrium condition of the experiments. The presence of carbon label in the glucose, and also in the DPGA region of the column effluents, confirms their origin to be from galactose.

Erythrocyte galactose-1-phosphate uridyl transferase activity could not be detected by a UDPGlucose consumption assay[10] in any of the galactosæmic subjects investigated. The presence of carbon label in UDPHexose suggested either that the transferase assay used is not sufficiently sensitive to detect very low levels of transferase activity or that the UDPHexose was formed by some other route.

Isselbacher has described an alternate pathway for galactose utilization in liver by way of UDPGalactose pyrophosphorylase, an enzyme capable of converting galactose-1-phosphate to UDPGalactose in the presence of uridine triphosphate (UTP)[11]. Although Isselbacher has stated that this enzyme does not occur in erythrocytes[12], it was desirable to re-examine the possibility in the present connexion. Hæmolysates of erythrocytes from normal individuals, galactosæmia heterozygotes, and galactosæmics (including two of the three individuals with relatively high production of carbon dioxide) were incubated with galactose-1-^{14}C-1-phosphate, both in the presence and absence of UTP[11]. Labelled UDPHexose formed was measured and the results are shown in the first column of Table 2. No incorporation of label into UDPGalactose could be detected with hæmolysates from galactosæmic individuals. Incorporation was found with normal hæmolysates and to some extent with those from galactosæmia heterozygotes. By themselves, these results might indicate that UDPGal pyrophosphorylase activity is present in normal hæmolysates and in those from galactosæmia heterozygotes but not at all in those from galactosæmia homozygotes. If this were to be true, it

Table 2. PYROPHOSPHORYLASE ASSAY*

Hæmolysate	μmoles labelled UDPHexose formed per hour per ml. packed cells† Without G-1-P	G-1-P added (2×10^{-5} M)
Normals		
B	0·17	0·61
E	0·30	0·63
F	0·35	0·60
G	0·25	0·65
H	0·27	0·71
Heterozygotes		
2-father	0·08	0·25
6-mother	0·09	0·21
7-mother	0·16	0·35
10-father	0·19	0·44
10-mother	0·10	0·31
Homozygotes		
2*a*	0	0
2*b*	0	0
7*a*	0	0
9	0	0
10	0	0

* Incubation mixture contained 0·2 μmoles Gal-1-^{14}C-1-P (15,000 c.p.m. in liquid scintillation system), 0·4 μmoles UTP, 5 μmoles $MgCl_2$, 0·4 m.moles glycine buffer (*p*H 8·1), Glu-1-P at final concentration as indicated, and 0·3 ml. of freshly prepared 50 per cent hæmolysate with total volume of 1 ml. Incubation period was 30 min at 37°. Isolation of UDPHexose by charcoal adsorption was in accord with a previous paper (ref. 16).

† Net increase over control without UTP.

would be necessary to postulate an additional enzymatic defect in galactosæmia.

A more likely explanation can be based on the conditions of the assay. Isselbacher has pointed out that apparent UDPGalactose pyrophosphorylase activity can result from a sequence of reactions involving glucose-1-phosphate, UDPGlucose pyrophosphorylase and transferase[11].

$$\text{G-1-P} + \text{UTP} \xrightarrow[]{\substack{\text{UDPG}\\ \text{Pyrophosphorylase}}} \text{UDPG} + \text{PP}$$

$$\text{UDPG} + \text{Gal}^{*}\text{-1-P} \xrightarrow{\text{Transferase}} \text{UDPGal}^{*} + \text{G-1-P}$$

The labelled UDPGal* produced would be indistinguishable from that arising from the reaction:

$$\text{Gal}^{*}\text{-1-P} + \text{UTP} \xrightarrow{\substack{\text{UDPGal}\\ \text{Pyrophosphorylase}}} \text{UDPGal}^{*} + \text{PP}$$

Since UDPGlucose pyrophosphorylase activity was found to be present in the hæmolysates used in this investigation[13], controls containing added glucose-1-phosphate were included (second column of Table 2). The addition of glucose-1-phosphate had no influence on the assay with galactosæmia hæmolysates, but it led to a marked net increase of incorporation (that is, in the presence of UTP) of galactose-1-^{14}C label into UDPHexose both with normal and with galactosæmia heterozygote hæmolysates at a glucose-1-phosphate concentration of 2×10^{-5} M. These results suggest that the incorporation found reflects the presence of galactose-1-phosphate uridyl transferase and that it does not represent a separate UDPGalactose pyrophosphorylase activity in erythrocytes. The difference in values between the normal and the galactosæmia heterozygote groups further suggests that this is the case. An estimated native glucose-1-phosphate level of 5×10^{-4} M in erythrocytes[14] could account for the incorporation recorded in column 1 of Table 2.

The present results are concordant with Isselbacher's earlier statement concerning the lack of UDPGalactose pyrophosphorylase activity in erythrocytes. The formation of labelled UDPHexose on incubation of galactosæmia erythrocytes with galactose-1-^{14}C must rest on some other basis. It appears that the enzymatic block in galactosæmia is incomplete. Any other explanation for the presence of carbon label in UDPHexose (and DPGA) under the experimental conditions seems remote. On the basis of the data, it cannot be decided if galactose-1-phosphate uridyl transferase activity is greatly reduced or if a modification in the enzyme is involved.

Recently, Segal *et al.* have discussed the possibility that during childhood some galactosæmia patients acquire an ability to metabolize galactose[15]. The present findings suggest, however, that galactose-1-phosphate uridyl transferase activity may be present in small, but varying, amount in all galactosæmics, possibly from birth. The ability of erythrocytes of some galactosæmics to produce carbon dioxide from galactose appears to be an inherited rather than acquired character.

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REDUCTION IN RATE OF GROWTH OF HAIR IN MICE AS AN INDICATOR OF EXPOSURE TO CHRONIC LOW DOSAGE IONIZING RADIATION

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PRESENT-DAY biological criteria for assessing damage caused by chronic exposure to ionizing radiation include evaluation of one or more of the following: life-span shortening, mortality rate, cataract formation, hæmatological changes, incidence of tumours, and changes in certain enzyme activities[1–4]. Many of these changes develop slowly over long periods of time and require prolonged study of large numbers of animals. In this study observations are reported which indicate that, in mice, the rate of regrowth of hair after plucking is a sensitive, rapid and simple, dose-dependent biological index to damage caused by chronic, low dosage ionizing radiation.

CF No. 1 female mice which were 7–8 weeks old at the onset of irradiation were used in all experiments. Every time animals were taken out of the irradiation facilities for examination of hair growth, a suitable number of non-irradiated animals were removed from the next room to serve as controls.

Details of the chronic fast neutron and γ-radiation facilities, and the dosimetry for the various exposure positions within each facility, have been described previously[5,6]. In brief, the γ-exposures were given by means of a 10-c. cobalt-60 point source, and the fast neutron exposures were administered by using a 100-c. plutonium–beryllium line source. In the work described here, the animals in groups of 12 were exposed for 10 h each day (9 p.m. to 7 a.m.) in metal or plastic cages placed radially around the sources. The radiation exposure was initiated by elevating the source from its storage pit into the centre of the cage-array, and the duration of each exposure was controlled by appropriate timing mechanisms which, in turn, controlled the source-hoisting motors. The animals remained in the exposure facility during the 14-h period when the sources were shielded, since Health Physics surveys indicated that radiation exposure was negligible under these conditions. Food ('Rockland Laboratory Diet') and water were available at all times. The radiation-dose at each of the exposure positions in the γ facility was determined with Victoreen ionization chambers, which were calibrated against a radium standard and against a National Bureau of Standards calibrated chamber. Various chemical dosimeters were also used to verify the radiation dosimetry within this facility. The fast neutron dosimetry is based primarily on the specifications provided by the manufacturer, although additional measurements have been carried out with chemical dosimeters, with foils, and with a Hurst proportional counter. The γ-contamination within the fast neutron facility was estimated by using a neutron-insensitive chemical system, and proved to be negligible for this study.

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* Operated by the University of Chicago for the U.S. Atomic Energy Commission.

In all animals, a 2 cm × 1·5 cm area on the mid-dorsal region was epilated by hand-plucking to induce anagen, that is, the growing phase of the hair cycle. The animals were then observed daily until a uniform sheen of new hairs appeared on the skin surface, which ordinarily occurred after 8–10 days. A small area of uniform new hair growth on each animal was outlined with a coloured marker, and 6 days later the hair was again plucked by hand from within the demarcated area. 300–400 of the plucked hairs were then placed in a Petri dish and floated on a small amount of water. Under a dissecting microscope, the four hair types of the mouse as indicated in Fig. 1 could easily be identified[7]. Intact, awl hairs were isolated and picked up with ophthalmological forceps and arranged parallel to one another on a standard 3·25 × 4 in. lantern-slide glass plate. A 4 cm × 1 cm strip of translucent graph paper (Keuffel and Esser No. 359*T*-14*LG*) was also placed on the glass plate which was then covered with another plate of the same size. After taping the two plates tightly together, the resulting 'slide' was projected on to a screen giving an approximately 19-fold image enlargement.

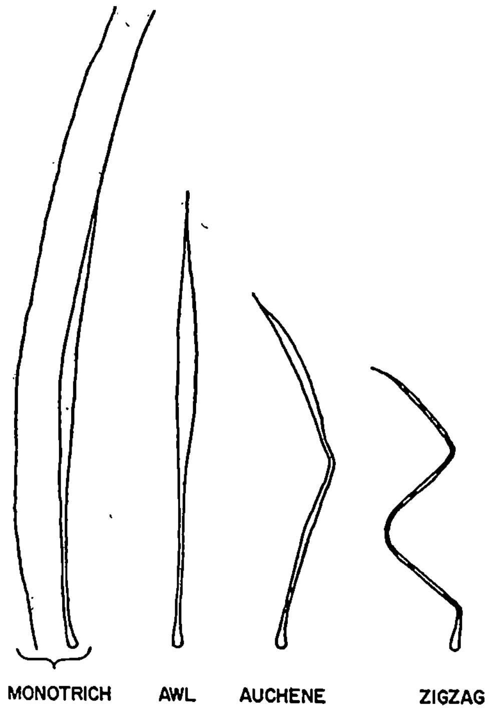

Fig. 1. Dry's four types of hair in mice

In the magnified images, hairs damaged slightly in plucking could easily be recognized. About 10 per cent of the mounted hairs were so damaged and excluded from measurement. For each animal, the lengths of 35 straight hairs with intact anagen bulbs were measured with a Swiss map plan measure (Charles Bruning Company Inc., Mount Prospect, Illinois, Catalogue No. 2375). Actual hair lengths were calculated by correcting their measured magnified lengths according to the magnification factor of the projected unit scale.

The animals irradiated with the cobalt-60 source received 31·3 r. daily doses of γ-radiation for varying periods of time. At the time of the first epilation, that is, 24 h after termination of irradiation, different groups of animals had accumulated total doses of 783–3,881 r. at the rate of 31·3 rads/day.

Table 1 shows that significant impairment of hair growth after induction of anagen is first observed after a total dosage of 1,064 r. has been accumulated, and increases in dose-dependent fashion at higher total dosage levels.

Table 1. Retardation of Hair Growth in Animals given 10-h Daily Doses of 31·3 r. γ-Radiation in the 10-c. Cobalt-60 Irradiation Facility

Group No.	No. of animals examined	Total dose of radiation received (r.)	Mean length of hair for group (mm)	Percentage retardation
GN-C	48	Nil	7·113 ± 0·036	—
G-I	8	783 (in 25 days)	7·033 ± 0·077	—
G-II	7	1,064 (in 34 days)	6·531 ± 0·061	8·1
G-III	7	1,314 (in 42 days)	6·045 ± 0·026	14·9
G-IV	4*	2,254 (in 72 days)	5·713 ± 0·054	19·6
G-V	7*	3,881 (in 124 days)	5·230 ± 0·030	26·4

* Three of 7 animals irradiated in Group *G*-IV and 7 of 14 in *G*-V died before completion of the experiment.

The animals irradiated with the 100-c. plutonium–beryllium source received varying doses (35–595 rads) of fast neutron radiation at the rate of 3·18 rads/day. Table 2 shows that, in these animals, the rate of hair growth is clearly retarded by fast neutrons at a total dose of 80 rads. From there on, the hair growth rates are progressively impaired as total dose increases.

Table 2. Retardation of Hair Growth in Animals given 10-h Daily Doses of 3·18 rads Fast Neutron Radiation in the 100-c. Plutonium–Beryllium Irradiation Facility

Group No.	No. of animals examined	Total dose of radiation received (rads)	Mean length of hair for group (mm)	Percentage retardation
GN-C	48	Nil	7·113 ± 0·036	—
N-I	9	35 (in 11 days)	7·081 ± 0·051	—
N-II	8	80 (in 25 days)	6·650 ± 0·042	6·4
N-III	8	117 (in 37 days)	6·529 ± 0·061	8·2
N-IV	7	229 (in 72 days)	6·228 ± 0·081	12·4
N-V	7	394 (in 124 days)	5·926 ± 0·023	16·6
N-VI	6	595 (in 187 days)	5·574 ± 0·034	21·5

Variations in length of hair in individual animals were always less than 1·5 per cent of the mean length and variations of the mean hair lengths between different animals in any one group were always under 1 per cent.

In the lower dosage ranges, where the general condition of the animals remained good, the relative biological effectiveness of chronic fast neutron radiation in retarding mouse hair growth was about 9 times that of γ-radiation. At the higher dose-levels this relative biological effectiveness was observed to drop sharply to about 4. The generally impaired nutritional state of these animals, together with smaller increments of hair growth retardation at these higher dosages, may account for this difference.

The quite substantial retardation of hair growth, at a dose-level as low as 80 rads of fast neutron radiation given in 25 days, is noteworthy in view of the absence of other gross signs of radiation damage in these animals. It is also of interest that microscopic examination of hairs at all dosage-levels studied failed to show dysplastic changes[8,9].

This work indicates that measurement of hair growth rates serves as an early, sensitive and simple, dose-dependent index to chronic low dosage radiation. Further studies of the duration and degree of reversibility of these hair growth effects might be of interest. In addition, manifestation of these effects can be recognized early, and large numbers of animals are not required for statistically valid data.

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EMPEROR PENGUINS AT CAPE CROZIER

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THIS article discusses recent changes in the colony of emperor penguins (*Aptenodytes forsteri* Gray) at Cape Crozier, Ross Island (77° 25′ S., 167° 50′ W.), in the Ross Dependency, Antarctica. The colony was discovered by Lieut. Skelton, of Scott's party, in 1902. Its position on sea ice at the foot of the Ross Barrier was recorded first by Scott[1] and later in more detail by Wilson[2]. The subsequent history of the colony has been summarized by Caughley[3] and Budd[4], who have also discussed reasons for changes in its status and success. My own censuses of the colony in 1961–63 suggest that the changes are more marked than earlier evidence indicated, and are closely linked with movements of the main Barrier cliff at Cape Crozier. These investigations are part of a long-term conservation programme sponsored by the University of Canterbury.

Table 1. EMPEROR PENGUIN ADULTS AND CHICKS AT CAPE CROZIER, 1902–63
Numbers of dead chicks are shown in brackets

Date	Chicks	Adults	Ref.
18.10.02	30 (80)	400	1 (490)
8.11.02	0	—	1 (490)
12. 9.03	150 (18)	1,000	1 (491)
2.11.03	30 (63)	400	2 (10)
20. 7.11	25–50	100	10 (39)
23. 9.57	100–270	700–800	3 (252)
26.11.58	523 (58)	618	3 (255)
25.10.61	1,325 (101)	Over 2,000	Stonehouse (in the press)
4.12.62	1,250 (30)	1,000–1,200	,, ,,
20.11.63	805 (12)	1,050–1,300	,, ,,

Table 1 summarizes counts of chicks and adults on the colony since 1902. Even in its present state of prosperity the colony is small compared with some of the more northerly ones[5]: during the first decade of the century it may have been close to extinction. Comparisons of counts from different years may be made only with caution. Adult numbers vary with time of year, being high during courtship (late May and June at Cape Crozier), low in incubation (July to September) when only males are present, higher again when the females return in August and September, and variable in spring and early summer when chicks are being fed, young non-breeding adults are present, and casual visits are paid by parents which lost egg or chick earlier in the season. The adults, which are highly social, enter and leave the colony in groups, assemble in parties away from the colony (where they may easily be missed in a rapid count) and tend to travel only on fine days. Some further difficulties encountered in censusing adult emperor penguins are described by Budd[4]. Chick counts are more reliable, especially toward the end of the breeding season (October to December) when counting is easy and mortality low. Their value as indicators of population size increases when figures for two or three consecutive seasons are known, as winter mortality of eggs and chicks can be high in certain seasons: thus at Cape Crozier in 1903, September and October blizzards killed more than two-thirds of the chick population[2]. A reasonably stable population should, during a number of years, provide a consistent upper level of chick numbers late in the season, representing a minimal value for the number of breeding birds in the colony. Ideally, both chicks and adults must be counted as accurately as possible over a number of years before the true status of a colony can be determined.

Cape Crozier can now be reached in 30 min by helicopter from New Zealand and American bases in McMurdo Sound, and the Canterbury unit visits each spring for counting. In fine weather (a prerequisite of a flying visit) the assembly of chicks is herded gently into a slowly moving column and counted by at least two observers; a confirmatory count is taken as the chicks return to their original site. The mean value is likely to be accurate within 2–3 per cent. Dead chicks and abandoned eggs are also counted, but others may remain undetected under snow drifts below the ice cliffs. Adults are counted on the ground and from the air, but their dispersal over several square miles of sea ice may lead to counting errors of up to 20 per cent. Interesting comparisons with earlier accounts of the colony are already emerging from these brief annual visits.

When first described in 1902, the colony was very small and situated on sea ice under the high, seaward-facing cliff of the Ross Barrier. A shallow bay in the cliff offered some protection from southerly and south-easterly winds, but the colony site was open to the north and vulnerable to the disrupting influence of swells with a northerly component. The 1902 position appears in Skelton's photograph[6], and details of the junction between ice cliff and Ross Island appear in Debenham's report (ref. 7, map II inset), from which Fig. 1 of this article is taken. New sea ice fills the foreground of Skelton's photograph. The small number of chicks and disproportionately large number of adults present on the colony in both 1902 and 1903 were noted by Wilson[2]; his interpretation, that only one in ten of the breeding birds laid each year, has not received support from observations in other colonies[8,9]. The position of the breeding group at the edge of older sea ice suggests that a break-out in winter had already removed some of the breeding birds and chicks, and many of the bystanders were therefore likely to be bereaved adults whose eggs or chicks had drifted away and perished. Budd's[4] estimate of an original breeding population of 500–1,000 pairs covers our scant knowledge of the movements of adults in these circumstances. Assuming that each chick in September 1903 was accompanied by a single parent, and that the remaining adults represented three-quarters of the bereaved parents, a breeding colony of some 700 may be postulated for that year. The 1902 population is unlikely to have differed by more than 5–10 per cent, so that catastrophes involving between 75 and 95 per cent of the annual crop of eggs and chicks must have occurred between June and October of both years.

There is more direct evidence for a similar catastrophe in 1911. During the intervening years the ice cliff advanced more than a quarter of a mile at Cape Crozier (Fig. 1), eliminating the shallow bay and leaving the colony more than ever exposed to the sea and to the disrupting effect of swells. From his July visit, Wilson[10] concluded that the ice supporting the colony had already broken and re-formed, probably with the loss of part of the annual crop of eggs. The three eggs collected during his visit yielded embryos which were estimated by Glenister[11] to have been laid about the beginning of July, close to the peak period of laying postulated by Stonehouse[8] for this colony.

Thus the early position of the colony under the face of the Ross Barrier appears to have been precarious in the extreme. It is doubtful if the colony could for long have maintained its numbers in these circumstances, and the population may well have declined between 1902 and 1911. Its position before 1902 cannot be determined with any certainty, although the disparity between numbers of chicks and adults in that year suggests that breeding success had been higher in the immediate past. Wilson[6] compared the ice cliff with that shown in Bernacchi (ref. 12, plate 17), taken in 1899, and directed attention to remnants of shelf ice under the cliffs at Cape Crozier; the two pieces of evidence indicated to him that the barrier had recently retreated and the small bay of 1902–03 was newly formed. Bernacchi's map[12], based on Admiralty charts, certainly shows the end of the Barrier in a position well to the north of the cliffs at Cape Crozier, but the scale is small and its accuracy cannot be assumed. Colbeck's chart from the same expedition[13] shows the Barrier starting well to the south of 'Cape Crozier', although the outline of the land cannot be reconciled with modern maps.

The colony was visited by Balham in 1957 and Caughley in 1958. Both observers found it more favourably situated, in a deep enclave of sea ice behind the main cliff of the Ross Ice Shelf[3]. Many more chicks were present than on any of the earlier visits. Caughley estimated that the shelf had advanced two miles since 1911. The western end of the Barrier, which in 1903 lay to the south of the Knoll (a prominent point overlooking the colony site) and in 1911 pressed hard against the steep cliffs of Cape Crozier, had by 1958 passed north of the knoll and developed a prominent east–west split, partly separating a large frontal block from the main body of shelf ice. The advance has continued to the present day, and a second split or enclave has developed. Fig. 2, based on a recent map (N.Z.M.S. 175/16, 1962), shows the position of enclaves and colony in 1960 and 1961. The 1962 and 1963 positions of the colony were similar, and correspond with the position shown by Caughley for 1958. The more southerly of the two enclaves, which was not present in 1958 (Caughley, personal communication), may have widened slightly since 1961. The Barrier face now lies some 2·75 miles north of the Knoll, marking a total advance of nearly four miles from the 1902 position.

Since 1958 (and possibly some years before) the colony has formed each year under the north-facing cliff of the wider enclave, where it finds shelter from prevailing southerly and south-easterly winds. An interesting feature is its annual movement away from the sea during the incubation or guard stages, as shown by a succession of guano-stained patches on the smooth sea ice. In 1958 Caughley noted that the nucleus of the colony had moved in stages some 1,500 yards westward from its original site by late November, and a similar succession of sites was apparent in each of my visits in 1960–63. Thus the birds retreat toward the apex of the triangular enclave, possibly on to firmer ice which is less likely to disperse before December or January. Similar movements of a breeding colony have been described by Prévost[14] at Pointe Geologie.

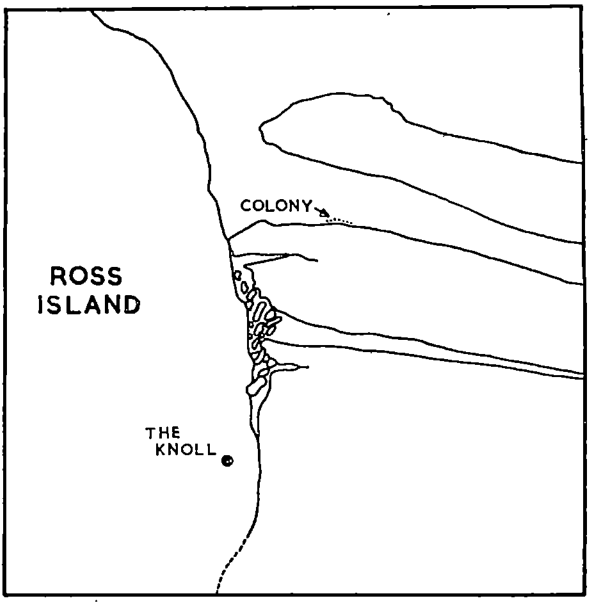

Fig. 2

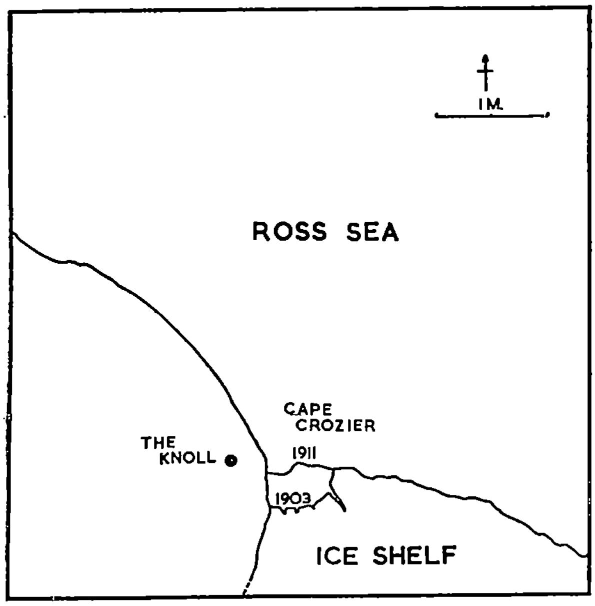

Fig. 1

In comparison with its situation earlier in the century, the colony now enjoys better protection from blizzards and a much longer period of stability late in the breeding season, as the sea ice is unlikely to disperse from the enclaves before mid-December. It is also completely protected from northerly swells early in the breeding season, thus avoiding the possibility of heavy losses through dispersion in June and July. Although sea ice north of the Barrier can probably be dispersed by wind and swell at any time of the year, ice within the enclaves is unlikely to suffer more than slight compression and tension from movement of the shelf blocks on either side. Its surface in early summer is usually buckled, but extremely solid and stable. Snow drifts build up in the lee of the cliffs, but the main spread of sea ice in the enclaves remains remarkably snow-free.

Observations at other colonies have shown that, at least toward the northern edge of their range, chicks are able to complete their moult before the group disperses[5,9], and both Caughley and Budd have questioned Wilson's[2] suggestion that chicks from Cape Crozier complete their moult after the dispersal of the sea ice in summer. However, it is clear that events in the breeding cycle at Cape Crozier occur about a month later than at the more northerly colonies; thus chicks at Pointe Geologie begin their moult early in November, while those at Cape Crozier show the first indications in late November or early December (Caughley in ref. 5 and personal observation). In 1962 at Cape Crozier only about 5–10 per cent of the chicks had lost down from their flipper tips by December 4; more than 50 per cent (including many only half to two-thirds grown) would not have completed their moult before late January or early February. In 1958 open water extended far down the enclave by mid-December, and it seems unlikely that firm ice would normally remain *in situ* after mid-January at the latest. Emperor penguin chicks, like those of king penguins[15], may be able to complete their moult long after their parents have left them, and large, well-fed chicks would not necessarily be at a disadvantage if their colony drifted out to sea in December or January. I see no reason, therefore, for discounting Wilson's suggestion on present evidence.

The census figures for 1961–63 indicate that the Cape Crozier colony is now enjoying a higher breeding success than at any time in its recorded history, and that the

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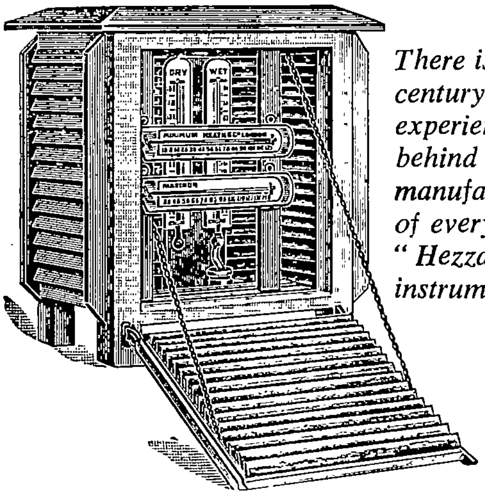

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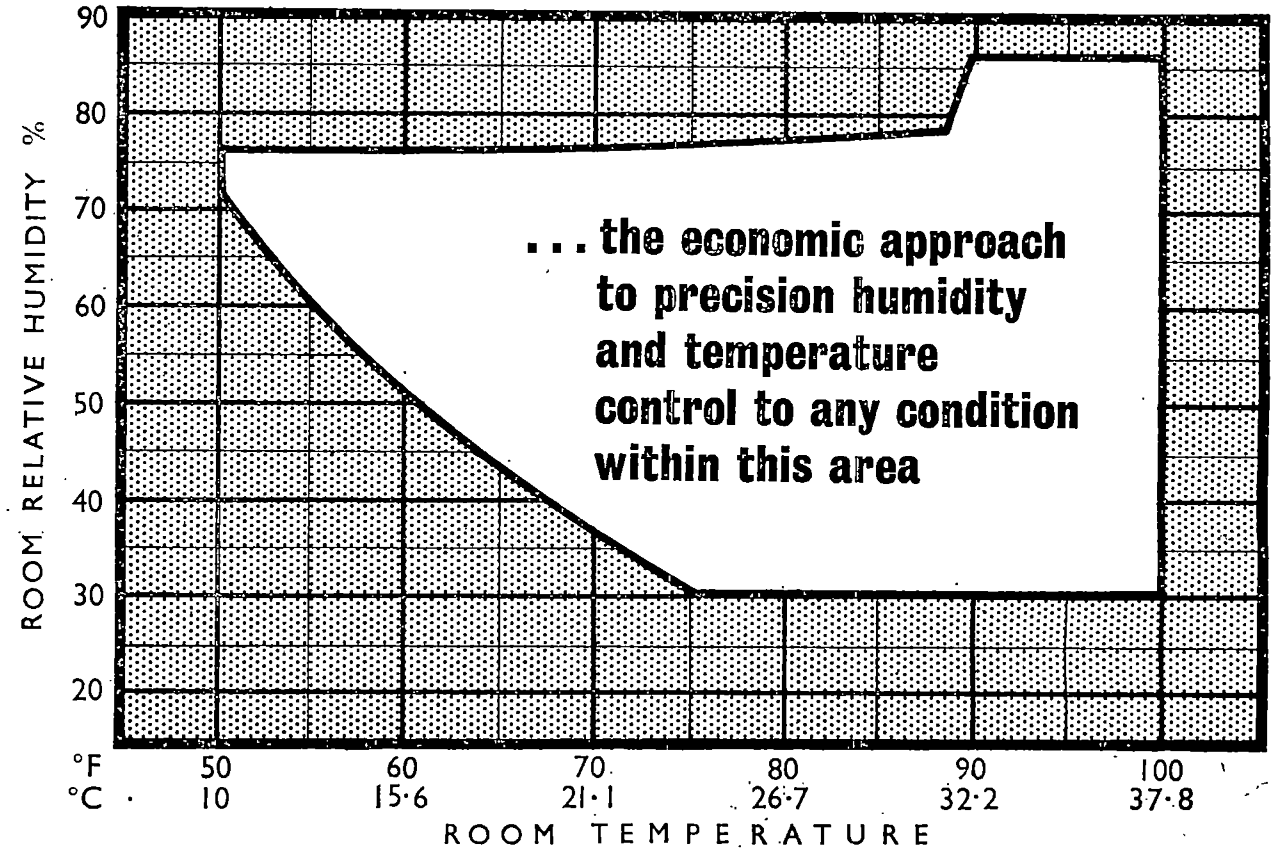

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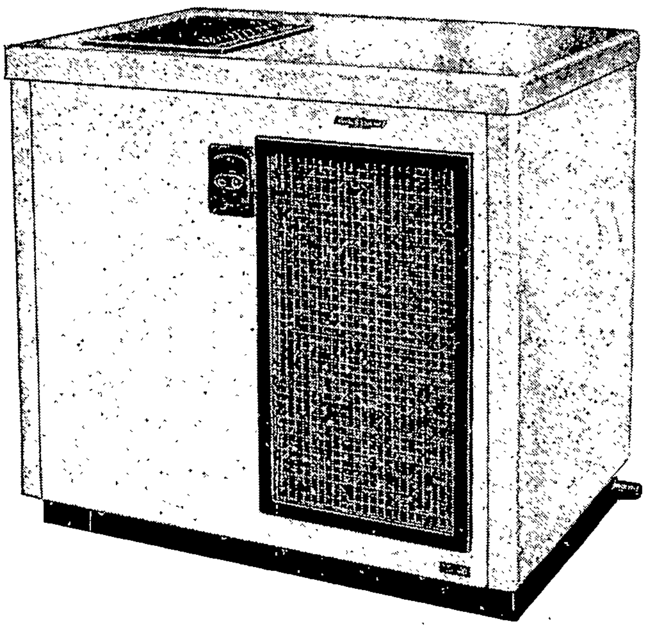

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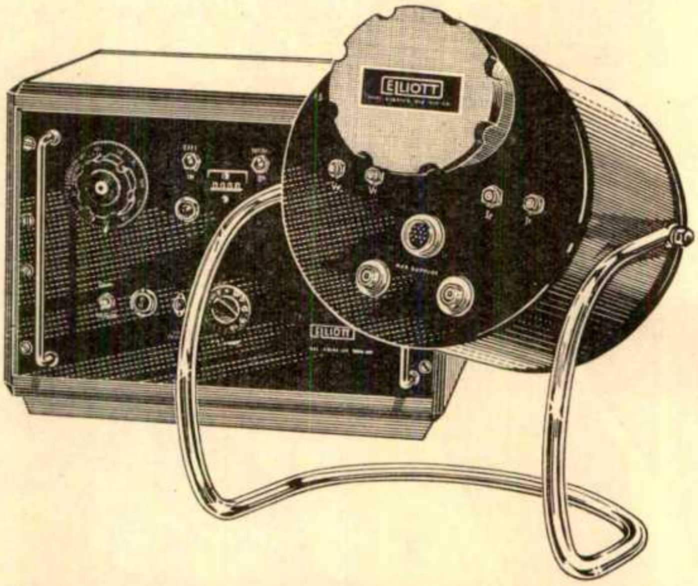

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breeding stock has also increased considerably in the past few years. At least 1,450–1,500 pairs bred on the colony in 1961 and 1962, and a similar number may have laid in 1963, suffering higher mortality of eggs or chicks. The colony is visited frequently by members of New Zealand and United States expeditions in McMurdo Sound. While there is no evidence to suggest that normal helicopter movements within the area affect the colony adversely, low-flying aircraft early in the season could inflict serious losses by raising panic in incubating or brooding birds; recommendations are therefore being made against casual visits before the end of October, and flying regulations to cover the remainder of the season are being drafted for submission to New Zealand and American authorities. Plans for future investigations include a programme of chick banding, and reconnaisance flights late in the year to investigate the dispersal and breeding success of the colony.

I thank Prof. G. A. Knox (Department of Zoology, University of Canterbury) for his advice, T. Jacobs, O. Sutherland and I. Spellerberg, who helped with observations, and the Research Committee of the New Zealand University Grants Committee for financial support. Field help was most generously given by *VX* 6 Squadron, U.S. Navy, and the good offices of the Antarctic Division, Department of Scientific and Industrial Research (Mr. G. W. Markham), and the Ross Dependency Research Committee are gratefully acknowledged.

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SIMILAR EFFECTS ON TOMATO PLANTS OF *Azotobacter* INOCULATION AND APPLICATION OF GIBBERELLINS

By Dr. R. M. JACKSON, MARGARET E. BROWN and SUSAN K. BURLINGHAM

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INOCULATION of seeds or seedlings of several kinds of crop plant with *Azotobacter* cultures can increase yields significantly[1–3]. Earlier flowering of wheat following *Azotobacter* treatment has also been observed by us and other workers[4,5]. Experiments with tomato (*Lycopersicum esculentum*) have now shown that, although the final yield of fruit may not be affected, *Azotobacter* treatment advances early growth and flowering.

Shaken liquid cultures of *Azotobacter chroococcum* were grown for 14 days in a nitrogen-deficient medium with sucrose as the carbon source (no sucrose remained at the end of the incubation period). Tomatoes, variety 'Money Maker', were treated either by allowing the seed to imbibe in the undiluted culture for 1 h before sowing in unsterilized potting compost, or by dipping the roots of seedlings into the culture before transplanting. Control seeds or seedlings were treated with nitrogen-deficient medium without added sucrose.

During growth, the plants were given nutrient solution at fortnightly intervals. They were graded for leaf number and length, height to growing point and first flowering truss, and for flower and fruit development, fruit ripening and yield. Developmental curves were constructed for each flowering truss by assigning a value to each stage of flowering and plotting the total score against time. Fig. 1 shows flowering curves of inoculated and control plants.

Seed or root inoculation significantly accelerated internodal stem elongation and leaf development during the first weeks of growth (Table 1), but as the first truss developed these effects became less. Treated and control plants produced the same number of leaves before the first flower buds appeared. Flower and fruit formation on the first truss were advanced by a week or more (Fig. 1), but the fruits neither ripened faster nor did their final yields differ from those of untreated plants. The second and third trusses developed sooner on treated than on untreated plants but by only 2–4 days. Four additional weekly applications of *Azotobacter* culture to plants grown from treated seed had no further effect.

These developmental effects closely resemble those resulting from application of gibberellins to tomato plants. Rappaport[6] found that single applications of 2·5–50 μg of a mixture of gibberellins 1 and 3 to the first expanding leaves increased internode length during early growth. Repeated treatments with small doses produced no further

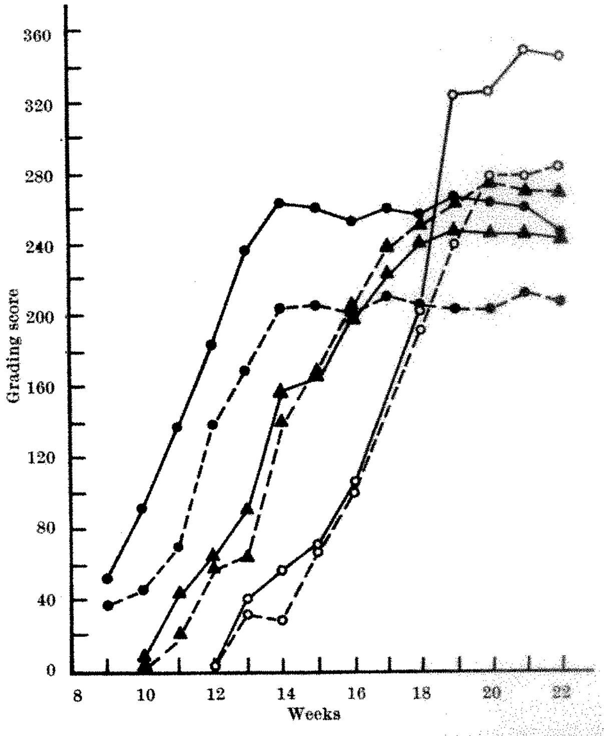

Fig. 1. Development of three trusses of tomatoes treated with *Azotobacter* (——) or control medium (– – –). ●, First truss; ▲, second truss; ○, third truss

Table 1. PERCENTAGE INCREASE IN INTERNODAL LENGTH AND LEAF SIZE OF TOMATOES INOCULATED WITH *Azotobacter* (*Az*) OR TREATED WITH GIBBERELLIC ACID (GA_3)

	Leaf number	Root treatment *Az*	Root treatment GA_3	Seed treatment *Az*	Seed treatment GA_3
Internodal length	2	—	—	—	—
	4	—	—	38·7*	25·2*
	6	20·8*	29·2*	13·5	11·3
	8	8·6	10·4	—	—
	9	—	—	9·4	8·9
	10	4·4	6·1	—	—
Leaf size	2	15·7*	22·5*	8·1*	17·3*
	4	—	—	20·0*	9·6
	6	2·6	24·6*	6·7	6·2
	8	6·2	11·2*	—	—
	9	—	—	11·9	7·3
	10	2·6	6·7	—	—

* Denotes significance at 5 per cent level.

effects, but with more than 25 μg per plant leaves often became abnormal. Flowering was hastened by 3–6 days without affecting the number of nodes; fruit set was rapid but ripening was poor.

Because of these similarities we compared *Azotobacter* inoculation and treatment with gibberellic acid. Gibberellic acid was applied to the seeds or roots of 5·0, 0·05 or 0·005 μg per plant. Table 1 shows that the early growth effects on tomatoes from 5 μg gibberellic acid and from *Azotobacter* inoculation were similar, except that 5 μg gibberellic acid produced leaf abnormalities and *Azotobacter* treatment did not. Smaller amounts of gibberellic acid had no significant effect. Most data on the effects of gibberellic acid come from treatments applied to growing plants. A report of seed treatment[7] suggested that concentrations below 5·0 per cent do not affect tomato growth; 5 μg per seed, which produced significant effects in our experiments, was equivalent to a 0·3 per cent concentration of gibberellic acid. The small effects produced by 0·05 μg gibberellic acid were not statistically significant.

Gibberellic acid and indolyl-3-acetic acid have been detected in *Azotobacter* cultures[8,9]. Cultures of the *Azotobacter* strain used in our experiments were assayed for both these growth regulators. Indolyl-3-acetic acid was found in amounts that increased from 0·05 μg/ml. in a 2-day-old culture to 1 μg/ml. in a 30-day-old culture. The method of extraction and chromatography described by Katznelson *et al.*[10] also detected gibberellins in small quantities, which increased with increasing culture age. Chromatograms were cut into sections and eluates from these tested for gibberellin-like substances on dwarf pea plants[11]. Internodal extension was significantly accelerated by eluates from sections of the chromatogram corresponding to the marker gibberellic acid, also by eluates from an unknown substance at R_F 0·85–0·9 in isopropanol/ammonia/water (10 : 1 : 1). The latter substance gave a pale green fluorescence after spraying with 3 per cent methanolic sulphuric acid[12]; the gibberellic acid spot from the *Azotobacter* cultures did not fluoresce because this method will not detect less than 5 μg.

Three mechanisms have been proposed to explain the effects of *Azotobacter* on plant growth: (1) nitrogen fixation; (2) antagonism towards pathogenic or toxin-producing soil organisms; (3) the production of growth regulators in the rhizosphere. Our results provide some evidence for the third mechanism, although it has yet to be determined whether the effects described are caused by the gibberellin-like substances formed by *Azotobacter* in the culture fluid, or in the rhizosphere, or by other micro-organisms that produce growth-promoting substances and are stimulated to grow by *Azotobacter*. There is no evidence that indolyl-3-acetic acid causes the growth effects we describe.

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SELECTION FOR HOST PREFERENCE IN *Anopheles gambiae*

By M. T. GILLIES*

East African Institute of Malaria and Vector-borne Diseases, Amani, Tanganyika

IN certain parts of Africa, notably Swaziland and Southern Rhodesia, the application of residual insecticides to houses has led to an apparent change in behaviour of the main vector of malaria, *Anopheles gambiae* Giles, from resting indoors and biting man to resting outdoors and feeding on cattle or other animals. Two explanations have been advanced for this situation: (*a*) that two sibling species of the *A. gambiae* complex may originally have been present in the area, one of which was eliminated owing to its domestic habits and consequent greater opportunity of coming into contact with chemically treated surfaces; (*b*) that there has been a change in behaviour within a single species due to selection by the insecticide[1]. Active research into the validity of the first possibility is going on at present, the available evidence suggesting that in this area, at least, there has been a replacement of one form of the *A. gambiae* complex by another[1]. However, the possibility of the occurrence of true behavioural changes within a single form of the species cannot be ignored, and with this object in mind an attempt was made to alter the feeding preferences of the species by artificial selection in the laboratory.

It should be stressed that the preference shown by a particular species of mosquito for one mammalian host or another is likely to be strongly influenced by environmental conditions. In experimental work, particularly, it is obvious that the manner in which the hosts are presented can profoundly influence the feeding responses of hungry mosquitoes. Because of this, only comparative data can be obtained and no special significance should be attached to the actual proportion feeding on one host or the other in a particular set of experiments. Furthermore, to obtain reliable information it is necessary to compare the behaviour of two strains of mosquito simultaneously. This avoids the effect of temporary or secular changes in the relative attractiveness of the two hosts as well as of other uncontrollable elements in the experimental set-up.

Selection experiments were carried out at Muheza in the coastal lowlands of northern Tanganyika in 1962–63, starting with eggs from a pool of wild-caught females. *A. gambiae* is known to be highly anthropophilic in this area, cattle and other domestic animals being largely absent[2,3]. No treatment of houses with residual insecticides had been carried out at any period. Previous observations had shown that form *A* of *Anopheles gambiae* (= Group *A* of Davidson and Jackson[4], Species *A* of Paterson[5]) was present in the district. As described

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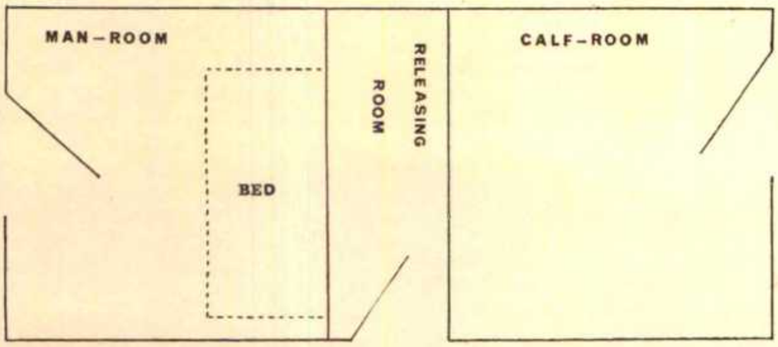

Fig. 1. Diagram of experimental hut used for selection experiments with *Anopheles gambiae*

here, it was confirmed at the end of the experiments that only one form of the complex was involved.

The technique adopted was based on the experiments of Hadjinicolaou[6], working in Southern Rhodesia, who had found that when *A. gambiae* females from different sources were released at night inside a large mosquito net containing a human volunteer and a calf, consistent differences in the feeding behaviour of the two strains could be detected. In the work recorded here the method was modified by using a specially built experimental hut of mud and thatch, containing two separate, partly communicating rooms in which the baits slept. The lay-out of the hut is shown in Figs. 1 and 2. The outside walls were 6 ft. 6 in. high, ventilation being provided by open eaves 4 in. high all the way round, which were screened off with netting to prevent the escape of mosquitoes. The dividing walls of the releasing room were of mud up to a level of 4 ft., the space above that being closed with netting except for a gap of 4 in. at the top which was left open to provide the mosquitoes with free access to both the other rooms. The whole hut was fitted with a cotton ceiling to prevent them flying up into the thatch. One room contained a bed on which an adult man slept, while the other was provided with a bedding of dry grass for the calf.

The two strains of mosquitoes used in each test were marked with coloured dusts before release so as to make possible their subsequent identification. They were introduced into a cage with a sliding lid and placed in the releasing room during the course of the afternoon. They were released at about 9 p.m. local time after both baits had settled down for the night. The sleeper wore shorts and a shirt and was allowed to cover himself with a thin cotton sheet, previous experience having shown that this covering did not prevent hungry mosquitoes from feeding. The insects were caught by hand in the morning after the baits had left, and the numbers of gorged specimens collected in each room recorded. The validity of the method, as a means of assessing host preference, depended on the assumption that the mosquitoes had actually fed on the occupant of the room in which they were caught and not simply flown across from the other room. Precipitin tests, done on stomach smears of females that were not used for breeding purposes, showed that 6·8 per cent of 369 mosquitoes from the calf-room had fed on man, while 2·3 per cent of 709 from the man-room were positive for ox blood. Assuming that both lines showed the same degree of mobility, it follows that the true total of females feeding on man was something like 4·5 per cent higher than the figures given in Tables 1 and 2 suggest. Mixed feeds amounted to less than 0·5 per cent of the total tested. Thus relatively little movement was occurring, and it may be concluded that the room catch gave an accurate picture of the feeding preference of the mosquitoes under test.

Only those caught in the man-room or calf-room and with fresh blood in their stomachs were retained for egg-laying. An outdoor insectary was built adjacent to the hut. This was divided into two separate sections, one for each selection line, each completely screened and entered by a separate door from the exterior. At the beginning of the experiments eggs from females caught in houses from the surrounding district were obtained, and the females that emerged from them were released in the experimental hut. This represented the parental generation. On recapture they were divided into parental-man and parental-calf strains, according to the room in which they had been caught, and returned to the insectary for egg-laying. The eggs they laid represented the F_1 generation of the two lines, the adult females of which were then released and treated in the same way. In each generation the mosquitoes were tested as soon after emergence as possible, usually when 1–3 days old, although older females were occasionally used. During this period they received sugar-water only.

Two series of experiments were conducted, 6–7 generations being reared and tested in the first and 4 in the second. Each series ran for a period of 5–6 months, since, although each generation only took 2–3 weeks to come through, difficulties were encountered in rearing the larvæ in adequate numbers, so that it was necessary to get repeated egg-batches from the same females after testing. The calf strain was rather more refractory in this respect, and in both series the man strain tended to get a generation ahead of it. Consequently it was not always possible to compare the behaviour of the same generation in the two strains.

The results of the experiments are shown in Tables 1 and 2. In order to simplify the tables only the percentage of those caught in the calf-room is shown separately. 4·1 per cent of the overall total of fed females were collected in the releasing room. Routine precipitin tests were made on all of these throughout the first series, and those positive for ox blood (about one-third of the releasing-room catch) have been added to the appropriate calf-room catches shown in Table 1. It will be seen from the results that by the F_2 a well-marked difference between the two lines had developed in the proportion caught in the calf-room. This was maintained during tests on the F_3. At this stage selection of the calf line was relaxed for a generation so as to use all the females for building up the numbers to an adequate level. When selection was resumed with the F_5 it was found that the difference in behaviour had virtually disappeared. However, in the F_6 a clear-cut difference had again developed between the two lines. It will also be seen

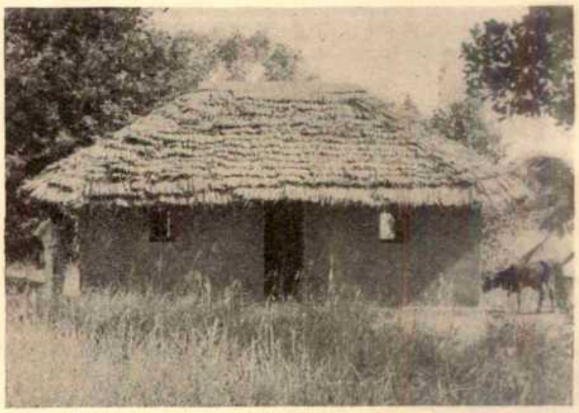

Fig. 2. Experimental hut used for host preference experiments with *Anopheles gambiae*

Table 1. Results of Simultaneous Releases of Two Selection Lines of *A. gambiae*, First Series of Experiments

Strain	No. of replicates	No. of fed mosquitoes	Per cent in calf-room*	Strain	No. of replicates	No. of fed mosquitoes	Per cent in calf-room*	χ^2	P†
F_1-calf	13	231	32·2	F_1-man	13	235	35·3		
F_2-calf	17	168	43·4	F_2-man F_3-man	15 2	284 55			
					17	339	25·1	17·7	< 0·001
F_3-calf	14	216	48·6	F_2-man F_3-man	2 12	53 264			
					14	317	22·1	41·0	< 0·001
F_4-calf	Not tested								
F_5-calf	19	312	23·7	F_4-man F_5-man F_6-man	6 5 8	118 111 205			
					19	434	19·7	1·64	0·2
F_6-calf	7	150	28·7	F_5-man F_6-man F_7-man	1 4 2	34 84 54			
					7	172	16·8	6·43	< 0·02
Parental	8	183	45·4	F_4-man	8	140	31·4	6·45	< 0·02
Parental	5	111	30·5	F_5-man	5	162	18·5	5·39	0·02

* This figure includes small numbers of calf-fed females caught in the releasing room.
† Estimated for the differences in behaviour between the two lines for each generation tested.

Table 2. Results of Paired Releases of Two Selection Lines of *A. gambiae*, Second Series

Strain	No. of replicates	No. of mosquitoes	Per cent in calf-room	Strain	No. of replicates	No. of mosquitoes	Per cent in calf-room	χ^2	P†
F_1-calf	18	262	17·6	F_1-man	18	402	12·4	3·36	> 0·05
F_2-calf	17	263	40·0	F_2-man F_3-man	13 4	315 107			
					17	422	34·4	2·16	> 0·1
F_3-calf	8	132	47·0	F_3-man F_4-man	6 2	181 41			
					8	222	54·1	1·7	0·2
F_4-calf	5	112	58·0	F_3-man F_4-man	1 4	38 94			
					5	132	34·1	14·0	< 0·001
Parental	5	131	16·8	F_5-man	5	162	16·0	—	—

† Estimated for the differences in behaviour between the two lines for each generation tested.

that there were significant differences in behaviour between the F_4 and F_5 of the man strain when tested against the parental stock. In a second series the difference in behaviour only became apparent in the fourth generation, on which only 5 replicate tests could be made before the experiment had to be terminated. However, it may be noted that in all 5 releases the calf strain showed a relatively higher proportion feeding on the calf than did the man strain. Thus, taking both series together, it is clear that within a very few generations it was possible to select out strains that differed significantly in the preference shown for feeding on man rather than on a bovine.

At the end of a series of experiments both lines were crossed with each other and the hybrid males that resulted were found to be fully fertile. Thus it appeared that both selection lines belonged to the same mating group. Further tests showed that the man line was fully interfertile with the 'Kisumu' laboratory strain, and without grossly distorted sex-ratios in the hybrids, and so belonged to Group *A* of the *A. gambiae* complex[4].

A number of points should be raised in connexion with these results. First, the fact that the feeding preferences of both lines often varied in the same direction from one test to the next, or from one generation to the next, provided ample evidence of the sometimes overriding importance of external factors, of which temperature and wind direction were probably the most important. Secondly, since the heritable component of host selection might be relatively slight compared with environmental effects under experimental conditions, it follows that negative results in a particular series of experiments would be of little significance. Thirdly, a difference in behaviour between the two lines was elicited after surprisingly few generations, despite the fact that selection could only be applied to the females. It is concluded that the experiments described give support to the view that natural populations of the form of *Anopheles gambiae* studied show some degree of polymorphism for host preference, thus providing a basis on which natural or artificial selection for behavioural changes can operate.

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LETTERS TO THE EDITOR

PHYSICS

Theory of Striations in Gas Discharges

So far, a satisfactory theory for the well-known phenomenon of striations in gas discharges has proved elusive[1]. This communication delineates the basic elements of a theory for the stationary striation with a fuller account to be offered later encompassing the moving variety as well[2]. The central mechanism has been provided in relation to the manifestation of Liesegang rings[3], which represents the underlying regularity producing repetitions of nodes and antinodes proposed earlier by Morgan[4]. While the two physical phenomena entail entirely different processes, it happens that the analytical formalisms are very much alike. Indeed, the non-stationary striation has no equivalent in the formation of periodic precipitates, involving as it does much more complex processes.

A signal outcome of the present theory is the explanation of Goldstein's law[5] relating the spacings of the stationary striations l with the ambient pressure p:

$$l \propto p^{-m} \qquad (1)$$

where m is very nearly 1/2. In an analogous development of the governing Poisson equation as for the Liesegang phenomenon[3], it can be shown that the spatially periodic behaviour of the potential as well as the electron distribution entail the vital Debye-length λ_D so that the spacing between striations obeys:

$$l \propto \lambda_D, \; \lambda_D^2 = \frac{kT}{4\pi n q^2} \qquad (2)$$

where all quantities have their usual meaning. Hence, it is more sensible to associate the distance between striations with the carrier density n, that is, $l \propto n^{-1/2}$. The temperature dependency is somewhat involved for partial ionization but can be described in terms of the Saha equation; for complete ionization a $T^{1/2}$ dependence arises.

The connexion between l and the pressure itself is not as direct since it very much depends on the nature of the gas and its degree of ionization. For simple gases like hydrogen, it may be seen that:

$$P = (n_n + 2n)kT \qquad (3)$$

where n_n denotes the number of neutral atoms per unit volume and $n = n_i = n_e$, n_i and n_e being the related concentration of the ions and electrons, respectively. In the limit of a strongly ionized system, $n_n \rightarrow 0$ and P is just proportional to n, whereby it follows that $m \sim 1/2$, but not precisely so for departures from complete ionization.

Since striations have been customarily observed in weakly ionized gases, it may be well to assess the question of incomplete ionization more fully. For simple systems such as:

$$A \rightarrow A^+ + e \qquad (4)$$

let N be the total neutral particle density which admits:

$$n = \frac{P}{kT} - N \qquad (5)$$

From equation (2), it follows that:

$$\lambda_D^2 = \frac{kT}{4\pi q^2 \frac{P}{kT}\left(1 - \frac{NkT}{P}\right)} = \frac{(kT)^2}{4\pi q^2 \, . \, P}\left(1 - \frac{P_0}{P}\right)^{-1}, \quad P_0 = NkT \qquad (6)$$

Since $1 > P_0/P > 1/2$ over the entire range of ionization:

$$l \propto \lambda_D \simeq \frac{kT}{2\sqrt{\pi}\, q P^{1/2}\left(1 - \frac{1}{2}\frac{P_0}{P}\right)} \qquad (7)$$

which accounts for slight departures of m from 1/2 even for weakly ionized gases; the formula has no meaning for $P = P_0$.

Clearly, it is far simpler to relate the spacings of the striations directly with the electron density, and in this regard the spacings can be either too large or too small for visual observation. In discharges having an ambient temperature somewhat below 1,000° K, an electron density of $n = 10^{14}$/c.c. would have striations spaced a fraction of 1 mm apart. At $n = 10^{20}$/c.c., the expected separation would be about 10^{-7} cm. In any event, intermediate ranges of electron density are essential for ready detection of the striation phenomenon.

It has been tacitly supposed that the periodic luminous appearance of the discharge somehow reflects the spatial fluctuations of the electron density. Without necessarily entering into the details of the recombination process ultimately leading up to the emission of radiation, at some stage associated with the release of photons:

$$\frac{dn_i}{dt} = \frac{dn_e}{dt} = -k n_i n_e \qquad (8)$$

Hence, since the photon production relates to an intensity

$$I \propto \frac{dn_i}{dt} \qquad (9)$$

a periodic variation in I will result so long as n_e itself is periodic.

Perhaps it should be mentioned that the reaction rate equation (8) may not merely be representative of volume recombination but also of the radiative step in the three-body mechanism recently discussed by D'Angelo[6]. The latter appears more favourable than the dissociative recombination process formerly viewed as dominating over direct volume recombination.

A further interesting aspect is next noted regarding the distinctness of the striations in terms of the electron density. The presence of a large number of electrons tends to enhance light output while simultaneously providing a greater variation of intensity across the width of a striation. But as already mentioned, high concentrations of carriers produce a bunching or crowding of the striations. Thus the periodic glow of a discharge is optimizable.

As for the Liesegang phenomenon[3], non-linear effects may arise in which strict periodicity of the striations fails to occur. Our present description applies in the linear limit. The non-linearity will be covered in a more comprehensive development incorporating the non-stationary behaviour[2].

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A Hydrogen Singing-flame Ionization Detector

The sensitivity of an ordinary hydrogen flame ionization detector to hydrocarbons has been found to be two to three times higher when the detector flame 'whistles' or 'sings'. Further investigation of this phenomenon has disclosed the probable reasons for the enhanced sensitivity and has uncovered other useful characteristics of the singing-flame detector. 'Singing' hydrogen diffusion flames were first noted more than 160 years ago[1], shortly after the discovery of hydrogen. A number of investigators[2,3] have since investigated the characteristics of such flames; however, no practical application of this unusual phenomenon appears to have been made.

A drawing of a hydrogen flame ionization detector of the type used in the present investigation is given in ref. 4. However, the rod electrode was replaced by a cylindrical platinum gauze electrode surrounding the burner tip in order to improve the ion-collection efficiency. Sample gases containing known concentrations of propane in nitrogen were used for calibration. With the optimum hydrogen and air flow rates, the ionization detector in both the singing and quiescent stages gave a linear response up to at least 15,000 p.p.m. propane in nitrogen. However, the ion current from the singing flame was 2·7 times higher than with the normal detector flame over the entire range of propane concentrations.

Of primary interest is the reason for the greatly enhanced sensitivity of the detector when resonating. Time exposure photographs of our detector flame showed it to be spherical when quiescent and elongated when singing. In 1866, Töpler[5], using stroboscopic methods, found that the singing hydrogen diffusion flame was, in fact, an oscillating flame. Drawings of his observations are given by Gaydon and Wolfhard[6]. In brief, Töpler's work showed that each oscillation was caused by a small flame at the burner orifice growing into a tongue of flame which separates from the burner tip and burns out; a small flame remains in the burner orifice for the next cycle. The output from our singing-flame detector was shown on a cathode ray oscilloscope to have a periodic waveform with a frequency of about 1,600 c/s, the same as that of the emitted note. Higher ion production in the singing flame, therefore, probably results because the flame surface area is larger and because the vibrating flame is ejected clear of the burner tip, thereby preventing quenching of the flame at the burner rim as occurs with the normal diffusion flame.

Besides the increasing sensitivity of the singing-flame detector, which would be particularly useful for trace hydrocarbon analysis, the wave-form output of the detector also makes it amenable to a.c. amplification with its inherent advantages (stability, cost, compactness, etc.) over d.c. amplification. The rectified output of the singing-flame detector is exceptionally steady in contrast to the erratic response of a turbulent or premixed flame[7].

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Energy Gap of β Silver Selenide

An interesting variation of apparent measured intrinsic band gaps of β (low temperature) silver selenide prepared by different methods has been noted. Melt-grown silver selenide has been determined to have a band gap of 0·04–0·1 eV at about 300° K[1,2]. When prepared as a powder at temperatures under 100° C[3] silver selenide has a gap of 0·07–0·1 eV. This same silver selenide when sintered at 200° C (above the β–α phase transition) for 15 min and then remeasured in the same temperature range exhibits a different band gap, 0·2 eV.

Another method developed by us in which basic solutions of silver and selenium are reduced by hydrogen at pressures in excess of 2,000 lb./in.^{2} at temperatures of approximately 200° C yields silver selenide having an apparent band gap of 0·2 eV that remains constant on sintering at 200° C.

Measurements on silver selenide prepared by the two methods were carried out in the same manner. Powder samples were compacted into pellet form (1·27 cm diameter with thickness up to 0·4 cm) using pressures of 70,000 lb./in.^{2} and the electrical resistivities and Hall effects were measured using the Van der Pauw technique[4] in the range of temperature from $1,000/T = 4$ to about 2·7/°K.

Our results tend to confirm the statements of Simon *et al.*[2] (who investigated melt-grown silver selenide) that the use of Hall or electrical resistivity measurements as a function of temperature in the range of temperatures from $1,000/T$ of 4 to 2·6/°K tend to give slopes from plots of $\ln RT^{3/2}$ versus $1,000/T$ or $\ln \rho$ versus $1,000/T$ which, assuming no degeneracy and intrinsic conditions, are not readily interpretable in terms of simple intrinsic energy gaps.

While Simon *et al.*[2] have mentioned the difficulty in associating a definite meaning to 'energy gap' determinations, they have not mentioned a factor which may influence the interpretation of the slopes determined as above, namely, the effect of dissolved silver, that is, the electrical state of small amounts of silver in the β silver selenide lattice. It is conceivable that the changes in the silver concentration change the Fermi level and that the actual band gap remains constant. Our results above may suggest that the state of silver in the silver selenide could change on undergoing a phase transition, and, as inferred in previous publications[5,6], the role of silver either in undergoing a phase transition or the change in solubility with decreasing temperature may give rise to precipitation of silver (especially on cooling) or to silver in interstitial sites. It is suggested that the effect of excess silver and its role in the lattice could account for the different 'activation energies' observed, especially as varying degrees of order or disorder in the lattice are possible.

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RADIOCHEMISTRY

Thermal Annealing of Tellurium resulting from Isomeric Transition in Solid Sodium Tellurate

Little attention has hitherto been directed to the chemical effects of radioactive decay in ionic crystals. Two papers are devoted to β-decay. Baro and Aten[1] have examined the distribution of arsenic-77, formed by β-decay of germanium-77 in GeO_2, between tri- and penta-valent states, and Andersen and Knutsen[2] the distribution of antimony-125, formed by β-decay of tin-125 in K_2SnCl_6. In both papers a small annealing effect has been reported. The yield of arsenic and antimony in the pentavalent state increased on heating. Campbell[3] has found that on heating sodium bromate crystals containing bromine-80*m* the distribution of bromine-80 resulting from isomeric transition is not changed.

In this communication experiments are described which show that thermal annealing does influence the yield of tellurium-127 as Te(IV) and Te(VI) from isomeric transition of tellurium-127*m* in labelled sodium tellurate.

The $Na_2{}^{127m}TeO_4$ crystals were prepared using tellurium dioxide irradiated for 6 months with a neutron flux of 10^{12} n cm^{-2} sec^{-1}, and then cooled for another 6 months. Almost all the activity was that from the tellurium-127*m* (103 days) → tellurium-127 (9·3 h) pair. The active tellurium dioxide was dissolved in 30 per cent sodium hydroxide, and the sodium tellurite oxidized with 15 per cent hydrogen peroxide. The fresh crystals of sodium tellurate, which had been precipitated while heating the solution, were washed with cold water and ethanol, then dried *in vacuo* and kept for two days at liquid air temperature. After two days they were subjected to thermal annealing treatment, followed by dissolving in cold 4 N hydrochloric acid and separation of the Te(IV) and Te(VI). This separation was done by extraction with 20 per cent tributylphosphate in kerosene from 4 N hydrochloric acid solution. The yield of ^{127}Te(IV) was determined by a well-known method, which consists of comparing the organic phase activity measured immediately after the extraction with that of an aliquot of the parent solution taken before extraction.

In Fig. 1 the retention (that is, the yield of ^{127}Te(VI)) is plotted against the time of heating at 20° ± 0·1° C, 60° ± 0·1° C, 120° ± 1° C and 190° ± 2° C. It can easily be seen that the retention increases quite markedly at the higher temperatures, the plateau being reached within 10–15 min. Even at 20° C the annealing effect can be observed; however, the ΔR value in this case is comparatively low (about 7 per cent), and the plateau is reached more slowly (within 1 h).

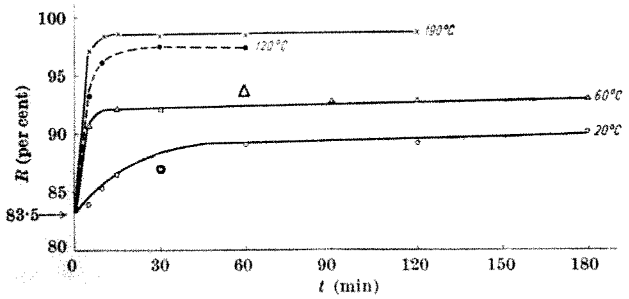

Fig. 1

Investigations of the chemical effects following isomeric transition in solids seem to be of interest, since in this case there are no radiation-induced defects in the crystals. Moreover, applying external irradiation with ionizing radiation, electrons, etc., the defects can be introduced in a well-controlled way. Experiments along these lines are being continued.

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METALLURGY

Low-temperature Alloy Synthesis

The low temperature reduction of metal ions to the finely divided free metal by sodium borohydride reduction has been reported recently[1]. In addition, the preparation of a number of inter-metallic compounds from mixed salt solutions using boiling hydrazine and hypophosphorous acid solutions as reducing agents has been accomplished[2]. It was of interest to examine the sodium borohydride reduction of solutions of two metal ions as a possible method of low-temperature synthesis of finely divided solid solution alloys. Such materials would be of particular interest in catalyst investigations, where high-surface area alloys are often difficult to obtain. Work on such reactions have, therefore, been carried out, using metals where alloy formation might reasonably be expected with suitable preparative conditions. Those chosen for initial investigation were combinations of platinum–gold, platinum–iridium, and palladium–gold. All three metals form face-centred cubic lattices, have atomic radius ratios well within the Hume–Rothery limit, and form solid solutions with each other over the entire composition range.

Table 1. Chemical and X-ray Data for Alloy Formation

Compositions (Atom %)		Unit cube dimensions (Å)*		Composition corresponding to experimental lattice spacing
Starting solutions	Reduced powder	Experimental	Literature	
Pt-40 Au	Pt-39·9 Au	3·970	3·982 (ref. 3)	Pt-32 Au
Pt-60 Au	Pt-63·0 Au	4·016	4·017 (ref. 3)	Pt-62 Au
Pt-80 Au	Pt-77·2 Au	4·036	4·038 (ref. 3)	Pt-75 Au
Pt-40 Ir	Pt-19·6 Ir	3·906	3·900 (ref. 4)	Pt-12 Ir
Pt-60 Ir	Pt-36·1 Ir	3·896	3·885 (ref. 4)	Pt-26 Ir
Pt-80 Ir	Pt-61·0 Ir	3·901, 3·848	3·865 (ref. 4)	Pt-82 Ir, Pt-19 Ir
Pd-50 Au	Pd-47·8 Au	4·013	3·971 (ref. 5)	Pd-70·5 Au

* Those values given in *kx* units have been changed to Å by the conversion factor 1·002.

The procedure followed for all syntheses consisted of adding 2·0 ml. of a mixed salt solution to 20·0 ml. of 1·85 M sodium borohydride solution at 50° C. The total salt concentration was held at 0·5 M, with the individual salt concentrations adjusted to the ratios shown in column 1, Table 1. The chloride salts were analytical reagent grade and the sodium borohydride was of 98+ per cent purity. All solutions were made with demineralized water. Additions were made in a steady stream from a pipette, while stirring the borohydride solution. In every instance a vigorous reaction occurred and a black precipitate formed immediately. The powders thus obtained were separated by centrifugation and washed thoroughly in 3·7 M sulphuric acid and water.

It was found that these systems indeed formed alloys by the foregoing procedure. Determination of their unit cube-lengths by X-ray diffraction and of their compositions by chemical analysis confirmed, in the case of platinum–gold, near stoichiometric alloy formation. As shown in Table 1, the compositions of these powders were within ±5 per cent of the starting solutions. Furthermore, the lattice spacings agreed, within their experimental error of ±0·2 per cent, with the chemical compositions. Thus complete alloy formation is indicated. As an example, the nominally 60 atom per cent gold alloy analysed chemically as 63 atom per cent gold. Our lattice spacing of 4·016 Å is in excellent agreement with the literature value for 63 atom per cent gold of 4·017 Å (ref. 3), and corresponds to a composition of 62 atom per cent gold, well within the experimental error. The other two powders also exhibited stoichiometric reduction with the expected precision, although the nominally 40 atom per cent gold sample apparently contains some free gold in addition to the alloy phase. As confirmation of the foregoing results with platinum–gold, physical mixtures of these two metals were examined by X-ray diffraction and gave two sets of peaks corresponding to the 3·914 Å lattice spacing of platinum and the 4·078 Å spacing of gold.

The platinum–iridium and palladium–gold powders, in contrast to platinum–gold, showed alloys the compositions of which did not agree with their original salt solutions. The nominally palladium-50 atom per cent gold, although analysing as 47·8 atom per cent gold, gave a lattice constant corresponding to 70·5 atom per cent gold. Thus unalloyed palladium was also present in the powder. The platinum–iridium systems uniformly showed lower than stoichiometric reduction of iridium and alloys of still lower iridium content. The nominally 80 atom per cent iridium sample in addition showed two sets of X-ray patterns, formed by a platinum-rich alloy and an iridium-rich alloy.

The varying results obtained with the three systems studied can be qualitatively understood in terms of two characteristics of the reduction reaction. First, the metal salts vary widely in their reactivity with sodium borohydride. We have observed the reduction rates of the single metals to be in the order $\mathrm{Pt} \simeq \mathrm{Au} > \mathrm{Pd} > \mathrm{Ir}$. Thus the near stoichiometric reduction and alloy formation of platinum–gold is due to the nearly equal reduction rates of these two metals. On the other hand, iridium, reduced much more slowly than platinum, is less likely to enter the lattice during crystal growth than is platinum. Hence a platinum-rich alloy is the result. Unalloyed iridium continues to be formed following completion of platinum reduction, but only until the borohydride is spent. This brings up the second pertinent property, the catalysis of borohydride hydrolysis by certain metals. Brown and Brown[1] reported a catalytic order of $\mathrm{Pt} > \mathrm{Ir} > \mathrm{Pd}$. We have confirmed this and also found that gold is still less active than these three. Since platinum is an excellent catalyst for the hydrolysis, the borohydride, even though initially present in large excess, is exhausted before all the slowly reactive iridium ions are reduced. In the case of palladium–gold, neither metal is an active hydrolysis catalyst and complete reduction of both metal ions is achieved. However, owing to the more rapid reduction of gold, the alloy formed is rich in this component and free palladium is, therefore, also present.

Surface area measurements by nitrogen adsorption gives areas of approximately 5 m^2/g. Using this number, an average particle size of about 600 Å is calculated. Attempts to fix this number by X-ray line broadening yield 50 Å as the average particle size. However, this latter method is uncertain since the line broadening may be due to some inhomogeneity in the alloy. Chemical and spectroscopic analysis of these samples revealed no significant impurities other than several tenths of one per cent oxygen as surface oxide. Boron, which might be expected to form metal borides or be present as an adsorbed impurity, was found in less than 10 p.p.m. concentration.

Tests of the generality of this technique are in progress. Preliminary results indicate that platinum forms alloys with non-noble transition metals such as cobalt and nickel, and also with copper. Other combinations of noble and non-noble metals are also being investigated.

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CHEMISTRY

Theory of the *p*H-stat Titration of a Polyfunctional Reactant by a Competitive 'Ligandee'

IN their recent extensive investigation of the reversible reaction of deoxyribonucleic acid (DNA) and bivalent mercuric compounds[1], Yamane and Davidson[2] introduced a useful new kind of *p*H-stat titration which they applied both to their investigation of the DNA–Hg^{II} interactions and to the examination of DNA–Ag$^+$ interactions[3].

Their results with Hg^{II} taken together with earlier investigations[1,4] have since been used as the basis of a mechanism for the reaction of this ion with double-stranded polynucleotides[5,6].

Although the purpose of their *p*H-stat investigations was different, it is intuitively clear that this kind of *p*H-stat titration can, in many cases, provide desirable quantitative information with relatively simple means, so I have thought it worth while to derive the theory of the titration.

Experimentally, one adds a small amount, ΔM_t moles, of the metal salt to an unbuffered solution of the polyfunctional reactant (or polyvalent ampholyte) at a predetermined *p*H. If the salt or its ions release protons into the solution, it can be back-titrated with hydroxyl ion to restore the original *p*H—thereby determining (by equivalence) a quantity ΔH_r. The ratio of these two quantities (constant temperature and pressure being understood) is, in the limit, equal to $(\partial H_r/\partial M_t)_H$, where the symbols are: H_r, concentration of hydrogen ion released; M_t, total added metal concentration; H, hydrogen ion concentration of the solution (maintained constant as described).

More generally, M_t need not be a metal ion but can be any kind of chemical species which is bound by a macromolecule. A good deal of verbiage can be saved by referring to the entity which is bound as the 'ligandee'.

If the ligandees do not interact, that is, if each ligandee is bound independently of the others, one can show that:

$$\left(\frac{\partial H_r}{\partial M_t}\right)_H = \frac{\sum^{m} \dfrac{a_i H^{a_i} K_i [D_i]}{(H^{a_i} + K_i M_f)^2}}{1 + \sum^{m} \dfrac{H^{a_i} K_i [D_i]}{(H^{a_i} + K_i M_f)^2}} \tag{1}$$

which is implicitly a function of:

$$M_t = M_f + \sum^{m} \frac{K_i M_f [D_i]}{H^{a_i} + K_i M_f} \tag{2}$$

The symbols not yet defined are: M_f, the free concentration of that ligandee which is added; a_i, the stoichiometric coefficient corresponding to the equilibrium:

$$D_i + M \rightleftharpoons D_i M + a_i \mathrm{H}^+ \; ; K_i \tag{3}$$

in which D_i is a binding site (ligand) of type i, M is the ligandee and K_i may be called the 'stoichiometric' intrinsic constant. It differs from the conventional intrinsic constant[7] in that its *p*H dependence (so far as stoichiometry is concerned) is explicitly stated. The remaining symbols are: D_i, the concentration of sites of type i; m, the total number of kinds of sites.

If the approximation of independent sites can be sustained, equations (1) and (2) are undoubtedly applicable to the binding of Ag$^+$ by DNA (ref. 3). The expressions for the binding of Hg^{II} by DNA (or polynucleotides) are somewhat more complicated because both CL$^-$ (if HgCl$_2$ is used) and OH$^-$ 'bind' Hg^{++} as well as DNA.

From the form of (1) and (2), it is obvious that in favourable cases (that is, when $a_i \neq 0$) the *p*H-stat titration itself may be used to determine the K_i. It is also obvious on physical grounds that the 'skyline' type of curve which results experimentally[3] when $(\partial H_r/\partial M_t)_H$ is plotted versus r (where r is defined as moles Hg^{II} or Ag$^+$

added per mole nucleotide; not to be confused with the subscript r on H_r which stands for 'released') is implicit in the set (1) and (2). Thus, a_i determines the maximum possible height of the 'buildings' as well as its presence ($a_i \neq 0$) or absence ($a_i = 0$); D_i determines their width and the K_i determine both the squareness (K_i large) and whether the 'buildings' merge into one another (two or more K_i of similar value) or are distinct (K_i or greatly dissimilar value). The set (1) and (2) is quite capable of reproducing the step-function used previously in discussing the mechanism of the Hg^{II}–DNA reaction[5,6].

Especially when the binding is very strong, as in the case of Hg^{II} and DNA, there is a definite heuristic advantage in plotting the results as $(\partial H_r/\partial M_t)_H$ versus r (rather than versus M_t). Such a plot facilitates the determination of a mechanism, as evidenced in detail by its recent use[6] in the mechanism proposed for the reaction of Hg^{II} and double-stranded polynucleotides. These remarks apply equally well to the case of Ag$^+$ and DNA.

The theory is by no means restricted to DNA or to macromolecules, but applies quite generally to those numerous situations in solution chemistry in which an added reagent (the ligandee, M, or a molecular form containing it) competes for a site on a polyfunctional reactant normally occupied by another ligandee (in the particular case of DNA, protons). The advantages of carrying out the experiment at constant pH are, no doubt, readily apparent to those engaged in the investigation of the interaction of ligandees and biological macromolecules, polyelectrolytes, and the like.

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Electron Spin Resonance Investigations of Ultra-violet and X-irradiated Bovine Serum Albumin

WE have already reported[1] that the radicals of methionine produced by X-rays at room temperature may be quenched by ultra-violet light. Corresponding investigations with bovine serum albumin show a quite different behaviour, and initial results with this substance are now reported.

Polycrystalline native bovine serum albumin (Behring Werke, Marburg, Germany) with a bulk volume of 540 cm^3/100 g was sealed under vacuum ($< 10^{-3}$ torr) and irradiated at room temperature. The electron spin resonance measurements were carried out under the same conditions. The irradiation with X-rays was carried out with a 180-kV source (half-value layer : 0·90 mm copper, dose-rate : 450 r./min, dose : 100 kr.). The irradiations with ultra-violet were performed with a mercury low-pressure lamp (*NN* 15/44, Quarzlampengesellschaft, Hanau Germany), which emits mainly in the 254 mμ line or with a mercury high-pressure lamp (*Q* 700, from the same firm). The ultra-violet light from the latter was filtered with a 2-mm filter-glass of the type *WG* 5 (Schott and Gen., Mainz, Germany) to give only ultra-violet of longer wave-lengths ($\lambda > 300$ mμ). The intensity of the low-pressure lamp and of the high-pressure lamp at the sample tube amounted to 9×10^3 ergs cm^{-2} sec^{-1} and $1 \cdot 1 \times 10^4$ ergs cm^{-2} sec^{-1} respectively. The electron spin resonance spectra were recorded with a Varian spectrometer which operates in the X-band. To avoid saturation effects in the irradiated sample the microwave energy of the klystron was sufficiently attenuated (20 db). All the other technical details are described in the aforementioned publication[1].

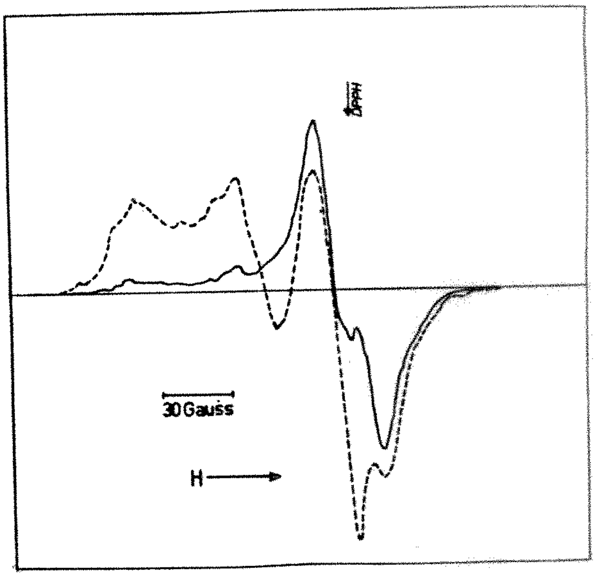

Fig. 1. Electron spin resonance spectra of irradiated bovine serum albumin. The spectra represent the first derivatives of the actual absorption curves. —, 100 kr.; – – –, 100 kr. + 5 h ultra-violet ($\lambda = 254$ mμ)

After irradiation of the bovine serum albumin, an electron spin resonance signal was obtained (Fig. 1, solid line) which is composed of a sulphur pattern and an unresolved doublet, described already by other authors[2,3]. The radical concentration of the X-irradiated bovine serum albumin was $3 \cdot 6 \times 10^{17}$ spins/g. For technical reasons, the X-irradiated samples could not be measured immediately after irradiation; they were stored for several hours at −17° C. On exposing the X-irradiated bovine serum albumin to ultra-violet from the mercury low-pressure lamp, the radical concentration increased immediately. By this process the X-ray induced signal was altered. With increasing irradiation time the spectrum became more and more 'cysteine-like' (Fig. 1, dotted line). On exposing bovine serum albumin only to 254 mμ-light, practically the same electron spin resonance pattern was observed. The results of these investigations do not give any information about the behaviour of the X-ray induced radicals under the influence of the 254 mμ-light. Therefore further experiments were carried out with ultra-violet of longer wave-lengths ($\lambda > 300$ mμ), which alone do not lead to any radical state detectable with our spectrometer. By the same irradiation a signal could only be detected in the presence of oxygen.

The irradiation with ultra-violet of longer wave-lengths does not alter the radical concentration of the X-irradiated bovine serum albumin (Fig. 2, upper curve, open circles and open triangles ; the two different signs stand for two sets of investigations under the same conditions). The slight decrease of the concentration with increasing irradiation time coincided with the radical decay of a control, which was only irradiated with X-rays (Fig. 2, open squares).

In the X-irradiated control, the maximal amplitude of the doublet showed the same decrease as the radical concentration (Fig. 2, upper curve, full squares). On the other hand, the signal-amplitude of the X-ray and ultra-violet irradiated bovine serum albumin led to a stronger decrease (Fig. 2, lower curve, full circles and full triangles). At the same time, the spectrum in the region of the lower

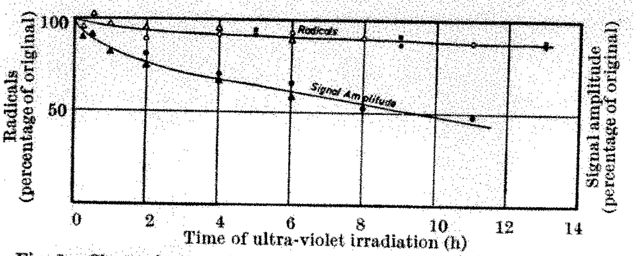

Fig. 2. Change in the relative radical concentration (open circles and triangles) and in the relative maximal signal-amplitude (full circles and triangles) in X-irradiated bovine serum albumin after exposure to ultra-violet of longer wave-lengths ($\lambda > 300$ mμ). The open squares and the full squares represent the time dependence of the radical concentration and the signal-amplitude respectively of a control which was not exposed to ultra-violet

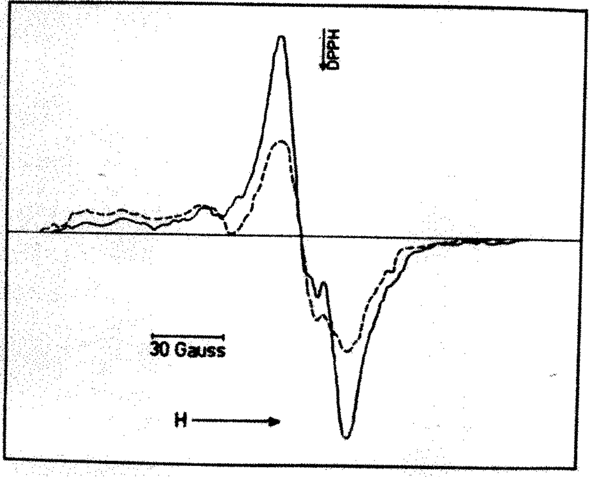

Fig. 3. Electron spin resonance spectra of irradiated bovine serum albumin. The spectra represent the first derivatives of the actual absorption curves. ——, 100 kr.; – – –, 100 kr. + 8 h ultra-violet ($\lambda > 300$ mμ)

magnetic field strength increased (Fig. 3; compare the solid line with the dotted one). This effect is not very large, but we believe that the site of the radical which gives rise to the doublet is shifted by the additional energy from the ultra-violet light. The irradiation with ultra-violet of 254 mμ and of longer wave-lengths formed also sulphuretted hydrogen.

This communication is dedicated to Prof. Brinkman (Groningen) on the occasion of his seventieth birthday.

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Thermal Decomposition of Barium Azide

The rate of decomposition of solids is a function of the rates of: (1) nuclear formation; (2) propagation of the reaction outwards from the nuclei. Both these components might be expected to be influenced by external stimuli. The formation of nuclei by bombardment of the solid with α- or β-particles, or by irradiation with ultra-violet light, X-rays and γ-rays should be possible in favourable circumstances, and by the same agencies the rate of propagation of the reaction might be accelerated. For example, the effects of pre-irradiation by high-energy γ-rays (~ 1 MeV) on the subsequent thermal decomposition of the permanganates of sodium, potassium, rubidium, cæsium and silver are to shorten the induction period and accelerate the reaction[1]. It was of interest, therefore, to examine the effect of pre-irradiation by γ-rays on the isothermal decomposition of barium azide. There have been several previous investigations of the effects of radiation on barium azide. Pre-irradiation with α-particles[2], X-rays[3], and ultra-violet light[4] accelerates the rate of subsequent thermal decomposition, but to our knowledge no study has been made of the effect of γ-rays.

Fig. 1 illustrates the pressure-time plot for the decomposition of dehydrated barium azide crystals pre-irradiated with ~ 1 MeV γ-rays. The decomposition of the unirradiated salt is also shown. It is noted that the induction period is practically eliminated and the acceleration of the decomposition is increased. The salt after irradiation was buff colour.

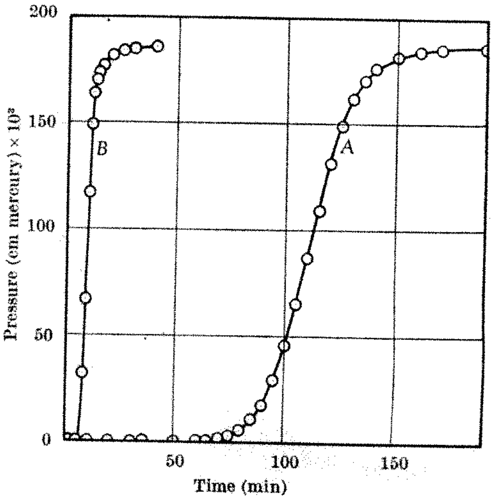

Fig. 1. Effect of pre-irradiation on thermal decomposition of barium azide. γ-ray dose, 20 Mrads; temperature of decomposition, 125° C. Curve *A*, unirradiated; curve *B*, irradiated

The pressure, p, after a time, t, for the decomposition of unirradiated dehydrated barium azide crystals has been given by Wischin[5] as:

$$p = Ct^x \tag{1}$$

where the value of x varied between 6 and 8. Thomas and Tompkins[6] introduced a correction, y, for the slow growth of nuclei when the pressure of nitrogen, expressed as a function of time of heating, t, is given by:

$$p = \text{const.}\ (t - y) \tag{2}$$

An analysis of random nucleation at potential nucleus sites, followed by three-dimensional growth, leads to the Avrami–Erofeyev[7] equation:

$$-\ln\ (1 - \alpha) = (k_1 t)^n \tag{3}$$

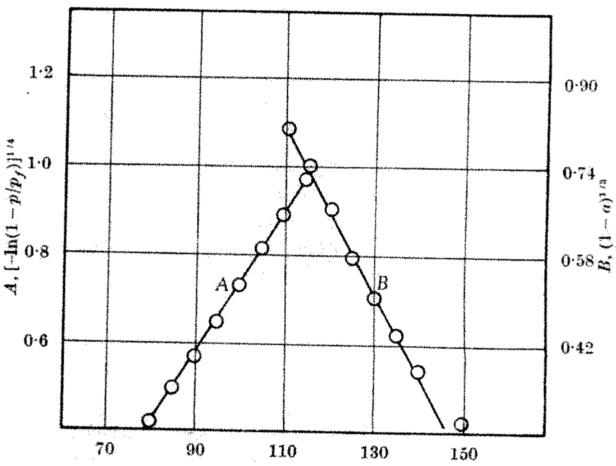

Fig. 2. Analysis of curve *A*, Fig. 1. Line *A*, $[-\ln(1 - p/p_f)]^{1/4}$ versus t; line *B*, $(1 - \alpha)^{1/3}$ versus t

where k_1 is a constant, and $\alpha = p/p_f$, where p_f is the final pressure.

We have applied the Avrami–Erofeyev equation to our pressure-time plots for the decomposition of unirradiated barium azide and find that with $n = 4$ the equation describes the acceleratory period excellently in the range 110°–140° C. The decay stage of the reaction is best described by the contracting sphere formula[8]:

$$(1-\alpha)^{1/3} = k_2 t + \text{const.} \qquad (4)$$

A typical analysis is shown in Fig. 2.

The activation energies calculated from the variation of the constants k_1 and k_2 with temperature are 26·8 and 24·3 kcal mole^{-1} respectively. The plots of log $k_{1,2}$ versus $1/T°$ K are shown in Fig. 3.

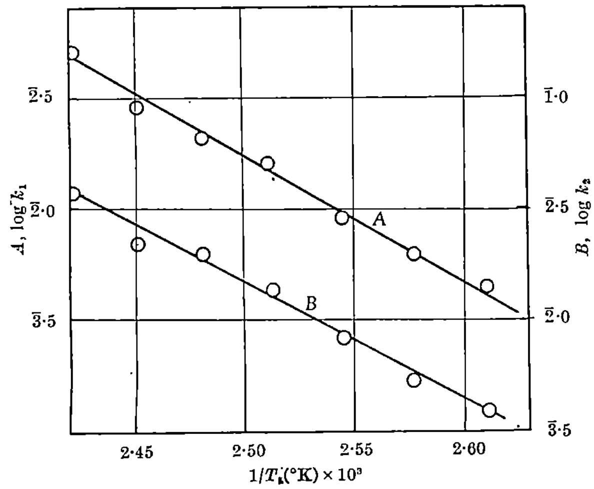

Fig. 3. Line A, log k_1 versus $1/T$(°K); line B, log k_2 versus $1/T$(°K)

We thank the South African Council for Scientific and Industrial Research for financial assistance, and Messrs. African Explosives and Chemical Industries for a research fellowship held by one of us (D.J.M.).

A more detailed account of this work, together with further observations, will shortly be published elsewhere.

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BIOCHEMISTRY

Extracts of Mould-infected Groundnut Samples in Tissue Culture

During recent years the toxicity of groundnut ingredients in feed rations has presented a general problem. It was soon discovered that the toxic effect is due to aflatoxins, produced by certain strains of *Aspergillus flavus* growing on groundnuts[1]. Up to now, however, research workers have failed to establish a practicable method for the elimination of the toxicity of infected groundnut batches. Therefore, methods enabling the demonstration of aflatoxin are of great importance partly for the identification of infected batches and partly for the determination of the non-toxic ratio of groundnut ingredient in the feed.

In the Tropical Products Institute, London, and in four other institutions in Great Britain, biological and chemical methods have been evolved for the assay of toxicity of groundnut samples[2]. Biologically, the samples are tested by feeding the toxic extracts to day-old ducklings. The degree of toxicity is estimated on the basis of mortality rate or liver lesions resulting from the toxic effect. The chemical test is based on the ultra-violet fluorescence of aflatoxins. The intensity of fluorescence is determined on aflatoxins separated by paper chromatography.

Platt *et al.*[3] injected extracts of groundnut samples into embryonated hen's eggs and found that the presence of toxin was indicated by the death of the embryo.

Here the demonstration of aflatoxins was attempted with the tissue culture inoculation technique.

Toxic extracts were obtained by the method of Sargeant *et al.*[4]. From the groundnut samples aflatoxins were extracted with methanol and purified by partition first between chloroform and water and next between methanol and petrol ether.

The purified extract was chromatographed with the ascending technique, using Whatman No. 1 paper and n-butanol/acetic acid/water solvent (4 : 1 : 5 by vol.).

The methanol fraction was inoculated to calf kidney monolayer cultures in amounts 0·5 ml. per tube. The results were read after 48 h of incubation. Toxicity was evaluated by the degree of cell destruction caused in the tissue cultures. As a control, extracts prepared in the foregoing way from non-infected groundnuts were used.

The extracts of groundnut samples infected with *Aspergillus flavus* proved remarkably cytotoxic to calf kidney tissue cultures. In the affected cells both cytoplasm and nucleus were destroyed.

If 1 ml. of the extract was equivalent to 10 g of groundnut cake, toxin concentrations of 0·1–0·5 p.p.m. caused cell destruction up to a dilution of 10^{-4}, and those higher than 0·5 p.p.m. up to a dilution of 10^{-5}. In the samples aflatoxin concentration was determined by the fluorescence test. The non-infected extracts used as control had no toxic effect on calf kidney tissue culture.

The petrol ether fraction was also examined in tissue culture. It was found that, in comparison with the methanol fraction containing aflatoxins, the cytotoxicity of the petrol ether fraction was about 0·1 of the former. It was demonstrated that the petrol ether fraction contained no aflatoxins and its toxicity was due to certain lipid-like components of the metabolic products of fungi.

From the methanol fraction, aflatoxins were separated by paper chromatography. The fluorescent spot was eluted with chloroform and assayed for toxicity in tissue culture. Chromatographic spots having R_F values higher ($> 0\cdot 8$) or lower ($< 0\cdot 8$) than aflatoxins were examined in a similar way. Substances with R_F values lower than that of aflatoxins proved non-toxic, whereas those with higher R_F values exhibited similar toxicity. Since the extracts of non-toxic groundnut samples have no toxic effect on calf kidney tissue, it is obvious that the toxic substances present in chromatographic spots other than aflatoxin are also metabolic products of fungi. Information on the biological role of these substances has as yet been inadequate, but it seems likely that it may also have lethal effect on the chick embryo.

Our results are indicative that besides aflatoxins other substances have a role in the cytotoxic effect of mould-infected groundnut samples. Nevertheless, as the extracts obtained from non-infected samples caused no cell destruction in calf kidney monolayer cultures, the tissue-culture test appears to give reliable information on the mould contamination and aflatoxin content of groundnut batches. Further examinations are in progress concerning the determination of the toxic titre of aflatoxin concentrations and the nature of the other cytotoxic agents present in infected groundnut samples. Further to this, comparative studies are being conducted on the toxin production of *Aspergillus flavus* and other moulds.

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Determination of the Aflatoxins

We report a new chemical method for determining the aflatoxins and, in particular, aflatoxin B_1. Previous methods have depended on the measurement of fluorescence[1,2] and are subject to certain errors, for example, quenching by impurities. Moreover, it is known that the fluorescence of solutions of aflatoxin B_1 in methanol varies with time[3].

All four aflatoxins have an ultra-violet absorption peak of high molar extinction at 363 mμ (ref. 4). The measurement of the optical density of this peak was chosen as the basis of an alternative assay procedure. This method is less sensitive than that depending on dilution to extinction of fluorescence, but it provides a more reliable means of assaying meals containing 1 p.p.m. or more of aflatoxin.

A brief outline of the procedure follows.

Groundnut meal (40 g) was extracted for 6 h with light petroleum (40°–60° C) in a Soxhlet extractor, dried and re-extracted for 6 h with methanol. The methanol solution was diluted with half its volume of distilled water, and continuously extracted with chloroform for 6 h. The residue from evaporation of the chloroform was made up to 10 ml. in chloroform, and 0·2 ml. portions of this solution were applied in a line across the bottom of a 'Kieselgel *G*' chromatoplate (thickness 750μ). This was developed in diethyl ether, dried and developed twice more in chloroform (2 per cent methanol). The chromatoplate was viewed under ultra-violet light (principal wave-length 365 mμ) and rejected if the bright blue fluorescent band of B_1 was not reasonably straight, clear of other fluorescent bands and running ahead of brown material near the origin.

The B_1 fluorescent band was then scraped from the chromatoplate treated for 5 min with cold methanol, the solution filtered and the silica washed with methanol. The methanol filtrate was made up to 5 ml. and the amount of B_1 calculated from the optical density of the solution at 363 mμ (ε 363 mμ = 22,000)[4].

In practice the ultra-violet spectrum was measured from 210 to 400 mμ on a recording spectrophotometer. A criterion of purity was obtained from the resemblance of the spectrum to that of pure B_1.

A number of extractions were made of a homogenized batch of groundnut meal which is being used as a standard for chemical and biological work. Several determinations were carried out on each extract, and the aflatoxin B_1 content of the meal was found to be 10·2 ± 0·19 p.p.m. (95 per cent confidence limits). Aflatoxin B_2 was detected as a second blue fluorescent band running behind B_1[4,5]. The amount present was determined by pooling the extract of this band obtained from several plates and was estimated as 0·2 p.p.m. in the original meal. The completeness of the removal of aflatoxin by 6 h Soxhlet extraction was checked by extraction of the meal for a further 6 h with methanol; this yielded no more B_1 or B_2. The possibility of loss of B_1 in the stages after the methanol extraction was checked by the addition of known quantities of pure B_1 to methanol extracts of the standard toxic meal and of a non-toxic meal and assay of the resultant products. There was good agreement between the found and calculated values for these samples.

The foregoing method has also been used for the determination of aflatoxins B_1 and G_1 in crude crystalline aflatoxin isolated from both synthetic media and sterilized groundnuts inoculated with *Aspergillus flavus*.

We thank Mr. A. B. Wood for the measurement of the ultra-violet spectra.

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An Ion-exchange Method for the Estimation of Strontium-90 in Milk

The classical method of Bryant *et al.*[1] for the estimation of strontium-90 in milk is both tedious and hazardous. Davis[2], Farabee[3] and other workers[4,5] have all reported separations of strontium from calcium using ion-exchange methods, and such techniques would appear to offer promise for the estimation of strontium-90 in milk.

In the investigation recorded here, ion-exchange columns 22 cm long and 1 cm in diameter of the sodium form of 'Zeo-karb 225' (52–100 mesh) were set up and the resin conditioned by the passage of three bed volumes of a 2 per cent solution of the disodium salt of EDTA at pH 5. The flow rate through the column was limited throughout the investigation to three bed volumes per hour.

Samples of milk ash (10 g) were dissolved in 10 M nitric acid, inert strontium carrier (50 mg) added and the mixture heated until evolution of brown fumes ceased. 5 M sodium hydroxide was added until alkaline, the paste dissolved in 10 per cent EDTA solution (100 ml. disodium salt) and the pH adjusted to 5·00 with 5 N hydrochloric acid.

The solution of ash was passed down the column, which was washed with a 2 per cent solution of EDTA (disodium salt, 200 ml.) at pH 5·25 and, finally, with distilled water (200 ml.). The strontium was eluted from the column with 5 N hydrochloric acid (70 ml.), followed by distilled water (100 ml.). The total eluate volume was reduced to less than 100 ml. by evaporation and the pH adjusted to 5 by the dropwise addition of 5 M sodium hydroxide. After heating to 80° C, '880' ammonium hydroxide (3 ml.) was added, followed by an excess of ammonium carbonate solution. After cooling to room temperature the suspension was centrifuged, the supernate discarded and the precipitate washed several times with distilled water. The precipitate was dissolved in the minimum volume of 6 M nitric acid and inert yttrium carrier (10 mg) added. The solution was left to stand for 14 days[6] to allow the yttrium-90 daughter to grow in.

The solution was made alkaline with carbonate free ammonium hydroxide, the time noted and the precipitate coagulated by heating on a water bath. The suspension was centrifuged, both precipitate and supernate being retained. The precipitate was dissolved in the minimum volume of 6 M nitric acid, diluted to 10–15 ml. and the yttrium reprecipitated with ammonium hydroxide. After centrifuging (the supernatant liquid being added to that obtained earlier), the precipitate was again dissolved in the minimum volume of 6 M nitric acid. The solution was heated to 80° C and the yttrium precipitated by the addition of an 8 per cent aqueous solution of oxalic acid (20 ml.). The precipitate was rendered granular by heating on a water bath for 10–15 min, and after cooling to room temperature was filtered off through a 1·5 cm No. 42 Whatman filter paper mounted in a 'Perspex' filter. The precipitate was washed several times with distilled water, then with three portions of methanol (each 10 ml.) and sucked dry. The filter paper with the precipitate was secured to an aluminium counting tray with a little Gelva adhesive and the yttrium-90 decay followed for at least 60 h to establish radiochemical purity using a Philips's low-level counting assembly[7]. Strontium recovery was assessed gravimetrically by precipitation as carbonate from the combined supernates remaining after the precipitation of yttrium as hydroxide. The calcium content of the milk powder was determined by precipitation as oxalate. The amounts of strontium-90 in seven milk samples (chosen for their differing degrees of contamination) were determined in duplicate and the results corrected for decay from the time of sampling. The results were expressed as 'strontium units', that is, as picocuries of ^{90}Sr/g calcium. Yttrium recovery was assessed by detaching the filter paper and precipitate from the counting tray with a few drops of methanol, heating in a porcelain crucible at 850° C for 2 h and weighing as yttrium oxide.

The results are given in Table 1, and in Table 2 are compared with those obtained from other samples of the same milk powders in which strontium-90 had been determined using the nitric acid method.

Table 1

Sample No.	Sr recovery	Y recovery	% Ca	Strontium units
1*a*	90·79	87·10	15·28	6·8
b	90·44	87·90		6·7
2*a*	91·65	82·25	15·16	13·5
b	88·69	86·29		13·3
3*a*	89·04	83·87	15·34	14·8
b	86·13	86·29		14·7
4*a*	90·91	91·12	15·76	16·2
b	91·96	90·32		16·5
5*a*	90·10	88·70	15·89	19·8
b	89·71	86·29		19·2
6*a*	90·79	88·70	15·92	24·8
b	89·97	89·51		24·5
7*a*	89·51	91·94	15·81	33·9
b	90·79	92·74		34·3

Table 2

Sample	Strontium recovery		Strontium units in sample	
No.	This method	HNO_3 method	This method	HNO_3 method
1*a*	90·79	88·0	6·8	6·9
b	90·44	89·62	6·7	7·2
2*a*	91·65	77·04	13·5	13·0
b	88·69	75·87	13·3	13·4
3*a*	89·04	88·58	14·8	14·5
b	86·13	89·63	14·7	14·5
4*a*	90·91	80·19	16·2	16·1
b	91·96	42·07	16·5	16·2
5*a*	90·10	88·93	19·8	19·5
b	89·71	78·90	19·2	19·8
6*a*	90·79	78·09	24·8	24·3
b	89·97	81·35	24·5	25·1
7*a*	89·51	87·66	33·9	34·6
b	90·79	87·65	34·3	34·8

It can be seen that the method is accurate, more rapid and less hazardous than that of Bryant and is easily adapted to analyse many samples concurrently.

Our thanks are due to Dr. R. F. Phillips, head of the Department of Applied Chemistry, Loughborough College of Technology, and to Stewarts and Lloyds, Ltd., for a scholarship for one of us (D. S.).

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Effect of *N*-Ethylmaleimide on the Starch-liquefying Enzyme from Soy-flour

Information regarding soy-bean enzymes catalysing the breakdown of starch is contradictory or at best conjectural. The biological function of amylases in soy-beans is obscure as the beans contain very little starch. The consensus of opinion seems to be that soy-bean seeds are a good source of β-amylase. While some workers[1–3] feel that soy-beans contain no or only traces of α-amylase, Teller's investigations[4] and evidence from the effects of soy-flour on starch-viscosity measurements[5–7] point to the presence of a starch-liquefying enzyme in non-germinated soy-beans. Recently[8], the presence of a starch-liquefying and of a starch-saccharifying enzyme in soy-flour was demonstrated by differential thermal inactivation. This communication deals with the effect of the SH-blocking reagent *N*-ethylmaleimide (NEMI) on the starch-hydrolysing enzymes of soy-flour. It is known[9] that none of the α-amylase so far investigated contains free sulphydryl groups essential for enzymatic activity. β-Amylase purified and crystallized from soy-beans has been reported[10] to contain —SH groups essential for enzymatic activity.

Starch hydrolysis by 0·2 per cent calcium chloride extracts of soy-flour[8] (centrifugates of 10 per cent suspensions shaken for 1 h) was followed by determining both reducing sugars (by the ferricyanide method) and starch liquefaction[11]. Soy-flour extracts were kept for 1–4 h with various concentrations of NEMI and their saccharifying and liquefying actions determined. Reduction in formation of reducing sugars depended on the concentration of NEMI in the mixture with soy-flour extract and on the length of incubation. Enzyme inactivation was consistent but small, and even incubation of equal volumes of a 10 per cent soy-flour extract and a saturated solution of NEMI resulted in a decrease of only about 25 per cent in the level of reducing sugars formed. The effects of different concentrations of NEMI and variations in incubation time with the —SH blocking reagent on starch liquefaction by soy-flour extracts are shown in Fig. 1.

Incubation of ten α-amylase preparations[12], at levels comparable in their liquefying action to soy-flour extracts,

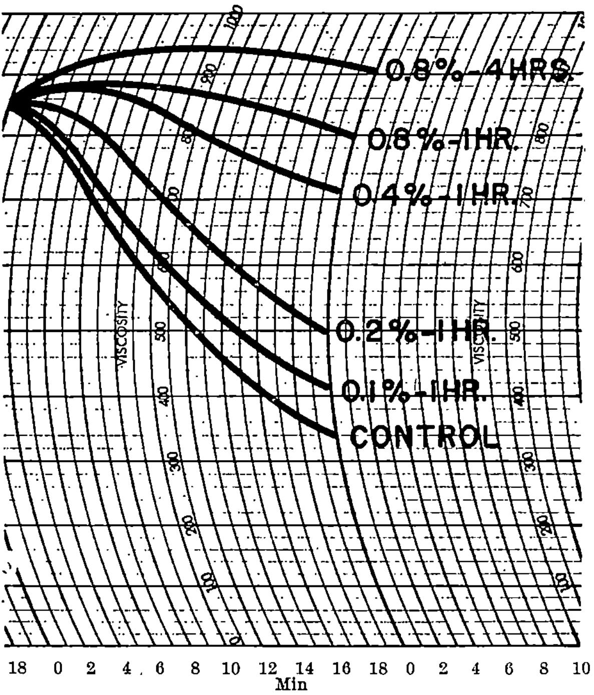

Fig. 1. Liquefaction of pre-gelatinized waxy maize starch by soy-flour extracts incubated with various concentrations of NEMI for 1–4 h

with 0·8 per cent solutions of NEMI for 1 h had no effect on the starch liquefying action of α-amylase from wheat or barley malt, fungi, bacteria, or pancreas. The results obtained confirm the absence of free —SH groups essential for enzymatic activity in cereals, micro-organisms, or pancreas. With regard to the starch-liquefying enzyme in soy-beans two possibilities seem to exist: (*a*) the starch-liquefying enzyme has free —SH groups in the active centre; (*b*) starch liquefaction by soy-flour extracts is the result of an enzyme other than the α-amylase type.

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Absorption Spectrum of the Crystal of D-Amino-acid Oxidase Michaelis Complex

Under anaerobic conditions, we crystallized an intermediate complex of enzymatic reaction of D-amino-acid oxidase and a substrate, D-alanine[1,2]. Electron spin resonance examination demonstrated that free radicals are concentrated in the crystal[3]. The crystal was purple under anaerobic conditions, but on aeration it changes to yellow, accompanied by its conversion to equimoles of the oxidized holo-enzyme and the reaction product pyruvate. From these results, we considered that this crystal is an intermediate complex which was composed of equimolar amounts of the semiquinoid form of the holo-enzyme and the partially modified substrate; thus we designated this intermediate as Michaelis complex.

This communication briefly reports the absorption spectrum of this crystal.

The crystal of D-amino-acid oxidase Michaelis complex was prepared as follows. The oxidase was purified as the enzyme–benzoate complex[4]. Benzoate was driven off from the complex by adding an excess of D-alanine. The enzyme was then precipitated by ammonium sulphate at *p*H 6·1. The precipitate was dissolved in the minimum volume of pyrophosphate buffer (M/60, *p*H 8·3). Its final *p*H became 7·2 and saturation of ammonium sulphate, 0·05.

Then, considering the previous results[2] on the molar ratio of pyruvate to the enzyme in the mother liquid which produced large numbers of crystals, pyruvate was added to the purple-coloured solution so that the molar ratio of pyruvate to the enzyme was 200 :1. The solution was kept at 5° C for more than a week under anaerobic conditions. The purple-coloured crystals (about 150μ × 20μ) were gradually formed as shown in Fig. 1. The shape of the crystals is hexagonal prism. They are far larger than the crystal in the previous report[1,2].

The crystals thus obtained were placed on a deck glass and sealed with petrolatum and a cover-glass[3]. Under these conditions, the crystal was stable at least for one day at room temperature. The absorption spectrum at visual wave-lengths of the crystal was measured by using an Olympus microspectrophotometer at 20° C. The diameter of the pinhole was 14μ. The measured absorption spectrum of the crystal is shown in curve 1 in Fig. 2. The absorption spectrum of the purple-coloured mother liquid was obtained under anaerobic condition using Beckmann *DU* spectrophotometer, as shown in curve II in Fig. 2. The absorption spectrum of the crystal is similar to that of the mother liquid, and their peaks are equally located at 492 mμ.

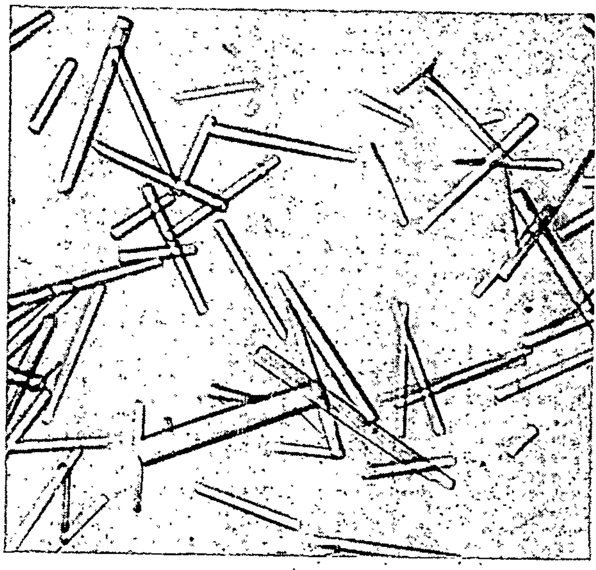

Fig. 1. Crystals of the Michaelis complex of D-amino-acid oxidase (× 200)

They are quite different from both the absorption spectrum of the holo-enzyme crystal (curve III) and that of the holo-enzyme solution (curve IV)[5].

Concerning the absorption spectrum of the semiquinoid form of flavin enzyme, Beinert[6,7] has already reported the transient appearance of a broad absorption band

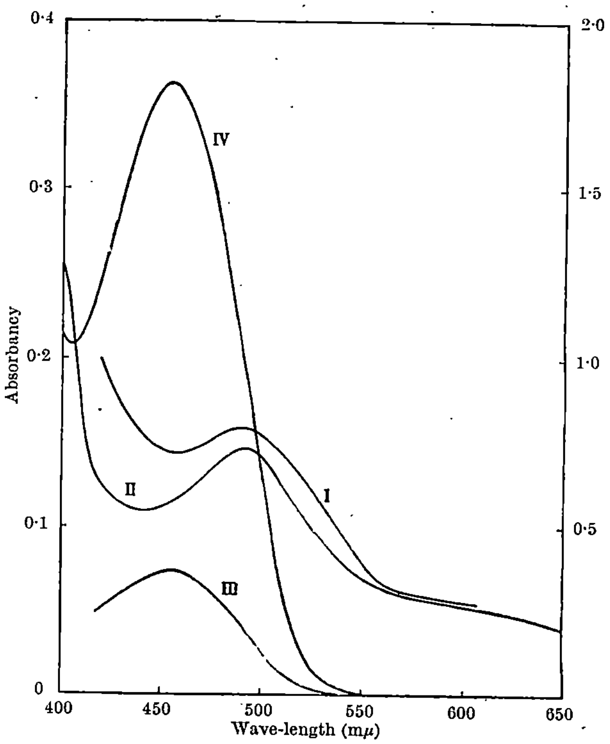

Fig. 2. Absorption spectrum of the Michaelis complex of D-amino-acid oxidase. Curve I, the crystal of the Michaelis complex; curve II, the mother liquid of the Michaelis complex crystal (the enzyme concentration, $1 \cdot 64 \times 10^{-4}$ M); curve III, the crystal of the holo-enzyme; curve IV, the holo-enzyme solution ($1 \cdot 64 \times 10^{-4}$ M in pyrophosphate buffer, *p*H 8·3). The left scale of the ordinate is for curves I and III, and the right for curves II and IV

with its maximum at 565 mμ accompanying the free radical formation for a number of flavin enzymes. Ehrenberg and Ludwig[8] reported the shift of the maximum peak of the old yellow enzyme about 10 mμ toward longer wavelengths (465–475 mμ) accompanying free radical formation.

The difference between the result reported here and those reported by other workers may be attributed to the difference between the enzyme samples or some other reason.

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Metabolism of 2,4-Dichlorophenoxyacetic Acid ('2,4-D') by *Aspergillus niger* van Tiegh

Earlier papers dealing with the microbial degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) have indicated that fission may be the ultimate fate of the aromatic ring. Thus Rogoff and Reid[1] detected an almost quantitative production of chloride ion by an organism belonging to the genus *Corynebacterium*, isolated from soil, while Fernley and Evans[2] obtained α-chloromuconic acid as a metabolite using a soil *Pseudomonas* sp., although under certain conditions the latter workers found 2,4-dichlorophenol to be the main product.

By contrast, previous investigations of the metabolism of aryloxyacetic acids by fungi[3-5] have shown that the most notable feature was the predominance of apparently non-specific nuclear hydroxylation, without ring fission. The metabolism of 2,4-dichlorophenoxyacetic acid, using a replacement culture technique with *Aspergillus niger*, has proved different, however, in that not all vacant ring sites are hydroxylated. The major metabolite has been identified as 2,4-dichloro-5-hydroxyphenoxyacetic acid, but two very minor metabolites have been shown to be non-identical with the corresponding 3- or 6-hydroxy acids, the production of which might have been expected in the light of earlier work[4]. These compounds have been separated with some difficulty by partition chromatography using silica gel as the supporting medium for the stationary aqueous phase which was buffered to *p*H 10, the mobile phase being 50 per cent saturated aqueous chloroform/*n*-butanol (60 : 40).

One compound has now been identified by comparison of infra-red absorption spectra and mixed m.p. as the hitherto unknown 2,5-dichloro-4-hydroxyphenoxyacetic acid, an authentic specimen of which was prepared from 2,5-dichlorohydroquinone. The formation of this hydroxy acid from 2,4-D is novel in that it involves not only hydroxyl-chlorine replacement (as was found with 2-chlorophenoxyacetic acid[4]) but also a chlorine shift. This is reminiscent of the formation of homogentisic acid from tyrosine by *A. niger* and various *Penicillium* sp.[6], though in that case it is likely that the side-chain migrates.

2,5-Dichloro-4-hydroxyphenoxyacetic acid is also the sole acidic metabolite produced by *A. niger* from 2,5-dichlorophenoxyacetic acid, but careful scrutiny of the 2,4-dichlorophenol used to prepare the 2,4-D for use in metabolism investigations has not revealed any 2,5-dichlorophenol impurity. Moreover, 2,4-D recovered from the silica-gel column separation, when re-used in the replacement culture technique, resulted in further production of 2,5-dichloro-4-hydroxyphenoxyacetic acid. Final proof of the genesis of 2,5-dichloro-4-hydroxyphenoxyacetic acid was provided by the use of 2,4-D labelled with carbon-14 in the carboxyl group. Metabolism of this acid by *A. niger* gave a radioactive chromatographic spot with the same R_F value.

Thus the bona fide production of 2,5-dichloro-4-hydroxyphenoxyacetic acid from 2,4-D seems certain and the question remains whether its formation is in any way involved in the mechanism of formation of the major metabolite. This appears to be unlikely in view of the fact that no corresponding 4-hydroxy compound is formed in the metabolism of 4-chloro-2-methylphenoxyacetic acid ('MCPA') by *A. niger*, where the previously unknown 4-chloro-5-hydroxy-2-methylphenoxyacetic acid has been identified as the major metabolite.

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Utilization of Labelled 'Myleran' for the Determination of the Size of DNA Molecules in Cells

The mutagenic action of the electrophilic alkylating agents has been attributed to many different causes. For Alexander and Stacey[1], the alkylation of the DNA phosphates could be sufficient to induce mutations; but these could also be the consequence of the hydrolysis of the very unstable triester phosphate formed, with rupture of the macromolecule followed by large deletions. Lawley and Brookes[2] have postulated that the 7-alkylation of guanine makes this base couple with thymine instead of cytosine and so is responsible for transitions. But the alkylation of the guanine and adenine residues can also be followed by the loss of the alkylated base[3]: this depurination might act in two ways: the hole left gives the opportunity for transitions, transversions or nucleotide deletions when the DNA replicates, or the DNA sugar-phosphate 'backbone' can break with loss of a part of the chain. All these chemical mechanisms damage only one helix of the DNA molecule exposed to the mono- or bi-functional alkylating drug and the mutation ought to appear only in one half of the genomes of the following generations.

Bridge formation between the two helices by a bifunctional alkylating agent would prevent the DNA replication and lead to the deletion of the cross-linked macromolecule which will have no descent in any genome of the following generations[4]. Besides this qualitative difference, mutation following bridge formation must be quantitatively the most important for bifunctional alkylating agents because the target for the drug is then the whole DNA molecule carrying the competent gene instead of a few sensitive triplets inside this gene.

In a previous publication[4], we tried to evaluate the size of a DNA molecule carrying a known genetic locus by the use of a bifunctional alkylating reagent labelled in its alkyl moiety. Cells of *Chlamydomonas eugametos* Moewus, sex minus, haploid and spontaneously resistant to streptomycin, were treated with 'Myleran' (1,4-dimethanesulphonyloxybutane) labelled with tritium in the butanediol moiety and a correlation was found between the frequency of cells becoming sensitive to streptomycin and the radioactivity of the isolated DNA. Starting from the hypothesis mentioned earlier that, when the mutation reaches all the cells issued from the treated one, the chemical damage must be a cross-linking between the complementary helices of the DNA molecule, and, taking into account the fraction of alkyl groups attached on the DNA which is utilized for cross-linkings, it must be possible to calculate the size of the DNA molecule carrying the competent locus. In the case of *Chlamydomonas eugametos* and the resistance to streptomycin, we tentatively evaluated for the DNA molecule specifically concerned a size of about 3,000,000 A.M.U.; this figure was only provisional in view of the several rather arbitrary corrections introduced in the arithmetics and that must be checked.

The reliability of the labelled 'Myleran' method for the estimation of the weight of an *in situ* DNA molecule biologically characterized depends on two things: (*a*) the stability of the alkyl groups bound to the DNA; (*b*) the knowledge of the percentage of bound alkyl groups forming bridges between the complementary strands of the macromolecule. The examination of these two problems was the purpose of the work reported hereafter.

Calf thymus DNA and 'Myleran' labelled with tritium in positions 2 and 3 of the butanediol moiety (20 mc./m.mole) were used throughout these experiments which were carried out at 22° C. They were dissolved separately before being put together; the final concentration of 'Myleran' was always 0·1 mg/ml. The radioactivity determinations were performed by liquid scintillation counting after suitable preparation of the sample.

(*A*) In a first set of experiments, the DNA was left for 3 h in contact with the T-'Myleran' before precipitation (addition of solid sodium chloride to bring the concentration to 1 M, then of an equal volume of 95 per cent ethanol). The fibres were washed three times with absolute ethanol and afterwards with anhydrous acetone until this solvent did not extract any more radioactivity. At this stage the alkylated DNA radioactivity was 9,100 d.p.m. mg.

This alkylated DNA was redissolved in water and DNA reprecipitated from aliquots at various time intervals. Fig. 1 shows that there was a sudden drop of the DNA radioactivity followed by a plateau.

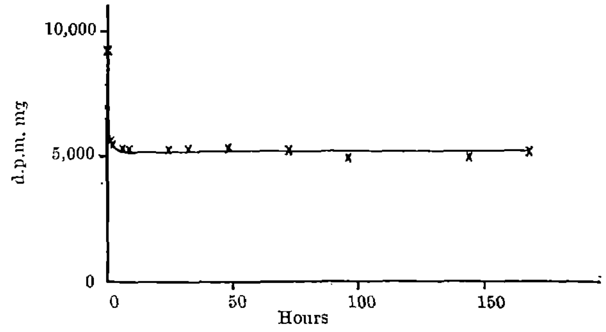

Fig. 1

To understand the meaning of the initial drop, the supernatant was percolated through an 'Amberlite IR-120(H)' column: 80 per cent of the activity came with the effluent and the water washings, while 20 per cent were retained and could be eluted with N ammonium hydroxide. The 20 per cent are thus alkylated bases. The 80 per cent (2,800 d.p.m. mg DNA) are due either to the hydrolysis of alkyl phosphates[5], or to a contamination by unreacted 'Myleran'. It is noteworthy that, when the DNA is precipitated from the solution immediately after it has been put in contact with the T-'Myleran', the activity bound to the DNA is precisely 2,800 d.p.m. mg.

Of the total of 9,100 d.p.m., we see that only 700 d.p.m. was lost as hydrolysed alkylated bases. Of course, there could also be some depurination during the 3 h contact between DNA and T-'Myleran': but the large radioactivity of the unreacted 'Myleran' prevents the detection of alkylated bases in the supernatant after precipitation of the DNA.

Most important from our point of view is the fact that, after the initial drop, the specific activity of the alkylated DNA is completely stable, around 5,000 d/min mg in our experiment. At this stage, the DNA can be dissolved and precipitated many times in succession without change of the specific activity: this proves that the precipitation procedure does not differentiate between alkylated and non-alkylated DNA.

This alkylated DNA of stable specific activity was hydrolysed for 30 min in 98 per cent formic acid at 175° C[6] and the hydrolysate evaporated to dryness under vacuum. The residue was dissolved in N hydrochloric acid and filtered. After substitution of water for N hydrochloric acid, the solution was passed through a column of 'Amberlite IR-120(H)' and the ion-exchanger washed with water; the effluent contained 880 d.p.m. (per mg hydrolysed DNA) which was not bound to compounds absorbing at 260 mμ. The column was afterwards eluted with N ammonium hydroxide which carried away the weak bases and 3,500 d.p.m. per mg DNA. This shows that most of the radioactivity firmly bound to the DNA is in alkyl groups attached on the bases of the macromolecule. The base fraction was submitted to a two-dimensional paper chromatography (methanol/ethanol/conc. hydrochloric acid/water (50 : 25 : 6 : 19, by vol.) and n-butanol/conc. ammonium hydroxide/water (86 : 1 : 13, by vol.)): the ultra-violet showed the fluorescent spots of the four bases plus two faint extra ones at the sites given by Brookes and Lawley[7] for the 7-alkyl derivative of guanine ($R_F = 0{\cdot}48$ and 0·15) and the corresponding di-guanyl compound ($R_F = 0{\cdot}26$ and 0·00). Radioactivity was found only at the sites of these two latter spots: respectively 2,950 and 250 d.p.m. (per mg hydrolysed DNA). The conclusion is that the stable radioactivity fixed on DNA after reaction with the T-'Myleran' is mostly in alkylated guanines with about 8 per cent forming bridges between two neighbouring guanine residues.

(*B*) In a second set of experiments, the DNA was left continuously in the presence of the T-'Myleran' in a 0·1 M phosphate buffer solution of pH 6·6. The DNA was precipitated from aliquots at various time-intervals and the fibres carefully washed with anhydrous acetone before the radioactivity determination. The solid line of Fig. 2 shows a continuous but non-linear increase of the radioactivity bound to the DNA. (The radioactivity found at time 0 was taken as a contamination by unreacted 'Myleran' and all the results were corrected accordingly.) In the reaction kinetics, the DNA can be considered in excess since, at the highest point of the curve, there is only one alkyl group per 3,000 nucleotides. On the other hand, the half-life of the 'Myleran', at the same temperature and in the same buffer, was shown to be 132 h, which means that the k_1 constant of the hydrolysis of the di-ester to the mono-ester has a value of $8{\cdot}75 \times 10^{-5}$ min^{-1}. Taking into account only the hydrolysis of the 'Myleran' (and assuming no participation of the mono-ester), the radioactivity (d.p.m.) bound per mg DNA ought to be:

$$x = \frac{k_2}{k_1}(1 - e^{-k_1 t})$$

where $k_2 = 19{\cdot}2$ min^{-1} is read from the initial slope of the solid curve drawn on Fig. 2. The theoretical curve of the 'x' values is depicted by a dashed line on the same graph: it is above the experimental one. The difference might be due, at least in part, to the existence of labile pools of tritium bound to the DNA.

The foregoing results indicate that, after treatment of the DNA with tritiated myleran, several pools of bound tritium are formed: a very labile, which might be alkyl phosphates but the existence of which has not been proved unambiguously (it could be a contamination by unreacted 'Myleran'); a medium labile one of alkyl bases; and, quantitatively most important of all, a stable pool also of alkyl bases. The stably bound tritium can be used

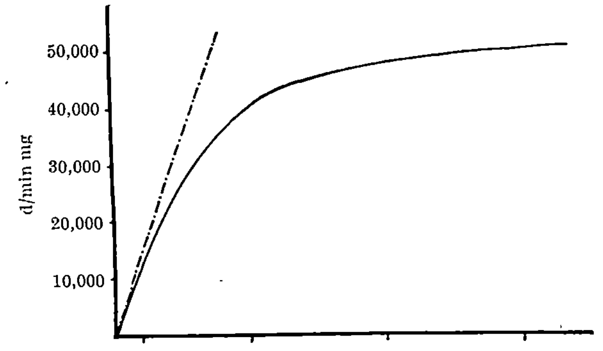

Fig. 2

as an index of the magnitude of the alkylation undergone by the DNA.

The analysis of the stably bound radioactivity has shown that it is mostly in alkyl groups attached to guanine residues with about 8 per cent linking together two guanines. We have no proof that these two guanines belong, in every instance, to the DNA complementary strands. Further work must be done to have the exact ratio between the stably bound alkyl groups and the bridges between the DNA helices. Even without this knowledge, relative molecular weights of *in situ* DNA molecules could be estimated by the 'Myleran' method; the ratio is, however, expected to be influenced by the base composition of the DNA's.

This technique of *in situ* evaluation of the weight of a DNA molecule functionally characterized by the use of a bifunctional alkylating agent has also some bearing on the elaboration of a reasonable model for non-bacterial cell chromosomes. The underlying hypothesis can be borne out only if the chromosomes of such cells are composed of independent DNA molecules glued together by more fragile linkers. On the other hand, the method actually provides a means to detect genetic loci carried by the same DNA molecule and to inquire whether each DNA molecule is an integrated operational unit[8].

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Accumulation of Acetoin by Myo-inositol-deficient Yeast

MYO-INOSITOL deficient cultures of *Saccharomyces carlsbergensis* strain 4228 (A.T.C.C. 9080) have been reported to grow both at a diminished rate and in abnormal cellular aggregates[1,2]. A comparison of the composition of deficient and normal cells revealed that myo-inositol deficiency led to an accumulation of glucans in cell walls[2]. This communication discusses another biochemical abnormality in myo-inositol deficiency resulting in the accumulation of enhanced amount of acetoin in *S. carlsbergensis* culture medium deficient in myo-inositol.

Aliquots (1 ml.) of a suspension of washed *S. carlsbergensis* cells (Klett Summerson reading of approximately 100, versus water blank, using No. 66 filter) were used to inoculate litre Erlenmeyer flasks containing 400 ml. of the myo-inositol assay medium of McKibbin[3] modified by deletion of casein hydrolysate and choline. Control flasks contained the foregoing medium supplemented with 5 mg of myo-inositol. The flasks were incubated at 28° C with shaking (New Brunswick gyrotory shaker, 143 oscillation min) and samples of growth medium were removed periodically. When supernatant fluids obtained from centrifuged resting phase cultures were analysed for glucose the myo-inositol-deficient media appeared to contain copper reducing material (by Clinitest, Ames Co., Inc., Elkhart, Ind.) which was not glucose, since it did not react with glucose oxidase test paper (Testape, Eli Lilly and Co., Indianapolis, Ind.). Afterwards, broth obtained from the deficient cultures was distilled *in vacuo* at 40° C and the distillate was shown to reduce alkaline copper solutions and to have an ultra-violet absorption maximum at 275 mμ. On addition of semicarbazide hydrochloride and sodium acetate the absorption maximum at 275 mμ disappeared and a crystalline precipitate with a melting point at 195° C was formed. This corresponds to the reported value of 193°–194° C for acetoin semicarbazone[4]. Elementary analyses were similar to those calculated for acetoin semicarbazone.

	C	H	N
Found	40·3	8·0	29·3
Calculated	41·3	7·6	29·0

Another derivative, the phenylosazone, melted at 234° C, in agreement with that reported for the biacetyl phenylosazone derived from acetoin[4].

Acetoin determinations, using the procedure of Westerfeld[5], provided data (Fig. 1) which showed that deficient cells accumulated approximately 5–8 times more acetoin than normal cells. The relatively high level of acetoin appeared sufficient to account for the previously observed copper reduction.

Acetoin accumulates in the broth during the period of most rapid growth of the deficient cells, and ceases when glucose is no longer present. This is consistent with mechanisms established for acetoin formation, in which the glucose metabolite, pyruvate, is a critical intermediate[6]. Further preliminary results indicate that other materials with reducing properties also accumulate in inositol-

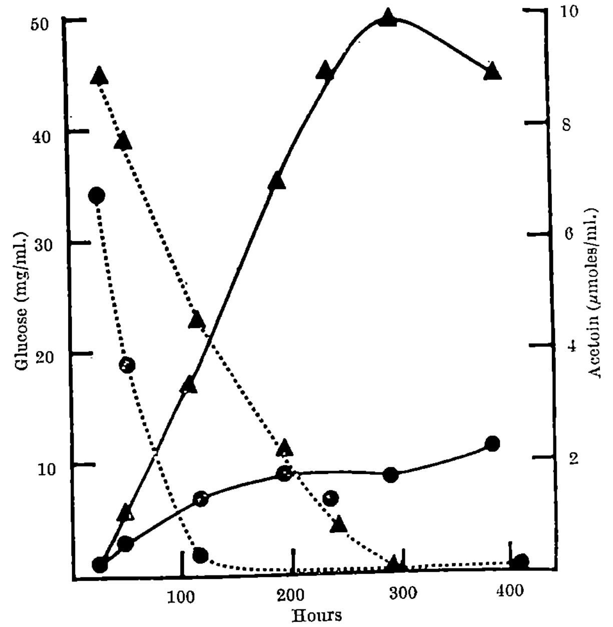

Fig. 1. Utilization of glucose and production of acetoin by *S. carlsbergensis* grown in the presence (●) and absence (▲) of myoinositol. Glucose (– – –) was determined using glucostat reagent (ref. 9), and acetoin (——) was assayed colorimetrically as described in the text

deficient supernatant broths. Analyses for acetaldehyde by the method of Desnuelle and Naudet[7] and by that of Barker and Summerson[8] modified by eliminating prior heat treatment indicate that this acetoin precursor may also accumulate in myo-inositol-deficient media. Paper chromatography of such media also demonstrated the presence of additional non-volatile compounds capable of reducing silver nitrate. The nature of these compounds, their interrelationships, and a connexion with the fundamental lesion or lesions of myo-inositol deficiency are undergoing further investigation in this laboratory. It is not clear whether the accumulation of the compounds described herein is a direct or indirect consequence of myo-inositol deficiency. It is believed that clarification of the nature of these reactions will lead to an understanding of the metabolic role of this vitamin.

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Thermal Changes in the *Nautilus* Shell

IN the shells of recent molluscs, the nacreous conchiolin appears in the electron microscope in the form of lace-like reticulated sheets or perforated leaflets[1,2]. Three patterns of structure (nautiloid, gastropod and pelecypod), differing by the size and the shape of the trabeculæ and by the disposition of the fenestration, have been provisionally recognized in these sheets.

Remnants of conchiolin have also been identified with the electron microscope in the shells of about 100 species of Palæozoic, Mesozoic and Cenozoic molluscs (refs. 3 and 4, and unpublished observations), some of them having retained the characteristic proteins of the nacreous conchiolin[5]. Well-preserved sheets of nacreous conchiolin were detected, for example, in cephalopodan shells buried in the Buckhorn asphaltic sandstones of Oklahoma[3]. In shells from other horizons, these remains were mostly modified in their structure. Examination of more than three thousand micrographs revealed that, in many fossil tests, the alterations in the conchiolin pattern consisted of flattening, thinning, shrinkage and coalescence of the trabeculæ, and conversely bloatings into vesicles of the protuberances protruding on these trabeculæ. In a more advanced stage of degradation, fragmentation of the trabeculæ resulted in formation of lenticular or rounded pebble-shaped segments or corpuscles of various sizes, generally clustered on the supporting film, and sometimes (as in an Oligocene *Aturia*[3]) appearing as riddled by tiny sieve-pits.

When the characteristic conchiolin pattern is not found in a sample, the nature of these structures is uncertain and confusion is possible with foreign contaminating matter (for example, fragments of bacteria, algae, fungi or sponges). However, several criteria, among which positive biuret tests, detection of transitional aspects between trabeculæ and pebbles in limited areas of well-preserved reticulated sheets, suggest that these structures are actually altered conchiolin.

Additional evidence for identification of these structures to conchiolin is furnished by the following observations. In order to appreciate the part played by temperature, one of the chief factors involved in diagenesis, in development of these structural alterations of conchiolin, fragments of mother-of-pearl from *Nautilus macromphalus* Sowerby and *Nautilus pompilius* Lamarck were exposed for 5 h in a dry oven to temperatures ranging from 150°–700° C. Other nacreous fragments were kept in the oven at 150° C for 11, 13 and 19 days. The results are summarized here.

Effects of temperature on preservation of organic constituents in shells (amino-acids) have already been examined in accelerated ageing tests by Abelson[6].

Mineral components. Mother-of-pearl is a hard substance, which breaks without cleavage, except on the outer margin of the fragments. Unpolished fragments are faintly iridescent, with pale pink-greenish hues. The effects of dry heat on that material consist of progressive increase in brittleness and easier cleavage into many thin flakes (200° C and above). At 500° C and above, the nacreous matter is transformed into a snow-white powdery substance.

A slight burnishing of the iridescence appears at 150° C and develops with elevation of temperature. Between 200° C and 300° C the cleaved flakes exhibit intense metallic colours and resemble closely the strongly variegated nacreous remnants of many Cretaceous cephalopodan tests (for example, Senonian *Eutrephoceras*, or tests from Ripley and Coon Creek Formations). Protracted exposure to 150° C produced the same changes. Iridescence disappears above 400° C.

X-ray powder diffraction analysis revealed the preservation of the original aragonite in the 150°–300° C samples, transformation into calcite in the 400° C and 500° C samples and into calcium hydroxide in the 600° C and 700° C samples, with traces of calcite.

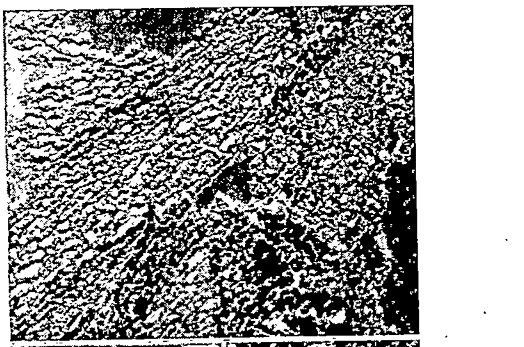
Organic components. The organic structures were examined on samples decalcified with a chelating agent (EDTAA disodium salt: 'Titriplex III' Merck). The samples heated in the range 150°–500° C dissolved rapidly, with intense emission of bubbles. At 200° C and above, a strong smell of petrol was noticeable, as in all the fossil samples treated in the same way. In the samples composed of calcium hydroxide heated to 600° C and 700° C, the chelating decalcifier produced a considerable effervescence for a short time. The mineral substance remained then inert as a white mud. Only a small portion of this mud could be dissolved in a 25 per cent solution of hydrochloric acid. It recrystallized soon afterwards.

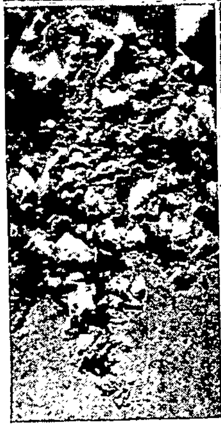
The conchiolin residues appeared as transparent shreds (in the 150° C samples heated for 5 h: as in the untreated material), as mahogany-brown (in the 200°–300° C samples) and brown-black particles (400° C and above samples). The two last-mentioned aspects are constant findings in the fossil shell material.

In all the samples, including those heated to 700° C, the biuret test showed under the conventional microscope pink and violet flakes corresponding to shreds of nacreous conchiolin.

Electron microscopy. The residues of decalcification were mounted on usual supporting films of 'Formvar', shadow-cast with palladium and examined with an R.C.A. electron microscope. The results are based on more than 400 micrographic records. Exposure to 150° C (for 5 h, 11, 13 and 19 days), to 200° C and to 225° C for 5 h altered only slightly the appearance of the reticulated sheets of nacreous conchiolin, consisting of sturdy trabeculæ with scattered protuberances, separated by an irregularly broad fenestration, characters of the nautiloid pattern (Fig. 1). In the 225° C sample, pebble-shaped swellings developed on the trabeculæ were the first signs of disintegration.

1





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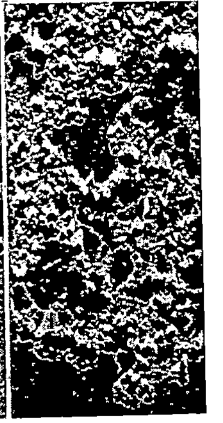

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Fig. 1. *Nautilus pompilius* Lamarck. Mother-of-pearl from the shell wall, heated before decalcification to 150° C for 5 h. Fragments of a reticulated sheet of conchiolin are shown (nautiloid pattern) (× *c.* 14,000)

Fig. 2. *Eoasianites hyattianus* (Girty) (Ammonoidea), Pennsylvanian, Buckhorn Asphalt, Sulphur, Oklahoma. Decalcified mother-of-pearl from an altered portion of the shell wall. Conchiolin remnants composed mostly of clustered, rounded bodies of various sizes (× *c.* 14,000)

Fig. 3. *Nautilus pompilius* Lamarck. Mother-of-pearl from the shell wall, heated before demineralization to 600° C for 5 h. Remains of reticulated sheets of conchiolin consist of rounded bodies of various sizes (× *c.* 14,000)

In the three figures, the shadows, directed upwards, behind the rounded bodies, appear as white triangles

Higher temperatures induced progressively disappearance of the nautiloid pattern by dislocation of the trabeculæ into rounded pebble-shaped fragments clustered at random by desiccation on the supporting films. These alterations, which precede transformation into calcite of the aragonite embedding the sheets, resemble those consistently recorded in the fossil samples of Palæozoic and Mesozoic mollusc shells (Figs. 2 and 3). They reflect probably morphological effects of degradation in some of the constituent proteins of the shell.

I thank Prof. William M. Furnish and Prof. B. F. Glenister, who supplied a fragment from *Eoasianites*, Mr. G. Casier, who performed the heatings of the material, and Mr. J. Grandjean, who recorded and analysed the X-ray diffraction patterns.

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Biosynthesis of Ethylene

It is the purpose of the present communication to describe some further results with carbon-14 precursors of ethylene, and to reconcile the pattern which emerges in these investigations using McIntosh apple tissue sections with that of the fungal system, *Penicillium digitatum*. The procedures have been described previously[1–3], and their reliability for measuring ^{14}C-ethylene and carbon dioxide commented on[3]; they were altered only in the use of a Cary–Tolbert ionization chamber in place of a Bernstein–Ballentine tube for measuring radiation. In the course of these experiments it was noted that alcohol vapour interferes with the assay for ethylene by dissolving in mercuric perchlorate absorbent where a slow acid-catalysed dehydration occurs. For example, when 10 µl. liquid ethanol was added to 20 ml. of mercuric perchlorate reagent, ethylene appeared at a linear rate for at least 6 h, by which time 0·02 per cent of the alcohol had been converted to olefine. Previously, Young *et al.*[9] reported that ethanol was not measured by the mercuric perchlorate manometric procedure, but they apparently took no account of the duration of contact between alcohol and perchlorate, and it should be noted that gas chromatography detects much smaller quantities of vapour than does manometry. In the work recorded here it was determined that a discernible error could occur only if at the end of a collection period in excess of 1 µc. of ^{14}C-ethanol had been formed in the tissue. In experiments with fumarate and acetate this would require that labelled ethanol form at approximately the same rate as $^{14}CO_2$, and with other compounds ethanol production would greatly have to exceed $^{14}CO_2$ evolution. Clearly, under the conditions of the assay, the results are not influenced by this factor except when ^{14}C-ethanol itself was administered to the tissue (Table 1), in which case the apparent transfer of label to ethylene must be dismissed as an artefact.

Table 1. CONVERSION OF CARBON-14 SUBSTRATES TO ETHYLENE AND CARBON DIOXIDE IN APPLE TISSUE

		Specific activity (d.p.m./µg C)*			
		Carbon dioxide†		Ethylene†	
Compound	^{14}C label	0–80′	80–200′	0–80′	80–200′
Glucose	U	77	114	67	143
Glycerol	1,3	416	752	24	214
Propionate	2	227	475	130	119
Pyruvate	2	508	536	20	—
Pyruvate	3	45	94	26	—
Acetate	1	22,000	34,150	89	92
Acetate	2	1,730	7,240	100	418
Glycine	2	163	502	2	11
Malonate	1,3	440	452	0	0
Malonate	2	26	50	0	4
Ethanol	1	80	89	776	215
Fumarate	1,4	9,550	7,190	218	365
Fumarate	2,3	1,800	2,610	257	584
Carbon dioxide‡	—	—	—	79	106

* Per 100 µc. administered ^{14}C of S.A. = 2 mc./mM.
† Times are intervals after the tissue was soaked for 60 min in the isotopic solution.
‡ $^{14}CO_2$ was present only during the 0–80′ collection period.

Previously, the conversion of the individual carbons of glucose to ethylene was evaluated in both *P. digitatum*[8] and McIntosh apples[3] with closely similar results. The relative ease of conversion to ethylene of carbon(s) 6 : 1 : 2 : (3 + 4 + 5) was 3·2 : 1·0 : 1·1 : 2·5 in apples and 2·4 : 1·3 : 1·0 : 1·9 in the fungus. To the extent that the hexose monophosphate pathway (HMP) functions in both tissues, triose will form preferentially from carbons 4–6 of glucose, and if ethylene arises from triose the 6-C of glucose should yield labelled ethylene more readily than the 1-C. In both tissues a significant contribution of the HMP pathway has been reported[3,7], and assuming ethylene to form from triose, the contribution to ethylene of the 6-C of glucose relative to the 1-C is quantitatively consistent with the respiratory data for apples[3] and would be so for *P. digitatum* if the 23 per cent estimate of the HMP pathway in that tissue[7] is a slight underestimate. Significantly, Katz and Wood[5] caution that the HMP calculation for *P. digitatum* may be in error since it fails to take into account carbon recycling and incomplete oxidation. Because of the aforementioned considerations, the 2-C of glucose cannot be equated to the 5-C as suggested by Wang *et al.*[8], but rather the ratio of ease of conversion to ethylene of the 1-C relative to the 6-C might be expected to be similar to that of the 2-C relative to the 5-C. If this were true, it can be calculated that the 3,4-C's of glucose do not contribute to ethylene formation in either tissue.

Carbon recycling by the HMP pathway would transfer label from the 2-C to the 1-C position of glucose-6-phosphate, but this cannot quantitatively account for the participation of the 2-C in ethylene synthesis because even at equilibrium in a tissue respiring entirely by the HMP pathway, after application of the 2-^{14}C-glucose the specific activity of the 1-C of glucose-6-phosphate would be only 1/3 that of the 2-C (ref. 5). Similarly, although in *P. digitatum* a significant conversion of the carboxyl carbon of acetate to the amino-carbon of aspartate occurs[6], this mechanism is not sufficient to explain the nearly equal yields in ethylene from 1- and 2-^{14}C-glucose. In apples, results with 2-^{14}C-pyruvate, 1-^{14}C-acetate, 1,4-^{14}C-fumarate and $^{14}CO_2$ (Table 1) emphasize that the 2-C of triose may be converted to ethylene by oxidative decarboxylation of pyruvate and metabolism of the resultant acetate in the Krebs cycle. It should be noted that even with 1-^{14}C-acetate, at no time did the $^{14}CO_2$ content surrounding the tissue rise above a level that could account for more than 6 per cent of the activity in ethylene, as judged by experiments with applied $^{14}CO_2$ (Table 1). Likewise, with *P. digitatum*, Gibson and Crane[4] reported that in 3 h experiments a significant conversion of 2-^{14}C pyruvate to ethylene occurred. Since during the first 80 min after isotope is administered to apples, carbons 1 and 2 of pyruvate are equally effective in forming ethylene, as are carbons 1 and 2 of acetate and 1,4 versus 2,3 of fumarate, it is likely that the 2,3-C's of triose are converted to ethylene as a unit, via acetate and Krebs cycle intermediates. This interpretation cannot be reconciled with the suggestion of Wang *et al.*[8] that ethylene forms by a double decarboxylation of fumaric acid, for such a mechanism demands that the yield from the carboxyl carbon(s) of fumarate and acetate be low, initially, perhaps increasing with time. There is no discrepancy between the behaviour of McIntosh apples and the *P. digitatum* culture used by Wang *et al.*, for as the duration of the experiments with apples is extended to 200 min (Table 1) the data resemble more and more the six-day cumulative results with the mould[8]; namely, carbon positions metabolically related to the methylene carbons of fumarate are preferentially used for ethylene synthesis. This phenomenon previously was noted[1] when 1-^{14}C-acetate and U-^{14}C-sucrose were found to be almost equally effective in forming labelled ethylene during 22 h after injection into the ovary of a whole apple, whereas in the next 36 h the specific activity of ethylene declined significantly in the acetate experiment, and increased markedly with sucrose. A major result of prolonging the duration of investigations of this type is to invite complication by isotope redistribution, and the different patterns obtained in short- and long-term experiments with organic acids or related compounds may be due to the fact that a carboxyl label is readily lost as carbon dioxide, whereas the 2-C of acetate and 2,3-C of fumarate tend to be recycled through the Krebs cycle and retained. For example, in experiments with apple tissue slices, about 45 per cent of the label applied as 1-^{14}C-acetate, but only 9 per cent of the activity fed as 2-^{14}C-acetate, appeared in carbon dioxide within 200 min. In the case of fumarate, 16 and 4 per cent of the administered activity was recovered as $^{14}CO_2$, respectively, from the 1,4- versus 2,3-C isotopes in the same period of time. When it is considered that only about 55 per cent of the ^{14}C-acetate and 30 per cent of the labelled fumarate entered the slices, the results indicate that depletion of the carboxyl carbon within the tissue limited its conversion to ethylene in experiments lasting several hours. Although figures are not available for *P. digitatum* under conditions exactly resembling those in which ethylene was assayed, it has been reported that the cells of *P. digitatum* retain the 2-C of acetate to about twice the extent of the 1-C (ref. 6).

With apples, the maximum specific activity thus far reported for ethylene amounts to only 0·3 per cent of that in the applied isotope (2,3-^{14}C fumarate), and for *P. digitatum* the highest average value is only 0·1 per cent (3,4-^{14}C glutamate). In apples it is certain that the low activity in ethylene cannot be explained by isotope dilution of the applied substance by a tissue pool because the carbon dioxide produced during 200 min with ^{14}C-acetate has a specific activity of between 13–20 per cent of that theoretically possible, and with 1,4-^{14}C-fumarate the value was 4–5 per cent. In fact, with all isotopes except glucose, the carbon dioxide acquired a significantly higher specific activity than ethylene, which suggests that although ethylene formation is related to the metabolism of Krebs-cycle acids as suggested by Wang *et al.*[8], still it is presumptuous to conclude that the relationship is very direct.

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Antioxidants isolated from Fermented Soybeans (Tempeh)

In an examination of the nutritional value of tempeh, which is prepared by the action of *Rhizopus oryzae* on boiled soybeans, rats fed tempeh showed better growth and greater resistance of red blood cells to *in vitro* dialuric acid-induced hæmolysis than rats fed plain boiled soybeans[1]. Hæmolysis of red blood cells induced by dialuric acid or by appropriate low concentration of hydrogen peroxide indicates in general a deficiency of vitamin E as a biologically highly effective antioxidant[2,3]. The comparative findings between boiled and boiled-fermented (tempeh) soybeans seems to support the preliminary conclusion that antioxidants were either formed *de novo* or liberated during fermentation through fungal activity from complex, originally inactive compounds, present in unfermented soybeans.

Paper chromatographic analysis by the procedure of Russell Eggitt and Ward[4] of alcohol and ether extracts of dried powdered tempeh indicated the presence of a substance at R_F 0·92 giving a positive ferric chloride reaction and Emmerie–Engel test. The spot did not correspond to α-tocopherol, or to *F* 84, isolated from yeast[5] and identified as 2-(6-hydroxy-2-methoxy-3,4-methylene-dioxyphenyl)-benzofuran[6]. An eluate of the section of the paper corresponding to R_F 0·92 prevented the hæmolysis of red blood cells of vitamin E-deficient rats with dialuric acid[7].

The *in vitro* test of hæmolysis-preventing activities of samples was carried out according to the procedure of Rose and György[2] as adapted by Forbes and György[8]. The activity was expressed as the number of 'hæmolysis prevention' units per mg; a unit was defined as weight

Table 1. PROPERTIES OF THE ANTIOXIDANTS FROM TEMPEH, *F*84 AND α-TOCOPHEROL

Substance	Colour reaction: Fluorescence	Colour reaction: $FeCl_3$	Colour reaction: Emmerie–Engel reaction	m.p. (°C)	λ_{max} in ultra-violet spectrum (mμ)	R_F on paper chromatogram: 25% EtOH (ascending)	R_F on paper chromatogram: BuOH (1)* EtOH (1) Borate (1) (ascending)	Hæmolysis preventing activity (ratio)
Factor 2	−	+	+	320 (decomp.)	260 327	0·25	0·72	1
Genistein	−	+	−	298 (decomp.)	262	0 (tailing)	0·86	0·05
Daidzein	+	−	−	323 (decomp.)	249 300	0 (tailing)	0·87	0·01
F 84	+	+	+	118	300	0	0·95	2
α-Tocopherol	−	−	+	−	292	0	0·99	1

* Coulson, C. B., and Evans, W. C., *J. Chromatog.*, 1, 374 (1958).

Table 2. PROPERTIES OF FACTOR 2 AND 6,7,4′-TRIHYDROXYISOFLAVONE

	m.p. (°C)	R_F value: 25% EtOH	R_F value: BuOH (1) EtOH (1) Borate (1)	R_F value: Bz (125) AcOH (72) H_2O (3)	Colour reaction: Fluorescence	Colour reaction: $FeCl_3$	Colour reaction: Emmerie-Engel	λ_{max} in ultra-violet spectrum (mμ)	μ_{max} in infra-red spectrum (cm^{-1})
Factor 2	320 (decomp.)	0·25	0·70	0·22	−	+	+	260 327	3,250 1,630 1,580 } 1,520 } 1,250
6,7,4′-Trihydroxy-isoflavone	320 (decomp.)	0·25	0·70	0·22	−	+	+	260 327	3,260 (OH)* 1,635 (pyrone C═O) 1,580 } (aromatic 1,524 } C═C) 1,253 (aromatic C—O)

* McMurry, T. B. H., and Theng, C. Y., *J. Chem. Soc.*, 1491 (1960).

of substance preventing hæmolysis as well as 0·2 μg of α-tocopherol.

An extract of control soybeans had very low activity. The activity approached that of tempeh after hydrolysis with hydrochloric acid.

After many attempts to isolate the active antioxidant from tempeh, three kinds of anti-hæmolytic substances were isolated in crystalline forms. For their isolation the following procedure was used[7]: (1) Powdered tempeh was extracted with 95 per cent ethanol and the alcohol extract was concentrated to syrupy consistence. (2) The syrupy residue was extracted with 50 per cent ethanol and the solvent was evaporated off. (3) The condensed oily material was extracted with ether and the ether of the extract was evaporated. (4) The residue was treated with 25 per cent ethanol. Crystals of m.p. 323° and 298° were obtained from 25 per cent alcohol-insoluble fraction. (5) The 25 per cent alcohol soluble part was fractionated by polyamide column chromatography as shown in Fig. 1. Peak 5 was shown by paper chromatography to contain daidzein and an unknown substance, called 'Factor 2'. (6) Factor 2 was separated from daidzein in a large-scale paper chromatographical experiment using butanol/ethanol/borate (1 : 1 : 1), and extracted from the paper with alkaline water, acidified, and transferred to ether. From the ether fraction the active substance was crystallized. (7) The crystals of m.p. 320° (decomp.) were obtained by recrystallization from dioxane. The yield was only 20 mg in a pure form out of 5 kg dried tempeh.

Crystals of m.p. 323° and 298° were respectively identified as daidzein (7,4′-dihydroxyisoflavone) and genistein (5,7,4′-trihydroxyisoflavone) by identity of melting points, elementary analysis, ultra-violet spectra, R_F value, colour reactions, and hæmolysis *in vitro* test with those of known specimens. Hæmolysis preventing activity of Factor 2 was much higher than that of daidzein and genistein. The activities of these substances are shown in Table 1 together with other properties.

From infra-red and ultra-violet spectra, colour reaction (ferric chloride and Emmerie–Engel), and melting point, Factor 2 was presumed to be the same substance as 6,7,4′-trihydroxyisoflavone, prepared originally from afromosin, which is present in *Afromosia elata*[9,10]. This working hypothesis was conclusively proved to be correct by direct comparison of pure 6,7,4′-trihydroxyisoflavone with pure Factor 2 (Table 2 and Fig. 2). (We thank Dr. T. B. H. McMurry for the specimen of 6,7,4′-trihydroxyisoflavone and Dr. K. Fukui, Dr. S. Shibata and Dr. N. Inoue for their advice.)

Daidzein and genistein are the aglucones of the known glucosides, daidzin and genistin. Enzymatic hydrolysis of the glucosides through the action of *Rhizopus oryzae* has been proved by the use of aqueous extract of the fungus on boiled extracts of unfermented soybeans (unpublished observations). The presence of 6,7,4′-trihydroxyisoflavone in natural products has not yet been demonstrated. Our investigations have to be extended to the demonstration of 6,7,4′-trihydroxyisoflavone in unfermented soybeans. In the absence of this information, the possibility of the *de novo* synthesis of 6,7,4′-trihydroxyisoflavone through fungal action cannot be excluded.

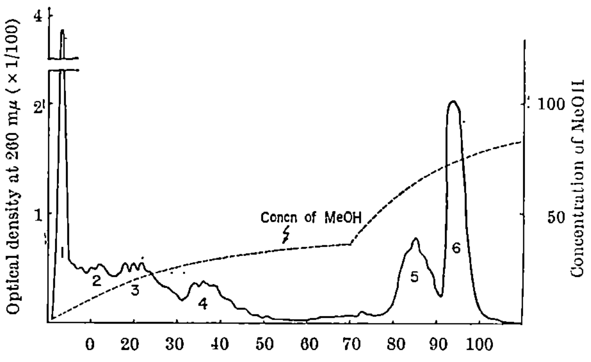

Fig. 1. Polyamide column chromatography of tempeh extract (gradient elution with water–methanol)

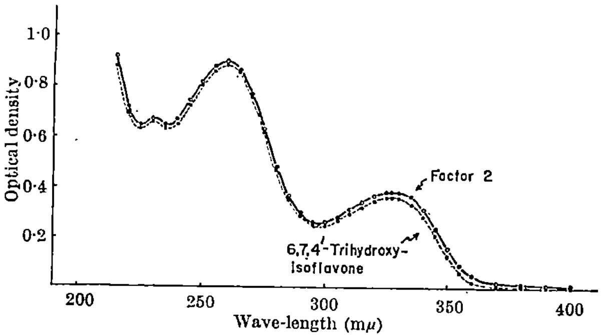

Fig. 2. Ultra-violet spectra of Factor 2 and 6,7,4'-trihydroxyisoflavone (10 μg/ml. in 99 per cent ethanol)

However, the high antioxidant activity of hydrochloric acid-hydrolysed extracts prepared from unfermented soybeans being of the same order as that of extracts obtained from fermented soybeans favours the conclusion that the highly active 6,7,4'-trihydroxyisoflavone is a normal constituent of soybeans.

The antioxidants liberated in tempeh act probably through their protection and preservation of the biologically eminently effective vitamin E in soybeans and not by direct biological action. When larger amounts of 6,7,4'-trihydroxyisoflavone become available, its antioxidant effect in vitamin E-deficient animals should be investigated.

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PHYSIOLOGY

Lability of the Action Potential and the Recovery Cycle from Isolated Motor Axons of the Crab, *Carcinus*

The action potential evoked from an artificially produced excitable node of isolated crab axon membrane[1] shows similar changes to those described for the isolated toad node[2], when currents are applied during the action potential itself. The rising phase of the action potential can be modified only by very considerable anodal currents (some 40 times rheobase), but the repolarization phase shows changes when weak currents of either polarity are applied (Fig. 1). Repolarization is delayed by cathodal current and is hastened by anodal current, when these are applied during the falling phase of the action potential. The divergence of the voltage profile of repolarization, produced by a constant current pulse of either polarity, is greater the later it is applied, as would be expected from the form of the change in membrane resistance that accompanies the voltage change of the action potential. The changes in the rate of repolarization are proportional to the strength of the applied current and its duration, so that complete repolarization can be achieved with strong anodal current, that is, the later phase of the action potential can be abolished.

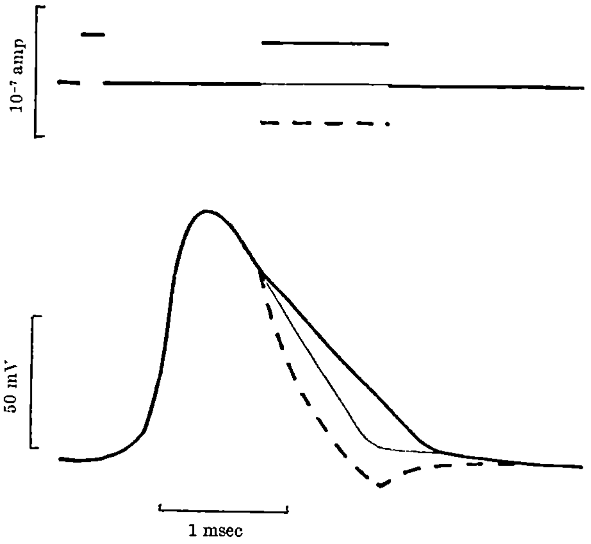

Fig. 1. Tracings from original records illustrating the modification of the form of the action potential when anodal current (dashed line), and cathodal current (solid line) are applied during the repolarization phase. The normal course of repolarization is shown by the fine solid line. Action potentials are evoked from an artificial node of excitable membrane, crab motor axon, 17° C

The form of the recovery of the spike generating mechanisms following an action potential is also modified by currents applied during the repolarization phase, as is shown by the recovery of spike amplitude, threshold current and potential threshold. An action potential shortened by anodal current is followed by a more rapid recovery, while one prolonged by cathodal current is

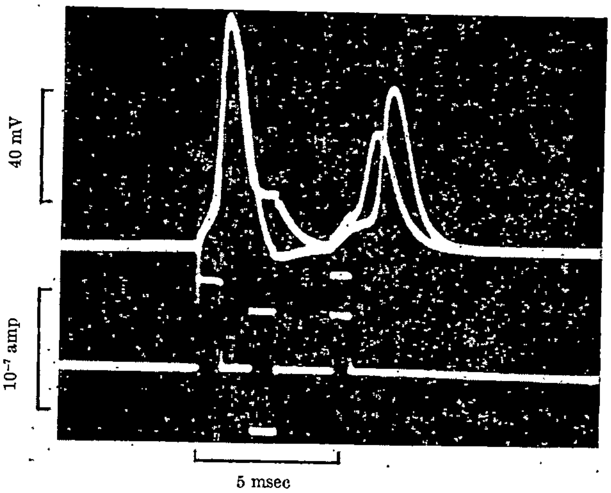

Fig. 2. Two superimposed records comparing the effects on recovery following an action potential, when that action potential has been shortened by anodal current or lengthened by cathodal current. The recovery is more rapid following treatment with anodal current, as is seen from the weaker threshold current, the greater spike amplitude, and the lower threshold potential. Artificial node of a crab motor axon, 17° C

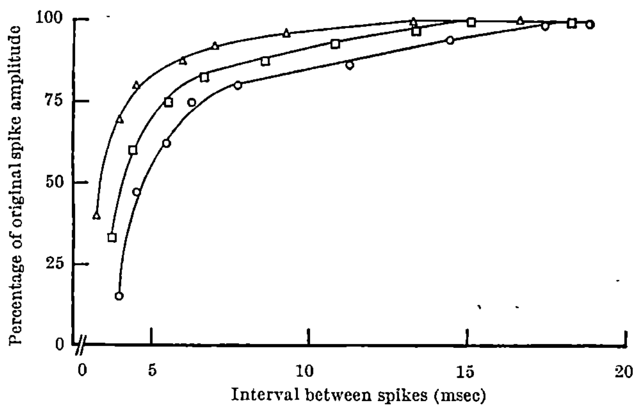

Fig. 3. The normal recovery cycle (□) is shorter in duration following treatment of the action potential with anodal current during repolarization (△), while prolongation follows treatment with cathodal current (○). Isolated crab motor axon, 17° C

followed by a slower recovery (Figs. 2 and 3), during which the spike amplitude, the critical level of depolarization for the spike, and the current threshold show parallel changes.

These findings are of obvious importance when the form of the response of these axons to maintained depolarization is considered. It has long been known that the form and duration of the recovery cycle cannot alone account for the nature of the response to maintained cathodal current in crab axons[3], if the recovery cycle remains unchanged throughout the response. Fuortes and Mantegazzini[4] on the eccentric cells of *Limulus* eye, and personal observations on crab axons, have shown that it is necessary to consider that sustained depolarization has an additional depressant effect on excitability, since higher discharge frequencies are obtained by stimulation with trains of short current pulses than are elicited by a maintained current of the same current strength, beyond a certain frequency. This depressant action of sustained depolarization can be equated with the prolongation of the recovery cycle which is found when cathodal currents are applied during the action potential.

The underlying changes associated with the modification of the action potential by anodal currents has been considered by Huxley[5]. He has shown that the Hodgkin–Huxley equations account for this behaviour since the sodium conductance is reversible during the action potential. The modification of the action potential in crab axons by applied currents can be interpreted similarly; a reversibility of inactivation and/or the potassium conductance can also be inferred from the changes in the recovery cycle.

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Biliary Excretion of Cæruloplasmin Copper

The importance of the mechanism by which normal subjects maintain zero balance of copper is seen in patients with Wilson's disease who, lacking it, accumulate copper to the point of toxicity. In these patients, the association of chronic copper toxicity and life-long deficiency of cæruloplasmin has aroused considerable speculation about a possible role of this copper-protein in the control of either absorption or excretion of copper[1]. Since earlier studies failed to indicate an effect of circulating cæruloplasmin on the absorption of copper[2], our attention has recently been directed on the alternative possibility that cæruloplasmin is involved in the excretory pathway of copper. Bile is a major route for the excretion of heavy metals; the question arose therefore whether cæruloplasmin copper passes directly into bile, as suggested by Jeunet, Richterich and Aebi[3]. This was answered by intravenously injecting cæruloplasmin labelled with copper-64 into rabbits, and correlating the disappearance of radioactivity from the blood with the appearance of radioactivity in the bile.

Cæruloplasmin labelled *in vivo* was isolated from the serum of rabbits which had received copper-64 acetate intravenously on the preceding day. The copper-protein was freed of non-cæruloplasmin copper with sodium diethyldithiocarbamate[4] and purified by chromatography of DEAE cellulose[5]. For each experiment, 14-60 μg of cæruloplasmin–copper-64 was injected intravenously into an adult female rabbit. The biological half-life of cæruloplasmin was measured in 3 rabbits by periodically determining the radioactivity of serum. Six other rabbits received similar doses of labelled cæruloplasmin. The bile ducts of two of these (rabbits Nos. 1 and 2) were immediately cannulated with No. 90 polyethylene catheters, and complete collections of bile were obtained for 6 and 18 h, respectively, at which time the animals were killed and the radioactivity in the liver determined. The same procedure was followed in another rabbit (No. 4) except for delaying cannulation of the bile duct for 20 h following injection of labelled cæruloplasmin and collecting bile for a total of 3½ h. In a fourth rabbit radioactivity of the liver alone was determined 19 h after injection. Complete collections of urine and fæces were obtained in the remaining two intact rabbits for 67 h after injection of labelled cæruloplasmin, after which they were killed.

Half-life of cæruloplasmin in the rabbit. The half-life was found to be 44, 58 and 67 h in the 3 rabbits examined, for a mean value of 56 h. This last figure was used as the basis for calculating in subsequent experiments the amount of cæruloplasmin cleared from the circulation during any time.

Excretion of cæruloplasmin copper. Following intravenous administration of labelled cæruloplasmin, less than

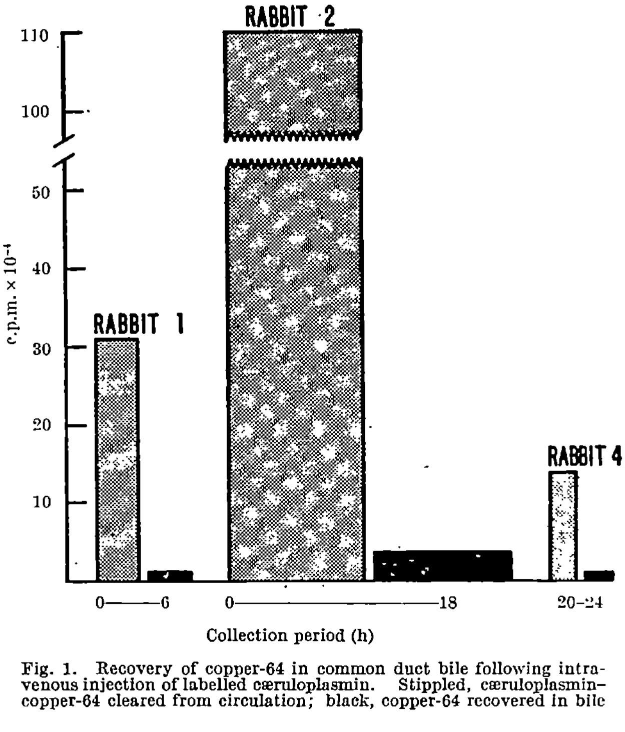

Fig. 1. Recovery of copper-64 in common duct bile following intravenous injection of labelled cæruloplasmin. Stippled, cæruloplasmin–copper-64 cleared from circulation; black, copper-64 recovered in bile

5 per cent of the fraction cleared from the circulation during the collection period was recovered in the bile whether cannulation was performed immediately following injection or delayed for 24 h (Fig. 1). When fæces and urine of 2 rabbits were collected for 67 h following administration of labelled cæruloplasmin less than 5 per cent of the radioactivity cleared from the circulation appeared in the combined collection from each animal.

Recovery of cæruloplasmin copper in liver. 17–70 per cent of the radioactivity cleared from the circulation during the interval between injection and killing was recovered in the liver (Fig. 2).

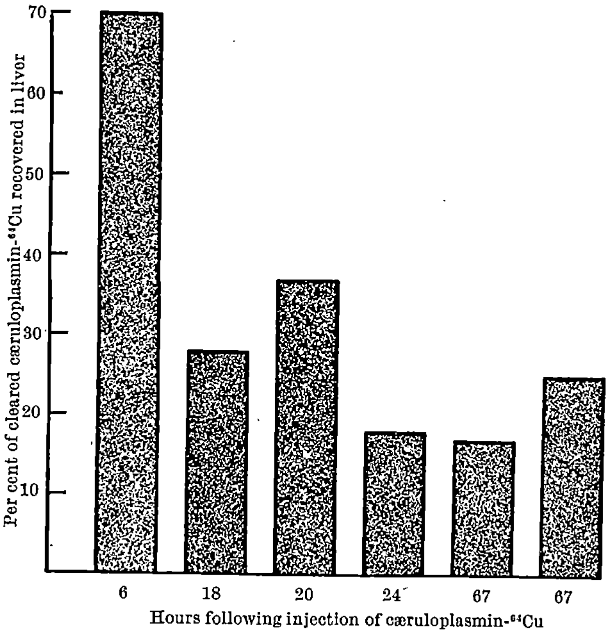

Fig. 2. Recovery of copper-64 in liver following intravenous injection of labelled cæruloplasmin

Because circulating cæruloplasmin does not renew or exchange its copper *in vivo*[4], the decline in radioactivity from the circulation following injection of copper-64-labelled cæruloplasmin is probably due to removal of molecules through metabolic degradation. Although a substantial fraction of the radioactivity cleared from the circulation was recovered in the livers of killed animals, only negligible activity was found in bile, fæces or urine. Thus, the copper of catabolized cæruloplasmin is not directly or preferentially excreted by hepatic, pancreatic, intestinal or renal routes in the rabbit.

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Auditory Habituation in Cats as a Function of Stimulus Intensity

HABITUATION of the electrophysiological response in the cochlear nucleus (CN) to repetitive auditory stimuli has been reported by a number of workers[1–3]. Similar decrements were not obtained in a more recent study[4]. Marsh *et al.*[5], while reporting a decrement in the CN response, have shown increased responses to repetitive stimulation in the inferior colliculus (IC) and the medial geniculate (MG).

This investigation was designed to examine the effects of repetitive stimulation on the CN, IC and MG, and to determine whether any habituation obtained was a systematic function of stimulus intensity.

Using methods previously outlined[6], chronic implantations with stainless-steel bipolar electrodes were made in the CN, IC and MG of 25 cats. After allowing 3–4 weeks for recovery, the animals were stimulated in a sound-attenuated test box, which allowed behaviour to be monitored through one-way glass. The evoked auditory responses were recorded by an Offner EEG machine. Each trial consisted of presenting a 1,000 c/s tone of 20 msec duration every 2 sec for 95 min. Each animal was tested twice with four levels of intensity (55, 65, 75 and 85 decibels (db) as measured by a Dawe Sound Level Meter. Background sound pressure-level within the test box was approximately 45 db with respect to 0·0002 dynes/cm^2). To reduce any possible long-term effects, stimulus intensity was randomized over trials and the interval between consecutive trials was at least one week.

Recordings were taken every alternate 5 min and the overall amplitude of each evoked potential was measured and a mean score calculated for every 5-min period. These means were then expressed as a percentage decrement of a control value which was the mean of the initial four responses of a given trial.

Fig. 1 shows the mean plot for each placement as a function of intensity-level. Over all intensity-levels, it can be seen that the CN habituates less than the IC, which in turn habituates less than the MG. When the results for each centre are examined as a function of intensity-level, habituation in the CN and the IC is an inverse function of stimulus intensity. In the MG, the trend of the lower centres is reversed, and the greatest habituation occurs to the most intense stimulus.

Several conclusions can be drawn from these observations. (1) The nearer a placement is to the cortex, then the greater the degree of habituation. (2) Very little habituation occurs in the cochlear nucleus, and any marginal decrements obtained are restricted to tones of low intensity. (3) By using this method of repeated sampling over time, it can be seen that most of the habituation effect occurs in the first 40 min. In fact the reason for using a control period of the four initial responses was based on some preliminary experiments in which significant decrements were shown in the first minute of a trial.

It could be argued that our results are a function of the duration of our recording session. That is, the dramatic decrements obtained by other workers could be a function of longer periods of stimulation. However, experiments[7] in progress indicate that if the duration of a test session is extended to 8 h or 14,400 consecutive stimuli, then there is little increase in the level of habituation for a given stimulus intensity. Perhaps this apparent conflict with other results can be explained by observations made by Garcia-Austt *et al.*[8], who have pointed out that unless continuous averaging over time is used in the examination of habituation, then it is possible that "if . . . responses in the peak of a waning are compared with the superposed or averaged responses of the waxing peak of the subsequent cycle, the response amplitude may be the same or even higher". We have observed waxing and waning of response amplitude over time, and if only small

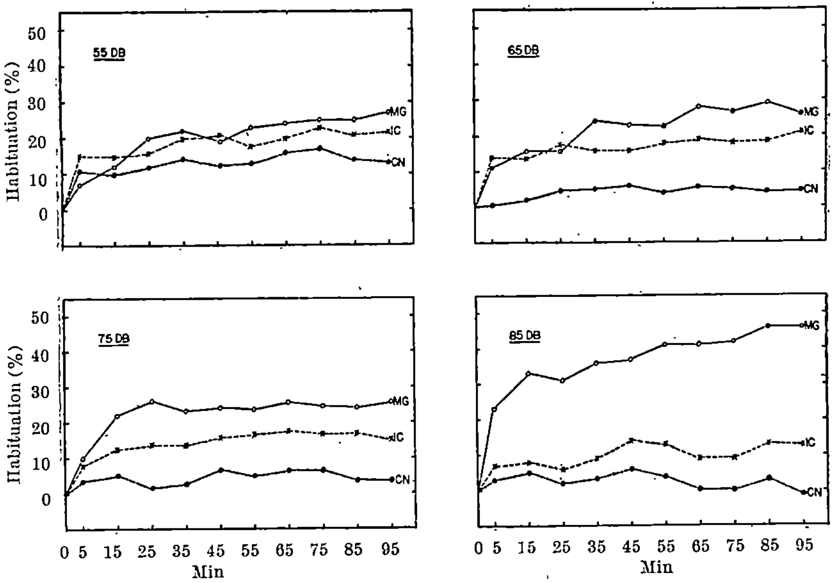

Fig. 1. Degree of auditory habituation, observed in the medial geniculate (MG), inferior colliculus (IC), and cochlear nucleus (CN) during 95 min of repetitive auditory stimulation at different sound intensity-levels

numbers of select responses are measured, then this could lead either to dramatic decrements or to significant increases over control-levels.

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Synaptic Transmission at Taste Buds

Previous investigations have shown that a variety of sensory receptors can be stimulated by the application of acetylcholine[1], this sensitivity being enhanced in mechanoreceptors by the addition of 10^{-5} g/ml. physostigmine. Excitation by acetylcholine was blocked by tubocurarine or hexamethonium (10^{-7}–10^{-5} g/ml.)[2], but the response of the receptor to normal mechanical stimuli was unaffected by the presence of these blocking agents[1,2], and Gray and Diamond conclude, therefore, that acetylcholine has no physiological function as an intermediary in the initiation of nerve impulses at sensory receptors[1]. Landgren *et al.*[3], applying drugs to the surface of the frog's tongue, found, however, that high concentrations of anticholinesterases initially increased the spontaneous activity in the lingual nerve and afterwards blocked taste sensitivity. Tubocurarine also reduced the response of the taste buds to gustatory stimuli and these authors suggest that acetylcholine is concerned with the initiation of impulses at chemoreceptors.

Taste buds differ from mechanoreceptors in that a synaptic discontinuity exists between the chemoreceptor and its afferent neurone, the synapse having now been demonstrated by electron microscopy[4]. Davis[5] has presented a model for sense-organ excitation in which he suggests that, where such a synapse exists, stimulation produces a receptor potential which causes the liberation of a transmitter. On its arrival at the post-synaptic side, this transmitter produces a generator potential which initiates the action potential in the neurone. The differing results obtained with mechano-[2] and chemo-receptors[3] might, therefore, be explained by the action of acetylcholine as a transmitter in the latter, a hypothesis which is supported by recent histochemical localizations of cholinesterases in mammalian taste buds[6].

In the present experiments, recordings were made from the lingual branch of the glossopharyngeal nerve of frogs (*Rana temporaria*), as described by Landgren *et al.*[3], but the drugs were perfused through the lingual artery. Gustatory stimuli were applied to the surface of the tongue.

Perfusion of the tongue preparation with 10^{-4}–10^{-2} M 62*C*47 or with 10^{-5}–10^{-3} M iso-*OMPA* for 25 min did not reduce the sensitivity of the tongue to gustatory or tactile stimulation. Preparations perfused successively with 62*C*47 and iso-*OMPA* still responded normally. 62*C*47 and iso-*OMPA* are regarded as specific inhibitors for acetylcholinesterase and pseudocholinesterase respectively and the highest concentrations studied are 1,000 times those required for complete inhibition of these enzymes[7].

The general cholinesterase inhibitors physostigmine (10^{-7}–10^{-4} M) and neostigmine (10^{-6}–10^{-4} M) did not affect gustatory sensitivity when perfused for 15 min. However, the response to chemical stimulation was blocked by using either $1\cdot25 \times 10^{-3}$ M physostigmine (15 min) or the general esterase inhibitor, diethyl *p*-nitrophenyl phosphate (*E*600), at a concentration of 10^{-3} M (7 min). More dilute solutions of *E*600 did not have this effect. The blocking actions of both physostigmine and *E*600 were readily reversible.

d-Tubocurarine (10^{-3}–10^{-5} M), hexamethonium (2×10^{-2} M) and decamethonium (10^{-2} M), which are blocking agents for muscle endplates and autonomic ganglia, failed to reduce taste sensitivity on perfusion for 20 min.

It is impossible to determine the concentration of the drugs at the synapse under these conditions, but the taste buds respond readily to other substances injected in a similar fashion[8]. The concentrations of esterase inhibitors at which a block of taste sensitivity was obtained were very high indeed and probably reflect the block produced by high concentrations (2×10^{-3} M) of physostigmine in non-myelinated nerve fibres[9].

It is, therefore, unlikely that cholinergic transmission operates at the synaptic connexion between the taste bud and its afferent neurone. Possibly, some other form of specific chemical transmitter is liberated, or alternatively transmission may be electrical, as in the synaptic connexion in the abdominal ganglion of crayfish between the lateral giant axon and the segmental motor fibre[10].

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PHARMACOLOGY

Effect of Excess of Antigen on the Active Anaphylactic Reaction *in vitro*

The similarities between the effects of the classic anaphylactic reactions and the reactions induced by antigen–antibody complexes suggest that there may be a similarity in the mechanisms responsible for these reactions. It is known that the ability of soluble complexes to produce anaphylactic reactions is governed by the quantitative relationship between antigen and antibody: the complexes prepared in slight-to-moderate excess antigen are most effective in producing anaphylactic reactions, whereas in greater than moderate antigen excess this effectiveness decreases with an increase in the amount of antigen added[1–3]. Recently, it has been shown that the passive anaphylactic reaction is inhibited by very large excesses of antigen[4,5]. In the present investigation on the release of histamine from actively sensitized peritoneal mast cells of the rat, it is shown that increasing excesses of antigen decrease the intensity of the anaphylactic reaction.

The rats were actively sensitized to ovalbumin using h. pertussis as an adjuvant. After 20 days at least of sensitization, heparinized saline or Tyrode's solution was injected intraperitoneally, the peritoneal fluid collected after gentle massage, and used after adequate dilution (about 20 mast cells per mm^3) in the experiments. In some cases, the peritoneal fluid was centrifugated and the sediment suspended again in Tyrode, or the mast cells were isolated from the other cells by differential centrifugation over a 30 per cent bovine serum albumin (BSA) solution. The antigen, 'Sigma' or laboratory ovalbumin crystallized three times, was added to the diluted mast cells suspensions after incubation for 15 min at 37° C. After a further 15-min incubation with the antigen, the cells were centrifuged, the supernatant completely drained and the sediment re-suspended and heated at 100° C for 3 min. The histamine released from the mast cells was biologically assayed both in the supernatant and in the sediment extract and expressed as a percentage of the total mast cell histamine content. The spontaneous histamine released was subtracted from the antigen one, each value being the mean of two tests.

The results of some typical experiments (Fig. 1) show that there is an optimum in the concentration of antigen, an excess of it inhibiting the histamine release. Controls involving the addition of a non-specific protein to the antigen solution (Fig. 1, III) showed that the inhibitory action was not caused by an excess of protein, but specifically by an excessive amount of the homologous antigen. Experiments showing that excess of ovalbumin had no effect on the release of histamine by various concentrations of 48/80 also confirmed the specificity of action of ovalbumin as an antigen. The inhibitory effect of excess of antigen was obtained in the same way with the isolated mast cells: in a typical experiment where concentration of the mast cells was 16 cells/mm^3 and purity in relation to other cells 90 per cent, 1 μg ovalbumin released 26 per cent histamine, 50 μg, 29 per cent and 2·5 mg, 4 per cent. It was verified that increasing the concentration of the mast cells removed the inhibition caused by the antigen excess (Fig. 2). Analysis of variance gave highly significant values of F for the effect of antigen, the effect of mast cells concentration, and the effect of interaction between them, suggesting that the effect of excess antigen depends on a quantitative relationship between the antigen added and the mast cells concentration.

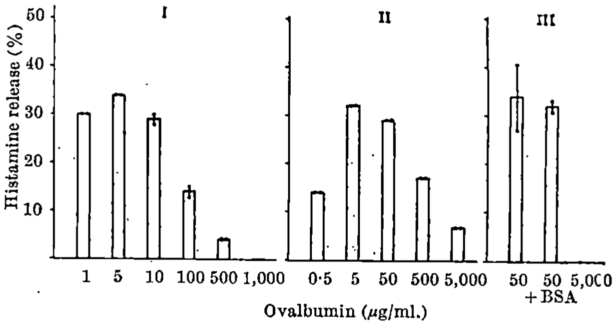

Fig. 1. Effect of antigen concentration on histamine release from actively sensitized rat peritoneal mast cells. Means with individual values are shown. Mast cells concentration = 19 cells/mm^3 in I, 33 cells/mm^3 in II, and 23 cells/mm^3 in III. BSA addition, 5 mg in III

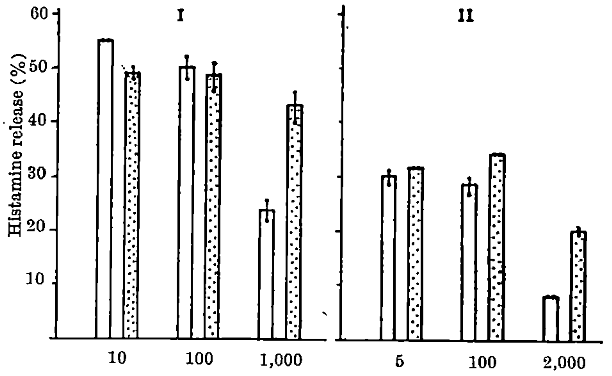

Fig. 2. Effect of antigen and mast cells concentration on histamine release from actively sensitized rat peritoneal mast cells. Means with individual values are shown. Mast cells concentration = 8·5 cells/mm^3 (white) and 85 cells/mm^3 (stippled) in I, and 7·7 cells/mm^3 (white) and 154 cells/mm^3 (stippled) in II

We did not succeed in obtaining an inhibition of the anaphylactic reaction in guinea pig or rat lung actively sensitized, nor the Schultz–Dale reaction, by addition of much larger excesses of antigen (25–100 mg/ml. for 100–200 mg tissue).

It has been demonstrated that antigen–antibody complexes formed with antigen concentrations more than 20–50 times that at equivalence are biologically inactive, while the most active ones are those formed in a zone where antigen is from 2 to 10 times in excess[1–3]. The ability of these complexes to release histamine from rat mast cells has been discussed by some authors[6,7]. In our experiments, it is not justifiable to compare the antigen/antibody ratios with those found in the soluble complexes, since the antibody was fixed to the tissue, and the antigen initially free in solution. Nevertheless, there is a strong suggestion that the anaphylactic reaction in the actively sensitized rat peritoneal mast cells would be initiated by a mechanism similar to that of the formation of a biologically active antigen–antibody complex.

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Combination Chemotherapy with Actinomycin *D* and Ribonuclease : an Example of Complementary Inhibition

Two mechanisms have been suggested by which combinations of drugs may depress the intracellular availability of a given metabolite to a level that becomes limiting for cell survival. Potter[1] has applied the term sequential inhibition to the action of two or more inhibitors on different enzymes in a limited portion of a multi-enzyme sequence (equation 1), and Elion, Singer and Hitchings[2] have denoted as concurrent blockade

$$A \xrightarrow{\overset{I_1}{\downarrow}} B \xrightarrow{\overset{I_2}{\downarrow}} C \tag{1}$$

the simultaneous retardation, by two or more agents, of alternate biochemical routes to the same end-product (equation 2).

$$\begin{tikzcd} A \arrow[r, "\overset{I_1}{\downarrow}"] & B \arrow[dr] & \\ & & C \\ D \arrow[r, "\overset{I_2}{\downarrow}"] & E \arrow[ur] & \end{tikzcd} \tag{2}$$

An additional mechanism can be visualized whereby drugs capable of binding and inactivating macro-molecules (for example, nucleic acids and enzymes) within the cell are used in conjunction with other agents capable of altering biosynthetic pathways leading to the formation of an end-product that has been rendered partially or completely non-functional. This mechanism, termed complementary inhibition, will be illustrated in the present report by the action of combinations of actinomycin *D* and bovine pancreatic ribonuclease on sarcoma 180 ascites cells. The function of the ribonuclease is to hydrolyse enzymatically some of the existing ribonucleic acid (RNA) molecules within the cell, and that of the actinomycin *D* is to retard metabolic repair processes that involve the re-synthesis of the catabolized RNA. The relatively specific inhibition of deoxyribonucleic acid (DNA)-dependent RNA synthesis by actinomycin *D* has been reported by several investigators and the mechanism involved has been reviewed recently[3,4]. Such metabolic insult results in an inhibition of neoplastic cell growth[3,4]; however, survival of cells may ensue through conservation and utilization of pre-existing RNA molecules for metabolic function. The addition of ribonuclease, an enzyme reported to arrest temporarily the growth of several transplanted or spontaneous rodent neoplasms[5], to the actinomycin *D* therapy depletes cellular RNA content further by depolymerizing some of the RNA formed prior to the actinomycin *D* blockade.

Table 1. EFFECT OF COMBINATIONS OF ACTINOMYCIN *D* AND RIBONUCLEASE ON THE SURVIVAL TIME OF MICE BEARING SARCOMA-180 ASCITES CELLS

Daily dosage* (mg/kg) Actinomycin *D*	Daily dosage* (mg/kg) Ribonuclease	Av. Δ wt.† (%)	Av. survival (days)	No. 50-day survivors ‡
0	0	+17·4	16·3	0/45
0	20	+18·8	14·3	0/10
0	40	+10·2	12·4	0/10
0	80	+10·3	14·4	0/15
0	120	+7·8	10·4	0/10
0	200	+6·6	15·3	1/20
0·015	0	+3·8	23·2	1/15
0·03	0	−2·9	21·3	0/20
0·045	0	−11·8	24·0	1/10
0·06	0	−8·8	27·6	1/10
0·10	0	−27·6	15·0	0/5
0·015	20	+1·7	19·2	0/15
0·015	40	−2·0	33·0	5/15
0·015	80	−1·7	39·3	9/15
0·015	120	+0·8	29·9	2/10
0·015	200	+1·7	38·2	5/10
0·03	20	−3·1	28·7	2/15
0·03	40	−4·1	26·1	2/15
0·03	80	−6·8	35·0	9/20
0·03	120	−6·6	39·3	6/15
0·03	200	−3·4	45·5	6/10
0·045	20	−9·6	21·6	0/10
0·045	40	−10·3	31·9	2/15
0·045	80	−7·6	27·6	3/15
0·045	120	−14·7	39·5	3/10
0·045	200	−7·6	44·9	6/10

* Administered intraperitoneally once daily for 6 consecutive days, beginning 24 h after implantation of tumour cells, with combined treatments given simultaneously. Mice were maintained throughout the experiments on drinking water containing 40 μg streptomycin/ml.

† Average weight change from onset to termination of drug treatment.

‡ Mice surviving over 50 days were calculated as 50-day survivors in determination of the average survival time.

The effects of such combinations on neoplastic growth were measured in adult *C3H* mice that had received approximately 4 × 10^6 sarcoma-180 ascites tumour cells by intraperitoneal injection as previously described[6]. Therapy was initiated 24 h after implantation of the neoplastic cells and was continued for six consecutive days. Therapeutic efficacy was determined by the prolongation of survival time afforded by drug treatments and by the number of mice surviving for at least 50 days. The percentage weight change from onset to termination of therapy was used as an indication of drug toxicity. The results in Table 1 indicate that commercial 3-times recrystallized bovine pancreatic ribonuclease, given intraperitoneally, did not prolong the survival time of sarcoma-180 tumour-bearing mice in daily doses up to 200 mg/kg of body-weight. Actinomycin *D* prolonged life over a range of doses; however, only a few mice survived for 50 days. Several combinations of the two agents resulted in pronounced synergistic inhibition of this neoplasm; toxicity as manifest by weight loss was comparable with that caused by actinomycin *D* alone. Combinations of actinomycin *D* with up to 200 mg of bovine serum albumin per kg, included as a control, yielded results comparable with those achieved with actinomycin *D*.

The results of experiments measuring the effects of drug therapy on the RNA content of sarcoma-180 cells are shown in Table 2. Since cells exposed to ribonuclease had a tendency to agglutinate, the average content of

Table 2. EFFECT OF ACTINOMYCIN D AND RIBONUCLEASE ON THE NUCLEIC ACID CONTENT OF SARCOMA-180 ASCITES CELLS

Treatment	Relative content, RNA/DNA Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
None	100	100	100	100	100
Ribonuclease	89	76	88	86	80
Actinomycin D	45	51	47	54	45
Actinomycin D + ribonuclease	38	39	40	44	39

Drugs were administered intraperitoneally to tumour-bearing mice beginning 4 days after implantation of ascites cells; three doses were given 48, 24 and 12 h, respectively, before analyses of nucleic acid content. Actinomycin *D* was used at 0·06 mg/kg/dose in all experiments, while ribonuclease was used at 240 mg/kg/dose in experiments 1, 2 and 3 and at 320 mg/kg/dose in experiments 4 and 5. Concentrations of DNA and RNA were determined by pentose analyses (ref. 10), using deoxyadenosine and adenosine as the standards, respectively. Untreated sarcoma-180 ascites cells contained 31·2 mμmoles of DNA deoxyribose/10^6 cells and 213·8 mμmoles of RNA ribose/10^6 cells.

Each value represents the average of results obtained from the separate analyses of ascites cells from 3–4 mice.

RNA per cell could not be calculated for every sample; the results, therefore, are expressed as the relative concentration of RNA/DNA. In samples in which accurate cell counts could be achieved, the DNA content of cells exposed to the various treatments were comparable with the control levels. In all the experiments, ribonuclease produced a slight depression of the RNA content, while actinomycin *D* caused a pronounced decrease in RNA. The combined treatment of ascites cells resulted in a depletion of the cellular RNA content that was greater than that caused by either drug alone. Although the decrease in RNA content induced by the drug combination was sub-additive, it is evident that such a level of depletion may well be critical for cellular survival and that synergistic growth inhibition may be the result. In contrast, bovine serum albumin (320 mg/kg/dose) did not augment the decrease in RNA content produced by the actinomycin *D*.

Other examples of the complementary inhibition, involving DNA, appear to be provided by the finding that combinations of uracil mustard (5-*bis*[2-chloroethyl]amino-uracil) with certain antimetabolites yield enhanced inhibition of the growth of several murine neoplastic cell lines[7,8] and by the report that synergistic inhibition of the growth of sarcoma-180 was achieved by combinations of mitomycin *C* with either 5-fluorouracil or 6-thioguanine[9]. Thus, theoretically, the use of complementary inhibition in the rational selection of combinations of drugs as potential synergistic inhibitors of neoplastic growth would appear to warrant further investigation.

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Interrelationships between Amphetamine and *Harmala* Alkaloids

It has been shown[1] that iproniazid increased the hypertensive effects of indirect sympathomimetic amines, without altering significantly those of the directly acting catecholamines. We suggested later[2] that monoaminoxidase inhibitors could facilitate the release of catecholamines induced by indirectly acting sympathomimetic amines. In this communication it is reported that two *Harmala* alkaloids, harmaline and harmine, which are both monoaminoxidase inhibitors with a rapid and brief action, can release catecholamines under certain conditions.

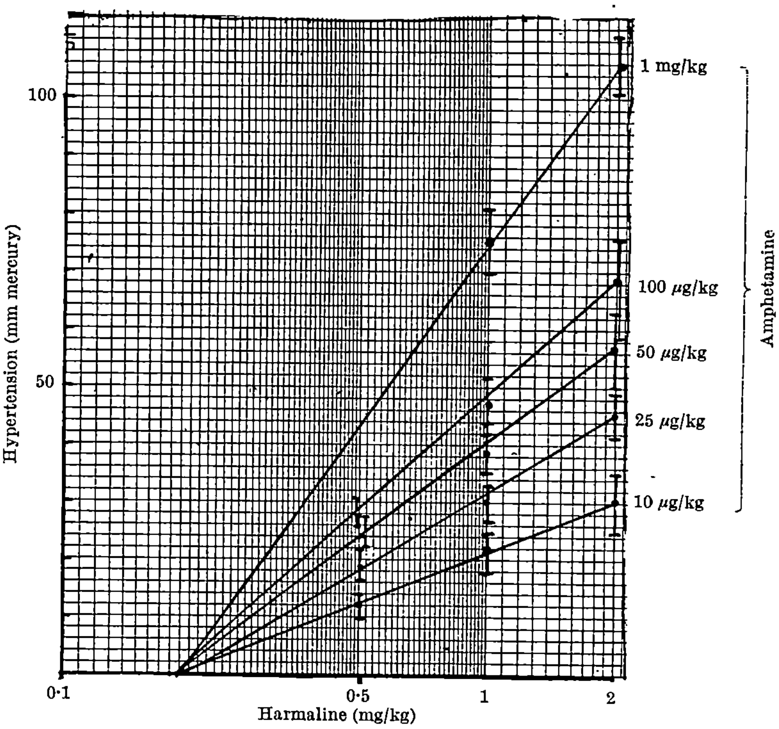

Fig. 1. Hypertensive effect of harmaline after various doses of amphetamine

Our experiments were performed on pithed rats prepared according to the method of Shipley and Tilden[3]. The blood pressure was recorded from a carotid artery using Condon's mercury manometer.

Injections of 0·5–2 mg/kg of harmine or harmaline potentiated very strongly the pressor responses to small doses of amphetamine but decreased the responses to large doses. A similar potentiation by harmaline was observed on pressor responses to phenylethylamine, tyramine, ephedrine, bretylium and guanethidine.

When harmaline and harmine were injected after the rats had been given a very small dose of amphetamine, they produced a marked increase in blood pressure, instead of the usual small one (8–10 mm mercury). Fig. 1 shows the relationships between the hypertensive effect of harmaline and the dose of amphetamine previously injected. The duration of this hypertensive effect of harmine and harmaline increased with the doses of amphetamine given. After producing a response by injection of a *Harmala* alkaloid, a second response could be produced by another injection of the alkaloid, but the second hypertensive response was smaller when higher doses of harmine or harmaline were used.

The increased hypertensive response to *Harmala* alkaloids could also be elicited by previous injections of bretylium (5 mg/kg), or guanethidine (5 mg/kg), or pheniprazine (2 mg/kg), but tyramine and phenylethylamine had no action.

The rise in blood pressure induced by either *Harmala* alkaloid after amphetamine was not changed by bilateral adrenalectomy; but it was abolished by treatment with phenoxybenzamine, and it could not be elicited in the rat pretreated 24 h previously with reserpine (10 mg/kg) in order to deplete the noradrenaline stores. From these observations it could be concluded that *Harmala* alkaloids release noradrenaline from peripheral stores after previous injection of amphetamine.

This noradrenaline-releasing effect of *Harmala* alkaloids does not seem to be related to their property of inhibiting monoaminoxidase for the following reasons: (*a*) Previous injections of harmaline and harmine (2 mg/kg) increased the pressor responses to amphetamine, but did not change the hypertension induced by *Harmala* alkaloids after amphetamine. Similar observations were made on the rat in which monoaminoxidase had been inhibited by previous administration of pheniprazine (5 mg/kg) or iproniazid (50 mg/kg, given 24 h previously). (*b*) Amphetamine did not increase but decreased the hypertensive effect of another monoaminoxidase inhibitor, pheniprazine.

In summary, harmine and harmaline can produce a release of catecholamines in rats previously given amphetamine; but this property seems to be unrelated to their action in inhibiting monoaminoxidase. We suggest that amphetamine not only induces release of catecholamines but also, even at very small doses, it changes the form of storage of catecholamines, making them available for release by *Harmala* alkaloids. We have previously shown that amphetamine has a similar action in making catecholamines more readily releasable by reserpine[2].

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HISTOCHEMISTRY

Localization of Invertase Activity in Small Intestinal Cells

As has been shown, the hydrolysis of disaccharides is accomplished by enzymes associated with certain structures of the intestinal epithelium cells. A more exact localization of the action of disaccharidases in the intestinal epithelium cells leads to some controversy between different authors.

Based on the investigation of hydrolysis of disaccharides it was suggested that the hydrolysis occurs inside the cells or in defined cytoplasmatic structures, which sediment together with the microsome fraction[1,2] or are located on "the inner aspect of the inner limiting membrane of microvilli"[3,4].

According to another point of view, the final stages of hydrolysis develop on the external surface of the cell membrane by means of membrane or contact digestion[5,6].

The concentration criterion which was successfully used by Rothstein[7] for the determination of invertase localization in yeast cells is a reliable argument for deciding which hypothesis is correct.

Indeed, if the hydrolysis occurs inside the cells, the accumulation of monosaccharides in tissue should be independent of its concentration in the medium during the course of incubation. If, on the other hand, the comparative examination of reaction products in tissue and in the solution reveals a higher concentration in the extracellular fluid, one would expect that the hydrolysis occurs extracellularly or at least on the surface of the mucosa. If we consider that the hydrolysis of disaccharides occurs on the mucosal surface, the uptake of monosaccharides should depend on the medium concentration.

The complete reliability of this criterion is only possible in cases of a smooth surface. In the case of the intestine, one has to keep in mind that the architecture of villi and microvilli hinders the diffusion of reaction products into the main volume of the incubation solution and contributes mainly to the passage of the substances into the cells.

A second restriction should be made: If the volume of the extracellular fluid largely exceeds the tissue volume the final concentration of hydrolysis products in the extracellular solution will depend not only on the localization of the hydrolytic enzymes but also on the dilution as well.

In the present investigation we repeated the data obtained by Miller and Crane. The extracellular and intracellular concentrations of hexoses after incubation in sucrose were compared. We developed a technique which enabled us to determine the concentration of hexoses in the immediate neighbourhood of the membrane.

Weighed pieces of everted intestine of white rats (Wistar strain), previously poisoned with sodium fluoride (0·02 per cent) to prevent active transport, were incubated in 2 per cent sucrose–Ringer's solution (*p*H 7). In order to increase the hydrolysis, the incubation solution was shaken. The concentration of reducing sugars both in tissue homogenates and in the extracellular solution was determined by the method of Nelson.

As we were technically unable to examine by this method reaction-rates for rather small extracellular fluid volumes (0·2–0·5 times the tissue volume), we changed this method as follows. An everted piece of intestine was dipped during 30 sec in a sucrose–Ringer solution (2 per cent) and the excess of fluid was discarded. The wet piece of intestine was laid on a plastic square with its wet mucosa uppermost, and covered with another plastic sheet to prevent evaporation. After a known incubation time (2–20 min) the cover was taken off and the mucosa was gently dabbed with a weighed piece of filter paper or lens-paper. (The histological preparation shows that the villi structure was not injured in these experiments.) The tissue was immediately homogenized and the wet paper was reweighed. In both tissue and paper the reducing activity was determined by Nelson's method.

By this method we were able to examine the incubation of small volumes of solution (up to 10 µl.) on 100–200 mg pieces of intestine and to compare the hexose concentration near the surface of the villi (mainly apical fluid) and in the upper part of the intervilli cavity with the hexose concentration in the tissue.

In the first part of this work, the experimental conditions used by Miller and Crane[3,4] were reproduced. Pieces of intestine were incubated in a sucrose solution which exceeds several times its own volume. In this case the observations of Miller and Crane were confirmed. We observed that the concentration of the hexoses formed in the tissue does not depend on the volume of the incubation medium. The concentration of hexoses in the surrounding medium is inversely proportional to its volume.

In the second part of this work we tried to determine the monosaccharide concentration in the immediate proximity of the external side of the membrane. In the case of the intracellular hydrolysis the concentration of hexoses in the external solution ought to be lower than the intracellular one (shaded area, Fig. 1). During a prolonged incubation time a relative increase in the hexose concentration in the surrounding medium was expected. This was shown by Miller and Crane[3,4]. However, as can be seen from Fig. 1, the concentration of monosaccharides in the extracellular fluid near the surface of the villi is higher than in the intracellular water. This fact can scarcely confirm the concept of intracellular hydrolysis of sucrose.

The dynamics of concentration ratio, at various incubation periods, showing a gradient reverse from that described by Miller and Crane, strongly supports the extracellular membrane digestion of sucrose. Indeed, in the first incubation minutes, the extracellular hexose concentration exceeds four times the tissue concentration. This fact can scarcely be explained as a consequence of diffusion of hexoses from the cell to the extracellular fluid against a concentration gradient of this strength.

The ratio decreases to a steady state of 2–2·5 for 20 min and remains constant due to the enzymatic activity.

We readily accept that this method cannot give a true image of the hexose content in the liquid which is present in the brush border pores. Our results are only an approx-

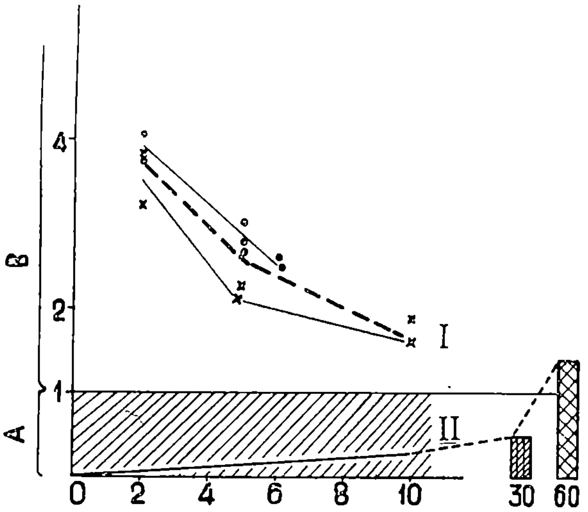

Fig. 1. Concentration ratio of hexoses liberated during sucrose hydrolysis in the incubation solution and in the intracellular fluid. Abscissa: time of incubation in minutes; ordinate: concentration ratio of hexoses in incubation solution versus intracellular liquid. I, Determination of hexoses in greater sucrose volumes according to the data of Miller and Crane; II, determination of hexoses in the immediate proximity of the cell surface. A, Zone typical for intracellular digestion. B, Zone typical for extracellular digestion

imation to the characteristics of the true concentration gradient. It is logical to assume that the hexose concentration on the external surface of the cell membranes should be higher than that we have observed in our experiments.

The results obtained by Miller and Crane rather reflect the dilution than the true distribution of substances on both sides of the cell membrane.

This concentration difference (solution/tissue) is independent of the length of the used intestine piece as we observed the same ratio for pieces of 1 cm as for pieces of 8 cm length (Fig. 2).

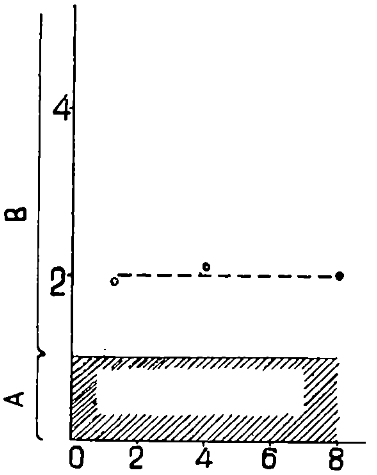

Fig. 2. Relationship between the concentration ratio of hexoses liberated during sucrose hydrolysis and the length of the everted pieces of intestine. Abscissa: length of intestinal pieces (cm); ordinate: concentration ratio of hexoses in incubation solution versus intracellular liquid

If one considers the disaccharides absorption mechanism to consist of at least three steps—the passive transport of disaccharides into the mucosal cells, its hydrolysis inside the cell and the active transport of monosaccharides through the cell into the portal blood—it can be postulated that, after inhibition of the active transport and preventing the destruction of reaction compounds *in situ*: (1) the accumulation of monosaccharides in the tissue is independent of the concentration in the medium; (2) the monosaccharides ought to appear first inside the cell and after a while, by diffusion, an equilibrium must be reached between hexose concentration on both sides of the membrane; (3) the accumulation of monosaccharides in the tissue depends on the amount of tissue used.

If one considers, on the other hand, the disaccharides absorption mechanism to consist of at least two steps—the membrane-bound surface hydrolysis followed by an active transport of monosaccharides—facts contrary to the first postulation ought to be expected.

In this series of experiments it can be observed that: (1) The hexoses appear first in the surrounding medium before they enter by diffusion in the tissue (Fig. 1). (2) A concentration gradient in hexose concentration between the bulk of the phase and the apical part of the mucosa was observed in the solution. (3) The accumulation of monosaccharides is independent of the length of the piece of intestine used (Fig. 2).

Using the reliable concentration criterion, we may assume that the true locus of sucrose digestion is on the external part of the limiting membrane of the intestinal epithelium, as has been shown by Ugolev *et al.*[8].

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HÆMATOLOGY

Interaction of Mercuric Ion with Hæmoglobin

The reactivity of the sulphydryl groups of human hæmoglobin has been examined in many laboratories. Recently, Cecil and Snow[1] have reported spectral changes in 6 M urea at *p*H 9 when 8 equivalents of $HgCl_2$ are added to oxyhæmoglobin (HbO_2) and metHb at 37° C. In the course of investigations in this Laboratory on the effect of $HgCl_2$, it was observed that the addition of more than 10 equivalents of mercury per hæmoglobin produces marked changes in the absorption spectra and the rotatory dispersion curves of both HbO_2 and metHb. The final product, in both instances, is similar to acid-denatured protein. Hardman and Gurd[2] have made essentially the same observations when copper or zinc reacts with metmyoglobin.

Hb*A* was prepared from cells washed with 0·9 per cent sodium chloride and lysed in water. The solution was deionized by dialysis and electrodialysis. Methæmoglobin was prepared by the method of Mirsky and Anson[3] with some modifications. Only 1·5 equivalents of $K_3Fe(CN)_6$ were used; the solution of metHb was deionized by dialysis against 0·05 M phosphate buffer *p*H 6·8 until the yellow colour was no longer visible, then dialysed against water, and finally electrodialysed. Absorption spectra were obtained at room temperature in a Cary, model 14, spectrophotometer. Rotatory dispersion curves were obtained using Bendix–Ericsson and Rudolph recording spectropolarimeters at room temperature (24°–25° C).

The absorption spectra obtained when HbO_2 was the starting material are given in Figs. 1 and 2. The addition of dilute acid to adjust the *p*H to 3 produced a marked decrease in intensity in the Soret region coupled with a shift to shorter wave-lengths (Fig. 1). The final absorption spectrum, obtained in about 2 h, had a single broad maximum between 370 and 390 mμ. This curve is virtually indistinguishable from that of hæmin (Fig. 2). When $HgCl_2$ was added to a solution of HbO_2 and the *p*H maintained between 5·5 and 5·8 during the addition of the mercury solution, the absorption spectra were similar to those seen in the early stages of acid denaturation. By maintaining the *p*H in a range where *p*H alone did not affect the absorption spectra it was therefore possible to separate the effects due to Hg (II) from those due to *p*H. The addition of $HgCl_3$ in ratios of 10–100/1 ($HgCl_2$/protein) gave similar results. With some variations, such as the degree of change observed at comparable ratios of Hg/protein, metHb gave the same general results. Sperm whale myoglobin gave the same spectral changes when $HgCl_2$ was added.

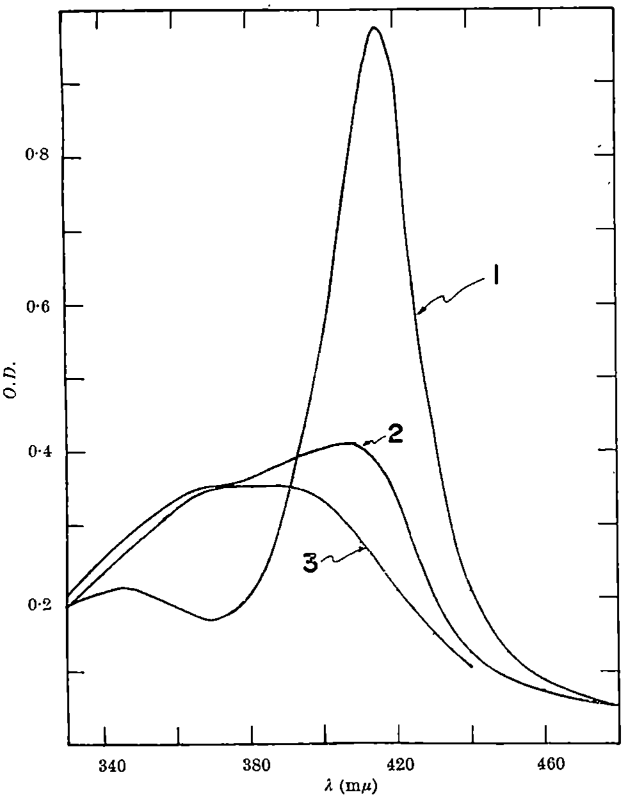

Fig. 1. Absorption spectra of HbO_2. Curve 1, *p*H 6·8; Curve 2, *p*H 3 after 30 min; curve 3, *p*H 3 after 1 h

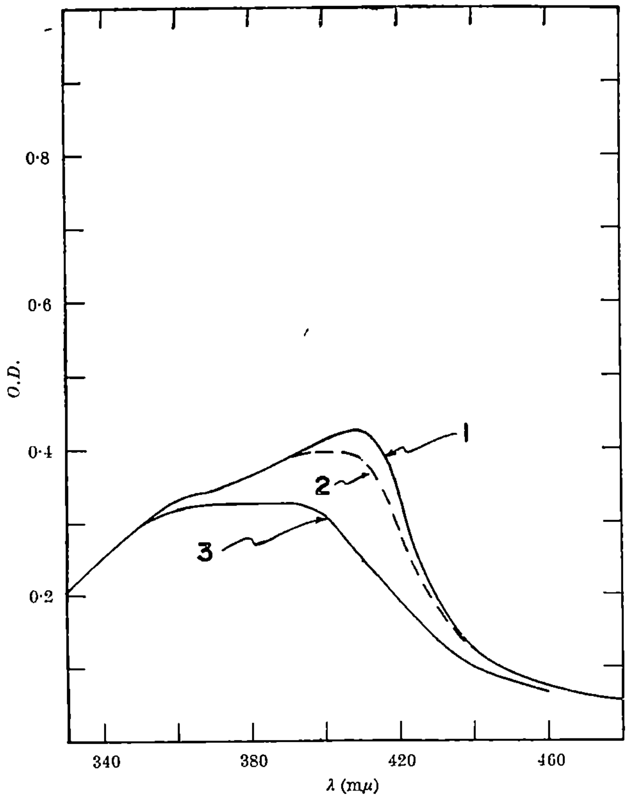

Fig. 2. Absorption spectra of HbO_2 plus $HgCl_2$ and hæmin. Curve 1, HbO_2 + $HgCl_2$ 40/1 after 30 min; curve 2, after 1 h, 3 h, and 9 h; curve 3, hæmin *p*H 5·8

The rotatory dispersion data indicated that the specific rotations of acid-denatured HbO_2, acid-denatured metHb, HbO_2 plus 50 equivalents of $HgCl_2$, and metHb plus 50 equivalents of $HgCl_2$ were all more negative than HbO_2 and metHb above 270 mμ and less negative below this wave-length. The values of the specific rotation at 233 mμ, which were obtained using the Bendix–Ericsson, are listed in Table 1. It can be seen that the addition of $HgCl_2$ as well as acid denaturation result in less negative values for the calculated parameter. In addition to these characteristics in the ultra-violet region, the Cotton effect in the Soret region is absent in acid-denatured protein and in protein plus $HgCl_2$.

Table 1

Protein	*p*H	Ionic strength	Hg/ protein	b_0*	$(\alpha)_{233m\mu}$
HbO_2	5·8	10^{-4}	—	−550	−9,200
	3	0·002	—	−75	−3,740
	3	0·002	—	−125	
	3→6	0·004	—	−550	−7,020
	5·8	10^{-4}	40/1	−417	−4,700
			90/1	−210	
MetHb	5·9		—	−517	
	5·9		—	−470	
	3	0·002	—	−25	
	3	0·2	—	−156	
	5·9	2×10^{-4}	50/1	−127	
MetMb	5·6	0·002	—	−550	
	5·6	0·002	20/1	−350	

* Calculated using $\lambda_0 = 212$ mμ.

From the data between 240 and 300 mμ Moffitt–Yang plots were drawn. The values of b_0 calculated from the slopes of these curves are listed in Table 1. It can be seen that both acid denaturation and the addition of $HgCl_2$ result in less negative values of b_0. Values calculated for human ferrihæmoglobin are similar to those reported by Beychok *et al.*[4] for horse ferrihæmoglobin. In addition, the effect of ionic strength is similar to that found by them. However, the ionic strength effect is not of sufficient magnitude to make any significant contribution to the changes seen in the presence of $HgCl_2$ under the conditions of these experiments and at the concentrations used (10^{-4} M). Similar observations have been made with sperm whale myoglobin (Table 1).

As evidenced from the absorption spectra both in the Soret region and at 630 mμ, metHb formed on the addition of $HgCl_2$. If the *p*H of acid-denatured hæmoglobin was raised from 3 to 5·8, both the absorption spectra as well as the rotatory dispersion indicated that there had been a 'renaturation'[5] of the protein.

From the changes in the absorption spectra, as well as the optical rotatory properties, it appears that Hg (II) produces effects that are qualitatively similar to those due to acid denaturation. This, coupled with the fact that the environment of the hæm group has been affected, suggests that histidine residues are involved[6,7]. Experiments in progress using nitrophenylacetate, the hydrolysis of which is catalysed by imidazole[8,9], indicate that imidazole residues are made unavailable when $HgCl_2$ is added to metHb. The rate of hydrolysis of the ester by metHb is 1·5–2 times more rapid in the absence of $HgCl_2$ than in the presence of it ($HgCl_2$/protein = 6/1). As a result, at the present time, it seems that $HgCl_2$ is capable of reacting not only with the sulphydryl groups but also the imidazole residues of hæmoglobin.

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PATHOLOGY

Effect of RNA on Vascular Permeability in the Rat

Previous workers have shown that certain nucleosides and nucleotides can increase vascular permeability and cause leucocyte emigration[1–4] and thereby directed attention to their potential role as mediators of inflammation.

An extract has recently been prepared from lymph node cells (LNPF) which causes an intense increase in vascular permeability and immediate and prolonged emigration of leucocytes and the deposition of a material resembling connective tissue fibrinoid[5]. Immediately following the injection of LNPF the inoculum reacts positively for RNA with the Unna–Pappenheim stain. After 20 min, however, the positive RNA reaction can no longer be obtained.

Because of these findings it was decided to investigate the ability of RNA and also DNA to reproduce the effects of lymph node extracts. Highly polymerized DNA from calf thymus (B.D.H.) failed to increase vascular permeability in the rat in doses of 15 µg to 5 mg/ml. On the other hand, a purified preparation of highly polymerized yeast RNA (B.D.H.)[6] was active in increasing vascular permeability over the range of 15 µg–1 mg/ml. This preparation of RNA also induced extensive emigration of leucocytes within 15 min of injection, 100 µg leading to the accumulation of about 50 leucocytes per high power field. By 24 h after an injection of 100 µg of RNA, large numbers of mononuclear leucocytes were infiltrating the inoculation site. Unlike LNPF, however, injections of RNA did not lead to the accumulation of an eosinophilic coagulum at the site of injection. The effect of RNA on vascular permeability could not be destroyed by prior incubation with 1·5 mg/ml. ribonuclease (Sigma 50 units/mg) at 37° C for 1 h.

Unlike nucleosides, the effects of which appear to be due to release of histamine[3,4], the increase in vascular permeability induced by RNA could not be abolished by systemic dosage with the antihistamine drug mepyramine maleate ('Anthisan' 1–2·5 mg/kg) or the 5-hydroxytryptamine antagonist '*BOL* 148'. However, incubation of RNA with guinea pig plasma led to a striking suppression of its effect on vascular permeability as did local administration of sodium salicylate 2·5 mg/ml.[7]. In its response to these antagonists RNA bore a close resemblance to LNPF[8].

Other forms of RNA were found at first not to share the activity of the highly polymerized preparation, thus freshly made solutions of soluble yeast RNA sodium salt (B.D.H.) and soluble yeast RNA Type III (Sigma Co.) both failed to increase vascular permeability significantly when tested in rats' skin. On the other hand, when these same solutions were allowed to stand at room temperature, Seitz filtered and tested in rat skin, there was a progressive increase in activity, until after 24 h their effects were as great as that of the highly polymerized preparation. The effect of the aged solutions could not be attributed to the appearance of histamine-releasing nucleosides[4] since dye leakage was not diminished by administration of mepyramine maleate (2·5 mg/kg). The true explanation of this phenomenon is not yet apparent.

Nevertheless the results so far are consistent with the view that RNA or a derivative, for example, an oligonucleoside, might be responsible for some of the phlogistic effects of extracts of lymph nodes and perhaps other tissues.

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Early Regressive Changes during the Tuberculin Skin Reaction

For an understanding of the pathogenesis of pathological processes, the initial stages of their development are usually most important. At first only the direct sequelæ of the causal agent play a part, and the position is not very complicated by regulatory and reparative processes aiming at the renewal of the normal state. The morphology of early stages of the hypersensitivity reaction of the delayed type has been examined by several authors[1–4], who directed attention mainly to the composition of the cell infiltrate. Because inflammatory infiltration is a result of tissue damage, I was interested rather in changes in tissues where the immunological reaction takes place.

I used white guinea pigs weighing 250–350 g, sensitized by injection of 0·1 ml. emulsion into both hind limbs of the following composition: 6 ml. paraffin oil, 2 ml. lanolin, 3 ml. physiological saline containing per 1 ml. 3 mg BCG vaccine killed by heat (1 h at 70° C). The skin tests were carried out 21–28 days after sensitization with 10, 1 and 0·1 µg PPD (Statens Seruminstitut, Copenhagen, Denmark) in 0·1 ml. physiological saline into the skin of the hip. The groups formed by five sensitized and one control guinea pig were killed 1/2, 1, 2, 3, 6, 12 and 24 h following the skin test. The sites of the skin test were examined in histological preparations stained with hæmatoxylin-erythrosin.

The inflammatory infiltrate was practically of equal composition to that described by Gell and Waksman[1,2]. The first changes, that is, leukostasis and leukodiapedesis, were found after 1 h around the subcutaneous venules. Gradually the infiltration of the subcutaneous tissue became diffuse and spread through the dermis underneath the epidermis. The composition of the infiltrate changed, depending on the dosage. When large doses were used neutrophils predominated; when small doses were applied an almost pure mononuclear infiltrate was formed around the subcutaneous venules. The changes in the subcutaneous fat were striking. Already after 2 h and sporadically even after 1 h I found lipophagic foci made up of macrophages surrounding, in a wreath-like fashion, the fat vacuole of the dead cell—partly of foreign body giant-cells, containing sometimes in their plasma optically vacant spaces after extraction of fat. In some larger lipophagic foci microabscesses were found, that is, an accumulation of neutrophils in a space corresponding to the size of the fat cell. The lipophagic foci appeared most frequently after medium or low doses of PPD or at the margin of the reaction to a larger dose. In the reaction with 10 μg PPD I found, after 2 h, several small foci of necrotic subcutaneous adipose tissue—an anuclear mass staining pale pink with erythrosin, with a sharp borderline at the adipose tissue which was only slightly infiltrated with polymorphs. Mononuclears were not represented. In subsequent specimens I did not find any necrosis foci; they were probably covered with a dense inflammatory cellulization. In non-sensitized controls there were neither necroses nor lipophagia.

The immunological reaction between sensitized cells and antigen takes place at the site where they meet. In the skin test these cells meet the antigen in the immediate vicinity of fine subcutaneous venules[5], which usually are next to the layer of subcutaneous adipose tissue. One of the consequences is damage of subcutaneous tissues which may manifest itself after larger doses of antigen by necrosis and after lower doses by a lipophagic reaction around individual dead or damaged cells. The observed regressive changes can be interpreted in two ways: (1) adipose tissue binds the antigen and the sensitized cells therefore attack it and break it down; (2) the subcutaneous adipose tissue is the only medium where the reaction takes place and it is damaged by the reaction products.

A hypothesis similar to the foregoing interpretation was elaborated by Waksman for the pathogenesis of experimental allergic encephalomyelitis. He assumes that sensitized monocytes (and histiocytes) attack and finally break down structures containing antigen[6]. It can be assumed that fat cells bind antigen; it cannot be concluded, however, from the fact that macrophages remove adipose tissue that they also attack and damage fat cells. The first hypothesis does not explain the observed early necroses, in the vicinity of which I did not find a striking accumulation of mono- or histio-cytes, but only a few polymorphs.

The second hypothesis has some points in common with the explanation of Goldberg *et al.*[7]. We assume that immunologically competent cells, after leaving the blood vessel, meet the antigen and liberate some biologically active substances which act as a mediator of the delayed hypersensitivity reaction. The mediator acts on the cells of the surrounding tissue and at higher concentrations it damages and kills them. The dead cells liberate further active principles, causing a reactive inflammation. In my own opinion, its character will not depend any more on the type of reaction which produced it but rather on its intensity rate, course and duration. Changes in adipose tissue are most striking because the reaction takes place in the immediate vicinity of fat cells and because adipose tissue is metabolically more active than fibrous tissue of the dermis. I believe that this hypothesis, though not yet confirmed, so far best agrees with the observed facts.

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Nucleolus-like Inclusions in the Cytoplasm of HeLa Cells treated with Puromycin

Cytoplasmic inclusions containing RNA have been noted in HeLa cells treated with puromycin[1], an antimetabolite which is a selective inhibitor of protein synthesis[2,3]. It is now clear that these inclusions appear in a large proportion of treated cells when high concentrations of puromycin are used, and that these structures bear a resemblance to nucleoli as regards morphological appearances and their dependence on continued synthesis of cellular RNA.

Inclusions were produced in HeLa cells grown in monolayer culture under conditions described by Wildy *et al.*[4]. Unless otherwise stated in the protocol, 100 μg of puromycin dihydrochloride (Nutritional Biochemical Corp., Cleveland, Ohio) was present in each 1 ml. of the culture medium and the duration of action was 24 h. The inclusions were observed under phase contrast optics in unfixed cells, but were best visualized by any standard staining procedure for RNA. In the experiments described here the coverslips were fixed in 5 per cent trichloroacetic acid for 10 min, and stained for 15 min in 0·1 per cent aqueous toluidine blue at *p*H 5·5. The cells did not stain by this method after pretreatment with ribonuclease.

The production of inclusions by puromycin was concentration dependent (Fig. 1). Prolonged treatment with puromycin at concentrations lower than 5 μg/ml. did not result in the appearance of inclusions, although protein synthesis is known to be approximately 80 per cent inhibited within 45 min of the addition of puromycin[5,6]. The inclusions were formed at concentrations of puromycin higher than 5 μg/ml., and their numbers increased with concentration of puromycin until a maximal effect was reached at 50 μg/ml. of puromycin. This concentration has been shown to produce maximal inhibition of protein synthesis in HeLa cells[5,6]. This suggests that the

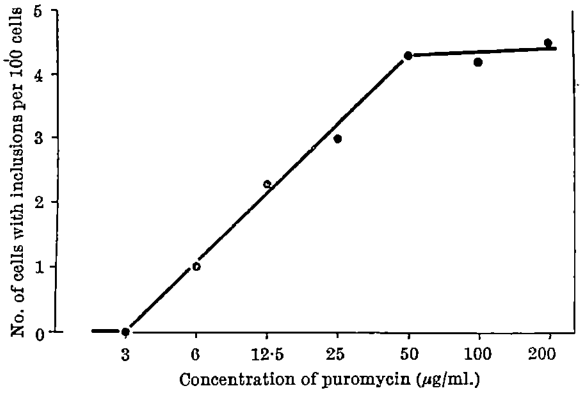

Fig. 1. Effect of concentration of puromycin on the frequency of cells with cytoplasmic inclusions. Duration of treatment was 4 h. The points shown are arithmetical means of values obtained in four separate experiments

appearance of cytoplasmic inclusions resulted from the interruption of protein synthesis.

The inclusions were first detected after treatment for 2 h with puromycin (Fig. 2). Their numbers increased gradually for 10 h, and at that time more than 30 per cent of the cells contained at least one inclusion. Thereafter, the proportion of cells with inclusions decreased slightly, and cells were being lost from the culture monolayer. Virtually all cells detached from glass after 48 h of treatment with puromycin.

The inclusions produced by puromycin are shown in Fig. 3. There was some variation in the appearance of cytoplasmic bodies produced by puromycin but a number of them were similar to the nucleoli in their size, in their ovoid or irregular shape, and in the intensity of staining with basophilic dyes. The inclusions were surrounded by an unstained area. In these respects they resembled cytoplasmic structures described by other workers as nucleoli extruded from the nucleus[7-10].

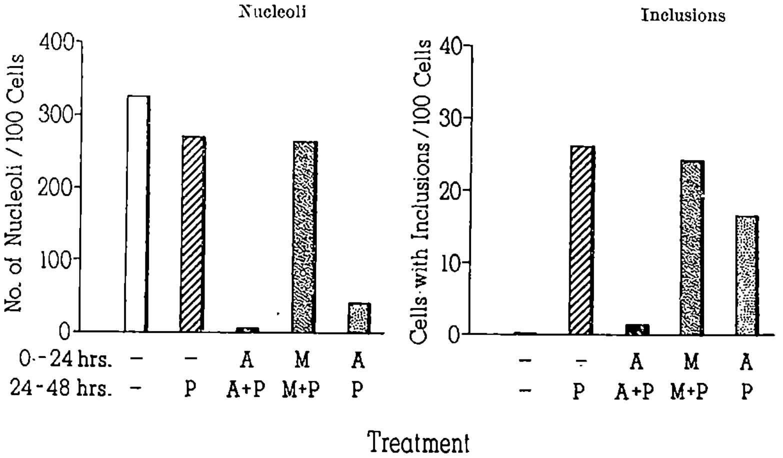

Fig. 4. Effect of pretreatment with actinomycin *D* (*A*) and mitomycin *C* (*M*) on the numbers of nucleoli and the production of cytoplasmic inclusions by puromycin (*P*). All cultures were stained after 48 h of treatment indicated below the histogram, and nucleoli and inclusions were enumerated at this time. 1,000 mononucleate cells were counted in each culture, and duplicate cultures were used in each experiment. The values shown are arithmetical means from four separate experiments. The concentration of puromycin was 100 μg/ml., actinomycin 0·03 μg/ml., mitomycin 1 μg/ml. Comparison of the two histograms shows a similarity of nucleoli and inclusions in their response to inhibitors of nucleic acid metabolism

While this view of the origin of the inclusions cannot be accepted or denied on the basis of morphological appearances alone, experiments with inhibitors of nucleic acid synthesis show another point of similarity between the inclusions and nucleoli. Treatment of HeLa cells with a low dose (0·03 μg/ml.) of actinomycin *D* is known to interrupt synthesis of ribosomal RNA (ref. 11) and to result in the disappearance of nucleoli after treatment for 24–28 h (ref. 12). Nucleoli gradually reappear, however, if actinomycin is omitted from the medium[12]. Fig. 4 shows that puromycin treatment did not result in the appearance of cytoplasmic inclusions if the cells were pretreated with and maintained on actinomycin *D* (Merck, Sharp and Dohme, Rahway, New Jersey). The inclusions did appear if actinomycin was withdrawn, or if mitomycin *C* (Calbiochem., Los Angeles, California) was used in place of actinomycin. Mitomycin is an inhibitor of DNA metabolism, with little direct effect on RNA synthesis[13], and Fig. 4 indicates that its presence in the medium does not lead to a gross reduction in nucleolar number. This parallelism in the behaviour of nucleoli and inclusions in response to inhibitors of nucleic acid metabolism emphasizes their similarity.

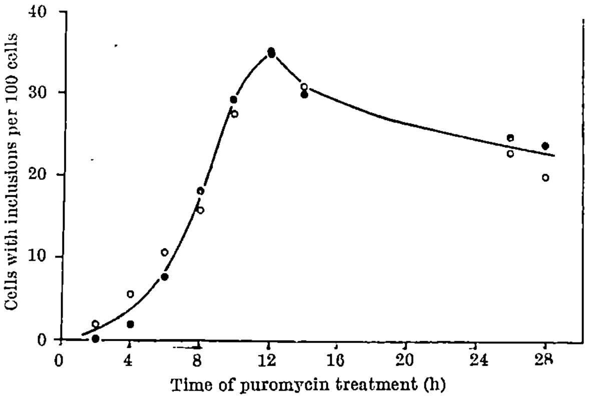

Fig. 2. Effect of duration of treatment on the production of cytoplasmic inclusions by 100 μg/ml. of puromycin. The values of two separate experiments are shown. ○, First experiment; ●, second experiment

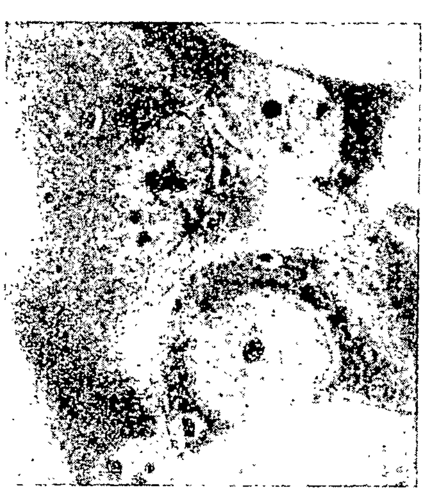

Fig. 3. HeLa cells stained by toluidine blue after 24 h of treatment with 100 μg/ml. of puromycin. Note the presence of multiple cytoplasmic inclusions (× 1,150)

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IMMUNOLOGY

Contact Reactivity to Azo Dye Carcinogens

Old *et al.*[1], in a recent publication, have shown that while three carcinogenic polycyclic hydrocarbons (3-methylcholanthrene, 3,4-benzpyrene and 3,10-dimethyl-1,2-benzanthracene) were capable of eliciting contact hypersensitivity in guinea pigs, the non-carcinogenic anthracene and phenanthrene were devoid of a similar capacity. They proposed that there might be a correlation between the capacity of a substance to elicit contact sensitivity and its carcinogenic activity as both seem to depend on interaction with proteins of the host. Further, they have suggested that in guinea pigs, resistant to chemical carcinogenesis and highly susceptible to delayed hypersensitivity, the response takes the form of contact sensitivity; in the rat and the mouse prone to chemical carcinogenesis, but with little or no ability to acquire contact sensitivity, the end result is tumour formation.

If this view is correct, carcinogens other than the polycyclic hydrocarbons should be capable of eliciting delayed hypersensitivity in guinea pigs, while their non-carcinogenic analogues should be devoid of activity. Indeed a few azo dyes, some of which are powerful carcinogens in rats[2], have been shown to be allergenic in man[3,4] and in guinea pigs[5].

In the experiments reported here 6 carcinogenic and 7 non-carcinogenic azo dyes were tested in guinea pigs. Those which are carcinogenic in the rat did induce a high degree of cutaneous reactivity while 6 of the 7 non-carcinogens were devoid of sensitizing capacity. The exception was 2-methyl-4-dimethylaminoazobenzene, the only compound bound to rat liver proteins without being a carcinogen[6].

The azo dyes used in these experiments were: azobenzene, *p*-aminoazobenzene, 4-dimethylaminoazobenzene (DAB), and DAB substituted at different positions with a methyl, or fluoro or hydroxyl, or trifluoromethyl or with an amino group. Azobenzene was purchased from Matheson Coleman and Bell, *p*-aminoazobenzene from Eastman Chemicals: both compounds were of the highest grade purity. All the other highly purified dyes were a gift of Dr. J. A. Miller.

The animals used were adult albino guinea pigs of both sexes. They were sensitized once with a total of 0·4 mg of azo dye (0·1 ml. in each foot pad) dissolved in complete Freund's adjuvant and emulsified with saline. Skin testing was performed 3 weeks after sensitization. It consisted of applying to the shaved dorsal skin one drop of the azo dye used for sensitization, at a concentration of 1, 0·1, 0·01 and 0·001 per cent. Cross-reactivity to other azo dyes was tested at the same time; the test dose used was 1 per cent. Each test compound was dissolved in a mixture of 4 parts acetone and 1 part olive oil. Animals injected with adjuvant alone served as control. The reactions were recorded 24–36 h after testing and were graded from 0 to + + + + as to their severity.

Six of the 7 carcinogens used evoked strong reactions (2 to 4+) in all guinea pigs sensitized. All 28 animals injected gave a reaction to a test dose of 1 per cent; 24, 17 and 5 of the 28 animals reacted when tested with the sensitizer in a concentration of 0·1, 0·01 and 0·001 per cent respectively. The 7th carcinogenic dye (MAB) sensitized only 2 of 5 animals injected, and reactions were obtained to test doses not lower than 0·1 per cent. The cross-reactions were as strong as the homologous reaction and seemed to depend on the sensitivity of the animal rather than on the compound used for testing.

The results obtained on the allergenicity of azo dyes are compared with Miller's results[2] on the carcinogenicity of the same compounds in Table 1. As can be seen, there is a good correlation between sensitizing capacity in the guinea pig (column 4) and binding to rat liver proteins (column 3). This correlation holds for carcinogenicity except for 2 methyl DAB: this is the only non-carcinogenic azo dye which was found to be bound to rat liver proteins[2].

Although it is generally accepted that low molecular weight compounds have to combine with proteins in order to induce sensitization, it is not clear whether elicitation of a contact reaction in a sensitized animal can be brought about by the chemical alone, or only by the complete antigen. Eisen *et al.*[7], working with dinitrofluorobenzene (DNFB) and its analogues, distinguished between two classes of compounds: those which could, and those which could not, elicit a reaction in animals sensitized to DNFB. They were able to show that the 'elicitors' could combine with bovine γ-globulin *in vitro*, and with guinea pig skin proteins *in vivo*; the non-elicitors did not interact with protein either *in vitro* or *in vivo*. On the basis of these results they suggested that for a compound to elicit a reaction in a sensitized animal it must first combine with protein to do so. The azo dyes which were shown to be devoid of sensitizing capacity in the present series of experiments were shown not to combine with rat liver proteins *in vivo*[2] nor to interact with bovine serum albumin (BSA) *in vitro*[8]. Nevertheless, two of these dyes (4'NH$_2$DAB and 3'CF$_3$DAB) were capable of eliciting reactions in guinea pigs rendered sensitive to any of the seven sensitizers used (Table 1, column 5). This latter finding could be interpreted either by postulating that the two compounds, although they do not interact with rat liver proteins *in vivo*, nor with BSA *in vitro*, nevertheless combine with guinea pig skin proteins, or that some free azo dyes can elicit an allergic reaction. Assuming that 4'NH$_2$DAB and 3'CF$_3$DAB can combine with guinea pig skin proteins one would still have to explain their inability to sensitize.

Table 1. SENSITIZING CAPACITY AND CARCINOGENICITY OF AZO DYES

Azo dye*	Carcinogenic† index (rat)	Binding† to rat liver proteins	Capacity‡ to sensitize	Capacity to elicit skin reactions§
DAB	6	+	100 (4)	100 (9)
3'CH$_3$DAB	10–12	+	100 (5)	75 (4)
3'F DAB	10–12	+	100 (5)	66 (15)
4'CH$_3$DAB	< 1	+	100 (5)	73 (15)
4'F DAB	10–12	+	100 (5)	80 (15)
MAB	6	+	40 (5)	33 (15)
2 CH$_3$DAB	0	+	100 (4)	60 (5)
4'NH$_2$DAB	0	0	0 (5)	75 (8)
3'CF$_3$DAB	0	0	0 (5)	50 (20)
2 OH DAB	0	0	0 (5)	10 (20)
2'OH DAB	0	0	0 (5)	10 (20)
p-Aminoazobenzene	0	0	0 (5)	5 (20)
Azobenzene	0	0	0 (2)	0 (12)

* For formulæ see text.
† See ref. 2.
‡ The numbers refer to the percentage of animals sensitized; those in brackets indicate the numbers of animals in each experiment.
§ Cross-reactions: the numbers refer to the percentage of animals reacting; those in brackets indicate the number of sensitized animals skin tested.

The results reported here are in agreement with those of Old *et al.*[1]: there is a parallelism both in the case of the azo dyes and the polycyclic hydrocarbons between sensitizing capacity in guinea pigs, and carcinogenicity in rodents. Experiments are being carried out to find out whether suppression of immunological reactivity in the guinea pig or intensive immunization in the rat might not modify the patterns of response.

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Immunological Distinction of *AKR* Thymocytes

In a previous communication we reported discovery of a strong antigen associated with the thymic lymphocytes of *AKR* mice[1]. Old, Boyse and Stockert have reported absorption of specific iso-antisera by thymus of *A* and *C*58 mice, and cytolysis of some leukæmias of *A* and *C57BL*/6 mice by the same iso-antisera. These results indicated presence of a common antigen (the *TL* antigen) in the tissues mentioned[2]. This antigen, and the antigens common to the Friend, Moloney and Rauscher viruses (the *FMR* antigens), are not related to the *AKR* thymic antigen[2,3].

In the present communication, a clear-cut serological differentiation between *AKR* lymphocytes of thymic and non-thymic origin is reported: thymic and splenic lymphocytes represent two antigenically different types of lymphocytes. The *AKR* thymic antigen is present to a high degree in brain and in some leukæmias of *AKR* mice, and to a lesser extent in other *AKR* tissues. The distribution of this antigen in thymocytes of other strains has been investigated. A preliminary report has been made[4].

Iso-antisera to tissues of *AKR* mice were prepared in *C3HeB*/*Fe* mice, that possess the same H-2 allele (H-2^{k} or H-$2^{k'}$) but presumably differ in other histocompatibility factors[5]; and in *C57BL*/6 mice, that possess the different H-2 allele H-2^{b} (ref. 6). The *AKR* tissues used were red blood cells, thymocytes, lymph nodes, spleen, marrow, liver; leukæmias *L*4946, *BW*5147, *S*775, and sarcoma *T*283. The resulting iso-antisera were titred by immune cytolysis[1,6,7] against the following *AKR* tissues: lymphocytes of thymic, lymph node, splenic and intraperitoneal origin, and marrow cells; leukæmias *L*4946, *BW*5147, *S*775; and against five different spontaneous leukæmias *RA*1 to *RA*5, which were transplanted intraperitoneally and tested within the first five transplant generations.

Absorptions of iso-antisera were carried out by a quantitative adaptation of the method of Möller[8]. Either a known number of viable cells, or a packed residue derived from a homogenate of a known wet weight of tissue, were used for absorption. The supernatant was titred by immune cytolysis against the same tissue as was used for preparation of the iso-antisera.

The cytolysis data are presented in abbreviated form (Table 1). All *C3HeB*/*Fe* iso-antisera cytolysed *AKR* thymocytes, while none cytolysed other types of normal lymphocytes (Table 1*A*). This represents clear-cut evidence of the antigenic distinction between *AKR* thymocytes and other normal *AKR* lymphocytes. Five out of eight *AKR* leukæmias tested (such as *RA*3) were highly sensitive to *C3HeB*/*Fe* iso-antisera, while three (such as *L*4946 and *RA*1) were not. This indicates that the five leukæmias sensitive to these iso-antisera are antigenically related to thymocytes (Table 1*A*).

In contrast, *C57BL*/6 iso-antisera showed a variety of reactions (Table 1*B*): *C57BL*/6 iso-antiserum against spleen gave strong cytolysis of spleen cells but very weak cytolysis of thymocytes. The iso-antiserum against thymocytes gave strong cytolysis both of thymocytes and of spleen cells. The relative potencies of iso-antisera involved in these reactions give evidence of wide quantitative differences in the surface antigens of thymocytes and of other normal lymphocytes. Marrow cells were resistant to all iso-antisera that were tested. Cytolysis of *AKR* leukæmias by the same iso-antisera showed that certain leukæmias reacted similarly to one or other of the normal cell types. For example, *L*4946 leukæmia reacted similarly to splenic lymphocytes (Table 1*B*).

Table 1. Cytolysis of Normal and Leukæmic *AKR* Cells by *C3HeB*/*Fe* and *C57BL*/6 Iso-antisera

	Potency* for cytolysis of					
	Normal *AKR* cells			*AKR* leukæmias		
	Thymus	Spleen	Marrow	*L*4946	*RA*1	*RA*3
(*A*) Iso-antisera prepared in *C3HeB*/*Fe* mice against *AKR*						
Thymus	450	< 2	< 2	(39% a.c.)†	< 2	96
Spleen	524	< 2	< 2	(10% a.c.)	< 2	97
*L*4946	340	< 2	< 2	(46% a.c.)	< 2	65
(*B*) Iso-antisera prepared in *C57BL*/6 mice against *AKR*						
Thymus	450	82	< 2	24	1·8	18·1
Spleen	1·6	710	(21% a.c.)	218	19·4	74
*L*4946	4	112	(21% a.c.)	78	5·4	14·2

* Potency is expressed as 100/cytolytic titre, where the cytolytic titre is the final concentration of iso-antiserum (per cent) that gives 50 per cent cytolysis

† The highest cytolysis obtained was 39 per cent above the control level.

Absorption experiments further substantiated the results of the cytolysis data. Absorption of a *C3HeB*/*Fe* iso-antiserum prepared against *AKR* thymocytes, by normal lymphocytes other than thymocytes, was low or negligible, except for adult splenic lymphocytes, which absorbed 5 per cent of the iso-antibody absorbed by the same number of *AKR* thymocytes. The *AKR* leukæmias which behaved similarly to splenic lymphocytes in cytolysis experiments absorbed between 4 and 14 per cent, while leukæmias which behaved similarly to thymocytes (Table 1) absorbed 39–95 per cent of the iso-antibody, calculated on the same basis. These results suggest that spontaneous *AKR* leukæmias fall into two types, those with and those without a high content of the *AKR* thymic antigen.

Residues of homogenates of various normal *AKR* tissues were tested for absorption of the *C3HeB*/*Fe* iso-antiserum against *AKR* thymocytes. Brain absorbed as much iso-antiserum as thymus, while appendix and lung absorbed approximately one-fortieth as much. Absorption by liver, skeletal muscle, kidney and testis was less than 1 per cent of that of *AKR* thymic residue derived from the same wet weight of tissue. These data do not indicate whether the *AKR* antigen is present on cells of spleen, appendix and lung, or whether presence of the antigen is due to migration of thymocytes to these tissues. However, the content of the antigen was far too high in brain to be ascribed to the presence of thymocytes.

Thymocytes of *RF* mice and of neonatal *AKR* mice were cytolysed by the *C3HeB*/*Fe* antiserum to *AKR* thymocytes at the same potencies as the homologous *AKR* cells, and the amount of iso-antibody absorbed by these cell types was only slightly lower. In contrast, thymocytes from *C3HeB*/*Fe*, *C3H*/*DiSn*, *C3H*/*St*, *A*, *A*/*He*, *BALB*/*c*, *C57BL*/6, *C57BL*/10, *C57L*, *C*58, *DBA*/1, *DBA*/2, *SWR* and 129 mice were not cytolysed, and did not absorb detectable amounts of iso-antibody. Comparison of these data with Table 10 of ref. 5 indicates that the *AKR* thymic antigen is not associated with any of the six known mouse histocompatibility factors *H*-1 to *H*-6.

An *AKR* iso-antiserum to *C3HeB*/*Fe* thymocytes cytolysed thymocytes from all the mouse strains mentioned here, except strains *AKR* and *RF*. These data exclude an association between the *C3HeB*/*Fe* thymic antigen and any of the six known mouse histocompatibility factors *H*-1 to *H*-6 (ref. 5). Quantitative absorption experiments showed that the tissue distribution of the *C3HeB*/*Fe* thymic antigen was similar to that of the *AKR* thymic antigen.

It is unlikely that either the present *AKR* or *C3HeB*/*Fe* thymic antigens are related to the *X* antigens of Gorer *et al.*, since *EL* leukæmias bearing the *X* antigens could not be cytolysed by iso-antibody[9]. Not only the *AKR* thymic antigen, but also the *C3HeB*/*Fe* thymic antigen, appears to be distinct from the *LT* antigen: *C57BL*/6 thymus, which possesses the *C3HeB*/*Fe* antigen, failed to absorb iso-antibody directed against the *LT* antigen[2]. In line with the suggestion of Amos[5] that apparently new serologically determined antigens be first designated by Greek letters, the names θ-*AKR* and θ-*C3HeB*/*Fe* are proposed for the two thymic antigens described in this investigation. The present results will be published elsewhere in detail.

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RADIOBIOLOGY

Shortening of the Span of Life of Rats by 'Myleran'

THERE are few reports on late somatic effects of radiomimetic chemicals, especially those dealing with span of life. In mice and rats the first observations on the effects of single and repeated doses of nitrogen mustards and myleran on span of life are rather conflicting and the dose-response relationship has not been established[1–5]. Following the administration of single doses of a radiomimetic agent the span of life shortening is less effective because the sub-lethal dose range of many of them is narrow. By repeated doses properly spaced to avoid the early mortality, a larger total dose may be administered and in this way a significant reduction of span of life obtained[3]; however, as more variables are introduced inherent to dose fractionation, the interpretation of the dose-response relationship may be complicated. On the other hand, the early mortality in rats associated with bone marrow aplasia induced by single doses of myleran can be successfully reduced by isologous and homologous bone marrow transfusions, and in those rats which survive 30 days the observation of long-term effects can be extended to single doses of myleran two or three times greater than the $LD_{50/30}$ (refs. 6, 7).

This communication reports the relationship between the reduction of span of life in rats and the dose of myleran. White male *L*-strain rats bred here by random mating were used; at the start of the experiment they were 3–4 months old, weighing 140–150 g. A group of rats survived the first 30 days to mid-lethal doses of myleran (12·1, 13·8 and 15·5 mg/kg). Another group is composed of 30-day survivors treated with bone marrow transfusions (32–128 × 10^6 nucleated cells on the 1st, 3rd or 5th day) following administration of increasing doses of myleran (from 15·5 to 48·3 mg/kg). A previous report from this laboratory described experimental methods and results on the 30-day survival[6]. The mean span of life and other criteria of longevity were established from the day of administration of myleran. Simultaneously until natural death a group of normal rats which served as controls has been recorded. Routine autopsies were performed on all animals.

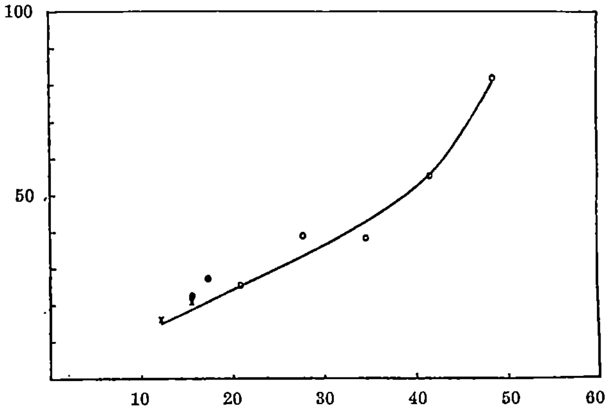

Fig. 1. The relationship between the shortening of span of life of *L*-strain rats and the dose of myleran. ×, rats receiving only myleran; ○, rats treated with bone marrow transfusions after myleran administration; ●, rats treated with bone marrow transfusions, data corrected for the mortality occurring during the 2nd and 3rd month

The results are summarized in Table 1. The mean, median and maximal spans of life at various dose-levels indicate that the long-term survival of rats is inversely related to the dose of myleran.

In the group of rats not treated with bone marrow transfusions, however, observed values for the mean span of life are not significantly different from control values. To demonstrate that the observed differences are statistically significant a greater number of animals had to be used and this fact emphasizes why definitive data at sub-lethal and mid-lethal dose ranges are difficult to obtain[4].

In the group of rats treated with bone marrow transfusions following myleran doses of 15·5 and 17·2 mg/kg the rats presented on the whole a more important reduction of span of life not in relation to dose of myleran used. Among them about 25 per cent died during the second and third month with symptoms indicating a secondary rejection of the bone marrow graft. This particular incidence of mortality does not occur among the rats which received nearest myleran doses without bone marrow treatment or in bone marrow treated rats following administration of higher myleran doses which depress more profoundly the immunological defence reactions of the host. By excepting the latter two dose-levels, the lowest myleran dose that resulted in a statistically significant decrease in span of life was 20·7 mg/kg, at the 0·01 confidence-level.

In Fig. 1, the shortening of life in rats expressed as a percentage of the normal span of life has been plotted against the doses of myleran on the ordinary scales. If

Table 1. LONGEVITY DATA OF *L*-STRAIN RATS SURVIVING 30 DAYS FOLLOWING ADMINISTRATION OF SINGLE DOSES OF MYLERAN WITH OR WITHOUT BONE MARROW TRANSFUSIONS

Experimental conditions	Dose of myleran (mg/kg)	No. of rats	Mean span of life ± *E.S.* (days)	Reduction of mean span of life (%)	Median span of life (days)	Maximum span of life (days)
Controls	—	24	551 ± 41	—	558	998
Myleran	12·1	21	461 ± 34	16·3	482	774
	13·8	12	482 ± 65	12·5	499	789
	15·5	13	436 ± 64	20·9	449	719
Myleran and bone marrow	15·5	106	324 ± 22 (427)*	41·2 (22·5)*	337	852
	17·2	75	303 ± 25 (401)*	45·0 (27·2)*	298	778
	20·7	99	410 ± 21	25·6	442	880
	27·6	119	336 ± 16	39·0	333	700
	34·5	80	340 ± 20	38·3	333	766
	41·4	29	246 ± 33	55·4	250	581
	48·3	2	99	82·0	99	128

* Corrected for the mortality occurring during the 2nd and 3rd month.

we assume that transplantation of bone marrow in rats is ineffective to reduce shortening of span of life, as it has been found in irradiated animals[8], the shortening of span of life by myleran is a non-linear function of the dose with an important reduction of span of life with the increasing dose. It is interesting that a similar dose-response relationship was found in animals exposed to single doses of whole body irradiation[9].

When compared in terms of the $LD_{50/30}$ doses, the ionizing radiation appears a more potent agent for reducing the span of life than myleran. However, the comparison on this basis may be misleading because the sensitivity of rat bone marrow tissue to myleran may be more important than to ionizing radiation and also relatively to the sensitivity of other tissues which could be involved in the shortening of span of life. For example, the sensitivity of bone marrow tissue to myleran, as reflected by the $LD_{50/30}$ doses, is more important in rats than in mice (respectively 14 and 56·7 mg/kg)[6]. If there are no species differences for the shortening of span of life by myleran, it can be predicted that single sub-lethal doses above 20·7 mg/kg of myleran would significantly reduce the span of life in mice.

In addition, routine autopsies of rats showed the presence of lens cataracts and testis atrophies with incidence rate and degree in proportion to myleran dose. The incidence of macroscopic neoplasms was not above the normal level observed in the strain of rats used. Terminal causes of death apparently did not differ from those found in normal controls dying spontaneously; but it is not impossible that systematic histological investigations now in progress would disclose other particular findings, especially in rats which received higher doses of myleran.

It may be concluded that single doses of radiomimetic chemicals share with ionizing radiations the property to reduce the span of life in mammals. It is hoped that further investigations in this field may shed more light on the mechanism of shortening of life by the agents described here and on factors which could modify their delayed somatic effects.

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Distribution and Fate of Soluble Proteins labelled with Iodine-131 from Rat and Rabbit Livers

Soluble proteins of rat and rabbit liver have been labelled[1] with iodine-131 using the chloramine *T* radio-iodination procedure[2,3]. In this communication the distribution and fate of liver ^{131}I-proteins in relation to the chloramine *T* and iodine monochloride[4] (ICl) procedures are described.

Soluble proteins were extracted from rat and rabbit liver, were dialysed and labelled with carrier-free iodine-131 ('*IBS*.3', Radiochemical Centre, Amersham) as previously described[1]. ^{131}I-tungstophosphoric acid (TPA, final concentration: 1·7 per cent in 0·68 N hydrochloric acid) soluble radioactivity as percentage of the original radioactivity was measured with carriers sodium iodide and bovine serum proteins.

The distribution of homologous liver ^{131}I-proteins was investigated in male Wistar rats of about 250 g weight having fasted for 26 h. The sample (100–120 μc.) was injected via the femoral vein into the ether-anæsthetized animal and its total body radioactivity was immediately counted. After a 15-min interval blood was withdrawn from the abdominal aorta and perfusion with 1 l. heparinized saline was started via the portal vein. Afterwards several organs were removed, enclosed in thin polythene bags and separately set, in their proper position, inside the thoracic and abdominal cavity of a non-radioactive rat carcass. In identical geometry, the rat was counted in order to assess the percentage of dose retained by the organs. The radioactive rat carcass and an aliquot of blood were also measured.

Results are shown in Table 1. A somewhat lower yield of protein-bound radioactivity was observed using higher amounts of iodine-131 (4 mc.). Raising the amount of chloramine *T* (5–20 mg) determined, after addition of the reducing agent, the appearance of an increasingly intense opalescence in the protein solution. Correspondingly an approximate proportionality between the percentage of dose retained by the liver and the amount of chloramine *T* used became evident, the higher uptake being due to some denatured proteins during the iodination. Heat-denatured[5] serum and liver ^{131}I-proteins gave 81 and 89 per cent retention, whereas normal serum ^{131}I-proteins gave about 1·2 per cent[3].

When liver proteins were labelled with iodine-131 by using the iodine monochloride method[4] (the final reagent

Table 1. Rat Liver Retention of Rat Liver Soluble ^{131}I-Proteins

Labelling techniques	Percentage total ^{131}I-recovery	Percentage of dose after 15 min from injection in: Blood	Liver	Carcass
Chloramine *T* (2 mg)	28·0	22·9 ± 1·3	51·5 ± 0·3	16·7 ± 0·8
Chloramine *T* (5 mg)	40·6	15·5 ± 0·5	61·4 ± 0·9 (+19·2%)	14·7 ± 0·2
Chloramine *T* (10 mg)	44·0	13·4 ± 2·4	66·7 ± 0·3 (+29·5%)	12·7 ± 1·1
Chloramine *T* (20 mg)	43·2	6·8 ± 0·2	74·7 ± 0·2 (+45·0%)	11·1 ± 0·7
Chloramine *T** (20 mg)	43·2	9·3	68·5 (+33·0%)	11·6
Iodine monochloride (126 μg)	4·5	22·1	51·9 (+0·8%)	17·9
Iodine monochloride (210 μg)	6·8	21·9	52·7 (+2·3%)	16·7

Each sample of liver proteins (29·4 mg) was labelled with 4 mc. iodine-131. Percentages of ^{131}I-TPA soluble radioactivity ranged between 3·5 and 6·7 per cent. Carrier liver proteins were added to prevent self-irradiation. About 10 mg of liver ^{131}I-proteins (in 2·0 ml. *p*H 7·6 phosphate buffer) were administered to the rats except one. Figures with ± *S.E.* represent mean of 3 animals. Figures between brackets represent percentages of higher liver retention.

* This rat received 59 mg liver proteins.

Percentage of dose in liver: *P* (2 mg : 5 mg) < 0·001; *P* (2 mg : 10 mg) < 0·001; *P* (2 mg : 20 mg) < 0·001.

Table 2. Rabbit Liver Soluble ^{131}I-Protein Recoveries using the Iodine Monochloride and Chloramine *T* Radioiodination Procedures

Labelling techniques	Opalescence of protein solutions	Percentages ^{131}I-recovery	^{131}I-TPA soluble radioactivity
Iodine monochloride			
126 μg	–	12·1	6·3
210 μg (*a*)	–	18·3	4·9
Chloramine *T* 2 mg (*b*)	–	43·8	5·8
Chloramine *T* 5 mg (*c*)	+	45·9	3·7
Chloramine *T* 10 mg (*d*)	+ +	48·5	3·5

The reaction mixture was made up with proteins (30·9 mg) in 0·15 M phosphate buffer, *p*H 7·6, with 5 mc. iodine-131 per each sample. Addition of carrier liver proteins prevented self-irradiation damage. Percentages of ^{131}I-TPA soluble radioactivity were estimated on the eluates.

a, *b*, *c*, and *d* refer to curves shown in Fig. 1.

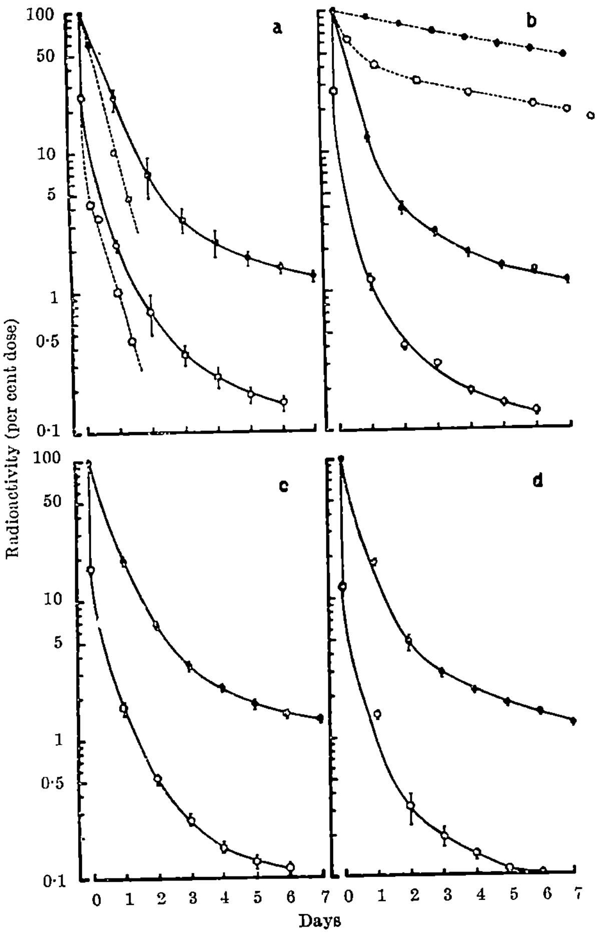

Fig. 1. Behaviour of soluble proteins extracted from rabbit liver and labelled with iodine-131 by the (*a*) iodine monochloride (210 μg) and chloramine *T*; (*b*) 2 mg; (*c*) 5 mg and (*d*) 10 mg, procedures and injected into rabbits. Labelling efficiencies of these samples are reported in Table 2. Vertical bars represent ± *S.E.M.* of 2 observations; ●, total body activity; ○, plasma activity as per cent of value at injection time. Dotted curves represent the behaviour in (*a*) of ^{131}I-sodium iodide, and in (*b*) of homologous normal serum ^{131}I-proteins

contained 0·42 mg of iodine as monochloride per ml.) 52 per cent ^{131}I-proteins were taken up by the liver, and this value is close to that obtained by using ^{131}I-proteins kept in contact with only 2 mg chloramine *T*.

Published reports[6] indicate that the *in vivo* behaviour of serum ^{131}I-proteins labelled by the ICl method is almost identical with that of the corresponding biosynthetically labelled ^{14}C-proteins.

Another test consisted of following (Fig. 1), in rabbits, the total body radioactivity and the intravascular (μc./ml. of plasma) radioactivity (all results being expressed as a percentage of the injected dose) of rabbit liver ^{131}I-protein samples (Table 2). Male rabbits, weighing about 2·75 kg, were used throughout and were separately housed in metabolism cages. In order to increase the rate of iodide excretion the drinking water contained 0·00034 M sodium iodide and 0·08 M sodium chloride for 15 days before and up to the end of the experiment. The liver ^{131}I-protein sample (about 500 μc. in 3·0 ml.) was injected into the marginal ear vein, and about 0·5 ml. of blood withdrawn 9 min later from the opposite ear vein. The activity to be injected and the syringe washings were counted and the injected dose obtained by difference. Plasma volume per each rabbit was measured[7] two weeks before by the injection of about 2 μc. rabbit ^{131}I-albumin. Thus by knowing the plasma volume and the dose the 100 per cent value (plasma activity) at injection time could be calculated. Total body and plasma radioactivities were measured as already described[3].

Curves were very complex as liver proteins are a heterogeneous mixture[8]. Fig. 1 shows that ^{131}I-proteins disappeared very rapidly from the circulation and that the clearing was even faster if, during iodination, 5-10 mg chloramine *T* were used. The slower fall of total body radioactivity was due, at least in part, to the diffusion into the extravascular fluid of free radioactivity liberated for the intense protein breakdown. Total body and plasma radioactivities half-lives for ^{131}I-sodium iodide (*a*) and serum ^{131}I-proteins (*b*) were shorter and much longer respectively.

Vertical starch-gel electrophoresis was performed according to Smithies[9] with a discontinuous buffer system[10] and a contact autoradiogram was taken with X-ray film on the bottom slice of the gel. It was noted that when chloramine *T* amounts were higher more ^{131}I-proteins remained near to or at the origin. The sieving effect of the gel on a modified protein molecular size, as suggested by the appearance of opalescence, might explain the result.

It is concluded that the reagents used in the radioiodination procedure, if not used under carefully selected conditions, can adversely modify the biological properties of liver ^{131}I-proteins whereas little, if any, protein damage might derive from either substitution of iodine-131 or from self-irradiation[11].

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Recovery from X-ray Injury and the Effect of Oxygen

Observations were reported recently[1,2] on a significant reduction of the extrapolation number of the survival curves of mammalian cell cultures which were exposed *in vitro* to varying X-ray doses under anoxic, as contrasted to aerobic, conditions. In view of the relationship between the extrapolation number and the extent of recovery from radiation damage, noted in connexion with treatment by fractionated doses of X-rays[3,4], fractionated treatment of anoxic cells can be expected to lead to a decrease or lack of recovery. This communication records the results of some preliminary experiments designed to test this possibility. Since access to oxygen is to a great extent reduced in neoplastic cell populations *in vivo*[5], the data should further clarify some of the factors which determine the result of tumour radiation therapy, as commonly practised according to various dose-fractionation schedules.

In vitro cultures of the *ELT* sub-line of *ELD*, an Ehrlich ascites tumour, were used as the cellular material[2,6,7]. In each test, a series of nine 'Pyrex' Petri dishes 6 cm in diameter, containing 4–6 ml. nutrient solution[2], were implanted with different dilutions of a suspension of single cells. This was prepared by trypsin treatment of cultures growing in a non-confluent monolayer. After 3 h incubation at 38° C in a moist atmosphere of carbon dioxide in air, the lids of the dishes were removed and the medium withdrawn to such an extent that a fluid

Table 1. Results of Tests of Survival of *ELT* Ascites Tumour Cell Cultures after Exposure in Argon or Oxygen to either a Single Dose of X-rays or to Irradiation with the Same Dose delivered in Two Equal Fractions separated by a Time-interval of 18 h

No. of repeat tests	Atmosphere during exposure	Plating efficiency of unirradiated cells*		X-ray dose (r.)	Irradiated cells			Difference between surviving fraction after single and fractionated irradiation with the same total dose**
		Mean	Range		Total No. exposed	Surviving fraction‡ Mean ± *S.E.*		
						Established from the tests§	Estimated statistically	
1	2	3	4	5	6	7	8	9
17	Oxygen	58	32–99	1 × 400	8×10^3	0·516 ± 0·015	0·525 ± 0·042¶	0·479 ± 0·021
				2 × 200†	2×10^3	0·995 ± 0·026		
18	Argon	45	25–69	1 × 400	8×10^2	1·391 ± 0·011	1·382 ± 0·044‖	0·011 ± 0·010
				2 × 200†	8×10^2	1·402 ± 0·014		
21	Argon	59	30–81	1 × 1,200	2×10^4	0·111 ± 0·016	0·138 ± 0·014‖	0·006 ± 0·008
				2 × 600†	2×10^4	0·105 ± 0·018		

* Expressed as the percentage of a standard inoculum of 2×10^2 cells which grew into macroscopic colonies in 10 days. In each test values were established from triplicate observations.

† Doses separated by a time-interval of 18 h.

‡ Expressed (in log units) as percentage of unirradiated controls in the same test.

§ In each test values were calculated from 3 irradiated and 3 control replicate dishes.

¶ Calculated from equation: $\log Y = 0{\cdot}2695 - 0{\cdot}00436\, X$, and the Y variance derived from the regression data of survival published in ref. 2 where Y indicates the surviving fraction and X the dose in r.

‖ Calculated from equation: $\log Y = 0{\cdot}0043 - 0{\cdot}00156\, X$, and the Y variance derived from the regression data of survival published in ref. 2 where Y and X are as indicated under ¶.

** Mean ± *S.E.* (in log units) calculated from differences determined separately in each of the tests.

layer of only about 1 mm was left to cover the cells which were now firmly attached to the bottom of the vessels. Afterwards, six dishes were exposed to X-rays and the remaining three dishes used as unirradiated controls, subjected to the same procedures as the irradiated ones. Half the cultures exposed to radiation received a dose of either 200 r. or 600 r., while the other half was treated with the corresponding double dose. After exposure, the dishes were refilled with fresh medium and reincubated for a period of 18 h. The cultures irradiated with 200 r. or 600 r. were then exposed to a repeated treatment with the same X-ray dose which they had received, while the other cultures were subjected to sham-irradiation. Afterwards the dishes were incubated for 10 days with medium changes taking place at 3–4 days intervals. Clones, fixed and stained *in situ*, comprising more than fifty cells were then counted. The fraction of cells surviving irradiation with retained capacity for reproduction, as judged by their clone forming capacity, was used as the measure of the radiation effect, and was calculated as the percentage of unirradiated controls of the same experiment.

The dishes were exposed to X-rays at room temperature in an air-tight plastic chamber. For 20 min prior to, and during, irradiation, the chamber was gassed with humidified oxygen or argon, both containing carbon dioxide to maintain a *p*H of 7·0–7·2 in the medium. The oxygen contamination of argon was less than 3 p.p.m. Radiation was generated at 190 kV and 15 m.amp. Details of the gassing system and the radiation procedure have been described previously[2].

The mean surviving fraction of cultures exposed in argon or oxygen to either a single dose of X-rays, or to irradiation with the same dose split into two equal parts, is presented together with standard errors in col. 7, Table 1. It can be seen that the size of the mean surviving fraction after a single exposure to a certain dose agrees closely with the value (col. 8) calculated from the regression data of the survival of *ELT* after irradiation under comparable atmospheric conditions[2]. The difference between the surviving fractions after the single and the fractionated irradiation with the same dose has been calculated in each of the tests separately. The mean of the differences and the corresponding standard errors are presented in col. 9. Fractionation led to minor and statistically insignificant differences only, when compared with the effect of single exposure in an anaerobic environment. In the presence of oxygen there was a clear differential effect, however. Single exposure was about three times more efficient in damaging the reproductive integrity of cells than fractionated treatment. The difference was statistically significant ($P < 0{\cdot}001$).

The increased survival due to fractionated irradiation under aerobic conditions suggests that a certain repair of the radiation damage occurs during the time interval which elapses between the treatments. Evidence presented by Elkind *et al.*[3] supports the view that such repair concerns the sub-lethal damage which is accumulated before a cell is lethally affected, and which has an expression in an extrapolation number of the survival curve larger than unity. The ratio of survival, following two exposures, compared with a single equivalent dose, was found[3] to have a value which is close to that of the extrapolation number if the length of the fractionation interval exceeds 15 h. This observation is in agreement with the result reported here, since the extrapolation number of the survival curve of *ELT* after aerobic irradiation has previously been found[2] to be about 2, that is, close to the survival ratio of 3 as calculated from the present results.

The lack of any difference in response due to dose fractionation in an anaerobic environment can be taken to indicate an absence of repair processes as regards cellular reproduction. After anoxic irradiation[2], the survival of *ELT* exhibited a nearly exponential dose-effect relationship with an extrapolation number close to unity. The present data support the postulate[3] that, under such conditions, the ratio of the surviving fractions, after a single, compared to fractionated, irradiation, will also nearly equal unity. Thus, the effect of anoxic treatment by sparsely ionizing X-rays resembles closely the effect of densely ionizing radiations, also characterized by an exponential survival pattern of cells without the involvement of any recovery phenomenon on dose fractionation[8]. Our result agrees with observations concerning the effect of fractionated gamma irradiation on *Trifolium repens* in the presence or absence of oxygen[9]. It is, however, at variance with findings made with human kidney cells in similar experiments[10]. The disagreement may be explained by the oxygen contamination of the nitrogen, used in the anaerobic assays, stated[10] to be about 0·1 per cent. Such an oxygen concentration has been shown to be sufficient to raise the radiosensitivity of bacterial cultures to half the maximal oxygen enhancement[11].

In summary, the results presented show a differential effect of fractionated irradiation with regard to the recovery phenomenon of the cells, depending on the presence or absence of oxygen during treatment. Considering the generally impaired oxygen supply of neoplasms as contrasted to their surroundings, it is thus conceivable that normal tissues, though enhanced in sensitivity, may nevertheless recover from radiation damage to a comparatively larger extent than neoplastic tissue in the course of fractionated X-ray therapy. Our result supports the view[12] that, in contrast to the idea of a purely quantitative dose-modifying influence of oxygen, there is also a qualitative difference in the lethal cellular damage caused by irradiation with X-rays under aerobic as compared with anoxic conditions.

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Effect of Alkylating Agents and Whole-body X-irradiation on Thymus

MANY investigations have indicated the disruption of the structure of DNA or nucleohistone by nitrogen mustards or ionizing irradiation[1,2]. The labilization of nucleohistone from thymus after irradiation has been measured by Hagen[3] by increased extraction of DNA by phenol treatment. Cole and Ellis[4] have reported an increase in soluble polynucleotide in spleen after irradiation. In investigations on the effects of these agents on amino-acid incorporation in thymus nuclei and cytoplasmic microsomal and 5·1*P* fractions[5], the presence of acid insoluble DNA material in the various fractions became apparent. The finding is reported primarily because of its importance in interpreting results of changes in enzyme activities in cell fractions from radio-sensitive tissues after treatment.

In investigations on effects of whole-body X-irradiation, 10–12-week-old male rabbits were exposed to 650 r., 260-kV radiation. 'Mustargen' (methyl-*bis*[β-chloroethyl]amine hydrochloride), dissolved in 0·04 M sodium phosphate buffer, *p*H 7·4, was injected through the ear vein (4 mg/kg body-wt.). Thymuses from 4–5 animals were pooled in each experimental group. Rabbit thymus nuclei and cytoplasmic fraction were made by slow stirring in 0·25 M sucrose–0·0033 M calcium chloride based on the procedure of Allfrey, Mirsky and Osawa[6]. The cytoplasmic fraction was centrifuged at 15,000*g* for 15 min. The microsomal fraction was prepared from the resulting supernatant by centrifugation for 1 h at 105,000*g*. The 5·1*P* fraction was prepared from this supernatant by the commonly used procedure.

The fractions were precipitated and washed with cold 4 per cent perchloric acid, alcohol and ether to obtain protein–nucleic acid powders for analysis. DNA content was determined using Webb's method[7]. The molecular status of the DNA material in these fractions is not known at the present time and is under investigation.

Results in Table 1 illustrate the presence of DNA in the microsomal fraction and 5·1*P* fraction at varying times after whole-body X-irradiation. DNA is present in both fractions 2 h post-irradiation. No DNA was found at 1 h, which would agree with the observation of Hagen[3] of a 1-h lag before a measurable change in phenol extraction of DNA from thymus. The amount of DNA in the fractions increases up to 6 h after irradiation. By 18 h the DNA content is decreased, probably due to the action of DNase and the phagocytic activity, which are known to be increased at this time after irradiation.

Table 1. EFFECT OF X-IRRADIATION ON DNA CONTENT OF ISOLATED THYMUS CYTOPLASMIC FRACTIONS
(Expressed as percentage dry weight of dry protein–nuclei acid residue)

	Hours after irradiation						
Fraction	0	1	2	3	4	6	18
Microsomal	0·96	0·90	2·3	4·6	9·4	15	3·4
5·1*P*	0·6	0·67	1·4	5·2	11	11	4·7

Similar observations (Table 2) are noted after treatment of rabbits with the nitrogen mustard, 'Mustargen'. Thus one early manifestation of the effect of both these agents on thymocytes is a labilization of the structure of the nuclei and the nucleohistone.

Table 2. EFFECT OF MUSTARGEN ON DNA CONTENT OF ISOLATED THYMUS CYTOPLASMIC FRACTIONS
(Expressed as percentage dry weight of dry protein–nucleic acid residue)

	Hours after injection					
Fraction	0	4	6	8	12	18
Microsomal	0·86	2·0	7·1	13·2	20·4	2·1
5·1*P*	0·84	1·2	5·0	12·4	14·5	5·5

These investigations do not permit the conclusion that the initial site of action of ionizing radiation or alkylating agents is directly on the structure of the nucleohistone. The observations reported here could be the result of disaggregation of chromatin because of loss of ions or proteins required for structure; this, in turn, could be caused by direct action of the agents or changes in nuclear membrane permeability[8]. It is also possible that the disruption of nucleohistone structure could be a primary effect. Peacocke and Preston[9] have measured the release of histone from nucleohistone in solution by γ-irradiation with no denaturation of the DNA. An initial change in the structure of the nucleohistone could make the DNA susceptible to the action of DNase, and it is the products in various stages of degradation which appear in the isolated cell fractions. Brookes and Lawley have studied the alkylation and subsequent change in structure of DNA[10]. Biesele[11] has discussed the effects of alkylation and emphasized that alkylations of RNA and protein may also be significant.

Regardless of mechanism, the gross observation reported here of the presence of nuclear products in cytoplasmic fractions at early times after treatment with these agents indicates that disruption of damaged nuclei must be considered in interpreting changes in enzyme activities in all cell fractions. It is also obvious that the presence of DNA breakdown products in the cell fractions must be considered in evaluating data on effects on various aspects of nucleic acid metabolism.

My results also indicate that the DNA material is removed from the microsomal fraction by the deoxycholate treatment in preparation of the ribosomal fraction. Data on incorporation of amino-acid by intact nuclei and the microsomal, ribosomal fractions, to be published in detail elsewhere, indicate that, at early times after X-irradiation or 'Mustargen' treatment when *in vitro* amino-acid incorporation by nuclei is inhibited, incorporation by the ribonucleoprotein particles in the ribosomal fraction is not inhibited.

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BIOLOGY

Responses of a New Hibernator (*Citellus variegatus*) to Controlled Environments

THE phenomena of hibernation and æstivation have both been recorded in the genus *Citellus*, and although it is not known whether the two phenomena are physiologically the same[1], nevertheless hibernation seems to be an adaptation to an unfavourable cold seasonal environment, and æstivation an adaptation to an arid hot environment with seasonal drought. It is of interest, therefore, to find that the rock ground squirrel, *Citellus variegatus*, the habitat of which is the arid south-west from Utah to southern Mexico[2], is capable of hibernating but does not appear to æstivate. Hibernation as used here refers to a mammal with a homothermic temperature of approximately 37° C, but which is able to lower this temperature to the environment and raise it again against the gradient.

In early June 1963, twelve animals of this species were trapped 10 miles west of Las Vegas, Nevada. They comprised seven adults and five juveniles. All survived well in captivity, and gained weight rapidly, the adults becoming extremely obese.

On August 5, one month after capture, groups of two animals were exposed to 3° C and 23° C environmental temperatures, with food ('Purina Chow') and water *ad libitum*, to determine whether spontaneous æstivation occurred as in *Citellus mohavensis*[3]. On August 15 two more groups of two were exposed to the same environmental temperatures, but only food was supplied. In the groups with food and water *ad libitum* no evidence of spontaneous æstivation was observed, and at the time of writing (December 1963) no evidence of spontaneous hibernation has been observed either. The latter is in contrast to *Citellus mohavensis* which æstivates and hibernates spontaneously[3] and *Citellus lateralis* which hibernates spontaneously[4]. The groups with food but no water lost weight gradually, but no drop in body temperature was observed in those in an environment of 3° C for 45 days when water was given, or the animals died. In those at 23° C there was no observed drop in body temperature for 50 days, but thereafter one animal did appear to become hypothermic on occasions for a few hours, body temperatures of 28° C and 31° C being recorded. During October food was denied to four more animals at an environment of 3° C, and in all cases they hibernated within 10 days. However, under these conditions the body temperature does not apparently fall to the environment like that of other hibernators[3,5] but is maintained at a low of 8° C in a 3° C environment. It seems that *C. variegatus* has some form of 'thermostat' set considerably above 0° C, which is confirmed by the fact that an animal in an environment of −18° C continues to hibernate successfully, but the body temperature is held at 8–10° C. The œsophageal temperature is slightly above the rectal.

This species also has a remarkable tolerance to dehydration. When free water is denied but food ('Purina Chow') permitted, the animals slowly lose weight, but some have survived more than 100 days at 23° C with no appreciable drop in body temperature. *C. lateralis* at the same season (that is, active one) will only survive some 20 days with no free water (Pengelley, unpublished results), but the percentage loss in weight before death is about 70 per cent in both species. There are, no doubt, differences in physiological adaptations to water deprivation in two such species (now being investigated), but there is an obvious behavioural difference. *C. lateralis* becomes highly active within 24 h of deprivation of water, whereas *C. variegatus* gives no response that can be observed and remains comparatively docile. Although the environmental conditions are probably not identical[6,7], *C. variegatus* may prove to have the longest survival time of any mammal yet examined which is unable to maintain body weight without free water (*Dipidomys merriami* can[6]); this is, of course, provided there is no lowering of body temperature in response to water deprivation.

The experimental results indicate that in *C. variegatus* the stimulus for entrance into hibernation is lack of available food, that æstivation does not occur spontaneously or in response to lack of water, and that while the animal is able successfully to lower its body temperature and raise it against the gradient, it does not in fact permit it to fall below 8° C. Thus, its behaviour is quite different from other species within the genus. Furthermore, it is possible to reason that the evolutionary adaptations to this animal's arid habitat are high tolerance to water deprivation during summer (the dry season throughout most of its range), and the ability to hibernate during winter in response to scarcity of food which it does not store, at least in captivity.

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Liver Mitotic Rates and Weights in Young, Rapidly Growing Rats after Serum Injection

THE systems which seemingly demonstrate control of cell division in rat liver commonly involve change in the composition of the blood. For example, Stich and Florian[1] found that single injections of normal serum administered to partially hepatectomized rats inhibited mitosis, while serum from rats which had been partially hepatectomized stimulated mitosis following a single injection. Recently, Moya[2] reported that single injections of both normal and post-hepatectomy serum prepared from arterial blood inhibited mitosis in regenerating livers. Moreover, Smythe and Moore[3] reported that weights of regenerating livers were decreased by repeated injections of normal plasma. These results, obtained in adult rats, raise the question of whether treatment with serum injections has the same effects on livers of young, rapidly growing rats.

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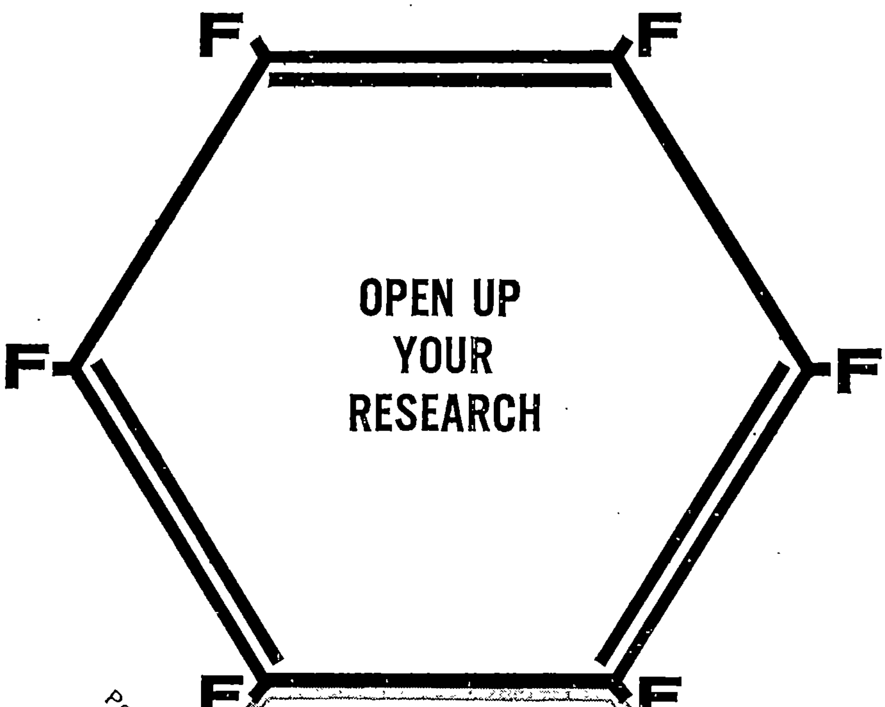

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Methylpentafluorobenzene
2,3,5,6-Tetrafluorophenol
1,2,3,4-Tetrafluorobenzene
Pentafluorothiophenol
Octafluorotoluene
Pentafluorophenol
2,3,5,6-Tetrafluoroaniline
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Table 1. EFFECT OF SINGLE AND REPEATED SERUM INJECTIONS ON LIVERS OF YOUNG RATS

Exp.	Group	Treatment—normal serum (1·5 ml. per 100 g body-wt.)	No. rats	Mitotic rate mean ± *S.D.* (mitoses/1,000 cells)	Liver weights Wet wt. (g) mean + *S.D.*	Liver weights Rel. wt. Mean + *S.D.*
1	*A*	Single injection	8	3·1 ± 1·61	—	—
	B	Untreated controls	8	3·2 ± 1·75	—	—
2	*A*	Daily* for 2 weeks	10	2·8 ± 3·24	9·9 ± 0·40	5·97 ± 0·11
	B	Untreated controls	15	5·4 ± 3·05	10·1 ± 0·34	5·91 ± 0·11
	C	Normal saline Daily* for 2 weeks	10	4·9 ± 3·45	10·3 ± 0·43	5·95 ± 0·16

* Except Sunday.

Male Sherman rats, 4 weeks old, were caged individually in an air-conditioned room (72° F), lighted artificially from 5 a.m. until 8 p.m. Ground 'Wayne Lab Blox' and water were available at all times. These young rats were injected with serum from adult rats of the same strain, prepared from blood withdrawn by direct heart puncture under light ether anæsthesia. Serum was separated by refrigeration for 1 h followed by centrifugation and was refrigerated until injected 3 h later. Treated rats received 1·5 ml. of pooled serum per 100 g body-weight by intraperitoneal injection. Rats of Exp. 1 (Table 1) received a single injection of serum and were killed 16 h later; rats of Exp. 2 were injected daily, except Sunday, at 5 p.m. (E.S.T.), for 2 weeks (13 injections). Fresh serum was prepared each day for rats of Exp. 2. Untreated rats, receiving no injections, served as controls for both experiments. A group of rats injected with saline served as additional controls in Exp. 2.

Rats were killed by chloroform inhalation between 8.30 and 11 a.m. (E.S.T.) following the last injections. Slices of liver from the median lobe were used for mitotic counts. In Exp. 1, the livers were fixed in Carnoy's fluid, and 10-μ paraffin sections stained with gallocyanin were used for counts. In Exp. 2, the livers were fixed in Bouin's fluid and the method of Ross and Flaharty[4] was used to stain the slides.

Counts of mitotic cells and the number of cells per field were made under oil immersion (× 1,250). All phases of mitosis were counted. Doubtful prophases and telophases were recorded; two doubtfuls were taken to equal one mitosis. Mitotic rates, expressed as the number of mitoses per 1,000 cells, were calculated from the number of mitoses in 100 fields and an estimate of the number of cells in 100 fields. Approximately 9,000 cells were examined in each rat's liver.

As shown in Table 1, the mean mitotic rate of the rats which received a single injection of serum was the same as that of the controls. In Exp. 2, the mitotic rate of the serum-treated rats was less than the other two groups, but the difference was not statistically significant. It might be said that insufficient rats were used, and that, if more rats had been used, it would have been possible to demonstrate statistically significant differences. The number of rats needed to demonstrate the statistical significance of specified differences can be calculated by Sillitto's method[5] using the standard deviation of the untreated controls. To demonstrate differences ($P = 0{\cdot}05$) amounting to 10, 25 and 50 per cent of the control mean, 106, 30 and 8 animals per group, respectively, would be needed. Thus, in the present experiment, sufficient rats were used to demonstrate a reduction of mitotic rate of about 50 per cent. Liver weights were also unaffected by repeated injections of serum from adult rats. The mean wet weights and relative weights of the livers of all three groups of Exp. 2 were nearly the same.

Thus inhibition of mitotic activity in livers of young, rapidly growing rats was not evident after a single injection of serum from adult rats or after prolonged treatment with serum. Similarly, Stich[6] found that injections of liver homogenate inhibited mitotic activity in regenerating livers but had no effect on growing livers of baby rats. The lack of effect in livers of young rats of treatment which inhibits mitosis in regenerating liver would seem to be important in view of Bullough's[7] recently proposed theory of mitotic control in adult mammalian tissue. Starting with the premise that any cell naturally prepares for mitosis unless diverted to differentiate, Bullough suggested that control of mitotic activity within a given tissue is accomplished by a tissue-specific mitotic inhibitor (chalone) which prevents further preparation for mitosis and promotes differentiation. It follows that this mechanism would not be operative in controlling mitotic activity in tissues of young, rapidly growing animals. Thus livers of young rats may fail to respond to treatments which inhibit mitosis in regenerating livers because differentiation cannot be promoted.

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Leptospira erinacei auriti in a Water Vole, *Arvicola amphibius*

DURING the course of an investigation made by the Central Veterinary Laboratory into the incidence of leptospiral infection in small wild mammals a number of strains were recovered. Most of these apparently belong to the *hebdomadis* group, but one strain, *W.V.*14, from a water vole, was serologically in the *autumnalis* group. It was finally identified in Amsterdam as *L. erinacei auriti* as shown in Table 1. An apparent relationship to grippotyphosa and butembo in cross-agglutination tests was not confirmed by absorption tests.

Table 1. ABSORPTION TESTS WITH STRAIN AND ANTISERUM *W.V.*14

Anti-serum	Absorbed with strain	Titres after absorption with strain, expressed as percentages of the reciprocal of the homologous titre *W.V.* 14	*Akiyami A*	*Rachmat*	*Bankinang*	*Erin. auriti*	*Djasiman*
W.V. 14	*Erin. auriti* 670	1	0	1	1	0	1
Erin. auriti 670	*W.V.* 14	0	1	0	0	1	0
Akiyami A	*W.V.* 14	0	100	1	100	1	0
W.V. 14	*Akiyami A*	33	0	10	1	> 10	
Rachmat	*W.V.* 14	0	10	10	10	0	
W.V. 14	*Rachmat*	10	0	0	0	> 3	
Bankinang	*W.V.* 14	0	100	10	100	0	0
W.V. 14-	*Bankinang*	33	3	3	0	> 10	33
Djasiman	*W.V.* 14	0	0	0	0	0	33
W.V. 14	*Djasiman*	100	33	100	> 33	100	0

This vole was one of a number involved in a population study on Fray's River near Uxbridge and was given to the Central Veterinary Laboratory by Mr. C. H. B. Worrall of the Infestation Division of this Ministry. Fray's River forms part of a complicated system of waterways joining the Thames to the Midlands.

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Occurrence of *Asellus* (Crustacea. Isopoda) in Esthwaite and Ullswater, English Lake District

IN a previous paper[1] *Asellus aquaticus* was recorded for Esthwaite from one station only, and it was also stated that *Asellus* was absent from Ullswater. Since then the situation has changed, as *Asellus aquaticus* is now established throughout Esthwaite and *Asellus meridianus* has been found at the south end of Ullswater.

The implications of this are of sufficient interest to warrant full publication, but the following points may be made now. In the case of Esthwaite, it seems from old records that *Asellus* was definitely recorded from the boat house and reed bed area at the north end in 1950, although it is possible that it may have been seen as early as 1944. Despite my own collections in Esthwaite in 1948 and 1951 and those of the Freshwater Biological Association supply department in intervening years, *Asellus* was not reported again until 1955—again around the boat house, one of the places where I collected in 1948. This raises the point as to whether it is more prudent to record a species 'as not found' rather than 'absent'. One has on several occasions, after prolonged and vigorous collections in the Midlands and in the Lake District, found a single specimen of *Asellus*. How often on other occasions must the odd specimen have been overlooked despite every care ?

Esthwaite is 1·5 miles long and less than 0·5 miles across at its widest, and after 14 years *Asellus* can be found around the whole lake and to a depth of 3–4 m. Whether this represents a genuine 'spread' of *Asellus* from a single centre, or the build-up from a population so diffuse that the collecting methods used failed to detect it, is a matter for consideration. It would be extremely interesting to re-examine the other lakes in the district where I previously failed to find *Asellus*. So far as the Ullswater district is concerned the only other known occurrence of *Asellus*, after many years collecting, is in three garden pools above the lake. One of these pools contains *A. aquaticus*, the other two *A. meridianus*, and *Asellus* is known to have been introduced with weeds. The pools are a mile away in direct line from the *Asellus* localities in Ullswater, and apart from these the nearest known occurrence is in Windermere, ten miles away over Kirkstone Pass.

I thank Mr. S. Nield and Mr. J. D. Allonby, of the supply department of the Freshwater Biological Association, for their help with the Esthwaite records and for the first record of *Asellus* in Ullswater.

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Antifungal Activity in Orange Tissue infected with *Aspergillus niger*

ORANGE fruits that had been wounded and inoculated at fixed points with conidia of *Aspergillus niger* became infected with *Penicillium digitatum*, and it was observed that growth and sporulation of the latter were halted some distance from the regions of the fruit colonized by *A. niger*. Cylindrical portions of the pulp from regions supporting the *A. niger* growth placed on plates of potato–dextrose agar seeded with the spores of various fungi inhibited the growth of several, but no such inhibition was obtained with pulp from healthy fruits (Table 1).

Furthermore, macerated orange pulp when inoculated with *A. niger* developed similar antifungal activity although potato–dextrose agar, which was supporting *A. niger*, did not. Therefore, in further investigations, peeled oranges were macerated in a Waring blender and the homogenate was distributed in 11-cm diameter Petri dishes in layers approximately 0·7 cm deep. After inoculating the surface of the fruit pulp at several points with a suspension of *A. niger* conidia in water, the dishes were incubated at 25° C for four days, by which time the surface was covered with a thick mycelial mat on which conidial heads were just developing. This was lifted, the adhering pulp brushed from its underside into the residual liquid and the mat discarded. Sufficient alcohol was added to bring the final concentration in the treated pulp to 80 per cent ethanol and the mixture was left at 4° C for 24 h to precipitate proteins. It was then filtered and the residue, which had no antifungal activity, was discarded. The alcohol was removed from the filtrate at 30° C under reduced pressure when lipid material separated. This was filtered off; it was non-fungitoxic and was discarded. The remaining clear aqueous solution was highly acidic, strongly antifungal to *A. brassicicola*, less so to *B. cinerea* and inactive against *A. niger*.

Table 1. DIAMETER OF ZONE OF INHIBITION (MM) CAUSED BY HEALTHY OR *A. niger*-INFECTED FRUIT (5 MM^3 PORTIONS)

Test organism	Healthy	Infected
Alternaria brassicicola	0	43
Aspergillus niger	0	0
Botrytis cinerea	0	0
Cladosporium cucumerinum	0	26
Glomerella cingulata	0	0
Penicillium digitatum	0	31

Laboratory investigations indicated that spores of *A. brassicicola* were acid-sensitive, and when samples of deproteinized aqueous extracts of both healthy and *A. niger*-inoculated pulp were passed through columns of ion-exchange resin ('Amberlite *IR*-4*B*(OH)') to remove acidic constituents fungitoxicity was lost. The adsorbed acids were eluted from the columns with 6 N formic acid which was then removed under reduced pressure. Two-dimensional chromatography of the eluted acids showed that citric acid was the predominant acid in both extracts. Titration against standard alkali indicated an equivalent of approximately 4,000 μg/ml. of citric acid in healthy pulp and 28,000 μg/ml. in infected pulp. The degree and range of antifungal activity exerted by citric acid solution at the above concentrations correlate closely with that of the supernatant from centrifuged healthy and *A. niger*-infected pulp (Table 2).

Table 2. DIAMETER OF ZONE OF INHIBITION (MM)* CAUSED BY 0·1 ML. OF JUICE OF HEALTHY OR INFECTED ORANGE AND OF TWO LEVELS OF CITRIC ACID SOLUTION

Test organism	Healthy	Infected	Citric acid (28 mg/ml.)	Citric acid (4 mg/ml.)
Alternaria brassicicola	0	41	45	15
Aspergillus niger	0	0	0	0
Botrytis cinerea	0	20†	20†	0
Penicillium italicum	0	0	0	0
Penicillium digitatum	0	27	26	0

* Diameter of filter paper disk, 13 mm. † Retarded growth.

Thus it would seem that much of the antifungal activity shown by *A. niger*-metabolized orange tissue towards certain fungi is due to citric acid production, though further experiments have indicated that other antifungal materials are present. The —OH resin-treated aqueous extract from infected pulp was of low activity, but an ethyl acetate extract was toxic to the spores of *A. brassicicola* at 250 μg/ml., though less active against *Glomerella cingulata* and *Botrytis cinerea* and inactive against *A. niger*. Similar extracts from healthy pulp were inactive below 5,000 μg/ml. against all the test fungi. Furthermore, some other fungi, for example, *Glomerella cingulata*, when inoculated into orange pulp, produce ethyl acetate-soluble antifungal materials without the large production of citric acid characteristic of *A. niger*.

The development of antifungal activity in plant tissues as a result of infection by pathogens has recently received considerable attention[1]. The resistance to further infection by orange tissue already infected with *A. niger* can therefore be considered in relation to the production of phytoalexins[2], substances defined as "antibiotics which are produced as a result of the interaction of two metabolic systems, host and parasite, and which inhibit the growth of micro-organisms pathogenic to plants"[3]. In this sense, citric acid, although present in healthy fruit at a non-toxic concentration, might be regarded as a phytoalexin since it reaches toxic levels through the activity of the invading pathogen. The ethyl acetate soluble antibiotic remaining after the removal of acids could likewise be considered as a phytoalexin.

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Thermal and Translocated Induction of Endophytic Mycelium in two Powdery Mildews

Endophytic mycelium of various biotypes of *Erysiphe graminis* was demonstrated by Salmon[1] in cereal leaves which had been mechanically damaged or heated to 50° C for short periods immediately prior to inoculation. Schnathorst[2] obtained endophytic mycelium of *E. cichoracearum* in lettuce leaves by stripping one epidermis and inoculating the exposed mesophyll. Yarwood[3] found that heat both predisposed cucumber cotyledons to infection by *Sphaerotheca fuliginea*[4] and made them less responsive to subsequent heat therapy. Yarwood[5] also demonstrated that heat treatment, abrasion or infection by *Uroymces phaseoli* rendered *Phaseolus vulgaris* susceptible to infection by *S. fuliginea*, by which it is not normally attacked, and Yarwood *et al.*[6] and Nienhaus and Yarwood[7] showed that some effects of heating one leaf of an opposite pair could be transmitted to the other, for example, increased susceptibility of bean and cowpea to a strain of tobacco mosaic virus.

Continuing this type of work with *S. fuliginea*, it was observed that when one of a pair of cucumber cotyledons, about 14 days old, was heated in water at 45°, 50° or 55° C for periods of $\frac{1}{2}$–120 sec, either 12 h before inoculation of the upper surface of both cotyledons, or up to 7 days after inoculation, the mycelium became endophytic in both cotyledons. Similar results were obtained in experiments made with the first true leaves of sunflower seedlings inoculated with *E. cichoracearum*.

Thermal induction of endophytic mycelium, first noted by Salmon[1], was confirmed in barley seedlings inoculated with the barley isolate of *E. graminis*, although here no translocated effect was observed.

In all cases, an intercellular mycelium was formed, and typical haustoria were formed in the mesophyll and palisade cells, in the parenchyma of the vascular bundles and in the opposite uninoculated epidermis. Conidiophores and conidia were formed externally on this and on the inoculated epidermis and also occasionally in the intercellular spaces, variously orientated to the leaf surface.

In sunflower and cucumber the effect in the heated leaf varied with the duration and temperature of the treatment and that in the opposite unheated leaf varied similarly, though to a lesser extent. As the duration of the heat treatment was extended a bimodal response became apparent in the curve relating effect to duration, and the development of endophytic mycelium was further affected by the time of day at which the treatment was made, by the time of year and by the age of the plant. Thus, in August, 11-day-old cucumber seedlings showed the greatest effect when one cotyledon was heated at 0700 h following inoculation at 1700 h the previous day, while in 14-day-old plants this occurred following heating at 1300 h, and the effect diminished progressively for the succeeding 5 months.

Salmon[1] found that cross-infection with isolates of *E. graminis* from various cereals and grasses became possible after heat treatment of the hosts; similarly, in the work recorded here, cross-infections with *E. cichoracearum* and *S. fuliginea* were observed. While, however, both isolates produced an endophytic mycelium in sunflower, *E. cichoracearum* from sunflower failed to become endophytic in cucumber. As Yarwood[5] demonstrated, *S. fuliginea* from cucumber infected leaves of *Phaseolus vulgaris* containing heat-killed mycelium of *Uromyces phaseoli*; further heat treatment of one of a pair of such leaves induced the mycelium of *S. fuliginea* to become endophytic, but in the rusted tissues only. This effect was again translocated to the unheated leaf.

When crude sap extracts were made from cucumber cotyledons at intervals after heat treatment, and applied to one cotyledon of other plants, which were then inoculated with *S. fuliginea*, an endophytic mycelium was formed, both in the treated and the untreated cotyledons. It is suggested that an active principle predisposing the cucumber cotyledons to this type of infection is formed as a result of the heat treatment and translocated; in bean two substances formed in response to heat and translocated have been demonstrated and to some extent characterized by English *et al.*[8,9].

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MICROBIOLOGY

Release of Cores from Bacterial Spores by Mechanical Breakage in Acidic Media

The dormant bacterial spore consists of a core surrounded by a structure called the cortex which in turn is bounded by a structure called the coat. The core contains cytoplasm plus nuclear material and the cortex contains mucopeptide[1]. The coat is mainly protein[2,3] with a high cystine disulphide bond content[4]. We have been unable to liberate cores by enzymatic digestion of spore coats using a variety of proteolytic enzymes including keratinase[5] before or after treatment of spores with reagents which rupture disulphide bonds[6]. We therefore investigated the possibility of releasing cores by mechanical breakage of spores.

Bacterial spores can be broken by shaking spore suspensions with Ballotini beads in the Mickle tissue disintegrator; but no recognizable cores are released by this process. The mechanical resistance of vegetative cells and protoplasts depends on the hydrogen ion concentration[7] and we therefore examined the effect of *p*H on the mechanical breakage of spores. The extent of breakage was followed by phase contrast microscopy. When bacterial spores (19 strains of *Bacillus* and 1 *Clostridium* strain) were shaken in the Mickle apparatus with Ballotini beads below pH 4·2, the cores became separated from the broken coats and existed as free phase bright bodies. The free cores of *Bacillus subtilis* spores were examined in detail. They were ovoidal, about 0·5μ long, and resembled small spores except that they were not so phase bright. The chemical nature of the buffer present during core production did not affect the appearance of the cores obtained, but the pH value was important, and no free cores were seen when spore breakage was performed above pH 4·2.

There was no change in the phase bright appearance and shape of cores made at pH 3 when the pH was raised to 4·2–6·5. However, at about pH 7·0 cores became swollen and phase dark; above pH 8·0 cores became still more swollen, pale and irregular in outline. These effects of pH change were instantaneous. Swollen cores at pH 6·5–9·0 shrank and became phase bright again when the pH was changed to 3·0. Spores that had just become phase dark during germination yielded phase bright cores when broken at pH 3·0 and these responded to pH in the same way as those prepared from dormant spores. Cores at pH 5·8 were not resistant to breakage even though they were in a shrunken and phase bright state.

Bacterial spores contain dipicolinic acid (DPA)[8] and calcium[9]. DPA forms 5–15 per cent of the dry weight of the spore and calcium occurs in slightly greater than equimolar amounts to the DPA[10]. The effects of DPA and calcium (25 and 50 mM) on cores were therefore tested; neither had any effect on the appearance of phase-bright cores at pH 6·0 and mixtures also had no effect (Ca : DPA ratios of 0·5, 1·0 and 2·0). Calcium ions (25 mM) in *tris* buffer pH 9·0 prevented the formation of pale swollen cores, but this protective effect was annulled when DPA (50 mM or 25 mM) or 'Versene' (1 per cent w/v) was also present. Excess calcium (50 mM) over DPA (25 mM) prevented the formation of pale swollen cores. Cores remained shrunken and phase bright in pH 8·2 *tris* buffer when 25–100 mM calcium was present. The protective effect of calcium ions was also shown by other divalent cations (25 mM), that is, barium, strontium and magnesium, by the trivalent cation lanthanum (25 mM), and by spermine (1 per cent w/v). The following monovalent cations (25 mM) were not protective: sodium, potassium, lithium, ammonium and cæsium. Inorganic or organic anions (Cl^-, NO_3^-, SO_4^{2-}, citrate, acetate, phosphate, veronal) had no observable effect on cores.

We suspected that the cores would be surrounded by some cortical mucopeptide, and we examined the effects on cores of agents known to complex with mucopeptide, that is, alcian blue (1 per cent w/v)[11] and auto-inhibitory concentrations of lysozyme (1–1·6 per cent w/v)[12]. Both reagents prevented phase darkening of cores as the pH was raised above 7·0. Complexing of lysozyme with cores was confirmed visually by using fluorochrome-labelled lysozyme which specifically stains mucopeptide[13]. Similarly, alcian blue stained cores but not coats. These staining reactions suggested that the cores contained mucopeptide. This was confirmed by analysis of the mucopeptide constituent hexosamine in core + coat fractions of *Bacillus subtilis* and *B. cereus* spores[14]. Depending on the species, 30–50 per cent of the spore hexosamine was rendered soluble during core formation at pH 3·3 and a further 20 per cent during phase darkening of cores at pH 6·6; the phase dark core + coat fraction at pH 6·6 retained 25–50 per cent of the spore hexosamine.

Core + coat fractions of *B. subtilis* and *B. cereus* spores contained less than 5 per cent of the spore DPA, estimated colorimetrically[15] and spectrophotometrically[16]. Thus most of the spore DPA was lost during the preparation of the coat + core fraction. This suggested that DPA may be located in the cortex of the spore.

Bacillus subtilis cores formed in the pH range 3·3–4·1 did not give rise to colonies when incubated on yeast glucose agar, nor did they grow when incubated in slide cultures and examined periodically under the microscope.

The effect of hydrogen ions on cores can be explained i terms of their effect on the relative numbers of positive and negative changes of the core macromolecules. I the pH region 2·0–4·2 the core is near its isoelectric point and so can exist in a contracted state which probabl accounts for the mechanical resistance of the core below pH 4·2. Above pH 4·2 and up to about pH 6·5 the core is still in a contracted state but is more susceptible t mechanical damage. Above pH 6·5 insufficient hydrogen ions are present to neutralize negative charges in the core so swelling occurs. Di- and tri-valent cations and spermin probably prevent swelling of cores by cross-linking pair of carboxyl groups. Monovalent cations are ineffective because they cannot form cross-links. Lysozyme and alcian blue may also prevent swelling of cores by a cross linking reaction that is specific for mucopeptide.

We think it likely that the cores of dormant spores are in a contracted state like cores below about pH 6·5 o like calcium-protected cores above pH 6·5. The possibility that the core of the spore is in a contracted state could help to explain the phenomenon of spore heat-resistance as suggested by von Daryáni[17]. How the core could get into such a state during sporulation is not known, but perhap it is by a mechanism similar to the contractile cortex suggested by Lewis, Snell and Burr[18]. Warth *et al.*[1 suggested that the mucopeptide cortex could behave like a weak cation exchange resin and that the spore calcium could cause the cortex to contract during sporulation. DPA is difficult to account for in this theory, because it chelates calcium and annuls the effect of calcium on cores. Thus DPA could annul any contracting effect of calcium during sporulation. However, the rigid spore coat may also be involved in maintaining contraction and the DPA may be located in a different region of the spore from the calcium. Investigations such as those of Alderton *et al.*[20], who demonstrated cation exchange reactions with spores and related them to heat resistance seem very pertinent to the elucidation of the roles of calcium and DPA in the structure and heat resistance of spores.

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Enumerating Viable Bacteria

For many purposes the number of viable organisms has to be known. In antibiotic assays, for example, the size of the inoculum influences the result of the test[1,2]. Optical densities of bacterial suspensions, not corroborated by viable counts, are misleading, as dead cells are also registered. Viable numbers are usually estimated from poured plates[3].

As plating has its limitations, an attempt was made to compare optical densities with viable counts, obtained by two other methods. The first method is based on the presence and absence of organisms in broth samples. The mean number of organisms in the broth tubes can be calculated from the Poisson series[4]. 155 test-tubes are needed, at the point of maximum accuracy, in order to obtain a standard error of sampling of 10 per cent. If the mean number of organisms per sample exceeds 2, this method becomes inaccurate. The second method is based on filtration through membrane filters, which retain bacteria. The membrane filters, after filtration, are laid on top of a broth-soaked pad, incubated for 24 h, and the colonies counted. This method, in contrast to the poured plate method, permits the enumeration of viable bacteria, even if only a few organisms are present in large volumes of fluid. Consequently, the same dilution can be used in both methods. For example, a mean of one organism per sample results in 36·79 per cent sterile tubes and in 63·21 per cent fertile tubes[5]. Filtering 100 ml. of this same dilution through a membrane filter should yield 100 colonies.

In experiments with 17–18-h-old cultures of *Staphylococcus aureus*, type 81, the mean bacterial numbers, obtained by both methods, differed, on the average, by 9·22 per cent. These results are based on 1,124 tubes and 31 filtrations.

The optical density of 17–18-h-old broth culture was determined at 640 millimicrons in a 1 cm cuvette. The concentration of this bacterial suspension was assumed to be unity. Dilutions were prepared, and their optical densities found. Sterile broth served as blank.

Optical densities were plotted versus relative bacterial concentrations. It was found that to points, ranging from about 0·3 to 0·7 optical density, a straight line could easily be fitted. It also turned out that readings obtained in this range are more accurate than readings obtained outside this range, because between 0·3 and 0·7 a smaller change in bacterial concentration corresponds to one scale part on the percentage transmission scale.

In this range a high and significant coefficient of correlation (0·9987) between relative bacterial concentrations and optical densities was found. The regression line was found to be:

$$x = 1{\cdot}0136\,y - 0{\cdot}09216 \qquad (1)$$

where x stands for relative bacterial concentration, and y for optical density.

Simultaneous determinations of optical densities and viable counts, by both methods, rendered it possible to assign absolute viable numbers to relative bacterial concentrations. Substituting for x the absolute viable count per millilitre, the right side has to be multiplied by a factor, f, in order to maintain the validity of equation (1):

$$\text{Viable number} = (1{\cdot}0136\,y - 0{\cdot}09216)\,f$$

whence

$$f = \frac{\text{Viable number}}{1{\cdot}0136\,y - 0{\cdot}09216} \qquad (2)$$

A mean value for f was determined by carrying out 31 comparisons of optical densities and filtrations, and found to be: $9{\cdot}05947 \times 10^{8}$.

Solving the equation for y, optical densities are obtained, which correspond to viable numbers:

$$y = \frac{\text{Viable number} + 0{\cdot}09216\,f}{1{\cdot}0136\,f}$$

or substituting for f its value:

$$y = \frac{\text{Viable number}}{9{\cdot}18268 \times 10^{8}} + 0{\cdot}09092 \qquad (3)$$

For a viable number of 1×10^{8}/ml., for example, the optical density, calculated from equation (3), becomes 0·19982, which is slightly outside the sensitive range. A 17–18-h-old broth culture was diluted to this optical density. From a 10^{-8} dilution of this strength two samples of 100 ml. each were withdrawn and filtered. One sample yielded 92 colonies, the other 98, instead of the 100 colonies expected.

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Transmission of Colicinogeny to *Providence* Strains

COLICINOGENY has been transmitted to non-colicinogenic strains of *Escherichia coli* and *Shigella*[1], *Klebsiella*, paracolons, *Salmonella*[2] and *Serratia*[3]. Hamon[2] tried unsuccessfully to pass this property to *Proteus* cultures. *Providence* strains (unpublished observations) are not bacteriocinogenic for genera of the family Enterobacteriaceae and are not susceptible to the various colicines.

The *Providence* strains used were N.C.T.C. Nos. 9204, 9248, 9250, 9260, 9268, 9294, 9295, 9278, 9283 and 9298. Standard colicinogenic strains and appropriate colicine-indicator cultures were kindly supplied by Prof. P. Fredericq and covered the range from *A* to *K* and *V*. Mutants resistant to 1 mg/ml. streptomycin of the *Providence* and colicine-indicator strains were selected and the transfer of colicinogeny from Fredericq's streptomycin-sensitive strains to the resistant *Providence* cultures was attempted according to the methods of Fredericq[1] and Ozeki *et al.*[4]. *E. coli* strain *CA*.23 transmitted colicine factor *D* to *Providence* strains N.C.T.C. 9260, 9278, 9283, 9295, 9298 and paracolon strain *CA*. 62 transmitted colicine factor *J* to strain 9295. Strain *CA*. 62 produces colicines *J* and *I*, but colicine factor *I* was not detected in 213 colicinogenic colonies of strain N.C.T.C. 9295 tested. No other colicine factors were transmitted to any of the strains and thus exactly half the strains accepted colicinogeny. Direct cell contact appeared essential for transmission of colicinogeny since the use of cell-free culture filtrates of donors was unsuccessful. The fraction of cells of the acceptor population which acquired colicinogeny was never more than 10^{-3} and usually the selection process had to be repeated on the growth picked off to obtain well-separated colicinogenic colonies. Control experiments with only the acceptor *Providence* strains never produced inhibition of the indicators. Ozeki *et al.*[4] reported a fraction of 10^{-4} for *S. typhimurium* cells accepting colicine factor *K* from *E. coli K*49.

The colicinogenic *Providence* strains have the same biochemical reactions and phage-susceptibility patterns[5] as corresponding non-colicinogenic cultures, and the colicines produced by the *Providence* cultures appear to be identical with those produced by the original strains. After storage for 9 months on agar slopes at room temperature the colicinogenic *Providence* cultures were streaked out for single colonies and tested for colicine production. All colonies were still colicinogenic. The colicinogenic property of the cultures is also not affected by exposure to acriflavine[6]. Attempts at intrastrain passage[7] of colicine factors *D* and *J* have been unsuccessful so far. In *Salmonella*[4] only factors *B*, *E*1 and *I* are capable of independent intrastrain transfer and these factors also promote the passage of other colicine factors present. In *Serratia* factor *I* behaves similarly[3]. None of these factors has yet been introduced into *Providence* strains and this may account for the failure of intrastrain transmission.

This work was aided by grants from the South African Council for Scientific and Industrial Research.

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A Factor required for Growth Initiation of *Pasteurella tularensis*

Previous investigations[1,2] established the essential requirements of *Pasteurella tularensis* (*P. tularensis*) for growth in both complex and chemically defined media. However, the media described sustained luxurious growth of *P. tularensis* when relatively large numbers of cells (about 10^6 to 5×10^6/ml. medium) were used for inoculation. Attempts to reduce the size of the inoculum by supplementing the media with a variety of vitamins, tissue extracts and other preparations noted for their growth-promoting ability, were by and large unsuccessful. The nutritional fastidiousness of *P. tularensis* appeared particularly puzzling in view of the notoriously extreme infectivity of this micro-organism, which is reputed to be capable of multiplying *in vivo* out of a few cells[3]. Furthermore, both highly virulent and completely avirulent strains exhibited the same pattern of growth requirements.

A clue for at least a partial resolution of this paradox was provided by an observation incidental on another line of investigation concerned with the exceptional susceptibility of low tonicity exhibited by *P. tularensis*. It was noted that a brief exposure of *P. tularensis* cells to a hypotonic environment resulted in a disruption of their permeability barrier, as evidenced by the leakage of intracellular constituents and the concomitant drastic decline of viability[4]. The viability of the so injured cells was found to be largely restored in the presence of a concentrate prepared from the suspending medium containing the material which leaked out of the osmotically shocked cell suspension ('leakage material'). Moreover, culture media fortified with the leakage material acquired the ability to support growth of the intact organism from apparently single-cell inocula, thus permitting the performance of viable counts which were otherwise not feasible with the strains tested by us.

The substance responsible for the growth initiating effect was found to be elaborated by the proliferating *P. tularensis* cells and to diffuse out in the course of growth into the surrounding medium. The active factor proved to be of low molecular weight, and owing to its relative stability and resistance to various chemical and physical treatments could be readily concentrated and extensively purified[5]. Work designed to procure sufficient quantities of the purified material for chemical analysis and biochemical studies is now in progress.

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CYTOLOGY

A Yeast Mitogenic Factor Active on Human Peripheral Leucocytes in Culture

We have observed that the addition of zymosan-treated sera to short-term human peripheral leucocyte cultures leads to a slight but significant increase in mitotic frequency. Inactivation of serum complement by zymosan[1] as an important factor in the increase in mitotic frequency was ruled out, and it could be shown that an active mitogenic factor was extracted from zymosan during treatment of the sera. It was found that a more active mitogenic factor could be extracted directly from whole frozen yeast (*Saccharomyces cerevisiae*) and this extract was then used exclusively. The method of extraction consisted of overnight incubation at 37° of equal volumes of saline washed cells and 0·2 N potassium hydroxide, followed by centrifugation at 2,000 r.p.m. for 30 min. The resulting supernatant was neutralized with perchloric acid, and then passed through a 'Sephadex' DEAE column which had been washed with 0·2 M *tris* buffer at *p*H 7·1. Before use, the yeast extract (*YAF*) was sterilized in boiling water for 30 min. Leucocyte suspensions were obtained by dextran sedimentation (2·5 ml. 6 per cent dextran per 10 ml. heparinized blood at 37°). Colchicine (0·02 μg/ml. culture) was added 8–10 h before collecting and chromosome preparations were made according to the method of Hungerford *et al.*[2].

The mitogenic factor in the crude 0·2 N potassium hydroxide extract is non-dialysable, acid labile (1 N hydrochloric acid for 20 h at room temperature), heat resistant, and is not retained by either an anion or a cation exchange column. The crude extract contains protein[3], phosphorus[4], and hexose[5] in approximately equimolecular amounts. It is resistant to digestion by ribonuclease, sensitive to digestion by alkaline phosphatase and lysozyme, and partially sensitive to trypsin.

The frequency of mitoses observed in more than 40 experiments varied from 0·4 to 3·0 per cent; the mitotic index always being significantly higher than that observed in control cultures, and lower than in cultures treated with phytohæmagglutinin (PHA). The peak of mitotic activity occurred between the fifth and sixth day of culture, although it was possible to observe occasional metaphases in cultures incubated for 20 days. Titration experiments with the same preparation of yeast extract always showed a limited region of linear mitotic response to increasing *YAF* concentration, followed at higher *YAF* levels by inhibition of mitoses and occasionally toxicity. It is possible that some of the responses of cultures to higher *YAF* levels might be due to impurities in the crude extract. This question can only be resolved when a purer preparation of the active factor is available.

An additive mitogenic effect was obtained when PHA and *YAF* were added to the same culture. This suggested that *YAF* was acting on a cell population different from that which was sensitive to PHA. To characterize this difference further, a comparative examination of DNA replication was undertaken in *YAF*, PHA, and *YAF* plus PHA treated cultures. Tritiated thymidine (Schwarz 1·5 c./mM, 0·2 μc./ml.) was added at the beginning of the cultures and left until collected, at which time slides were prepared for autoradiography (Kodak *NTB* 3 emulsion). After exposure for one week, the slides were developed, stained, and then scored for labelled and unlabelled metaphases.

Table 1. Percentage of Metaphases with Varying Grain Counts in Different Cultures

Cultures	No. of grains per metaphase 0	 1–10	 10–20	 20–30	 30–over	Total metaphases counted
PHA*	0	25	12	22	41	59
YAF†	67	23	7	2	1	105
YAF+PHA	6	15	25	21	33	33

* PHA, phytohæmagglutinin. † *YAF*, yeast extract.

The results of this work, given in Table 1, show that all the metaphases in the PHA culture were labelled, while most of the metaphases in the *YAF* culture were unlabelled. In the *YAF* plus PHA culture nearly all the metaphases were labelled (94 per cent).

The 100 per cent labelling of metaphases in the PHA culture confirms a previous report[6] and indicates that PHA sensitive cells are in the pre-DNA synthetic period (*G*1) prior to culture. The fact that most of the metaphases were unlabelled in the *YAF* culture suggests that most of the *YAF* sensitive cells are in a post-DNA synthetic period (*G*2) prior to culture. Since most of the metaphases in the *YAF* plus PHA culture were labelled and many heavily so, it would seem that inhibition of uptake and incorporation of thymidine by *YAF* is not responsible for the many unlabelled metaphases in the *YAF* culture.

Rather our results seem to indicate that the majority of the cells stimulated by *YAF* to divide have already undergone DNA replication before the initiation of the cultures, and that a small portion (lightly labelled) are apparently terminating DNA replication when the culture is started. Several authors have reported the existence of a small fraction of nucleated blood cells which undergo DNA replication while in circulation[7,8]. However, under physiological conditions, no mitotic figures are observed in peripheral blood smears. Although the frequency of the cells in active DNA synthesis varies considerably in the different reports (0·06–0·5 per cent), it is generally higher than the frequency of mitotic figures observed in cultures initiated without the addition of any agent.

It appears, then, that the circulating leucocytes consist of at least three populations with respect to their ability to complete cell division *in vitro*: (1) a major population of cells that are in their *G*1 phase and are stimulated to divide by PHA; (2) a minor population of cells that are in *G*2 and are stimulated to divide by *YAF*; and (3) a very small population of cells which are able to divide spontaneously.

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SOIL SCIENCE

Influence of Nitrogen on the Availability of Fertilizer Phosphorus

THE beneficial effects of additions of nitrogen on the availability of fertilizer phosphorus have been investigated with various techniques, such as when nitrogen is mixed with the soil and phosphate added as placement, when both nitrogen and phosphate are added separately or when nitrogen and phosphate are added in intimate association. With the view of investigating the influence of nitrogen on the availability of fertilizer phosphorus, under conditions of both nutrient sources being mixed with the soil, work described here was carried out on two soils (important characteristics given in Table 1) using wheat as a test-crop through greenhouse pot-culture experiments, involving the use of radiotracer techniques.

Table 1. MAIN CHARACTERISTICS OF SOILS

No.	Soil	*p*H (1 : 2·5 ratio)	% organic N (Kjeldahl's method)	Available P_2O_5 (lb./acre Olsen's method)*	% free $CaCO_3$ (Collins calcimeter)	Cation exchange capacity (m.equiv./100 g)
I	Alluvial soil I.A.R.I., N. Delhi	7·8	0·07	37·0	0·69	10·21
II	Alluvial soil Sabour (Bihar)	6·2	0·08	10·0	Traces	5·22

* *U.S. Dept. Agric. Circ.* No. 939.

Table 2. INFLUENCE OF UREA ON THE AVAILABILITY OF PHOSPHORUS FROM SUPERPHOSPHATE

	Soil I				Soil II			
Treatment	Total P (mg/pot)	% uptake	Fert. P (mg/pot)	% utilization	Total P (mg/pot)	% uptake	Fert. P (mg/pot)	% utilization
Control	3·30	—	—	—	1·88	—	—	—
P	3·56	49·43	1·74	8·89	3·78	56·65	2·15	10·87
N_1+P	5·21	48·02	2·74	13·82	3·91	62·27	2·35	11·89
N_2+P	5·32	63·05	3·48	17·06	4·32	64·68	2·40	12·12
S.E.M.*	0·30	2·22	0·24	1·19	0·23	1·87	—	—
L.S.D.† at 5% level	0·87	6·84	0·73	3·65	0·68	5·76	N.S.	N.S.

P, 40 lb. P_2O_5/acre as super: N_1 and N_2, 40 and 80 lb. N/acre as urea respectively.

* S.E.M., standard error of mean.

† L.S.D., least significant difference.

Three levels of nitrogen (0, 40 and 80 lb. N/acre) as urea were combined with two levels of phosphate (0, 40 lb. P_2O_5/acre) as superphosphate in a randomized design, and each treatment was replicated thrice. Superphosphate was tagged with phosphorus-32. The nutrient sources were mixed with the soil in an electrically operated twin-shell dry blender.

Dried composite samples consisting of three plants from each replicate were wet-ashed and analysed for phosphorus[1]. Radioactive phosphorus was determined by the method of Meckenzie and Dean[2].

In soil No. 1, the supplementary application of nitrogen as urea significantly increased the absorption of total phosphorus by wheat plants when compared with that due to phosphate application alone. Here the application of nitrogen significantly enhanced the amount of phosphorus in plants derived from the fertilizer source. In this soil, percentage utilization of applied phosphorus has also been found stimulated due to the presence of nitrogen along with the source of phosphorus. Table 2 shows that higher doses of urea significantly increased percentage uptake of fertilizer phosphorus in both the soils under examination.

Olson and Dreier[3] have shown the importance of association between nitrogen and phosphate sources in causing increased uptake of fertilizer phosphorus when applied in intimate association. Rennie and Soper[4] also found increased utilization of fertilizer phosphorus only when nitrogen was intimately associated with phosphate fertilizer.

It must be realized in the investigation recorded here that both nitrogen and phosphorus were homogeneously mixed with the soil and under these conditions the effect of nitrogen on the utilization and uptake of fertilizer phosphorus is revealed. In such cases, as here, the association between nitrogen and phosphate had definitely been maintained though to a lesser degree as compared with that in a band. Probably this association between nitrogen and phosphorus may be partly responsible for increasing the availability of fertilizer phosphorus to plants in such systems also.

I thank Dr. N. P. Datta, head of the Division of Chemistry, I.A.R.I., New Delhi, and Prof. J. Låg, director of the Institute of Soil Science, Norway, for their advice.

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MISCELLANEOUS

Sintering-Cobalt – Chrome – Molybdenum Alloys for Osteosynthesis

Of the materials which have been used for surgical prosthetics, the stellite alloys (based roughly on 65 cobalt, 30 chromium, 5 molybdenum) have excellent strength and adequate corrosion resistance[1]. Unfortunately these alloys are available only in the form of castings which, in the absence of any special techniques, develop a very large grain-size in the material. Small surgical implants have cross-sectional areas comparable to this grain size which may result in their mechanical properties being dependent on the random crystal orientation of a single grain to an applied stress. The strength of the implant may also be limited by weakness of the grain boundaries in the material. Any small amounts of impurity will segregate to the grain boundaries of the casting; when the grain size is large, the grain boundary area becomes relatively small and so the impurities may reach a high concentration. It is thought that both the foregoing factors are responsible for the wide variation and unpredictability of mechanical tests carried out here[2-4]. Therefore, a refined grain structure of these alloys should have beneficial effects by (*a*) giving a truly polycrystalline material, preferably of higher purity, and (*b*) spreading any impurities more uniformly throughout the material by increasing the grain boundary area.

Table 1

Property	Cast stellite	Sintered alloy
Density (g/c.c.)	8·298	8·3–8·4
Ultimate tensile strength (tons/in.^{2})	46·8	55
Percentage elongation	10	2·5
Fatigue limit (tons/in.^{2})	13·5	40
Young's modulus	$16·1 \times 10^3$	13×10^3

Powder metallurgical techniques appear to offer the best method of combining a fine grain size with high purity. In our work the following procedure was adopted. A master alloy consisting of 6 : 6 : 1 cobalt–chromium–molybdenum, respectively, was made up under a controlled atmosphere from the pure metal powders; pure cobalt powder was added to make up the correct proportions for the final alloy, and the mixture ground down to give a mean powder size of 5μ. The resultant powder was then cold-pressed hydrostatically at 10 tons/in.^{2} and sintered under vacuum for 10 h. At no stage in the process was air allowed to come into contact with the powder, and the contamination of the unsintered compact was minimized by keeping it coated with Analar methyl alcohol. The tests which are proceeding on the sintered alloy have shown considerably greater consistency of results and a definite improvement on the mechanical properties of the cast alloy. Results are given in Table 1, and comparative fatigue curves for the two materials shown in Fig. 1. Comparison of the different microstructures (Figs. 2 and 3) shows the accumulation of impurities in the grain boundaries of the cast material and their more uniform distribution in the sintered alloy.

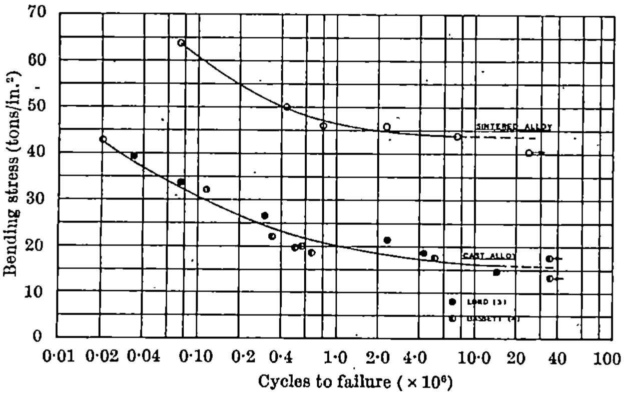

Fig. 1. *S–n* curves for the cast (●, Lord, ref. 3; ◐, Bassett, ref. 4) and sintered (○) alloys

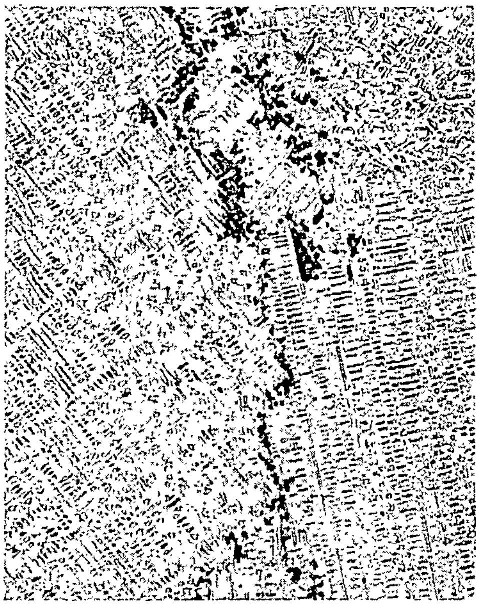

Fig. 2 Microstructure of cast stellite showing grain boundary inclusions and cored structure within grain. (× *c*. 28)

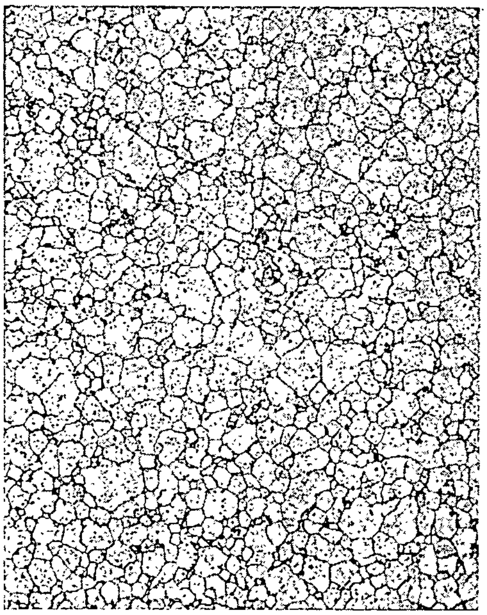

Fig. 3. Microstructure of alloy made up from powder; pressed at 10 tons/in^2, sintered in vacuum for 10 h at 1,250° C, showing great refinement of the grain size and absence of coring (× *c*. 100)

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Wednesday, August 26–Wednesday, September 2

British Association for the Advancement of Science (at Southampton)—126th Annual Meeting.

Wednesday, August 26

At 7.45 p.m.—Right Hon. Lord Brain, F.R.S.: "Science and Behaviour" (Presidential Address).

Thursday, August 27

At 10 a.m.—Sir Harry Melville, K.C.B., F.R.S.: "The Contribution of New Instruments to Progress in Chemistry" (Presidential Address, Section B).

At 10 a.m.—Dr. C. E. Lucas, C.M.G.: "Aspects of Marine Fisheries Research" (Presidential Address, Section D).

At 10 a.m.—Mr. G. B. Ryle, C.B.E.: "The Need for Forests in an Industrial Society" (Chairman's Address, Section K*).

At 10 a.m.—Dr. F. Lincoln Ralphs: "Responsibility and Education" (Presidential Address, Section L).

At 10 a.m.—Mr. Eric Rea: "The Age of Advice" (Presidential Address' Section M).

At 10.15 a.m.—Mr. H. G. Conway: "Engineers at the Crossroads" (Presidential Address, Section G).

At 10.15 a.m.—Prof. F. G. Young, F.R.S.: "The Biochemistry of the Endocrine System" (Presidential Address, Section I).

At 11.30 a.m.—Prof. B. R. Williams: "Economics in Unwonted Places" (Presidential Address, Section F).

At 2.30 p.m.—Prof. R. V. Jones: "Chance Favours the Trained Observer" (York Lecture).

Friday, August 28

At 10 a.m.—Mr. J. A. Ratcliffe, C.B.E., F.R.S.: "The Sun and the Ionosphere" (Presidential Address, Section A).

At 10 a.m.—Mr. A. A. L. Caesar: "Planning and the Geography of Great Britain" (Presidential Address, Section E).

At 10 a.m.—Prof. C. G. C. Chesters: "Fungal Friends and Foes" (Presidential Address, Section K, K*).

At 11.30 a.m.—Prof. H. J. Eysenck: "The Biological Basis of Criminal Behaviour" (Presidential Address, Section J).

At 11.30 a.m.—Sir Graham Sutton, C.B.E., F.R.S.: "The Resurgence of Interest in the Observational Sciences" (Presidential Address, Section X).

At 2.30 p.m.—Dr. N. Tinbergen, F.R.S.: "Why Do Animals Fight ?" (Young People's Lecture).

At 8 p.m.—Prof. W. J. G. Beynon: "The International Years of the Quiet Sun" (Evening Discourse).

Sunday, August 30

At 10.30 a.m.—Official Service in St. Mary's Church. Preacher: The Very Reverend the Dean of York, Canon Alan Richardson, M.A., D.D.

Monday, August 31

At 10 a.m.—Dr. W. Marshall: "The Exploration of Solids Using Thermal Neutrons" (Kelvin Lecture).

At 10 a.m.—Mr. W. H. Wilcockson: "Some Aspects of East African Vulcanology" (Presidential Address, Section C).

At 10 a.m.—Dr. C. A. Ralegh Radford: "History and Archæology" (Presidential Address, Section H).

At 11.30 a.m.—Prof. Lucy Mair: "How Small-scale Societies Change" (Presidential Address, Section N).

At 2.30 p.m.—Dr. H. A. Thomas: "Automation" (Young People's Lecture).

At 8 p.m.—Prof. A. S. Parkes: "Biological Aspects of the Population Explosion" (Evening Discourse).

Tuesday, September 1

At 2.30 p.m.—Dr. E. J. Ambrose: "Cells at Work" (Young People's Lecture).

Wednesday, September 2

At 10 a.m.—Dr. G. R. Coope: "Insect Populations in the Ice Age" (Darwin Lecture).

At 10 a.m.—Dr. V. B. Proudfoot: "Bringing Archæology to Life" (Lister Lecture).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Principal (normally national of the United Kingdom or the Republic of Ireland, with an honours degree in science or engineering, a recognized professional qualification, and extensive experience in industry, teaching and administration) of the Government Technical College, Enugu, Eastern Nigeria—The Council for Technical Education and Training for Overseas Countries, Eland House, Stag Place, London, S.W.1 (August 31).

Scientific Officer (graduate with a good honours degree in botany, biochemistry or chemistry, and preferably with research experience either in the physiology of higher plants or in plant metabolism)—The Director, Agricultural Research Council Unit of Plant Physiology, Imperial College of Science and Technology, London, S.W.7 (August 31).

Technicians (2) to assist with a programme of research into the effects of pathogenic bacteria on insects (one technician will be mainly concerned with the bacteriological aspects of the programme, and the other with the entomological aspects)—Prof. T. L. Bywater, Department of Agriculture, The University, Leeds, 2 (September 1).

Senior Research Assistant (graduate in science or veterinary science) to take part in research on respiratory disease in poultry in the Department of Veterinary Preventive Medicine at the Veterinary Field Station in Cheshire—The Registrar, The University, Liverpool, quoting Ref. CV/264 (September 5).

Research Assistant (either a physicist or an engineer with an interest in biology or a biologist with some knowledge of physics or engineering) at the Marine Science Laboratories, Menai Bridge, to work on the measurement of the forces of breaking waves on rocky shores—The Registrar, University College of North Wales, Bangor, North Wales (September 7).

Head (graduate, aged 30–45, with suitable university library experience) of the Periodicals Department—The Goldsmiths' Librarian, University of London Library, Senate House, Malet Street, London, W.C.1 (September 9).

Assistant Lecturer in Biochemistry in the Department of Physiology and Biochemistry, Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (September 12).

Fisheries Biologist (with a Ph.D. degree in biology, or postgraduate research experience of equivalent duration and standard, supported by satisfactory evidence of research ability, also postgraduate research experience in marine biology and/or fisheries biology, preferably with specialization in crustaceae, and a knowledge of statistics sufficient for biological research) in the Division of Fisheries and Oceanography, Commonwealth Scientific and Industrial Research Organization, Adelaide, South Australia—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 320/297 (September 12).

Research Officer (with a Ph.D. degree in physics or mathematics, or postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability, and preferably an interest in flying) in the Division of Radiophysics, Commonwealth Scientific and Industrial Research Organization, Sydney, N.S.W., Australia, for interesting work in the field of cloud physics—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 780/364 (September 12).

Post-doctoral Fellows (with a Ph.D. in physics or electrical engineering or other appropriate field of postgraduate research) in Physics and Electrical Engineering in the Division of Applied Physics, Commonwealth Scientific and Industrial Research Organization, located in the grounds of Sydney University, Australia—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 750/315 (September 14).

Research Assistant (with a Ph.D. or honours degree in zoology or equivalent qualifications) to Dr. N. W. Runham, to work on reproduction in slugs—Dr. Runham, Department of Zoology, University College of North Wales, Bangor, North Wales (September 14).

Senior Professional Officer (with at least a B.Sc. degree with appropriate postgraduate experience, suitably qualified in one or more of the following fields: herpetology, ornithology or marine biology, and preferably some museum experience) at the Port Elizabeth Museum—The Chairman, Museum Board of Trustees, P.O. Box 1359, Port Elizabeth, South Africa (September 14).

Head of the Bacteriology Department; and an Assistant Bacteriologist—The Secretary, School of Agriculture, West Mains Road, Edinburgh, 9 (September 15).

Research Assistant (graduate in zoology and preferably some research experience in insect physiology or a related field) in Insect Pathology for work in connexion with a study of the bacterial pathogens of insects—The Registrar, The University, Leeds, 2 (September 15).

Assistant Lecturers (2) (candidates for the one post should have special interests in the evolutionary field studies, and for the other in physiology) in Zoology—The Registrar, The University, Manchester 13 (September 18).

Chair of Fuel Technology and Chemical Engineering—The Registrar, The University, Sheffield (September 19).

Lecturer (with qualifications in one of the appropriate basic sciences) in Dental Materials in the School of Dental Surgery—The Registrar, University of Liverpool, Liverpool, quoting Ref. CV/265/N (September 19).

Fisheries Research Biologists (2) (with at least an honours Bachelor's degree) with the Fisheries Research Board of Canada, one to be in charge of pelagic fish investigations (capelin, herring), and the other to assist in marine invertebrate investigations (squid, scallop, clam, oysters)—The Director, Fisheries Research Board of Canada, Biological Station, St. John's, Newfoundland, Canada (September 25).

Lecturer in the Chatterton Astronomy Department of the School of Physics—The Registrar, University of Sydney, Sydney, New South Wales, Australia (September 25).

Chair of Applied Mathematics—The Registrar, The University, Manchester 13 (September 30).

Chair of Genetics—The Registrar, The University, Leeds, 2 (September 30).

Director of the Electronic Computing Laboratory—The Registrar, The University, Leeds, 2 (September 30).

Entomologist (with a good honours degree in zoology, with special emphasis on entomology, and an interest in, and aptitude for, taxonomic research) in the Commonwealth Institute of Entomology Identification Service, which is accommodated at the British Museum (Natural History), to work on the Coleoptera—The Director, Commonwealth Institute of Entomology, 56 Queen's Gate, London, S.W.7 (September 30).

Lecturer/Senior Lecturer in Physics in the School of Physical Sciences of the University of Adelaide at Bedford Park—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and the Secretary for Bedford Park, University of Adelaide, Adelaide, South Australia (Australia, September 30).

Professorial Fellow, a Senior Fellow, and a Senior Research Fellow in the Department of Sociology, Research School of Social Sciences, Institute of Advanced Studies, Australian National University—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

Chair of Biology at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 15).

Chair of Philosophy at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 15).

Assistant Lecturer or Lecturer in Experimental Physics—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, marking envelope "Application".

Biochemist (Basic Grade) at Black Notley Hospital, Braintree, for duties under the direction of the Group Biochemist, to assist with the routine and development work of the Group Laboratories—The Group Secretary, St. Helena Group Hospital Management Committee, 14 Pope's Lane, Colchester, Essex.

Chair of Plasma Physics—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, marking envelope "Confidential"..

Gassiot Research Fellows (2), one for research in seismology and the other for research in geomagnetism—The Secretary, Meteorological Office, London Road, Bracknell, Berkshire.

Herbicide Botanist (national of the United Kingdom or the Republic of Ireland with a degree in botany, preferably with postgraduate research experience) with the East African Common Services Organization, to undertake the botanical aspects of herbicidal and arboricidal work in East Africa with particular attention to weed pests in major East African crops—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, quoting Ref. RC 213/214/05.

Industrial Liaison Officer (man or woman with a good honours degree or equivalent in science or technology), to promote in local industry an appreciation and understanding of the value of technical, scientific and management development and to encourage the maximum use of the educational and advisory facilities of the College and of other local and national scientific and technical organizations—The Principal, Worcester Technical College, Deansway, Worcester.

Malacologist (national of the United Kingdom or the Republic of Ireland, with a first- or second-class honours degree in biology, and preferably appropriate relevant postgraduate research or experience) with the East African Common Services Organization, to study the aspects of snail life history, behaviour and distribution which affect their control by molluscicides and to study the effects of different molluscicides on snails—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/214/06.

Post-Doctoral Research Fellow (physical chemist or solid-state physicist with an interest and some experience in catalysis in gas/solid reacting systems) **in the Department of Chemical Engineering**—Dr. W. M. Crooks, Department of Chemical Engineering, The University of Birmingham, Birmingham 15, marking application "Research Fellow".

Professor of Anatomy and **Head of the Department**—The Dean, Faculty of Medicine, University of Manitoba, Cor. Bannatyne and Emily Streets, Winnipeg, Manitoba, Canada.

Protein Chemist to work on antibodies and antibody-like substances; a **Protein Chemist** (interested in pollen, mould and tissue antigens); a **Chemist** or **Biochemist** (B.Sc., Ph.D. or experienced technician) to take charge of fluorescent microscopy, mainly in connexion with work on antibodies; and a **Physical Chemist** to work mainly with microcell electrophoresis apparatus, in the Immunochemistry Unit for Research in Cancer and Allergy (Department of Bacteriology)—The Registrar, The University of Liverpool, Liverpool, 2, quoting Ref. CV/262/N.

Research Student (with a good honours degree in physics) **with the Spectroscopy Group in the Department of Physics**, for work on radiation characteristics of plasmas and spectra of highly ionized atomic species—Dr. W. R. S. Garton, Physics Department, Imperial College of Science and Technology, London, S.W.7.

Senior Research Associate in the School of Chemistry for research into reaction mechanisms in boron chemistry—The Registrar, The University, Newcastle upon Tyne.

Technical Assistant (with an ordinary degree, H.N.C. or equivalent qualification in chemistry) **in the Robert Robinson Laboratories, Department of Organic Chemistry**, to join a group investigating fungal biosynthesis—The Registrar, The University, Liverpool, 3, quoting Ref. CV/267/N.

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SCIENCE AND BEHAVIOUR

THE scientific study of human behaviour has been a recurrent theme in presidential addresses to the British Association during the past decade. In 1954, Lord Adrian, clearly perturbed by the implications of modern science for man and society, made a strong plea for the furtherance of the human and social sciences. In 1959, Sir James Gray reminded scientists that humanists have no monopoly of the maxim that the proper study of mankind is man. In 1961, Sir Wilfrid le Gros Clark, speaking on the theme of the humanity of man, was no less insistent on the need for fuller understanding of the springs of human action. While this concern may be ascribed in part to the growth and promise of what have come to be called the behavioural sciences, there is no doubt that it also reflects the reaction of many scientists to contemporary hazards, particularly the danger of all-out nuclear war. As Sir Wilfrid sombrely enquired: "Can the human species avoid at least partial extinction by the misapplication of its own ingenuity ?".

In his address on *Science and Behaviour* (see p. 943 of this issue), Lord Brain to some extent follows in the footsteps of his predecessors. Like Sir Wilfrid le Gros Clark, he lays stress on the comparative and physiological approach to the problems of behaviour. Like Lord Adrian, he shows himself well aware of the special problems created by man's evolutionary development and the importance of taking into account the social context of his activities. But he is much less evidently concerned with the prospect of imminent annihilation. As he sees it, the dangers ahead of us stem from the "frozen emotional attitudes of the past" rather than the lurid perils of the future. He advises us how to live with science rather than how not to die from it.

In his attempt to present a biological view of behaviour —including our own—Lord Brain draws together evidence from a variety of biological disciplines, including ethology, comparative neurology and experimental psychology. He also touches on criminology and the social sciences. Although only too well aware that "to try to give such a satellite view is to court attack from those over whose territory one flies", Lord Brain's astronautical essay is unlikely to provide a target for the specialist anti-missile missile. Indeed, workers in the behavioural sciences everywhere will derive much encouragement from his thoughtful survey.

Quite properly, Lord Brain takes his departure from the study of the lower organisms. As he rightly reminds us, animals with comparatively simple nervous systems may exhibit quite complex behaviour, but that in studying its properties it is much easier than in the case of man to isolate the factors involved. This leads him to consider the selective responses, often surprisingly elaborate, shown by insects and birds in their food-seeking and reproductive activities. Although he gives some consideration to the nervous mechanisms on which these patterns of behaviour depend, it is perhaps a pity that he does not discuss the ways in which more primitive nervous systems differ from those of the higher animals. For example, it now seems clear that the possibilities of integration are much more limited in the insect nervous system than in that of vertebrates owing to the inherent limitations of the insect neurone as an integrating unit. From this it follows that the nervous mechanisms which have been evolved to cope with essentially similar problems of adaptation may vary widely at different levels of the evolutionary hierarchy.

In turning to the behaviour of higher vertebrates, Lord Brain takes note of recent physiological work on the mechanisms of sensory discrimination in the receptor organs and the brain. This is already beginning to give us a fairly detailed picture of the ways in which sensory information vital to adaptive behaviour is taken in, coded and processed in the receptor systems. But he appreciates, too, that behaviour depends in large measure on endogenous factors, commonly referred to as instincts or drives. This leads him to consider the part played by certain phylogenetically primitive areas of the forebrain in the control of instinct and emotion. Here he touches on an important and rapidly advancing area of physiological knowledge, in which the classical techniques of neurophysiology have been fruitfully combined with the methods of experimental psychology. Indeed, the steady growth of interdisciplinary research in this field has already thrown much light on the brain envisaged as the instrument of behaviour.

Ever since Darwin, it has been traditional to enquire in what respects man most closely resembles his animal relatives. Lord Brain turns the question around. In what respects, he asks, do human beings, as animals, differ from other animals ? The uniqueness of man, in Lord Brain's view, lies in his outstanding learning capacity and, more significantly, in his possession of language. (Wisely, perhaps, he eschews the question of intelligence.) These evolutionary advances he relates to the outstanding development of the major regions of the human neocortex. Although the existence of a correlation between brain size and intellectual capacity has been accepted since the time of Gall, it must be admitted that its nature is still almost wholly obscure. We still do not know, for example, why the left temporal lobe of the human brain can sustain speech while that of the chimpanzee cannot. Here considerations of relative size alone are unlikely to be decisive. Nor do we yet know for certain whether human intelligence depends on circumscribed areas of the brain cortex or whether it is principally due to a mass-action factor, as Lashley and others have surmised. Until human cerebral organization is far better understood than it is at present, the biological uniqueness of man will remain an enigma.

It is the evolution of language, Lord Brain believes, allied with the growth of self-consciousness, that provides the impetus to seek the explanation of our own behaviour. Can man explain himself ? Is there a science of human behaviour ? Experimental psychology, which has now been in existence for almost a century, claims to provide such a science. Yet it has so far failed to produce a coherent body of explanation of the kind which might reasonably be expected of a scientific discipline. Nevertheless, Lord Brain appreciates the contribution which experimental psychology has made to our understanding of limited segments of human activity, for example, the study of sensory-motor skill. Where psychology has so far failed has been in the development of truly quantitative methods for the study of complex mental processes. The measurement of intelligence is a case in point. As Lord Brain points out, it is far from clear what the conventional intelligence tests are measuring or what interpretation should be placed on the results. There is, he suggests, a great need for fruitful hypotheses to direct genuine experimental attack on the problem of mental ability. This need is perhaps even greater in the sphere of emotion and temperament. Nevertheless, Lord Brain does not contest the value of psychological enquiry, even where woefully inexact. He has even a good word for Freud.

But experimental psychology can scarcely compass man in his relations with society. Here Lord Brain accepts the claim of the social sciences to study man scientifically, but is under no illusions as regards the difficulty of the task. This he illustrates vividly with regard to recent studies of crime and its causes, which might appear to be scientific in little more than name. Lord Brain suggests that in the social sciences generally we need not only more and better observations but more and better hypotheses. One might suggest that we also need more and better men. But how are we to find them ? At the present time, the social sciences tend to attract arts men without basic scientific training or scientists who have failed to make their mark in a more conventional field of scientific enquiry. How are we to attract to the social sciences men of the calibre of Darwin, Rutherford or Freud ? Money, encouragement and facilities are not enough. A positive lead must be given to find, educate and hold the right kind of men.

In conclusion, Lord Brain is led to express some general views of the proper use of our human resources in an age of rapidly expanding industrial technology. He urges that the speed of modern industrial development makes it essential that we should attempt to forecast the social changes which radical changes in technology will inevitably bring about: and this, in turn, will depend largely on our fostering, through education in the broadest sense, new attitudes to the rapidly changing social environment which science is creating. This problem is real and important. Actions may be changed more rapidly than attitudes, and every change in technology is liable to bring about some change in long-established concepts of status and responsibility. If individuals are unprepared for social change, serious loss in personal security and social stability may ensue. Education, even in the wide sense advocated by Lord Brain, is not enough. We need also much more serious study of human reaction to technological change and its impact on social structure. This, perhaps, is the most urgent challenge to the social sciences and the real lesson of Lord Brain's address.

"AND NEAR A THOUSAND TABLES PINED AND WANTED FOOD"

Man Must Eat

By Sir William Slater. Pp. ix+112. (Chicago and London : The University of Chicago Press, 1964.) 28*s*.

MAN Must Eat deals with the age-long problem of increasing the food supply to keep pace with the increasing population. It consists of four lectures delivered in the University of Chicago. These are augmented for publication with an introduction giving a general account of the main aspects of the problem.

The subjects of the lectures are on the means of food production, food processing, the problem in the developing countries, and the effect on mankind of the fall-out from nuclear tests. For those engaged in food research the most interesting are probably those on food processing and the fall-out from nuclear tests. These are excellent reviews of recent advances in knowledge in these subjects.

The lecture on food production in the developing countries shows the difficulties and indeed the possible dangers of giving technical aid on food production. Some of the technicians sent out to the food-deficit countries seem to think that all that is needed to modernize their agriculture is to give the local inhabitants the Western 'know-how'. Few of them have the 'know-how' needed for soil and climatic conditions and social and economic systems different from the Western temperate zone countries, and on tropical crops of which Western experts have little experience. Further, they are apt to forget that some of the countries, like India, for example, have colleges of agriculture and research institutes with well-informed senior officials, many of whom are trained in Western universities. They know as much about the principles of agriculture as foreign experts and, in addition, know the difficult and complex conditions to which modern methods must be applied. As Sir William Slater points out, the main difficulty is the lack of local industries to produce the fertilizers, equipment and other things needed for increasing production, and the foreign credits to import them.

Looking to the future, Sir William estimates that by the end of the present century, that is, only thirty-six years ahead, the population of Britain will reach 70 million—an appalling thought for the already crowded Islands. He also estimates that home food production can be increased by only about 40 per cent. So, with her increasing population, Britain will continue to depend on importing about one-third of the food she consumes. The population of the United States will also increase so much that by the end of the century it will have no surplus to export.

This raises the problem of where Britain can find her food imports. It will not be from the food deficit countries where, with all the technical and financial aid they receive, increased production can do little more than provide for the increase in population. During 1962–63 it failed to do even that, with the result that the amount of food per head of the world population actually fell. Second only to war, the accelerating growth of population, with a possible food shortage, is the most serious threat to Western civilization. It warrants more attention from leading politicians than the attempt to put a man on the Moon, or even which political party is going to win the next election.

It is not certain, however, that the threatened world food crisis will occur. The present rate of increase of population may begin to slow down as has already occurred in a few countries, notably Japan, where the number of births fell from 2,697,00 in 1949 to 1,607,000 in 1962. If the new oral contraceptive could be produced in sufficient quantities and brought within the purchasing power of the poorest, it may slow down the growth of population and the alarming estimated increase in population may never be reached.

Sir William warns us that it will be difficult, if not impos-ible, to provide sufficient food for the rapidly growing population. That is certainly true under present political and economic conditions. If, however, all Governments would co-operate in a world scheme for the production and distribution of goods to eliminate hunger from the world, then, with modern agricultural and engineering science, sufficient could be produced even though the population increased at its present rate for another hundred years.

This book on food problems by an authority of international repute deserves to be studied by scientists and also by Governments and their officials dealing with the national and international aspects of food production.

BOYD-ORR

THE COMPUTOR IN SEARCH OF OPTICAL NEURONES

Optical Processing of Information

Edited by Donald K. Pollock, Charles J. Koester and James T. Tippett. Pp. x + 286. (Baltimore: Spartan Books, Inc.; London: Cleaver-Hume Press, 1963.) 60*s*.

OPTICAL Processing of Information forms the proceedings of a conference of the 'cross-fertilizing' type, held in October 1962 under the sponsorship of the U.S. Office of Naval Research and the American Optical Company, at Washington. The purpose was to bring modern optical devices to the knowledge of computor experts, with a view to bigger, better and faster computors. Somewhat surprisingly, the first of the nineteen speakers, Prof. H. M. Teager of the Massachusetts Institute of Technology, poured cold water on these hopes by showing very convincingly that optical elements such as fibres, lasers, photoconductors, have, at most, a very slender chance of competing with already existing electrical components, such as tunnel diodes and thin films. This did not prevent the conference from going on cheerfully for another eighteen lectures. One cannot deny that these present a great deal of stimulating and even exciting material to the inventive mind; the sort of mind for which the difficulty of ever reaching the final objective is a challenge rather than a discouragement.

The past ten years have produced two outstanding optical developments; fibre optics and lasers. Fibre bundles, one-half inch in diameter, can contain one million fibres, and can conduct a picture with about 50 lines/mm over several yards, if required, over a very tortuous path. In fact, the information capacity of such a bundle is better than one logon per fibre, because more than a dozen orthogonal modes can be propagated in each (O'Brien, Snitzer, Koester of the American Optical Company). An interesting, unorthodox application of fibres is described by R. D. Hawkins in an enthusiastic article, perhaps a little too untainted by theory. Fibres are assembled into a brush with bristles of various lengths, and are made to vibrate by a complex sound. They reveal the spectrum of the sound by their amplitude pattern, a principle well known since the time of Frahm. What is novel is the idea of selecting speech sounds, even words, for recognition by putting a photographic plate in front of the fibres, exposed to the pattern which is to be recognized, which then serves as a selective mask. Somewhat more sophisticated pattern recognition principles are described in an article on the 'Perceptron' by Babcock, Friend and Heggs, but the results are somewhat disappointing in relation to the enormous gadgetry, and appear to be inferior to those obtained by W. K. Taylor (University College, London) by simpler means. André Maréchal (Paris) summarizes the interesting work of the French school on the improvement of images by optical filtering, that is to say, operating on the Fourier transform of the picture; and this is completed in an article by El-Sum, who has made important improvements in the double-diffraction method introduced by me fifteen years ago.

The rest of the contributions are mostly devoted to the production of light by lasers, and methods for its modulation. At the time of the conference, the neodymium–barium glass laser was the great novelty, and the gallium arsenide laser was just making its first appearance. The weakness of the laser schemes is not only in their inefficiency but also in the near-impossibility of devising really fast methods of modulation for what is essentially a high-*Q* device.

This book will be useful to all those who would like to keep up with the development of optical art, and for anybody who wants a vivid picture of the way in which the old art of invention is carried on in the modern world.

D. Gabor

EARTHQUAKES AND EARTH STRUCTURE

Earthquakes and Earth Structure

By John H. Hodgson. Pp. ix + 166. (Englewood Cliffs, N.J., and London: Prentice-Hall, 1964.) 24*s*.

DR. J. H. HODGSON, chief of the Division of Seismology, Dominion Observatory, Ottawa, is the famous son of a famous father—Ernest A. Hodgson. That he has written this modest book for the non-specialist should not only help to ensure that any non-specialist will henceforth be interested in seismology, but also that seismology will attract more scientists to its disciplines and more people in general to its support.

After the introduction, *Earthquakes and Earth Structure* is divided into four parts, the first two being approximately of equal length, and the final two each about half the length of the first. The first section is descriptive of nine well-chosen shocks, together with a selection of isolated interesting occurrences due to earthquakes. The second section is concerned with the observation of earthquakes and earth structure (seismology), the third with statistics, geography and causes, and the final one with seismic regionalization, earthquake engineering and international co-operation.

Of the earthquakes chosen for description, the earliest is the New Madrid series of 1811–12, and the most recent the swarm in Chile in 1960. This 1960 swarm in Chile began on May 21, the main shock coming about 11 min after 3 o'clock in the afternoon of the second day. The motion of this earthquake was smooth and rolling with smaller oscillations imposed on it, the maximum intensity being felt in the coastal areas from Puerto Saavedra to Isla Chiloé and along the Reloncavi fault in the Chilean Lakes region. There were thousands of landslides and seiches in the lakes with amplitudes up to 3 ft. and in the region silty clay flowed like water. On the Isla Mocha there was a land elevation of 7 ft., so that the foundations of the dock became clear of water. Near Valdivia there was a depression of the land so that salt water now floods land up-river from the city. The volcano Puyehue, dormant since 1905, erupted and sent a cloud of ash and steam 20,000 ft. into the air.

The principal shock produced one of the most spectacular tsunamis on record. Some minutes after the shock the sea receded from the coast, returning about 10 min later with great speed and with wave heights of as much as 20 ft. The periodic retreat and advance of the sea continued throughout the afternoon. In Puerto Saavedra, debris was later found nearly 2 miles inland. This tsunami crossed the Pacific Ocean, causing coastal damage and casualties. In Japan it arrived more than 22 h after the time of the earthquake that caused it, attaining a height of some 12 ft., and doing much damage. 180 Japanese died as a result of this tsunami. Tremendous damage was done in Chile by the earthquake, by permanent ground displacements at the time, and by the tsunami. Many were killed and injured and the economy of the area suffered a blow from which it will take years to recover.

Considerations such as these, Dr. Hodgson asserts in his book, make it imperative to do something about the matter. Two important ways, apart from pure seismological research, are opening up in many countries throughout the world. The first is called 'seismic regionalization', and the second 'earthquake engineering'.

'Seismic regionalization' is a term first introduced by the Russian seismologists. Seismic regionalization maps are usually contoured to show maximum earthquake intensity likely to be encountered at each point on the map. Such estimates are extrapolations based on all available evidence, present geo-tectonic as well as historical-seismic. Prof. V. V. Beloussov, with the assistance of the European Seismological Commission, has produced the first draft of a seismo-tectonic map of Europe, and other maps of a somewhat similar character have been produced for other countries and regions of the world. Dr. Hodgson gives maps for Canada and the United States in his book.

For engineers dealing with earthquake-resistant design, a knowledge of the maximum earthquake intensity likely to be encountered in any locality is only a beginning. Strain seismographs can indicate present trends, strong motion seismographs can record accelerations and periods, and from shaking-table experiments much can be learned since the shaking may now be made to simulate actual earth movement during the earthquake for which seismograms are available. In some areas of the world building codes are absolutely essential. Moreover, since the advent of atomic power stations into areas likely to be affected by earthquakes, the problem has become much more acute. In recent years in Britain a seismic engineering group has been formed. This would have delighted John Milne, the 'founder' of British seismology and, through the British Association for the Advancement of Science, the originator of the project of a world-wide network of unified seismograph stations; for Milne was a British professor of engineering in Japan, turned seismologist. Dr. Hodgson in his book deals with the North American attack on these problems.

Dr. Hodgson's book will prove a most valuable introduction to the observation of earthquakes for all who wish for a non-mathematical approach. It cannot fail to be interesting and informative, and the only drawback in Britain is the price; 24*s*. is rather expensive for a paper-back of 175 pages. E. TILLOTSON

A CHAPTER OF CHEMISTRY

Man-Made Transuranium Elements

By Prof. Glenn T. Seaborg. (Foundations of Modern General Chemistry Series.) Pp. viii + 120. (Englewood Cliffs, N.J.: Prentice-Hall, Inc., 1963.) 12*s*. paper; 32*s*. cloth.

NO part of chemistry, not even a paragraph, is ever complete; but this important chapter has now reached a stage where further developments can be expected to be more in the nature of refinements to the style, than exciting new changes or additions to the text.

Most books are written for a readily identifiable readership, first- and second-year university texts, authoritative monographs for research workers and a number of other clearly defined categories. It is pleasant to read a scientific work that avoids this classification. *Man-Made Transuranium Elements* was written as a consequence of the author's interest in the Chemical Educational Material Study Group in California. One presumes that it is intended, in part, for the more gifted secondary or high-school student. Although it would be surprising if many such students, even in California, could really understand everything in the book, it is certainly true that they would both profit from and enjoy reading it.

The author and the subject are so related that the book has a strongly autobiographical flavour. Never obtrusive, these touches heighten the reality of the historical sequences in the book and convey much of the excitement that was felt during many of these remarkable discoveries.

The story is unique; eleven man-made elements produced and identified in twenty-odd years, many of these in Prof. Seaborg's laboratory. But the proliferation of the elements, though interesting, is not the most important scientific aspect of these achievements. Now that one begins to read this chapter in the context of the rest of the developments in chemistry, it is, perhaps, possible to begin to pick out the most significant features.

First, the key to the identification of the later members of the group, the demonstration that these elements constitute a new series in which the 5 *f* orbitals are being filled. Such a series had long been predicted, even in such details as its differences from the lanthanide elements; but, as ever, the facts, while broadly confirming these predictions, threw up a host of new problems. Some of these, such as the unusual crystal structures and properties of the elemental forms of these metallic elements, are still unresolved.

The earlier investigations of the first five or six transuranic elements were a triumph for microchemical techniques. The tetrad, uranium, neptunium, plutonium and americium, with their several valencies has provided inorganic chemistry with a rich field of investigation for many years to come. The difficulty of the separation of these elements, especially those of the highest atomic number, has led directly to a much deeper knowledge of the processes of ion exchange and solvent extraction. Indeed, it is clear that, but for the close analogy between the ion-exchange behaviour of the actinide and lanthanide elements, it is unlikely that the elements of greater atomic number than curium would have been characterized so quickly. Present-day knowledge of their chemistry amounts to little more than their ion-exchange characteristics.

Besides these important purely chemical results, the production and investigation of the transuranium elements have yielded a rich reward of nuclear chemical information. A large part of our knowledge of fission derives from observations of isotopes of these elements.

The accumulation of a substantial body of fairly reliable data on the nuclear characteristics of the numerous isotopes of these elements revealed a number of these systematic features of nuclear stability, and this provided one of the lines of evidence on which our knowledge of nuclear forces has developed.

Some years ago, the present director of the Atomic Energy Research Establishment, Dr. R. Spence, proposed to me a measure of the importance of developments in natural science which is easily applied and generally reliable. The really important developments are those that, within two or three years, demand inclusion in the undergraduate courses. By this yardstick, this chapter of chemistry does indeed include a number of developments of fundamental importance. A. G. MADDOCK

INSTRUMENTATION FOR CHEMICAL ANALYSIS

Analysis Instrumentation–1963

Edited by Dr. L. Fowler, R. D. Eanes, and T. J. Kehoe. (Proceedings of the Ninth National Analysis Instrumentation Symposium held April 29–May 1, 1963, at Houston, Texas.) Pp. x + 261. (Pittsburgh, Pa.: Instrument Society of America, 1963. Distributed by Plenum Press, New York.) 12.50 dollars.

AS the bibliographic particulars indicate, this volume contains papers read at a symposium, and it follows the usual pattern, covering a very wide field. Unlike the *Proceedings* of earlier symposia of this series, the book is bound in hard covers.

There are thirty individual papers grouped under six headings:

"Keynote Session—Analysis Instrumentation Today" deals with the present status of analysis instrumentation from the point of view of the manufacturer, user and developer, and is discursive and non-detailed. Three pages devoted to humorous and apt cartoons enliven this section.

"Dynamics of Analysis Instruments" is concerned with detailed design of instruments for the control of dynamic industrial processes, for example, optimum settings for a cigarette machine and a conductivity bridge for tests on extraction apparatus and continuous flow empty and packed columns.

"Problems of Analyzer Applications" includes the calibration of gas monitors and process gas chromatographs and the application of flame ionization chromatography to raw motor-car exhaust gases and to the analysis of paraffin waxes. Space research is catered for by a paper on the design of a lunar gas chromatograph.

"Laboratory Instrumentation" has an interesting paper dealing with polymer molecular weight measurement by gel permeation chromatography: a potentiometric method detects as little as 2·5 p.p.m. of chloride in various salts: peroxyacyl nitrates, 'smog' air pollutants, are determined by gas chromatography. Other papers are concerned with instrument demonstrations and processing analytical data.

In the section dealing with "Electrochemical and Chemical Methods" we find described a sensitive instrument for continuous measurement of temperature changes on mixing two liquids, for example, dilute solutions of citric acid and sodium hydroxide. An all solid-state *p*H meter, a new method for instrumenting square wave polarography and an autoanalyser for the determination of chemical oxygen demand in water are also described. Two papers are concerned with the measurement of moisture, one based on the use of microwaves and one on a dielectric system.

"Radiation Methods of Analysis" contains two papers concerned with the use of infra-red in the determination of gases and toxic vapours: infra-red absorption data for 320 gases and vapours are tabulated. Lunar exploration receives attention in a paper describing neutron activation methods for compositional studies and another paper relates to the use of charged particles for the bombardment. Two instruments are described, a gamma-ray density gauge and a spectrodiffractometer, the latter an instrument for chemical analysis by both X-ray diffraction and emission.

With such a mass of information, detailed comment in a review is not possible.

The book should be of interest to all concerned with instrumental methods.

C. O. Harvey

NORTH AMERICAN ECOLOGY

The Ecology of North America

By Victor E. Shelford. Pp. xxii + 610. (Urbana: University of Illinois Press, 1963.) 10.00 dollars.

THIS large, well-illustrated book is the culmination of a lifetime of ecological research by Prof. Shelford, aided by numerous students, and is based on many field expeditions throughout North America. By combining the observations of early travellers with surveys of semi-natural areas, Prof. Shelford is able to attempt to describe the ecological situation in North America during the sixteenth century before the arrival of European settlers. He has succeeded remarkably well in this formidable task, but not everyone will agree with his description of primeval North America. It is strange, for example, that the effects of windblow receive scant attention, yet American ecologists at Yale and Harvard have emphasized that uprooting of forest trees by wind was of widespread occurrence, with tornadoes regularly cutting swathes through the forest sometimes over two kilometres wide. The effects of successive, ancient windthrows can be traced in the irregular forest floor of humps and pits and in the complex soil morphology that has resulted from repeated overturnings of the soil layers.

The Ecology of North America contains nineteen chapters, the first of which deals with the definition of ecological terms and concepts used in the book. Some of these, such as permeants and influents, are not generally used and are of doubtful value. Each of the remaining eighteen chapters is devoted to the description of a major ecological community, which range from the tundra biome of the north to the tropical rain forest of the south. The major communities are subdivided into regions and seral stages of development. A large list of literature cited is given but unfortunately many modern investigations, such as the description of the vegetation of Wisconsin by Curtis, are not included. Excellent locality and species indexes are provided but no general index, so that it is difficult to obtain readily a picture of the influence of fire or of Indians.

In describing the ecological communities, Shelford expertly brings together a great amount of information on the characteristic species of plants and animals present and also on their numbers and interrelationships at different seasons of the year. This information is set against the background of topography, soil, climate and human influence. The careful integration of knowledge gained from different disciplines provides an excellent insight into the complexity of natural biological situations and of the virtues of comprehensive investigations.

Undoubtedly the book will be invaluable to conservationists and to anyone concerned with management of natural resources, for it provides a unique historical perspective against which to view past and future developments in North America. It also serves to direct attention to the inadequacy of our knowledge of the interrelations between plants and animals and of the quantitative aspects of community dynamics. These deficiencies need to be remedied if ecologists are to contribute significantly to schemes of land use and to understand the impact of modern technology on Nature and natural resources.

J. D. Ovington

THE BIOSPHERE

Animal Worlds

By Marston Bates. Pp. 316 + plates. (London: Thomas Nelson and Sons, Ltd., 1963.) 84*s*.

ANIMAL WORLDS is an introduction to natural history by way of ecology. It makes no attempt at a systematic description of the animal kingdom but deals in turn with many different environments, their floras and faunas. Marston Bates has travelled widely and is well qualified to write on natural history from this angle; he writes skilfully and has produced a lively and interesting volume that will be appreciated both by the layman and the professional zoologist. In spite of the vast range of the subject, its salient points are covered and much illuminating detail is included within the moderate space of more than three hundred pages.

The ecological treatment gives scope for the consideration of many aspects of animal life, including not only the direct reaction of animals to the physical environment, but also their physiology, behaviour, special adaptations, migrations, breeding habits, relationship to other species of animals and to plants, to mention only a few. The result is a real natural history of the biosphere in which the wood is not obscured by the trees and which gives a good insight into the complicated relations between animals and their physical and biological surroundings. As Bates remarks in the foreword, a survey such as this is necessarily selective from the great mass of material available, but his selections are judicious and very much to the point.

The final three chapters deal with the relationships between man and the other animals, with domestication, extermination, parasites, pests and pets. Much of the surface of the Earth is rapidly being changed by man's activities; the changes eliminate some species of animals but provide favourable conditions for others which are able to reach a biological equilibrium. The important exception, which has not yet become extinct nor reached a state of equilibrium with the environment, is man himself. If he does not apply his knowledge of biology to solving his own biological problems on a very large scale without loss of time, he may well come to realize the truth of the sun-dial motto, "It is later than you think".

The style of this book, written and first published in the United States, naturally has an American flavour. The editing of this British edition is, however, irregular—the caption of the picture illustrating the capercaille gives the bird's name as 'wood grouse', and that of a skua has not been translated from the American 'jaeger'. The name 'cockroach' appears in all its horrid nakedness alongside 'roach', emasculated in deference to American prudery.

The beautiful photographic illustrations, many of them in colour, add greatly to the attractiveness of the book. But the author has suffered, as have many others, from the photographs having been collected, as he acknowledges, by the editors, who presumably have supplied the captions which unfortunately contain some errors. The 'living zooplankton' is manifestly a preserved dead sample; the nesting 'fulmars' are herring gulls; the Brazilian 'horned frogs' are toads and are not as stated 'strictly aquatic'; the flamingos in Lake Nakuru are feeding on surface plankton, not "reaching down into the mud"; the Amazonian tapir is not an "odd relative of the elephant"; the 'topi' in Nairobi Park are not topi (*Damaliscus*) but kongoni (*Alcelaphus*) . . . and so on. It is a pity that these blemishes have been allowed to disfigure an otherwise splendid and fascinating book.

L. HARRISON MATTHEWS

AT THE TURN OF A TAP

Salt Water Purification

By Prof. K. S. Spiegler. Pp. ix + 167. (New York and London: John Wiley and Sons, Inc., 1962.) 57*s*.

THE inhabitants of the British Isles are apt to take for granted the supply of fresh water on tap and are prone to complain about the excessive rainfall. Water of drinking quality is generally available for all domestic purposes and, at slight extra charge, for car-washing and garden watering. Probably ten times as much water as is thus consumed is used in addition for irrigation and for industrial purposes to provide us with the needs of an industrial society. It is therefore not surprising that Manchester, with its high concentration of industry, is forced, despite its reputedly high rainfall, to seek additional supplies of water from Wales and the Lake District. The fact that the rich farm lands of Essex would benefit from the equivalent of another eight inches of rainfall is probably less well known. The general shortage of water in this area may indeed limit the growth of new towns there. There is an evident need to conserve water by persuading industry of the necessity of recovering and re-using its process and rinse waters and by reducing to a minimum the pollution of the rivers and lakes from which supplies are drawn.

If conservation of water is important in Britain, it is still more vital for the arid areas of the world, which are being opened up in the search for natural gas, oil, and minerals, and in response to the ever-growing pressure of world population. Some of these areas are not far removed from the sea, but, although the oceans represent a vast reservoir of water, it is too salt to drink since it contains about seven times as much salt as can generally be tolerated for this purpose. At the same time, the rainfall which supplies our fresh water has resulted almost entirely from the evaporation of water from the sea and its subsequent condensation in the cooler parts of the atmosphere to form clouds. In seeking to supplement his supplies of fresh water in the arid regions, man has inevitably turned to local brackish waters or to sea-water as the source of supply. However, while the random supply of rain water at the whim of Nature costs us nothing, apart from impoundment and distribution, the separation of fresh water from salt water involves an irreducible minimum of energy, which is exceeded by a substantial margin in practice, thus making the process a doubtful economic proposition. Only where the need for fresh water is the overriding consideration, as in parts of the Persian Gulf area, Israel, or Libya, or where the problem of storage is the decisive factor, as in Guernsey, has much use been made of salt water sources.

Prof. Spiegler's book *Salt Water Purification* is concerned not only with the problems outlined here, and to an extent which reveals his concern for humanity, but also with the ingenuity that has been brought to bear on their solution. In this latter aspect he reveals the clarity of thought and exposition which characterizes him as a scientist. His aim of providing "an understanding of the essentials of salt water purification in simple terms for all those interested in the field" has surely been achieved. The scientist or engineer will certainly find in the book a useful overall survey of the problem, while the statesman or economist will find in it an adequate background to enable him to appreciate the fundamental principles involved and a guide to the jargon of the technologist. The text, diagrams, and tables are easy to follow and should not present any great difficulties to the layman.

The first three chapters deal with the basic problems of salt water purification, including the underlying thermodynamic considerations. In the fourth chapter the problem of scale-formation, which occurs in most of the distillation methods and in electrodialysis, is discussed in detail. Of the four chapters which follow, one is devoted to each of the methods of distillation (including multiple effect, flash, vapour compression, and solar), electrodialysis, freezing, and ion-exchange. In each case the practical considerations, the advantages, and the disadvantages are fully discussed. Miscellaneous methods, including solvent extraction, ultrafiltration, osmionis, and critical pressure distillation which, although feasible on a laboratory scale, have not for various reasons been felt of sufficient promise to justify large-scale tests, are briefly discussed in chapter nine. The final chapter of summary and conclusions underlines the problems of assessing the relative costs, not only of producing fresh water from salt as compared with the transportation of the former from the nearest available source, but also of the various processes of desalting. So much depends on the scale of operation and on purely local considerations such as the sources of energy available. An appendix gives a rather more detailed treatment of energy considerations and a table of unit conversions, and there is a useful bibliography.

Frequent references are made in the book to the extensive contributions to this field of the Office of Saline Water, U.S. Department of the Interior. Those who are surprised at the relatively few references to British work may be interested to read the report of a lecture given recently by Dr. Miller of the Water Research Association to the Royal Society of Arts (*J. Roy. Soc. Arts*, 112, 491; 1964) in conjunction with Prof. Spiegler's book.

I myself hope that many others will read this book with as much interest as I have done. Concern for our fellow men should prompt our interest in the problems of the arid regions, but the general problems of water conservation are coming even closer to our doorstep.

The importance of this topic is further underlined by the report (*The Times*, June 25) of the United States and Soviet Governments' joint research project.

J. E. SALMON

Selected Papers of Ernst Georg Pringsheim

Edited and with a preface by Prof. C. B. van Niel. Pp. ix + 331. (Published for the Institute of Microbiology. Distributed by Rutgers University Press, New Brunswick, New Jersey, 1963.) 6.50 dollars.

MICROBIOLOGY is now in a transitional stage in which the ideas of the older microbiologists, based as they were on morphology and simple physiology, have given way to those of the biochemist, the geneticist, and the statistician. Pringsheim belongs to the old school, and to read these collected papers is like turning back from the pages of a modern biology to a treatise on old-fashioned natural history. Here are arguments and discussions based almost entirely on morphology, and there is a wonderful sense of simplicity about all the micro-organisms discussed in these papers. That they are borderline forms between the blue-green algae and the bacteria is not disputed, but there is a remarkable—some might think commendable—lack of evidence of the kind we now expect and demand of our taxonomists.

This is a group of micro-organisms about which most of us know little or nothing, and they are described by a master of technique who has had the patience and pertinacity to make of them a most detailed study; here he tells us in simple terms what he has learnt in this work of a lifetime. They are probably not economically important organisms, they do not cause infections or poison food, but they are the happy hunting ground of an almost extinct kind of naturalist-microbiologist. This book is refreshing, for it shows how far we have come in the past few decades; it is stimulating, for it shows how right we are to question all the convictions of the classical taxonomists.

These papers show what has been done by good technique and careful observation; they should serve as object lessons to microbiologists to renew acquaintance with the light microscope and the simpler tools of their trade. With the advantage of hindsight we may be tempted to judge Pringsheim's opinions as those of a dogmatic teacher upbraiding the enquiring and unbelieving pupil. He is impatient with Buchanan and intolerant of opinions such as Pijper's on the mechanism of bacterial motility, but the simplicity of the arguments is like manna to the genetically and computer-sotted mind of the modern taxonomist.

S. T. Cowan

The Formation of Wood in Forest Trees

The Second Symposium held under the Auspices of the Maria Moors Cabot Foundation for Botanical Research, Harvard Forest, April 1963. Edited by Martin H. Zimmermann. Pp. xv + 562. (New York and London: Academic Press, 1964.) 114*s*. 6*d*.

"THE Physiology of Forest Trees" was the theme of the first Cabot Foundation symposium, and the second, held six years later in 1963, took as its topic "The Formation of Wood in Forest Trees", thus continuing the general subject of fundamental problems of tree growth. The papers and edited discussions are now available in this excellent publication.

In recent years, much research has gone into the various processes which are associated with the formation of wood and many of the results and some of the theories of this rather complicated subject have been dealt with by the 28 contributors from North and South America, Europe and Australasia. The papers are grouped into four parts. Part 1 is concerned with the cambium and its derivatives. Too often the cambium is treated in rather general terms, but it is evident that we need to know more about its rate of division during the growing season to prove or disprove certain theories associated with it. Phloem, one of its derivatives, becomes in its secondary state bark, but the characteristics of a particular bark are determined not only by the structure and development of the phloem but also by the structure of the periderm and by the timing, depth and frequency of its initiation in the bark. Advances in electron microscopy and photographic techniques have helped to provide new knowledge on the structure and formation of the cell wall; some excellent illustrations are provided in a comprehensive and detailed paper on this subject.

The complicated chemistry of the three main constituents of cell walls—cellulose, hemicellulose and lignin—is dealt with in Part 2. This is followed by Part 3, which contains five papers associated with the translocation of photosynthetic products to the cambium. Much of the recent knowledge on this subject has been brought about by newly developed carbon-14 techniques. One such series of experiments shows that there is a complex of processes involved in the translocation of organic material from the site of assimilation to the place of utilization, but that the physiological activity of the latter is probably the main factor in controlling translocation. The final part embraces some of the internal and external factors controlling wood formation, and it is emphasized more than once that more field experiments are required to elucidate the relationships which may exist between growth behaviour and environmental factors.

These papers will be welcomed by the various specialists in this complex subject, not only for their own worth and for points brought out in the associated discussions, but also for the lists of references. Editor and publisher have presented the material in a most commendable way.

C. J. Taylor

Green Medicine

The Search for Plants that Heal. By Margaret B. Kreig. Pp. 462 + 63 photographs. (Chicago and New York: Rand McNally and Company, 1964.) 5.95 dollars.

THE sub-title of this book, "The Search for Plants that Heal", roughly indicates its content. It is written in a popular and somewhat dramatic style by a professional American woman journalist with a scientific background —and also three young sons! The object of the book is to direct attention to the enormous amount of energy and money that is being applied in the United States, especially by the large drug firms, in attempting to produce new drugs from plants. Facts and figures quoted are likely to be an eye-opener for many readers, especially for those outside the United States. Some of the plants producing such well-known drugs as quinine and digitalis are also discussed.

There is much of interest in the book, such as the chapter on Mexican wild yams as sources of diosgenin, the chapter on *Rauwolfia* for reserpine and the remarks on the amazing narcotic plants of the Americas used for centuries by Amerindians. The writer gives reasons for the belief that in the next quarter of a century our knowledge of the drug plants of the world will increase greatly and that there will be much activity in their investigation.

F. N. Howes

Dissection of the Locust

By Dr. Joan G. Thomas. Pp. 72. (London: H. F. and G. Witherby Ltd., 1963.) 8*s*. 6*d*.

UNTIL about twenty years ago, the advanced school pupil and university undergraduate were almost always introduced to insect anatomy by dissecting the cockroach. This insect was readily available and so also were accounts of its anatomy and instructions for its dissection. However, improved standards of hygiene have made the cockroach more difficult to obtain, and the establishment of many laboratory cultures of the migratory locust in Britain has provided an alternative, and in some ways superior, species with which to teach insect anatomy. Until now, two obstacles have impeded the widespread adoption of the locust for this purpose: there has been no dissection manual, and supplies of the adult insects have not been available on a commercial scale. Dr. Thomas's book has removed the first of these, and the removal of the second will no doubt soon follow.

The book has been written for sixth forms in schools and for undergraduate students, and the author has struck a very suitable balance between the requirements of these two classes of reader. After introductory notes on the life history, and on rearing and preserving locusts, the book deals in successive sections with the external features and the dissection of all the principal systems. The text is written very clearly, with the practical instructions for dissection usefully marked off from the rest of the text by being printed in italics; accounts of microscopical structures are printed in smaller type. An appendix gives brief notes on the making of permanent preparations and suggests a number of simple investigations that can be made with living locusts.

The book is amply illustrated with clear, fully labelled diagrams, enabling the descriptions and instructions for dissection to be easily followed. It is well bound in a large sensible format, and can be recommended unreservedly to both students and teaching staff of the biological departments of schools and universities.

D. R. Ragge

The Two-nucleon Interaction

By Michael J. Moravcsik. (Oxford Library of the Physical Sciences.) Pp. viii + 154. (Oxford: Clarendon Press, 1963.) 18*s*.

The physics of the two-nucleon interaction has recently reached a breathing-space. Our empirical knowledge of the proton–proton interaction is now essentially complete throughout the elastic scattering region; a similar wealth of information on the neutron–proton interaction is much harder to win and will not all be available for several years. This review of the subject is therefore timely and welcome.

The book is divided into two equal parts. The first deals with the experimental information and with the simple theoretical apparatus (*S*-matrix, potential models, phase shifts) needed to describe it phenomenologically. The second half discusses the attempts that have been made to provide a fundamental theory of the nucleon–nucleon interaction (pion potentials, dispersion relations, exchange of pion resonances).

Within the restricted format of an Oxford Library volume it would of course be impossible to develop, for example, the theory of dispersion relations from scratch; nor would it be desirable in a monograph such as this. Enough is said, however, to indicate what sort of theories there are, their premises and their mathematical expression, the type of results to be obtained, and the difficulties involved. Together with the full experimental survey, this gives a good clear picture of our present state of knowledge of the interaction which lies at the heart of so much modern physics.

J. M. Soper

Intra-ocular Lenses and Implants

By Prof. Peter Choyce. Pp. x + 211. (London: H. K. Lewis and Co., Ltd., 1964.) 90*s*. net.

This is a beautifully produced book, well illustrated and liberally provided with coloured illustrations of a high calibre. The illustrations have the great merit of showing at a glance what they are intended to convey, and are self-explanatory to such an extent as to render the captions almost redundant. Prof. Choyce, a master equally in explaining basic principles and detailed operational techniques, surveys the brief history of lens prostheses and puts his weight firmly behind Strampelli's fixed-length acrylic anterior chamber implant. Various types of fixation are described and the special requirements of aniridic eyes carefully set out. If one wonders to what extent Gullstrand's schematic eye is truly representative of the optics of the non-existent average eye—witness only the dependence on age which is not negligible but which has heretofore been ignored in 'schemes'—one may, none the less, provisionally follow Prof. Choyce in the acceptance of his theoretical basis. It may be argued that, if the eye were not normal before the insertion of the implant, it would scarcely be so afterwards. His Tables 22 and 23 showing the residual spherical and cylindrical errors assuage one's fears and, at the same time, demonstrate the progressive improvements of the technique.

Implants can also be used in dealing with keratoplasty and for the restriction of vitreous prolapse into the anterior chamber, although the long-term effects following these, and other, operations remain to be established. Prof. Choyce believes that later editions of this monograph will be more helpful in throwing light on these questions. This leads to a feature of this book which he has done his best to prevent one from overlooking. In his commentary on Spinoza, Prof. Hampshire emphasizes the philosopher's severely analytical style, as typified by the exclusion of all adornment and personal traits: the writer's ego appears in his treatises no more than it does in Euclid's theorems. The very reverse is true of the monograph under discussion. If the reader is reminded of '57 Varieties' when reading references to "Choyce mark III, IV, V implants", he may well be pardoned for questioning the objectivity of this highly personalized account. Prof. Choyce's surgical skill may match that of his pen. He cannot quote his patients' opinions: we have to wait for those of his peers.

R. A. Weale

Radionuclide

Von D. Kurt Schmeiser. Zweite, völlig neu Bearbeitete und Erweiterte Auflage von Radioaktive Isotope. Pp. xii + 282. (Berlin: Springer-Verlag, 1963.) 59 D.M.

The book is primarily intended as a guide for biologists, doctors, chemists and other scientists who want to use radioactive nuclides for radiography or analysis. It is quite easy to understand, and the main emphasis is on practical applications. There are numerous literature references up to 1961 which permit a study in greater depth when this is desired.

In seven main sections the book deals with the basic principles of atomic structure and the production of radionuclides, the interaction of radiation with matter, radioactive decay, radiation detectors, source applications of radionuclides and radiation protection.

Three new items are added to the second edition: the determination of low-energy radiation (^{3}H, ^{14}C) with liquid scintillation counting, γ-spectrometry and low-level counting. The chapter on liquid scintillation counting gives on eight pages a survey about the techniques involved, the properties of some scintillators and solvents in use and the advantages and disadvantages of the method. Compared with its importance nowadays, the γ-spectrometry is discussed rather briefly (six pages). The chapter on low-level counting mainly centres on the techniques for reducing the background, including anti-coincidence shielding.

In greater detail are discussed the techniques of activation analysis (14 pages) and autoradiography (25 pages). A number of useful examples are described of the application of the various techniques in the fields of chemistry, biology and technology.

The book is well written and can be recommended as a useful tool to those familiar with the German language.

E. Glueckauf

VARRO AND THE SMALL BEASTS: A BIMILLENNIUM FOR MICROBIOLOGISTS

By Dr. D. F. CHEESMAN

Bedford College, University of London

TWO thousand years ago, probably in 36 B.C., the Roman polymath, Marcus Terentius Varro, published his treatise on agriculture and husbandry, known as the *De re rustica*. Varro's birth in 116 B.C. at Reate (now Rieti) in the Sabine country is well attested[1]. In the first chapter of the book, the author remarks that he must make haste with the writing: "since my eightieth year warns me to get my luggage together for my departure from this life"[2]. Pliny the Elder records that the *De re rustica* was published in Varro's eighty-first year[3]. The bimillennium may thus be placed without great error in 1965.

The book is the only surviving intact work of the man whom Quintilian regarded as the most erudite of all Romans. It is one of the very few technical hand-books which survive from classical times. Most significant of all, perhaps, is that the *De re rustica* contains the first mention in extant literature of invisible micro-organisms, with the statement that these may be agents of disease.

The treatise is in the form of a conversation between Roman gentlemen versed in the different aspects of farming. The most expert of them all, one Gnaeus Tremelius Scrofa, discusses the choice of the site for a farmhouse: "Care must be taken in swampy localities . . . since in such places there grow certain minute animals (*animalia quaedam minuta*) which cannot be followed by the eye and which are carried with the air into the body by way of the mouth and the nostrils, there giving rise to serious diseases"[4]. Scrofa later emphasizes that the house should be built in a high and well-ventilated place: "Being exposed to the Sun throughout the day, it is more healthy, since any small beasts (*bestiolae*) that are bred nearby and carried in either are blown away or quickly die from desiccation"[5].

Such doctrine, though novel, is quite reconcilable with the Hippocratic theory of disease. Varro himself successfully applied Hippocratic principles during an epidemic when he was with Pompey's army in Corfu[6]. His account of diseases in farm animals does not depart from orthodoxy[7]. It is thus of all the more interest to contrast the first passage cited with analogous passages by writers who came directly or indirectly under Varro's influence.

The architect Vitruvius, a younger contemporary, gives evidence that he read and admired Varro. He had probably at least considered Scrofa's remarks when he made his recommendation concerning the choice of high sites for public buildings: "The neighbourhood of a marsh is thus avoided. When the morning winds reach the town with the rising Sun and, combining with the marsh clouds, spray the bodies of the inhabitants with the venomous exhalations of the swamp animals, the place will become pestilential"[8].

Two generations later, Columella, another admirer of Varro, gives advice on the location of the farmhouse: "Nor should a marsh be close to the buildings . . . for in the heat it gives off an unwholesome stench and breeds animals armed with stings which fly upon us in dense swarms. It also gives off plagues of swimming and creeping creatures, poisoned by the mud and the decaying sludge, from which strange diseases are often contracted, the causes of which not even the doctors can understand"[9].

Finally, and in a similar context, Palladius, a fourth-century writer who owes much to Columella: "A marsh, however, should by all means be avoided . . . because of the pestilence or the hostile animals which it generates"[10].

Vitruvius persists, as indeed men persisted for another nineteen centuries, in a miasmatic theory of the ague; but so much concession is made to the marsh beasts that the miasma is said to originate in their breath. Columella, in his vivid picture of swamp life, comes fortuitously closer than Varro to identifying the vector of malaria; but his animals all remain visible. Palladius seems to find no causal relationship between the animals and the pestilence. It appears, at all events, that the invisible *bestiolae* were just too much to accept, however respected the mind which conceived them.

We have too little of Varro's writings to assess his competence as a natural philosopher. The *De re rustica* is full of common sense, all that might reasonably be expected of such a work. Superstition extends no farther than such deference to the established religion as would to-day be held respectable in a landowner. Here and there one encounters a passage startling in its insight. The following plea by Scrofa for the experimental method may well be without parallel in classical literature: "The earliest farmers established most techniques by trial; their successors, in the greater part, imitated them. We must do both: imitate and try by experiment to do some things differently, following not chance but some kind of reason"[11].

It is not difficult to suppose that the man who wrote this could have made the extrapolation from the very small insects which lodge, all too sensibly, in the body's orifices, to yet smaller organisms which, unperceived by the senses, pass unhindered into the body. This great imaginative feat appears to have made no impression whatsoever on biological or medical thought. It was not until the seventeenth century that the learned Jesuit, Athanasius Kircher, in his essay on the plague, suggested that the pestiferous atoms of Democritus and the Epicureans, which had inspired Fracastoro to elaborate his theory of the *contagium vivum*[12], were, in fact, living animals, worms so small that "you would call them atoms, unless they were made perceptible by the most exquisite of microscopes"[13].

Varro, who, as a believer in an "Efficient Cause"[14], was as monotheistic as any Jesuit, may himself have conceived the *bestiolae* as a counter to the atomic theory of disease. We shall perhaps never know, however, whether the concept derived originally from a Greek source. Further uncertainty comes from the fact that Gn. Tremelius Scrofa was not, as the learned editors of the Loeb edition of *De re rustica* assume, a fictitious character with a suitably funny name (*scrofa* = breeding sow), but a distinguished writer on agriculture to whom Pliny[15] and Columella[16] acknowledge their debt. Varro, as a good encyclopædist, may be attributing to his *dramatis personae* the opinions held by their prototypes.

Whatever the truth of these matters may be, Varro gives the earliest hint in surviving literature of a world of organisms too small for the eye to detect. It would be pleasant if, during the two-thousandth anniversary of the book, a few microbiologists were to drink to his memory.

[1] St. Jerome, *Eusebian, Chronicle*, ad ann. Abr. 1901.

[2] Varro, *De re rustica*, 1, i, 1.

[3] Pliny, *Naturalis historia*, 18, 23.

[4] Varro, *De re rustica*, 1, xii, 2.

[5] Varro, *De re rustica*, 1, xii, 3.

[6] Varro, *De re rustica*, 1, iv, 5.

[7] Varro, *De re rustica*, 2, i, 21.

[8] Vitruvius, *De architectura*, 1, iv, 1.

[9] Columella, *De re rustica*, 1, v, 6.

[10] Palladius, *De agricultura*, 1, vii.

[11] Varro, *De re rustica*, 1, xviii, 8.

[12] Pellegrini, F., *La dottrina Fracastoriana del contagium vivum* (Verona, 1950) *passim*.

[13] Kircher, Athanasius, *Scrutinium physico-medicum contagiosae luis*, 140 (Rome, 1656).

[14] Cicero, *Academica*, 1, ii.

[15] Pliny, *Naturalis historia*, 17, 199.

[16] Columella, *De re rustica*, 1, i, 12.

A SINGLE-GENE MUTATION OF *Chlamydomonas reinhardii* AFFECTING MOTILITY: A GENETIC AND ELECTRON MICROSCOPE STUDY

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IT is clear that recent progress in microbiology, molecular biology and biochemical genetics has brought us to a new frontier in the understanding of the organism and its organization: and we may now hope that within a reasonable period of time some at least of the principles of morphogenesis and differentiation will be disclosed. The analysis of these principles has been pursued for many years, chiefly in higher organisms[1,2]. Although recent investigations with micro-organisms now allow us to think of morphogenetic processes in terms of genes and macromolecules[3], there are many daunting problems facing those who would wish to apply these ideas to still more complex living systems. In higher plants and animals the distinction between somatic and reproductive tissue does, in fact, greatly hinder the direct experimental examination of the genetic control of differentiation in such organisms. Consequently, one valuable method of attack is to focus attention on some more limited objective, such as a distinctive and well-characterized organelle, the basic features of which seem to be of universal occurrence; or alternatively on phage, for example phage *T*4, in the head assembly of which 7 genes are already known to be implicated[4,25]. Many of the leading workers on the Protista[5,6] have emphasized and demonstrated that these organisms display important features of morphogenesis and differentiation. Externally in many genera there are cilia and flagella; and cortically their basal bodies, roots and other attachments. Some groups such as algae possess, in addition to flagella, another type of organelle of morphogenetic interest, namely, the chloroplast, which has also been the subject of genetic and fine-structure investigations[7,8].

We have chosen to examine the morphogenesis of cilia and flagella and their basal bodies, and this article presents an account of our first experiments. In due course it may be possible to express the morphogenesis of a flagellum in terms of its genetic determinants and to distinguish between processes of self-assembly[9,10] sequential (genetic) control and subsequent regulation. Our first concern, however, has been to establish an appropriate experimental system and explore its potentialities.

The main features of the fine structure of the mature cilium or flagellum are now well established[11], particularly in contrast to the comparatively meagre amount of information available on the development of these organelles. Recently, both Ehret and de Haller[12] and our own Laboratory[13] have given descriptive accounts of the sequence of development of basal bodies in terms of fine structure for *Paramecium* and *Stentor*, respectively, and Schuster has examined the transformation of *Amoeba grüberi* from amœboid to flagellate[14]. For various reasons these organisms are unsuitable for our present investigation. The alga *Chlamydomonas reinhardii*, a biflagellate member of the Chlorophyceae, has many of the attributes necessary for our purpose. The fine structure of the flagella of a related species, *Chlamydomonas moewusii*, has been examined by Gibbs, Lewin and Philpott[15]. Although the electron micrographs reproduced by these authors are somewhat lacking in detail, there seems to be no reason to suppose that flagellum structure in *Chlamydomonas* spp. differs markedly from that found in so many other organisms. *Chlamydomonas reinhardii* is also suitable for genetic investigations: it is normally a haploid organism, has a short life-cycle and is very convenient for tetrad analysis[16]. Non-chromosomal inheritance has been demonstrated in the species[17].

Mutants of *Chlamydomonas* spp. with impaired motility have been isolated previously, notably by Lewin[18], although no structural abnormalities of the flagella were detected when these were examined in the electron microscope[15]. We shall now describe results obtained from the examination of a mutant of *Chlamydomonas reinhardii* induced in the presence of proflavine.

(*A*) *Genetics*. Cultures of wild-type *C. reinhardii* were obtained from the Cambridge collection and originally from R. P. Levine's laboratory. Experimental cultures were maintained at 25° C at an intensity of illumination of 500 ft.-candles provided by tubular fluorescent lamps. The culture medium was based on Medium I described by Sager and Granick[19] in which ferric chloride was replaced by 0·01 g/l. ferric citrate and 0·1 g/l. citric acid.

Wild-type (mating type +) cells were grown for six days on solid medium containing 100 mg/l. proflavine hydrochloride. (Since no estimation of mutation rates in the absence and in the presence of proflavine has been made, the possibility of spontaneous mutation certainly can not be excluded.) Selection of non-motile, so-called paralysed, mutants from these cells was made in two stages:

(1) Non-motile cells sink in liquid culture and this provides a simple basis for a mutant-enrichment technique[20]. Therefore about 10^6 cells were transferred from the proflavine-containing plates to 100 ml. liquid medium in a separating funnel and afterwards incubated for a further three days. The vast majority of cells in such a culture are motile and are found close to the surface. In order to obtain an enrichment in the proportion of non-motile types, 1 ml. of the above culture was taken from the bottom of the funnel and used for the second stage of selection.

(2) This cell suspension was diluted and plated on semi-solid medium (containing 0·5 per cent 'Oxoid No. 3' agar) at a density of about 60 cells/dish. On this medium, non-motile mutants form rather small dark-green colonies in contrast to the large, paler colonies of wild type. One small colony was found to consist of cells with non-motile flagella of approximately normal length. The flagella of a few cells showed slight, unco-ordinated twitching which in no case resulted in any movement of the whole organism. Cells of this strain always sink to the bottom

in liquid cultures. The strain was re-isolated from a single colony and designated pro_1. This mutant strain was crossed to wild type and dissection of resulting tetrads carried out according to the method of Ebersold and Levine[21]. It was confirmed that gentle shaking favours mating of non-motile mutants[18]. The products of ten tetrads were cloned and classified for motility by two methods: by microscopic examination, and by their behaviour in liquid medium. In all cases, segregation for motility : non-motility was 2 : 2, which is in accord with a single gene control of the trait, although a chromosomal location for the gene has not yet been established.

(*B*) *Electron microscopy*. Examination of the fine structure of the flagella of the wild type of *Chlamydomonas reinhardii* and the mutant pro_1 was carried out in an RCA *EMU* 3*G* electron microscope using an accelerating voltage of 100 kV; the instrument was fitted with a 30μ objective aperture. Organisms in their normal liquid medium were fixed by draining the specimens of excess liquid and then adding a buffered solution of 1 per cent osmium tetroxide (*p*H 7·5) at room temperature for times varying from 5 to 50 min. Dehydration was achieved by passing the cells through a graded series of acetone solutions and then finally embedding in 'Araldite' in the usual way. Thin sections of the embedded cells were applied to copper grids coated with a carbon film and then double-stained as follows with the grids in an inverted position. The preparations were exposed to a saturated solution of uranyl acetate in equal parts of ethyl alcohol and water for 1 h at 37° C in a sealed chamber. The grids and sections were subsequently well rinsed in several changes of distilled water and the sections further stained by exposure to a solution of lead citrate for 35 min[22]. Preparations were then washed with a jet of 0·02 N sodium hydroxide and, afterwards, several times with distilled water.

The main features of the fine structure of cilia and flagella are well known, and the review already cited[11] discusses these in detail. Each flagellum consists (within a bounding membrane) of nine pairs of peripheral and apparently tubular fibres and a central pair. To one member of each of the outer nine pairs of fibres may be attached two short projections or arms. These extend in a circumferential direction towards the next fibre. Gibbons and Grimstone[23] consider that these arms all point in the same direction. The matrix of the flagellum, and particularly that region lying between the central and outer fibres, contains moderately densely staining components which have been interpreted in terms of subsidiary longitudinal fibres[23] and as radial lamellæ[24]. These finer details of flagellum structure, together with others recently observed in this laboratory, require further investigation and may be of importance in the wider concept of morphogenesis. In the present article we shall be concerned only with the main peripheral and axial fibres.

Fig. 1 shows a transverse section of a flagellum of *Chlamydomonas reinhardii*, wild type, in which the dispositions of the nine outer pairs and the central pair of fibres are clearly visible. In view of the appearance of flagella of the mutant now to be described it should be emphasized that the normal 'nine-plus-two' structure has been observed in all of many independent preparations of the wild type. Fig. 2 illustrates the appearance of the mutant (pro_1) flagellum in transverse section, and it is at once clear that the central pair of fibres is no longer present in its characteristic form, but has been replaced (in the section) by an irregular area of material of moderate density.

We have also examined the fine structure of the flagella of clones grown from the four members of a tetrad resulting from the cross between wild type and mutant. The structural abnormality observed in electron micrographs of flagella of the original mutant is also present in the clones grown from the two non-motile members of the tetrad. Conversely, clones grown from the two motile members have flagella of normal structure. Thus the gross phenotypic change to non-motility is correlated in the segregants with a significant change in fine structure.

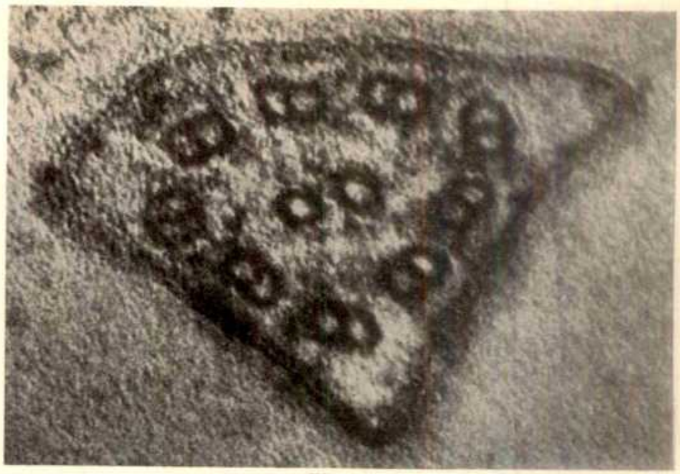

Fig. 1

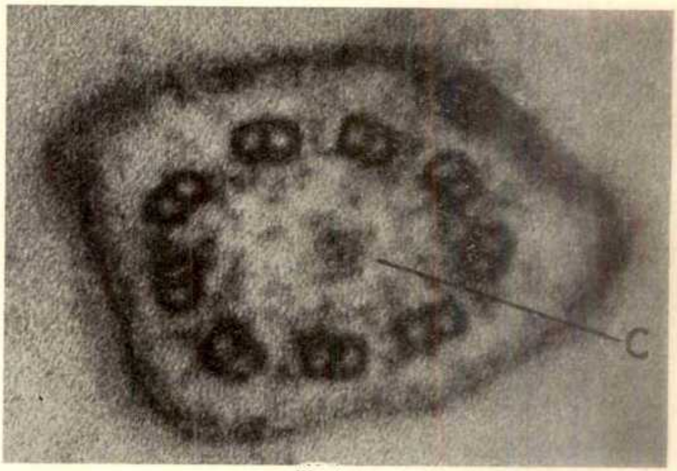

Fig. 2

Figs. 1 and 2. Electron micrographs of transverse sections of flagella of *Chlamydomonas reinhardii* (×225,000)

Fig. 1 shows the normal '9+2' fibre pattern observed in wild-type organisms; Fig. 2 illustrates the disorganized nature of the central pair of fibres in the mutant (as at *C*)

Discussion

It would be inappropriate to enter into detailed discussion of our results at this stage. Nevertheless a few comments can be usefully made. The association of non-motility and structural defect in a single gene trait is of direct and immediate interest. In the longer term sense it leads us to hope that other similar effects may be discovered which will lead to a better understanding of a specific morphogenetic process.

Examination of electron micrographs of the flagella of the mutant suggests that part, if not all, of the material of the axial fibres is still present and that it may sometimes appear in the form of small fibrils. Thus if protein units or even fibrils have been synthesized—and this is the obvious inference—their subsequent organization into the characteristic twin axial fibres of the normal flagellum has failed to take place. It is not yet known if the chemical nature of the axial material in the mutant is identical with that of the wild type. Clearly, the inability of the mutant flagellum to organize its chemical components in characteristic form could arise from differences in primary, secondary or tertiary protein structure. Further speculation along these lines is undesirable until it can be fortified by a more detailed knowledge of the chemical nature of the flagellum fibres.

Other possible differences between the fine structure of the flagella of wild type and those of the mutant are now being investigated. There are indications that the radius of the cylinder on which lie the nine outer pairs of fibres is larger for the mutant than for the control, but more measurements are required to justify this observation on statistical grounds. There appear also to be differences in the proportions of the complex basal body structure; these too require further examination.

Summary

(1) A mutant non-motile or 'paralysed' strain of *Chlamydomonas reinhardii* has been produced in the presence of proflavine and isolated.

(2) Electron microscope examination of the flagella of the mutant shows an abnormal structure, the most obvious feature of which is the apparent lack of a central pair of fibres in an organized form.

(3) Tetrad analysis of crosses between mutant and wild type has demonstrated a 1 : 1 segregation on the basis of motility and also in terms of fine structure (as described in (2)) in the electron microscope.

(4) *Chlamydomonas reinhardii* appears to be a suitable organism for the investigation of the morphogenesis of flagella. The mutant pro_1 of *Chlamydomonas reinhardii* is believed to be one of the first to be isolated with a clearly observable alteration in the fine structure of a well-characterized organelle, the flagellum.

We thank P. J. Hastings of the Botany School, University of Cambridge, for initiating us into various techniques of handling *Chlamydomonas*, particularly tetrad analysis.

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ELECTROMAGNETIC WAVES FROM VERY DENSE STARS

By Prof. F. HOYLE, F.R.S., Dr. J. V. NARLIKAR and Prof. J. A. WHEELER
Department of Applied Mathematics and Theoretical Physics,
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THE recent detection of X-rays from the region of the Crab Nebula and from a discrete source in Scorpius by Giacconi *et al.* and by Bower *et al.*[1–3] has much increased the interest in neutron stars (cf. Chiu[4] and Morton[5]). It has been considered important for the maximum mass of a stable neutron star to exceed that permitted for a white dwarf, about $1{\cdot}3\ M_\odot$. The original discussion of the problem by Oppenheimer and Volkoff[6], treating neutrons as a simple Fermi gas, gave a maximum mass of only $0{\cdot}7\ M_\odot$. However, allowance for a hard core nucleon potential increases the maximum mass and it is likely that in the stiffest possible situation with the pressure equal to the energy density the maximum mass for a neutron star does exceed that for a white dwarf[7]. This has been considered essential in order that neutron stars be formed during the course of stellar evolution.

We begin by pointing out that for actual stars possessing rotation the usual treatment of the maximum mass has no general validity. When gravity is balanced by centrifugal force in directions perpendicular to the axis of rotation there is no limit to the mass which may be supported in a disk-like structure. For a disk of radius a, thickness b, in which the material has angular momentum h per unit mass, we have the following relations:

$$h^2 a^{-4} = \omega^2 \simeq 2\pi G\rho \qquad (1)$$

$$P \approx 2\pi G b\rho . b\rho \qquad (2)$$

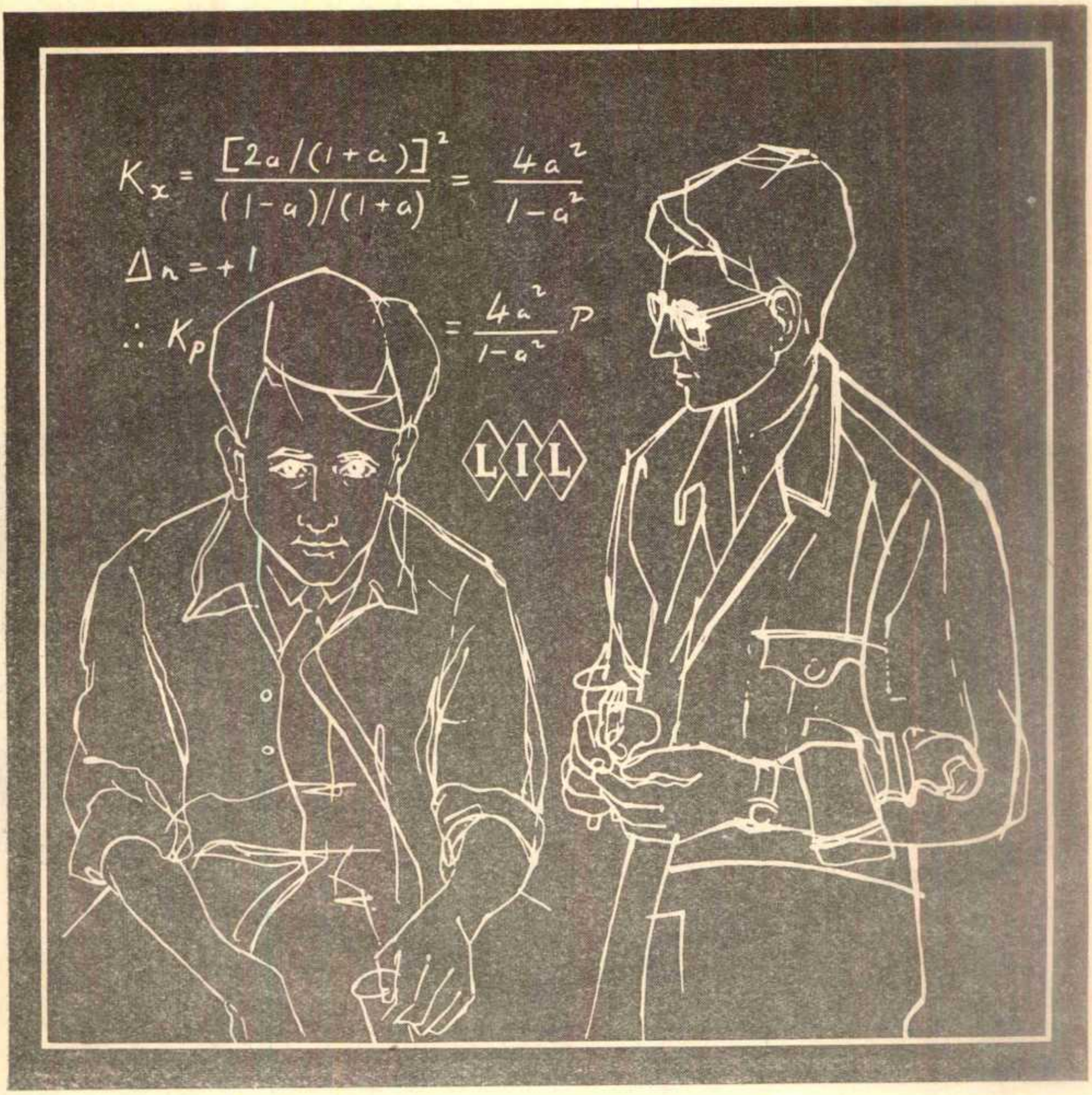
where P is the pressure. Equation (1) is necessary for the rotational support and equation (2) is necessary in order that the pressure shall balance gravity in the direction perpendicular to the disk. The mass M is given by:

$$M = \pi a^2 . b\rho \simeq \tfrac{1}{2} h \sqrt{\frac{P}{G^3 \rho}} \qquad (3)$$

in which a has been eliminated with the aid of equation (1) and b with the aid of equation (2). Whatever the dependence of the pressure on ρ, it is clear that M can be made as large as we please simply by increasing h. At constant ρ, b remains constant while a increases with h, so that b/a decreases with the increasing h and the disk-like property assumed here is enhanced as $h \to \infty$.

Our second point is that a neutron star is thought to be formed catastrophically, probably through the free fall collapse of a supernova. This means that such a star does not arrive gently at its stable configuration. Large dynamical velocities are developed and there must be oscillations about the stable configuration. For situations in which the dimensions of the equilibrium structure are not much greater than the critical Schwarzschild radius, the dynamical speeds are not much less than speed of light. Since for neutron stars the dimensions of the equilibrium structure are taken as not more than 3–10 times the Schwarzschild radius, the corresponding speeds are $\sim c/3$, and the associated dynamical energy is of order 10^{20} ergs gm^{-1}. Not until after this energy of oscillation has been dissipated can the stable structure be arrived at. The question we wish to raise is: How is the dynamical energy of oscillation, totalling $\sim 10^{54}$ ergs for a star of mass $5\ M_\odot$, dissipated? Our suggestion is through the emission of electromagnetic waves.

For definiteness we shall consider a neutron star with a mean internal density of order 10^{15} gm cm^{-3} and with dimensions $\sim 10^6$ cm. It seems unlikely that the magnetic intensity at the surface can be much less than 10^{10} gauss. Even a star with such a small dipole field as the Sun, 1 gauss at a mean density of ~ 2 gm cm^{-3}, would develop a polar field of $\sim 10^{10}$ gauss if it were catastrophically contracted to $\sim 10^{15}$ gm cm^{-3}. (The magnetic field would not be squeezed out of the star. Although neutrons dominate, electrons and protons are


$K_x = \frac{[2a/(1+a)]^2}{(1-a)/(1+a)} = \frac{4a^2}{1-a^2}$
$\Delta n = +1$
$\therefore K_p = \frac{4a^2}{1-a^2} P$
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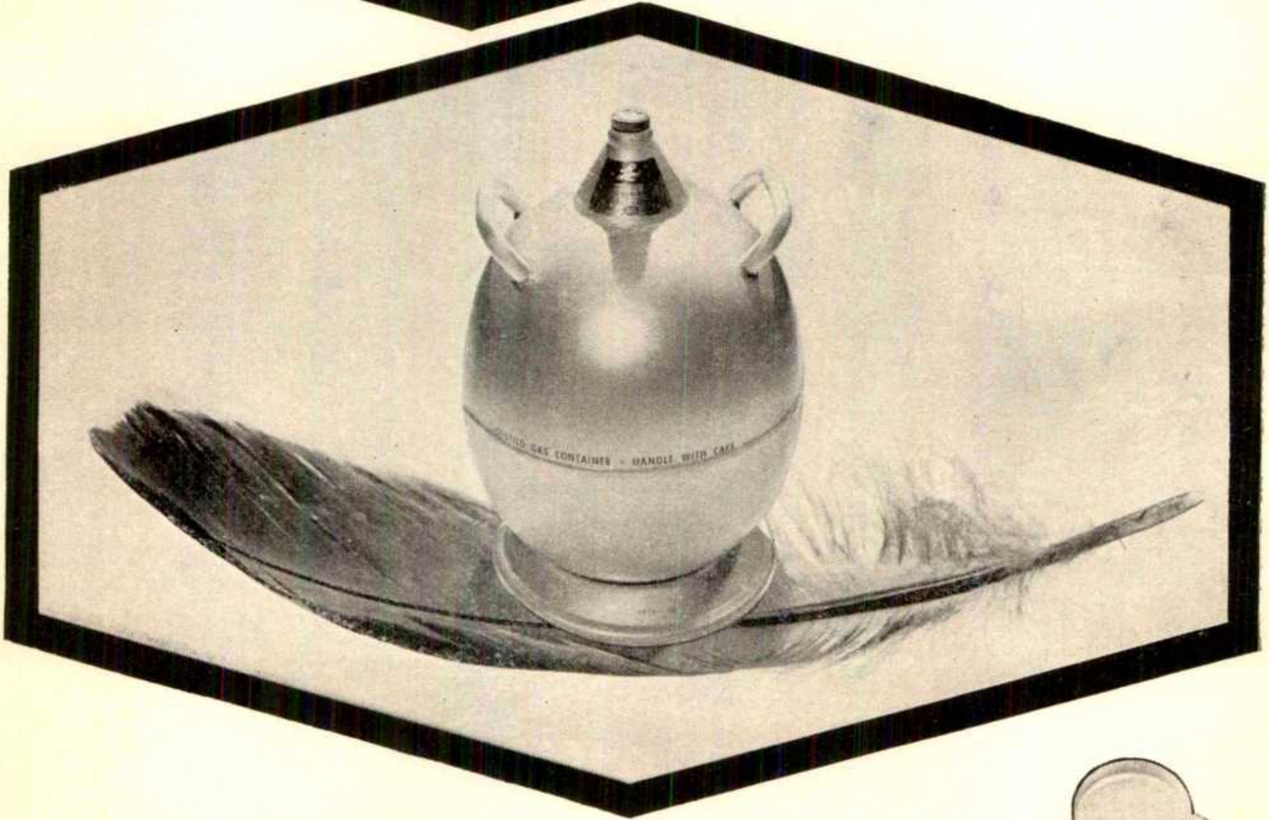

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WCWISE

also present, the sum of the top of their Fermi distributions being equal to that of the neutrons. The electrons have high conductivity.)

Dynamical oscillations of the main body of a neutron star evidently imply an oscillation of the associated magnetic field. The oscillations will be of a hydromagnetic character so long as the hydromagnetic wave speed $H\sqrt{4\pi\rho}$ is appreciably less than c, as it is in the interior of the star. But ρ falls steeply at the surface, the scale height being only a few centimetres. Evidently, for $\rho \simeq 1$ gm cm^{-3} we have $H/\sqrt{4\pi\rho} \sim c$ and the situation becomes electromagnetic rather than hydromagnetic, with the displacement current of crucial importance. Outside the immediate surface we expect electromagnetic waves to be generated, $\lambda/2\pi$ being of the order of the dimensions of the star. This follows because the frequency ν is $v/2\pi R$, where v is the dynamical speed of oscillation, $\sim 10^{10}$ cm sec^{-1}, and R is a characteristic dimension. Hence, $2\pi\nu \simeq 10^4$ c/s, and $\lambda/2\pi \simeq 3 \times 10^6$ cm. The simplest example is that in which an oscillating star with an initial non-uniformity of rotation produces oscillating variations in the winding of a toroidal magnetic field, requiring an oscillating component in the axial current, that is, a Hertzian oscillator. The emission of electromagnetic waves serves in this case to damp the initial non-uniformity of rotation. On the other hand, an oscillating component in the toroidal current would seem to be necessary in order to damp out the radial oscillations of the star.

The possible rate of emission of energy by electromagnetic waves is very large. The Poynting vector $c\mathbf{E} \times \mathbf{H}/4\pi$ has magnitude $cH^2/4\pi$, which gives $\sim 3 \times 10^{29}$ ergs cm^{-2} sec^{-1} for $H = 10^{10}$ gauss. The total surface area is $\sim 10^{13}$ cm^2, so that the total emission rate might be as high as 3×10^{42} ergs sec^{-1}, although a lower value, say 10^{41} ergs sec^{-1}, would be a more conservative estimate, since the oscillating component of the field is likely to be less than the total field. However, even 10^{41} ergs sec^{-1} is sufficient to dissipate the available store of $\sim 10^{54}$ ergs in $\sim 10^{13}$ sec $= 3 \times 10^5$ years.

It is important that no propagation difficulty arises. Electromagnetic waves are damped out in a few wave-lengths when the dielectric constant:

$$1 - \frac{Ne^2}{\pi m \nu^2} \qquad (4)$$

becomes negative. Here N is the electron density and e,m are the electron charge and mass. Putting $2\pi\nu = 10^4$ c/s, it is easily seen that (4) is zero for $N \simeq 3 \times 10^{-2}$ electrons cm^{-3}. While this is only very small compared with the internal density of a neutron star, it is much greater than the likely exterior density at a few radii out from the surface, simply because the radius exceeds the scale height by a large factor $\sim 10^6$. The point is that the strong gravitational field of the star creates a near-vacuum immediately outside itself, and the electromagnetic waves are free to travel out into space. Moreover, any residual gas outside the star would simply be swept away by the pressure of the electromagnetic waves.

How does the electromagnetic flux from an oscillating neutron star lead to observable effects? So long as the dielectric constant (4) is positive the waves pass essentially freely through space, but a critical condition arises if (4) becomes negative, that is, if N exceeds $\sim 3 \times 10^{-2}$ electrons cm^{-3}. The waves will be reflected from any cloud they encounter in which this condition is satisfied, and the cloud is then subjected to radiation pressure. The consequent compression increases N so that the pressure, once started, continues. Although radiation pressure is usually thought of as a weak effect—this is true for optical radiation—the electromagnetic flux from a neutron star can be so large that dramatic possibilities arise.

Consider the case of the Crab Nebula. The total mass of the gaseous filaments is about $0{\cdot}1\ M_\odot$, which is about the mass of interstellar gas to be expected in a volume of 1 pc^3, the volume of the Crab. The filaments are in outward motion with speed $\sim 1{,}000$ km sec^{-1}, so that the total outward momentum of the gas is $\sim 2 \times 10^{40}$ gm cm sec^{-1}. If this momentum has been derived from radiation, the energy of the radiation must have been 2×10^{40} c. Taken over 1,000 years the necessary mean energy output is $\sim 2 \times 10^{40}$ ergs sec^{-1}, close to the value 10^{41} ergs sec^{-1} estimated here. Hence it is possible that the observed outward momentum of the Crab has been derived from the electromagnetic flux.

Using 10^{41} ergs sec^{-1} for the total flux, the flux per unit area at $\sim 10^{17}$ cm from the emitting star is $\sim 10^6$ ergs cm^{-2} sec^{-1}. Reflected normally from a surface this flux exerts a pressure of $\sim 10^{-4}$ dyne cm^{-2}. Since reflexion occurs in only a few wave-lengths, in $\sim 10^8$ cm, the reflecting zone can indeed be thought of as a very thin layer in which the internal pressure must build up to match that due to the reflexion, $\sim 10^{-4}$ dyne cm^{-2}. If the electron density is 3×10^{-2} cm^{-3}, a mean electron energy of 10^{-2} erg $\simeq$ 10 BeV is necessary to supply this pressure. Although this estimate would not be correct if the electron density were to increase to much more than 3×10^{-2} cm^{-3} in a distance of $\sim 10^8$ cm, it is clear that any steep increase of density must imply violent non-uniformity in the thin-surface zone, and that particle acceleration could well take place in the ensuing fine scale motions. The critical behaviour of the refractive index would therefore appear to provide a mechanism for the generation of the high-energy electrons necessary to explain the synchrotron emission of the Crab. If the electrons were derived from the star itself it would be hard to understand why their energies were not immediately degraded through synchrotron emission in the very high magnetic fields close in by the star.

Turn back to the formation by collapse of a neutron star: why should this phenomenon show up as a supernova? Hoyle and Fowler[8] considered that, while the inner regions of a supernova collapse, the outer regions are thrown explosively off into space through a rapid release of nuclear energy, but these considerations apply only to supernovæ of Type II. For Type I supernovæ there may well be no explosive emission of an outer shell. There is the possibility that the optical radiation of Type I supernovæ comes from circumstellar clouds, distant perhaps as far as 10^{16} cm, that are activated by the ensuing electromagnetic flux.

We end by enquiring whether a similar process might occur on a still larger scale. Ginzburg[9] has considered the problem of the magnetic fields associated with massive objects, 10^6–$10^8\ M_\odot$. The problem appears essentially different, however, so long as one is dealing with a single coherent object. The wave-length would in any case be much larger, $\sim 10^{13}$ cm, and there would be a propagation difficulty similar to that aforementioned for ordinary stars. On the other hand, there is the possibility[10] that rotation may cause a massive object to break up into a swarm of neutron stars and/or white dwarfs. In such a situation each neutron star would behave essentially independently of the others, and with $\sim 10^7$ such stars the intensity of the phenomenon would be multiplied by this factor. The total energy available for electromagnetic radiation would then be $\sim 10^{61}$ ergs. A process of high efficiency, converting this energy into cosmic-ray electrons, would be necessary if the strong radio sources were to be explicable in these terms.

It is of considerable interest that the momentum associated with this total energy efflux is $\sim 3 \times 10^{50}$ gm cm sec^{-1}, which is sufficient to endow 10^{43}gm ($5 \times 10^9\ M_\odot$) with a recoil speed of 300 km sec^{-1}. The possibility must therefore be considered that a marked disturbance in the whole of the interstellar gas within a galaxy could be produced. Indeed, the possibility must not be excluded that the interstellar gas might be entirely expelled from a galaxy, or—if the gas were a sufficiently large fraction of the total mass—that a whole galaxy might be disrupted and scattered.

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OBITUARIES

Prof. James Franck, For.Mem.R.S.

PROF. JAMES FRANCK died on May 21 at the age of eighty-one years. Born in Hamburg on August 26, 1882, Franck went to school there with indifferent success—as he was fond of relating—because he had trouble with classic languages. He began to study chemistry and geology in Heidelberg, but soon turned to physics and moved to Berlin where he graduated as Dr.Phil. in 1906, with a paper on ion mobility in gases. In 1907 he became research assistant to Prof. H. Rubens, in whose colloquia I first met him in 1908. I had the great good fortune to win his friendship and that of his family and to retain it for nearly sixty years. Only recently I was able to spend some weeks with Franck, visiting old friends together all over Europe, until a few days before his sudden death in Göttingen, on May 21.

In the First World War Franck went out as a private, was wounded, decorated and returned as a lieutenant. He was then assigned to a group led by the chemist F. Haber, who later, in 1918, made him a division head at the Kaiser-Wilhelm Institute for Physical Chemistry in Berlin-Dahlem.

How well I remember his famous lecture on May 22, 1914, "On the Excitation of the Mercury Resonance Line 2536 Å by Electron Impact" in which he reported his joint work with Gustav Hertz, which gave strong support to Bohr's new theory of the atom. Einstein said to me: "It's so lovely, it makes you cry!" Many a discussion between Franck and Otto Stern about those experiments I have listened to during the War years, whenever Franck happened to be in Berlin.

In 1920 Franck was called to a chair in Göttingen. Haber's farewell speech—at a party which Otto Hahn and I arranged—ended with the words: "There are three stages in the life of a scientist: becoming, being and representing; may representing come easy to you". I think Franck did not enter that last stage until a few months before his death, and it was not easy for him, despite the many honours that have come his way, among them the Nobel Prize in Physics in 1926 jointly with Hertz, the Max Planck Medal in 1953, the Rumford Medal in 1955, membership in many learned societies, and the election, in March 1964, as Foreign Member of the Royal Society.

Göttingen saw Franck's happiest and most fruitful period; he remained attached to it, received its honorary citizenship in 1953, and it is an odd turn of fate that he died there. There he did all his work on the transfer of energy in molecular collisions, as in the processes called quenching and sensitized fluorescence. Franck discovered the method of determining the heat of dissociation of diatomic molecules from band spectra by extrapolating the vibrational levels. His discussion of the coupling between electron transitions and vibrational motion led him to formulate the principle which was later put in wave-mechanical form by Condon and is known as the Franck–Condon principle. Thus the intensity distribution in band spectra and the existence of continuous molecular spectra came to be understood. The influence of those ideas has been far-reaching, particularly in photochemistry. His many pupils, still attached to Franck in affection and devotion, include such famous names as Blackett, Condon, Kopfermann, Kroebel, Maier-Leibnitz, Oppenheimer and Rabinowitch, to mention some of them.

Those happy days ended when Hitler came to power. When the racial laws were announced, Franck published a courageous open letter in which he resigned his chair. In view of his distinguished war service he might have been allowed to stay, but he did not wish to serve under a Government which treated his fellow Jews as outcasts. After an interlude at Copenhagen, with Niels Bohr, he went to the United States, first to the Johns Hopkins University and then to the University of Chicago, as professor of chemistry. Here his efforts were devoted to the elucidation of the most important of all photochemical reactions, the photosynthesis performed by green plants. It is a borderline problem between physics, chemistry and biology, and one of appalling complexity. Franck's last paper on that subject, in collaboration with J. L. Rosenberg, went to press only a few weeks before he died.

During the War he interrupted his own work for two years to direct the Chemistry Division of the Metallurgical Laboratory of the University of Chicago, a part of the Manhattan Project. He saw it as his duty to help develop a nuclear weapon as long as Hitler might be doing the same. But he felt strongly that the United States should not be first in using it, and other scientists felt similarly. Two months before Hiroshima, a joint letter which became known as the "Franck Report" was sent to the U.S. Secretary of War, Henry L. Stimson, presenting strong and wise arguments against the unannounced use of that dreadful new weapon and suggesting that its power be demonstrated by publicly exploding it over an uninhabited area. Perhaps the nuclear arms race could have been avoided if that letter had been heeded.

Franck enjoyed talking about his problems, not so much to explain them to others as to clarify his own mind. Once a problem had aroused his interest he was completely captivated, indeed obsessed by it. Common sense and straight logic were his main tools, together with simple, mostly home-made apparatus. His research followed an almost straight line, from his early studies of ion mobilities to his last work on photosynthesis; it was always the energy exchange between atoms or molecules that fascinated him.

Franck was equally interested in people. His kindness and generosity, not only to his friends and family but to everybody who needed help, were known to all who knew him. He was the most lovable of men because he loved people; kindness shone from his eyes. There must be many beside myself who now feel they have lost one of their best friends.

I wish to thank O. R. Frisch and H. G. Kuhn for valuable help in writing this notice. LISE MEITNER

Prof. Isadore Fankuchen

THE death of Prof. I. Fankuchen on June 27 at the age of fifty-nine leaves a great gap among crystallographers in the United States and particularly, also, in Britain. He was a figure very well known and loved in both countries.

His life shows him as a man devoted to research and teaching in his chosen field of crystallography. Educated at the Cooper Union, he passed on to Cornell University and thence to various other appointments in the United States. He came to England to work with Sir Lawrence Bragg at Manchester in 1934. He had already started his interest in crystallography, although fundamentally he was a physicist, and it was the physical interest rather than the chemical or biological that inspired his work. He found that in X-ray investigations he was able to apply the physics in more and more refined ways to problems of interest in industry and biology.

From Manchester, he joined me in Cambridge at the very exciting time, in 1936, when the first protein crystals were being examined by X-rays. His point of entry was the examination of the sterols which, in the form of vitamin D and its analogues, has just come into biological and pharmacological interest. His earliest work was on the structure of the uranyl compounds and he might well have gone on with this, for he was able to detect by the somewhat primitive methods of the time the presence and structure of the uranyl complex, UO_2, as an independent element in the uranium salts. Here he co-operated with Dr. Dorothy Crowfoot and me in an extensive investigation reported in the chemistry of the steroids (*Phil. Trans. Roy. Soc.*, A, 239, 135; 1940).

However, very soon after he arrived at the Cambridge laboratory his interest was diverted to the first examination of tobacco mosaic virus solutions, prepared by Bawden and Pirie. These turned out to be something quite different from anything that had been examined before; they are composed of long particles with a strong tendency to parallel packing which we now know is typical of the rod-shaped viruses. Before the development of the electron microscope this all had to be worked out by X-ray and optical methods.

Fankuchen threw himself into this examination. He excelled in the devising of apparatus specially tailored for the purpose. One problem was that of examining the liquid crystals at very low angles, and for this monochromatic X-rays were essential. He devised an X-ray monochromator made of a pentaerythritol crystal sliced in such a way that it could give a very narrow beam of strictly monochromatic radiation of high intensity. With this he investigated the variation of the photographs for tobacco mosaic virus solutions where the mutual distance between the rod-like particles was a function of the ionic strength, the *p*H and the dilution of the solutions. This examination dealt, not so much with the virus itself, but with the long-range forces between virus-like particles and, indeed, any kind of colloidal particles in solution. His work here was finally recorded some years later in the X-ray and crystallographic studies of plant virus preparations (*J. Gen. Physiol.*, 25, 111; 1941).

In 1938, Fankuchen left Cambridge and came to Birkbeck College, London, where I had just become professor of physics, and continued his virus investigations. The War intervened and he returned to the United States where he was later appointed professor of physics in the Polytechnic Institute of Brooklyn, a post which he held until his death.

During the War he took an active part in the investigations concerned with the use of snow and ice for military tactics, particularly in the project for airborne snow vehicles (weasels) and for the necessary studies to set up the *Habakkuk* ice ship which was conceived but never built on the full scale.

It was at Brooklyn Polytechnic, an extremely democratic, lively organization, that "Fan" (as he was known to all) found his true vocation as a teacher and director of research. The great growth of the use of X-ray analytic methods in the United States, particularly in industry, is to a very large measure due to his inspiration. He was able to imbue generations of students with the same devotion to the subject he himself had. This was not only in the Polytechnic itself, but also at Woods Hole in what he called his "Summer Clinics", where every year he taught a set of volunteers from industry in a fortnight all they needed to know of X-ray method. Fankuchen thus played a large part in spreading the ideas of crystallography and the benefits of crystallography to American industry, where it is more securely based than it is in Britain.

He was also extremely active in promoting the organization of crystallography in the United States and internationally, being one of the founders and, ultimately, the president of the American Crystallographic Association. He became the chairman of the National Research Council Committee on X-ray Crystallography.

Not only was he a devoted teacher, but he was a strongly critical supervisor; no shoddy work was to be tolerated. He further demonstrated this in his work as an editor of the *Acta Crystallographica*, the international crystallographic journal. Fankuchen helped greatly to strengthen and perpetuate the links between British and American crystallography. He was one of the effective founders of the International Union of Crystallography in 1945.

He will be remembered not so much for his scientific achievements as for his personality and inspiration. His ebullient good humour, friendliness and helpfulness, and his zest for life, made him one of the best-loved figures in American science.

Fankuchen died as he had lived. Stricken by cancer in 1962, he continued, in spite of two operations, all his lecturing work at Brooklyn and carried out his "Summer Clinic". He gave the last three-hour lecture at this Clinic on June 24 and then collapsed. He was taken to hospital and died only three days later, a life well spent. He leaves behind a wife and son.

J. D. BERNAL

NEWS and VIEWS

Physical Chemistry in the University of Oxford: Prof. R. E. Richards, F.R.S.

ON October 1, Dr. R. E. Richards will succeed Sir Cyril Hinshelwood as Dr. Lee's professor of chemistry at Oxford (see p. 958 of this issue of *Nature*). Since 1947 he has been a Fellow and Tutor of Lincoln College, in which capacity he followed the late N. V. Sidgwick. Since 1948 he has worked, first as a University demonstrator and later as reader, in the Department over which, in its recently somewhat reorganized form, he will preside. Dr. Richards was educated at Colyton Grammar School, Devon. He was an Exhibitioner of St. John's and later Senior Demy of Magdalen College. In 1954 he was awarded the Corday–Morgan Medal of the Chemical Society and in 1955 he was a Research Fellow at Harvard. He was elected to the Royal Society in 1959. His first research work was in the field of infra-red spectroscopy, but for the past fifteen years or so he has worked mainly on magnetic phenomena. It soon became evident that measurements of nuclear magnetic resonance were capable of throwing a great deal of light on very varied chemical problems, and Dr. Richards has been one of the foremost leaders in its development and application, winning thereby world-wide recognition. Dr. Richards takes over the Department at a moment of considerable expansion of its already vigorous activity, and it is fortunate to be passing into the charge of one with such eminent qualities.

The Torry Research Station: Dr. G. A. Reay, C.B.E.

Dr. G. A. Reay, who retires from the directorship of the Torry Research Station, Aberdeen, in October, was born on May 31, 1901, in Aberdeen. From Robert Gordon's College he went to the University of Aberdeen, obtaining his M.A. degree in 1921 and his B.Sc. in 1923, winning a senior Kilgour scholarship with which he commenced research in biochemistry. After a further year at Aberdeen he moved to Emmanuel College, Cambridge, and continued his research under the late Sir Frederick Gowland Hopkins. He was awarded a Carnegie research fellowship in 1926 and in 1927 obtained his Ph.D. In September 1927 Reay joined the staff of the Department of Scientific and Industrial Research Food Investigation Organization, becoming one of a small team of enthusiasts who set out to investigate and improve the handling of fish at sea. The Torry Research Station was not yet in existence, but Reay and his colleagues successfully demonstrated the value of adequate washing and proper icing procedures, and also the benefits of freezing fish at sea. The Torry Research Station was opened in October 1929, Reay being one of its first scientists and concentrating on the mechanism of protein denaturation in frozen fish. He soon began stressing the greater importance of low storage temperatures than of rapid freezing, a view at variance with much contemporary thinking. On the retirement in 1937 of the Station's first Superintendent, Mr. A. L. Lumley, Reay was appointed officer-in-charge, becoming superintendent in 1947 and director in 1958. He was elected a Fellow of the Royal Society of Edinburgh in 1955, and was awarded an O.B.E. in 1949 and a C.B.E. in 1958. Dr. Reay is an able musician and a keen angler.

Dr. J. A. Lovern, O.B.E.

Dr. J. A. Lovern, who has been appointed to succeed Dr. G. A. Reay as director of Torry Research Station as from November 2, was born in Liverpool in 1906. Dr. Lovern gained a first-class honours B.Sc. degree in chemistry at the University of Liverpool in 1927 and a Ph.D. in 1929 for investigations of the chemistry of fish oils under Prof. T. P. Hilditch. Thus began his professional acquaintance with fish, and also with Aberdeen, for he had to prepare his oil samples on board the research vessel of the old Fishery Board for Scotland. Joining the staff of the Torry in April 1930, when the Station was but six months old, Dr. Lovern gained international fame before the Second World War for his outstanding and varied chemical and biochemical investigations of fish oils. The University of Liverpool recognized this achievement by awarding him a D.Sc. in 1937. Two and a half war years in Washington, D.C., with the British Food Mission allowed Dr. Lovern to become thoroughly acquainted with the United States and Canadian fish industries and related research. Returning to Torry in 1945, Dr. Lovern enhanced his academic reputation by his notable work on the more complex lipids and the lipoproteins of fish, being promoted senior principal scientific officer on special merit in 1947. At the same time he became responsible for the Station's by-products research, especially on fish meal, and as a result he has become increasingly engaged in advisory work and sponsored research for the Association of Fish Meal Manufacturers and, since 1959, for the International Association of Fish Meal Manufacturers, of the Scientific Committee of which he has been chairman since its inception. Dr. Lovern was elected a Fellow of the Royal Society of Edinburgh in 1960 and received an O.B.E. in 1962. His hobbies include hill walking, natural history and alpine plant culture.

Royal Greenwich Observatory: Dr. O. J. Eggen

Dr. O. J. Eggen, formerly of the Lick Observatory, California, has joined the staff of the Royal Greenwich Observatory at Herstmonceux (Sussex). An authority on photoelectric photometry, he has put forward views on moving groups of stars considered most significant in relation to the history of the galaxy. He worked for five years at Herstmonceux before returning to the United States, where for three years he has been a professor in the California Institute of Technology and had use of the 200-in. telescope on Mount Palomar. Dr. Eggen will work as a temporary senior principal scientific officer at the Royal Greenwich Observatory.

Government Expenditure on Science

In reply to various questions in the House of Commons on July 2, total expenditure in the United Kingdom on all branches of oceanography and marine biology in 1964–65 was estimated by Sir Edward Boyle at about £2·17 million; Government expenditure on medical research in 1963–64 at £18·5 million, of which £7 million was for the Medical Research Council. Expenditure by the Department of Scientific and Industrial Research on seven basic research projects in scientific documentation at universities and colleges was estimated at £100,000, excluding research at the National Physical Laboratory. The new grant arrangements with the Association of Special Libraries and Information Bureaux would allow for an expenditure of up to £20,000 a year for a research programme in this field.

Channel Tunnel

In a statement in the House of Commons on July 1, the Minister of Transport, Mr. E. Marples, announced that the British and French Governments had decided that the further geological survey for the Channel tunnel should begin this summer. Arrangements were being made for the Channel Tunnel Study Group to conduct the survey on an agency basis under the general supervision of the British and French Governments, who were to appoint a Joint Commission of Surveillance for this purpose. Half the cost, which was expected to be about £1·25 million, would be met by the British Government and half by the French Railways which, at the instance of the French Government, were participating in the contractual arrangements. The survey arrangements were entirely without prejudice to decisions yet to be made by the Governments on the organization and financing of the project, but it was the present intention of both Governments that eventually the cost of the survey should be charged to whatever organization might be set up for the tunnel itself. Mr. Marples added, in reply to questions, that this was the first of many investigations which would have to be made before the tunnel was built. In the House of Lords, on the same day, Lord Chesham said that Col. D. McMullen, chief inspecting officer of railways in the Ministry of Transport, would be co-chairman of the Joint Commission of Surveillance, of which Dr. C. J. Stubblefield, director of the Geological Survey, would also be a member. He hoped that the survey would be completed within a year.

Problems of Water Supply in Britain

In reply to a question in the House of Lords on July 15, the Joint Parliamentary Secretary, Ministry of Housing and Local Government, Lord Hastings, said that large quantities of water were already being obtained by exploiting the natural storage capacity of underground aquifers, but in some areas their full capacity was unknown or not fully utilized. The Thames Conservancy was exploring the potentialities of the chalk in the Thames Basin, and since the publication of *The South-East Study, 1961–81*, a preliminary investigation of the chalk in the Great Ouse Basin had been arranged. The Water Resources Board and the new river authorities would investigate the suitability of geological conditions in Britain for the large-scale use of artificial recharge to augment natural storage capacity, but he did not think that this could be a complete panacea for all water supply problems. In a written answer in the House of Commons on July 16, the Secretary of State for Industry, Trade and Regional Development, Mr. E. Heath, stated that if the preliminary assessment now being made by the North-West Study Group of the suggested Morecambe Bay and Solway barrage schemes showed that the expenditure would be justified, the examination would include a full technical and economic investigation of these projects.

Large-scale Distillation and De-salting Plants

Replying to a question in the House of Lords on July 15, the Joint Parliamentary Secretary of State for Education and Science, the Earl of Bessborough, said that the Atomic Energy Authority's preliminary investigations of the use of nuclear reactors for providing heat for sea-water utilization plants indicated that, to be economic, the reactors would need to be very large, and this function would have to be combined with the production of electricity. In addition to producing base load electricity, such a reactor station might provide a considerable quantity of fresh water as a by-product at a price within the range of interest for domestic and industrial users in certain areas. The necessary continuous demand for electricity on a large scale limited the number of areas in which such dual-purpose reactors could be economic. Further development in the technology of de-salting plants was required and the Authority was in close touch with the Department of Scientific and Industrial Research. It was also represented on the panel of experts set up by the International Atomic Energy Agency to investigate the possibilities of these plants in developing areas. British industry was taking a leading part in the construction of large-scale distillation plant and provided some 70 per cent of land-based plants in the world.

Planning and Amenities

The annual report of the Sheffield and Peak District Branch of the Council for the Preservation of Rural England directs attention to ways in which the importance of the preservation of amenities, in central planning, is being undermined (Pp. 26. Sheffield: Council for the Preservation of Rural England. Sheffield and Peak District Branch, 1964). Difficulties of æsthetic control were aggravated by the Central Development Order of 1950, and in the same year the Schuster Report tended to lessen the importance of æsthetics in the training of qualified officers. Another serious blow followed in 1951, when the word 'Planning' was left out of the Ministry's title and "The order and seemliness striven for so long began to disintegrate again". Now, alarmed by the unexpected rise in population, the Minister has hinted that 'Green Belts' are not inviolate, and has thus loosened the restraints on ill-sited development of rural land in a way that may be bitterly regretted by coming generations. "We are thus losing at a greater pace than ever a countryside that has been called 'the loveliest in the world' ". Now, at last, influenced by experienced planners, we are beginning to recognize that regional planning is a necessity. "This has been not unfavourably received by urban authorities, so long as it is understood that their own town or city should be the central influence in the region."

British Mammals

The Identification of British Mammals, by G. B. Corbet, published by the British Museum (Natural History), is an excellent guide to the classification and identification of the sixty-odd species of mammals found in the British Isles (Pp. iii+46. London: British Museum (Natural History), 1964. 4*s*.). It is intended mainly for the amateur naturalist, but will be equally useful to the professional zoologist. The principles of classification are first explained and then follows a list of the British mammal fauna, giving the synonymy and status of each species. The main part of the pamphlet consists of keys for identifying the different species by external features—field identification is not included. There is an interesting list of possible additions to the fauna, a select bibliography, and a good index.

The Geological Museum, London

The fourth edition of *A Short Guide to the Exhibits* of the Geological Museum in South Kensington starts with a brief history of the Museum and then gives a summary of the general arrangement of the exhibits (Pp. 36. London: H.M.S.O., 1964. 2*s*. 6*d*.). The visitor is then taken on a short tour of the galleries and is thus able to obtain a broad idea of the scope and nature of the display collections. The well-known dioramas in the Museum are illustrated, and with these and other pictures even a visitor not acquainted with geology should be able to view the exhibits intelligently. The text of this useful and handy pamphlet is clearly written, with few technical phrases.

Nuclear Magnetic Resonance Current Literature Service

A new literature searching service, dealing with papers on nuclear magnetic resonance, nuclear quadrupole resonance and electron paramagnetic resonance, is to be established by Butterworths, the publishers of *Documentation of Molecular Spectroscopy*. This will take the form of a numbered and coded literature list which is searched by means of a peep-hole index of coded punched cards. The coding has been devised to enable searches to be made from two main starting points: (1) from the general contents of the paper, the nature of the problem investigated, and details of the techniques and apparatus described; (2) from the chemical classification of the compounds examined. The literature lists will give the title, author, journal or other source of publication, date and other relevant information, including, where necessary, a brief note on the content. It is hoped that, as they will be issued four times a year, they will offer: (*a*) an up-to-date and comprehensive source of rapid information which is easily retrievable by the use of the peep-hole index containing 105 cards per issue; (*b*) a current documentation of the entire field of magnetic resonance spectroscopy. At the end of the subscription period of two years the peep-hole index cards provided with each issue will be replaced by a consolidated peep-hole card index, thus making possible the searching of the literature in compact two-year batches. An alphabetical list of authors will also appear. A detailed *Instruction Manual* will be provided giving particulars of the coding. The service will cover the literature published since the beginning of 1963. It is also planned to produce, in due course, a collection of references covering the years 1946–62. A service similar to that described here, but covering infra-red, Raman and microwave spectroscopy, has been in operation as part of *Documentation of Molecular Spectroscopy* since the beginning of 1963. This is supplied to subscribers as part of their subscription but is also available separately. Full details of these services are available from Butterworths, 88 Kingsway, London, W.C.2.

University News: London

Dr. A. O. Betts, University lecturer at the School of Veterinary Medicine, Cambridge, has been appointed to the chair of veterinary microbiology and parasitology at the Royal Veterinary College. Dr. J. Edelman, reader in enzymology at the Imperial College of Science and Technology, has been appointed to the chair of botany at Queen Elizabeth College. Dr. F. G. Foster, reader in statistical computing at the London School of Economics and Political Science, has been appointed to the chair of computational methods at that School. Dr. W. W. Schwabe, a member of the staff of the Agricultural Research Council Unit of Plant Morphogenesis and

Nutrition at Wye College, has been appointed to the chair of horticulture at that College from October 1, 1965. Prof. R. A. J. Coutrez, "Professeur extraordinaire" at the Free University of Brussels and astronomer at the Royal Observatory of Belgium, has been appointed Belgian Visiting Professor for the session 1964–65.

Oxford

THE annual report of the Curators of the Bodleian Library for the year ended July 31, 1963, refers to the completion of the structural work on Duke Humfrey's Library early in 1963 (Supplement No. 9 to the *University Gazette*, May 1964. Pp. 43. Oxford: The University, 1964. 2*s*.). Attention is directed to the progress of development and rearrangements which will greatly increase the efficient administration of the Library and provide space for 24 more readers, if alternative exhibition rooms are found in the Divinity School and the Proscholium is converted into a new entrance hall serving both the Divinity School and the Old Library. The annual grant from the University General Fund was £221,644, nearly £9,000 less than the previous year; but the 107,071 books and manuscripts fetched for readers and 28,653 for staff represents an increase of more than 20,000. Accessions to the Department of Printed Books totalled 44,826 volumes and 121,276 parts, compared with 44,203 and 115,844, respectively, in the previous year.

The Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme

AWARDS under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have been made as follows: Dr. Margaret C. Anderson, Botany School, Cambridge, to enable her to work on light penetration at various levels in pine forest in the Section of Agricultural Physics, C.S.I.R.O. Division of Plant Industry, Canberra, for about five months from November 1964; Dr. R. L. W. Averill, senior research officer, New Zealand Medical Research Council's Department of Endocrinology Research, to assist him to learn techniques of hypothalamic investigation at Oxford, for ten months during 1964–65; Dr. D. J. Eve, lecturer in chemistry, University College of Rhodesia and Nyasaland, to enable him to carry out research on the spectroscopic characterization of inorganic co-ordination compounds at Southampton, from September 1964 until June 1965; Dr. K. M. King, associate professor, Soils Department, Ontario Agricultural College, to enable him to study in the field of micrometeorology and evaporation at the C.S.I.R.O. Division of Meteorological Physics, Melbourne, for five months from December. Dr. Alicja M. Kirkien-Konasiewicz, lecturer in chemical engineering, University of the West Indies (Trinidad), to enable her to learn techniques in high-resolution mass spectrometry at University College, London, from January until April 1965; Dr. S. E. Livingstone, associate professor of inorganic chemistry, University of New South Wales, to assist him to work in the field of co-ordination chemistry at University College, London, for nine months from September; Dr. B. Morris, Professorial Fellow, Department of Experimental Pathology, Australian National University, Canberra, to enable him to investigate at Oxford the role of the lymphatic system in the absorption of fat in fishes, for six months from September; Prof. K. C. Mukherji, professor of electrical engineering, Indian Institute of Technology, Bombay, to enable him to carry out investigations on electrical machines at the University of London King's College, for a year from October; Prof. R. A. Reyment, professor of geology, University of Ibadan, to assist him to study the ostracods of the marine Tertiary of Australia, for three months in the autumn; Dr. G. G. E. Scudder, assistant professor of zoology, University of British Columbia, to enable him to acquire knowledge of type material of Rhyparochrominae and techniques for insect blood analysis at the Imperial College of Science and Technology, London, for a year during 1964–65; Dr. R. L. Stanton, senior lecturer in geology, University of New England, to enable him to examine naturally deformed sulphide ores, at Imperial College of Science and Technology, London, and Durham, from September until November, in connexion with investigations on the significance of surface tensions in the final development of grain shapes in such ores. Dr. L. B. Thrower, senior lecturer in botany, and Mrs. S. L. Thrower, senior demonstrator in botany, both of the University of Melbourne, to assist them in their investigations on the effect of sporulation on the pattern of movement of nutrients in fungal hyphæ at Birkbeck College, London, and to attend a course on radiobiology, and to enable her to investigate the physiology of lichen growth at Birkbeck College and the British Museum (Natural History) during 1965.

Announcements

PROF. F. G. YOUNG, Sir William Dunn professor of biochemistry in the University of Cambridge, has been appointed the first Master of the newly formed post-graduate Darwin College at Cambridge. Prof. Young is president of Section I (Physiology and Biochemistry) at the Southampton meeting of the British Association for the Advancement of Science (see p. 952 of this issue of *Nature*).

AN index to the survey *Manchester and its Region*, prepared for the British Association meeting held in Manchester in 1962 (*Nature*, 195, 890; 1962) has now been compiled by the Manchester and District Division of the Association of Assistant Librarians under the editorship of Mr. M. J. Harkin. To the public spirit of him and his colleagues warm thanks are due from all who appreciate the merits of the admirable survey originally issued for the British Association by the Local Committee.

THE fourth Louis Rapkine Memorial Lecture entitled "La Proliferation et Differenciation des Cellules Hematopoietiques" will be delivered by Dr. Louis Siminovitch (Ontario Cancer Research Institute) at the Pasteur Institute, Paris, on October 7.

A CONFERENCE on "Electron Emission", arranged by the Electronics Group of the Institute of Physics and the Physical Society, will be held in the University of Keele during October 1–2. During the conference, the Guthrie Lecture for 1964 will be delivered by Prof. Martin Ryle. Further information can be obtained from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

THE National Conference of Food Hygiene, organized by the Institute of Nutrition, Budapest, will be held in Budapest during October 29–31. Topics under consideration will include: the effect of extraneous substances in food on the human organism; the hygiene problems of pesticide residues; the hygiene problems of contaminants of technical origin (metals, plastics). Further information can be obtained from the Institute of Nutrition, Gyáli ut 3/*a*, Budapest IX.

THE annual meeting of the American Documentation Institute on "Parameters of Information Science" will be held in Philadelphia during October 5–8. Subjects under consideration will include: symbolization and transformation of information; information-seeking behaviour; information in decision procedures; analysis, correlation and indexing; document media, storage and display; memory organization and search; information sciences; goals in education; the profession; information centres and networks. Further information can be obtained from A. W. Speakman, American Documentation Institute, 2000 P Street, N.W., Washington, D.C. 20036.

AUTOMATION, LEISURE AND YOUTH SERVICES

TWO short debates in the House of Commons on June 19 and June 22 complement earlier debates in the House of Lords on automation on April 8 and on the problem of leisure on May 13 (see *Nature*, 202, 629; 1964; and 203, 1; 1964). In the first of these debates, a motion of Mr. C. Curran was agreed, calling on the Government to state its policy for improving still further the educational facilities provided for less-gifted children who may otherwise be excluded from an automated labour market. This motion had been framed in view of the social consequences that follow when automation increases production by using a smaller labour force and when people below a certain minimum standard of ability and education may consequently find it hard to obtain employment. As the motion indicates, Mr. Curran was concerned with the social consequences of automation and with the less-able children with whom the Newsom Report was specially concerned (see *Nature*, 201, 1; 1964). He thought it would be most unwise to limit their secondary education to technical instruction: lack of literacy and the ability to communicate was likely to be a much more serious handicap to them than any lack of acquired skill. This found strong support in the debate; for example, Colonel Sir Harwood Harrison paid a warm tribute to the work of the county civic colleges, and pointed out that all education should seek to help a boy or girl to live a fuller life and to be more self-reliant. Mr. R. E. Prentice stressed the need to develop within the framework of Government techniques of forecasting man-power which would enable us to discern where redundancies and shortages were likely to occur. Britain's education and training policies should be geared to this kind of forecasting. He anticipated in future a much larger demand for skill and education in the working population as a whole and thought that there was much potential talent at present unused. He urged the importance of expanding the youth service and asked whether the Government had accepted the interim target of an increase of 50,000 day-release places a year for the next five years recommended by the Henniker Heaton Committee of the Ministry of Education. Mr. Dudley Smith, like Mr. Prentice, referred to the importance of leisure and sport, stressing the need to inculcate a better sense of civic purpose and responsibility in the community as a whole, while Sir Barnett Janner described the successful working of youth clubs in Leicester.

The Joint Under-Secretary of State for Education and Science, Mr. C. Chataway, replying on the debate for the Government, while welcoming the motion, discounted the idea that automation was likely to lead to any serious shortage of work. After referring to the work of the Interdepartmental Committee dealing with the general theme of 'the schools and society', Mr. Chataway said that one of the tasks of the new Schools Council, to be set up as an independent representative body with Sir John Maud as chairman, would be to foster the circulation of ideas in the schools. Its first meeting would be in October and the main function of the body would be to keep under review curricula, teaching methods and examinations in Britain's primary and secondary schools. He thought that the new Council was likely to stimulate much more research and development, and he pointed out that the context within which the recommendations of the Newsom Report must be pursued had been subtly altered by the Government's acceptance of the main recommendations to raise the school-leaving age. He also stressed the importance of the problem of relevance between school and work. Much more was now required to bridge the gap between school and work, and he thought a major co-operative effort involving the Government, trade unions, employers and many other bodies and organizations, besides the Schools Council, was needed if this problem was to be tackled seriously. We should know much more than we do about the qualities school leavers ought to possess if they were to move easily from school to work and from one form of employment to another in later life.

In opening the debate on leisure and sport, Mr. J. P. W. Mallalieu pressed for better facilities for enjoying all forms of leisure. Besides greater support for the National Playing Fields Association, the Central Council for Physical Recreation and the Youth Hostels Association, as well as for music and drama, he pressed for more regional surveys of the needs and facilities for enjoying leisure, but centrally co-ordinated. Mr. Chataway, who followed, said that the building programme for youth clubs was now running at £4·5 million a year, compared with less than £1 million before the Albemarle Committee Report, and by this spring about 700 projects had been finished. By midsummer 1963 Britain had nearly 1,000 youth leaders against the target of 1,300 set by the Albemarle Committee for 1966 and a further 200 were in training at recognized centres. A major need was to widen the appeal of the Youth Service and to devise and extend new approaches to the less purposeful kind of youths. Since 1960–61, capital expenditure on facilities for sport and recreation had risen from £5·6 million to an estimated £22·5 million in 1964.

In replying to a later debate, the Secretary of State for Science and Education, Mr. Q. Hogg, referred to the importance of seeing that, when Government expenditure on youth services was increased, it was done so intelligently and purposefully. Discussing Mr. Mallalieu's suggestion for a senior Cabinet Minister responsible for leisure, Mr. Hogg thought that this was unlikely to provide a satisfactory solution to the problem. He had originally been attracted by the idea of a sports development council, but now was moving away from it—for administrative reasons. He thought that the present arrangements, with a small central organization to co-ordinate the work of the voluntary bodies. the local education authorities and Government departments had won general confidence and were working well. Developments had been rapid both on the arts side and in sports. Expenditure on the arts had risen from £3 million in 1951 to £13·5 million, and developments in museums, entertainments, adult education and educational broadcasting had enlarged almost beyond recognition the spectrum of culture and recreation in leisure in Britain. On sport, he said that there was a more liberal policy of grants and capital expenditure to amateur sports clubs; these grants are now available to single-purpose, and not, as before, multi-purpose authorities. Grants to composite bodies, such as the Councils for Physical Recreation and National Playing Fields Association, were increasing and Britain had liberalized her loan policy to local authorities. It was also hoped to have a series of sports development councils on a local basis throughout Britain.

THE MELLON INSTITUTE, PITTSBURGH

THE fifty-first annual report of the Mellon Institute, Pittsburgh, covering the year ended February 29, 1964*, includes the usual list of publications and records expenditure on independent and sponsored research amounting to 6·4 million dollars. At the end of the year the Institute had a staff of 541, including 347 professional and technical workers. During the year arrangements were made for much closer working with the Carnegie Institute of Technology and the University of Pittsburgh: a non-profit corporation was formed under their joint sponsorship, with the major objective of promoting closer contact and co-ordinated efforts among members of the staffs and local industry.

In theoretical chemistry, the judicious application of the principles of molecular orbital theory and its simplification, ligand field theory, can yield a striking clarification of the various colours exhibited by transition metal ions with the architecture of the crystal lattices housing them. Work on the nature of a chemical binding in transition metal cyanides has been resumed and a general investigation initiated of the relative placement of energy-levels in other families of transition metal elements. In continuum physics, mathematical methods were developed for finding the restrictions which the Second Law of Thermodynamics places on constitutive equations, and solutions have been obtained for some unsteady flow problems in the theory of a second-order fluid, a fluid the behaviour of which approximates that of general viscoelastic fluids in slow flows. The coefficient of thermal expansion was determined for several copper germanium alloys with hexagonal close-packed structure by investigating their lattice spacings as a function of temperature between 24° and 600° C. The influence of temperature, strain rate, and interstitial impurities on the plastic deformation of poly-crystalline niobium has been investigated, and results from a thermal activation analysis supported the view that the mechanism controlling the rate of flow of dislocations at low temperatures is that of overcoming the Peierls–Nabarro barrier by the nucleation of kinks on dislocations. Electron spin resonance investigations continue to provide very detailed information on the structure of free radicals. An investigation of internal rotation in molecules by low-frequency infra-red spectroscopy has taken two new directions, one involving torsions of methyl groups attached to a phenyl ring, and the other, torsions round the central single bond in the butadiene glyoxal and acrolein systems.

A new vacuum ultra-violet spectrophotometer with low dispersion is being used to obtain the absorption spectra of systems which have electronic transitions of interest beyond the range of most commercial instruments. Studies on the chemical bonding of *tris*-2,2-dipyridyl complexes with transition metal ions in unusual low oxidation states continued, while an investigation of the different intermediate products isolated during the chemical or electro-chemical reduction of solutions containing tungstates was started. In a direct investigation of hydrogen content of acidic catalysts, it was found the total hydrogen content could be accurately determined by exchange with deuterium. The isomerization of *cyclo*propane to propylene has been examined over series of alumina and silica–alumina catalysts, and spectroscopic investigations of the chemisorption of triphenylmethane and of polynuclear aromatic hydrocarbons continued.

Other work was concerned with the total synthesis of diterpenes and diterpene alkaloids, and the effect of ring size on the nuclear magnetic resonance shifts of the saturated cyclic hydrocarbons from *cyclo*propane to *cyclo*heptodecane was investigated. A survey of the chemical and physical characteristics of the asphaltic fractions of some 40 crude oils and other bituminous materials was completed, while a broad research programme of the separation, concentration and identification of volatile components of potential food flavour import was continued. A soluble poly-*cyclo*pentadiene of relatively high molecular weight has been obtained using a new homogeneous catalyst system conveniently formed *in situ*, and the theoretical investigation of branched polymers in dilute solution was extended to include a statistical mechanical calculation of the second virial coefficient in the equation of state for 'comb' molecules. A detailed investigation was undertaken of the crystallization, kinetics and morphology of poly (tetramethyl-*p*-phenylene) siloxane. A rapid method of electrophoresis on cellulose acetate strips has been found for developing the electrophoretic components of brome-grass mosaic virus.

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* Mellon Institute. Fifty-first Annual Report of the Institute for the Fiscal Year ended February 29, 1964. Pp. 39. (Pittsburgh, Pa.: Mellon Institute, 1964.)

MIRIDS AND OTHER INSECT PESTS OF COCOA

IN view of the common problems which prevail in the field of agricultural research in West Africa, the Governments of the then British and French Territories decided to institute a series of conferences on cocoa (*Theobroma cacao* L.), oil palms, rice, groundnuts and other crops to which the Belgian, Liberian, Portuguese and Spanish Governments would be invited. The first of these conferences was on cocoa and was held at the West African Cocoa Research Institute (now Cocoa Research Institute (Ghana Academy of Sciences)) at Tafo in Ghana in December 1953 (ref. 1). Apart from a conference on Cacao Breeding[2], held in Ghana in 1956, no further conferences on cocoa occurred in this series until August 1963, when a conference on Cacao Mirid Control[3,4] was held in Ghana. At this conference information was released on the existence within Ghana of populations of the mirid (= capsid) *Distantiella theobroma* (Dist.) (Hemiptera; Miridae) resistant to lindane (gamma-BHC) and to other *cyclo*diene insecticides.

At the same conference, data suggesting the existence within Nigeria of a similarly resistant mirid, *Sahlbergella singularis* Hagl., were presented. *D. theobroma* and *S. singularis* being the most important insect pests of cocoa in West Africa, it was the feeling of the 1963 conference that the problems of resistance and control should be the subject of another meeting in March of the following year when the results of experimental work in the intervening season of high mirid populations would be available. The opportunity was also taken to include other insect pests of cocoa, and the conference was held during March 24–27, 1964, at the West African Cocoa Research Institute (Nigeria) at Ibadan, Western Nigeria. In view of the prevalence of mirid attack on cocoa in many producer countries outside Africa and of other common entomological problems, this conference was given an international bias.

The emphasis of the conference was naturally on mirid resistance and control, but Mr. P. F. Entwistle and Mr. A. Youdeowei presented a general, but preliminary, world review of cocoa mirids. Attack on cocoa by a mirid (*Helopeltis antonii* Sign.) was first noted in Ceylon in 1863 (ref. 5), and since then at least 34 more species have appeared on cocoa throughout the world. The only significant producing area which appears to have remained entirely free from attack is the Caribbean archipelago. New adaptations of mirids to cocoa have occurred in recent times; for example, during the past ten years, five species have appeared on cocoa in the Territory of Papua and New Guinea (1954–57), one species in Madagascar (1960), and two species in the State of Sabah (Malaysia) (1961–62). In addition, *H. antonii* appears to have been completely replaced on cocoa in Ceylon by *Helopeltis ceylonensis* De Silva, a species not described until 1957. *S. singularis*, which at one time appeared to be the dominant species in Ghana, has, since the wide-scale use of lindane, become much less common than *D. theobroma*[4]. This dynamic situation extends to behaviour within the crop; for example, *D. theobroma*, which now attacks both mature and seedling trees, was at one time restricted to seedlings. It is not easy to make a world assessment of the status of mirids as pests, but there is little doubt that attack is most severe in West Africa. In the New World, attack tends to be locally restricted, and this is also true of Papua and New Guinea and of Sabah, but not of Ceylon and Java, where attack is by species of the genus *Helopeltis* (which have a very wide host–plant range).

Mr. G. R. Conway reported that in Sabah a hitherto undescribed mirid species, *Platyngomiriodes apiformis* Ghauri, and *Helopeltis clavifer* Walk. (which first attacked cocoa in Papua and New Guinea in 1954), has recently begun to attack cocoa. *P. apiformis* initially confined its attacks to pods, but later began to damage stems severely and is considered a potentially more serious pest than *H. clavifer*. Mr. G. S. Dun submitted notes on a list of 249 cocoa pests in the Territory of Papua and New Guinea, prepared by Dr. J. J. H. Szent-Ivany, among which were five species of mirids which did not begin to attack cocoa until 1954.

Mr. M. Wessel presented a paper on the climate of the cocoa-growing areas of Western Nigeria, in comparison with some other producer countries in West Africa, which provided a necessary background to discussion of species distribution and the design of regionally adapted spray programmes. M. J. E. Decelle and Dr. G. Nonveiller reported there was no resistance in the Ivory Coast or Cameroun, though one population of *D. theobroma* in the Ivory Coast showed a bimodal distribution of kill when exposed to *cyclo*diene insecticides. Mr. J. N. Telford said *D. theobroma* was found to be resistant in a large area of Ghana comprising a portion of Ashanti and Eastern Region and also in a number of smaller isolated areas within these two Regions. The intensity of resistance had not been fully measured, though three areas tested contained 90–100 per cent resistant individuals. While no indication of resistance in *S. singularis* had been found in Ghana, Mr. Entwistle reported that a population of this species containing resistant individuals has been located in Western Nigeria. *D. theobroma* occurs sparingly within the area of resistance of *S. singularis* in Nigeria, but does not itself appear to be resistant.

In the Ivory Coast, M. Decelle reported that nine phosphoric esters were tested in the laboratory on cocoa mirids and the action of dichlorovos (DDVP) was noted to be particularly fast, though in comparison with 'Sumithion' it gave poor control in field trials at 0·3 per cent. Mr. G. Prins described the laboratory assessment of 16 organophosphates, five carbamates, one vegetable and one vegetable insecticide plus a synergist sprayed on to various parts of anæsthetized fourth and fifth instar nymphs of *D. theobroma* in Ghana. He assessed his results by the

$$\text{'Index of effectiveness'}\left(= \frac{\text{oral } LD50 \text{ to rats}}{LD50 \text{ to insects} \times 1{,}000}\right)$$

which is an attempt to combine safety to humans with insecticidal activity, and is used primarily by the United Nations World Health Organization. He found eleven compounds to have a higher index than lindane. Dr. B. M. Gerard suggested that 'index of acceptability' would be a better name and thought it best to ignore the index altogether when the insecticide is 'obviously safe', and to consider only its efficiency and cost. On this basis, and from some additional data, Dr. Gerard considered only seven or eight of these candidate compounds worthy of field tests.

Dr. D. G. Peterson and Mr. E. F. Bond described the final phase of their field control experiments in Ghana with 'Sumithion' and carbaryl ('Sevin 85-*S*') which, when applied by motor-powered knapsack machines adjusted to deliver 0·19 gal. per min, were equally effective, in contrast to laboratory findings which suggested that, when applied in acetone solution, carbaryl has eight times the efficiency of 'Sumithion'. In Nigeria, Mr. Entwistle, Mr. Youdeowei and Mr. W. Eguagie conducted trials with these two insecticides applied with pneumatic knapsack machines fitted with spray lances 5 ft. long, a type which many farmers already possess. These machines have a shorter distance of throw (about 5 ft. with the model used) and distribute the spray deposit less satisfactorily than motor-powered ones. Two oz. carbaryl or 1 oz. 'Sumithion' in 5 gal. water per 100 trees gives approximately 86 per cent control. At the same toxicant level, but at lower volumes, less control was achieved, and increasing the toxicant by 50 per cent, at the 5-gal. rate, did not appear to improve control. Hence volume, probably as coverage, seems to be the limiting factor.

Dr. G. E. Marshall commented on some of the problems inherent in the conduct of mirid control trials. Among variables are the mechanically disturbing effect of counting on mirid populations, recorder and spray application efficiency and differences in coverage resulting from the use of different types of machine. He pointed to the necessity for developing both intra- and inter-nationally standardized experimental procedures which will allow a comparison of findings. There is a need for developing additional screening steps between purely laboratory assessment and full-scale field trials.

Extension work in mirid control was described by Mr. J. O. Akinwolemiwa for Western Nigeria, where 600,000 farmers have been trained in mirid control during 1956–63, and by Dr. Nonveiller for Cameroun, where the 1964 anti-mirid campaign is designed to treat 350,000 acres.

Dr. J. Miyamoto reported that the poorer penetrability of 'Sumioxon' than methylparaoxon (the oxygen analogues of 'Sumithion' and methylparathion) into brain, together with the poorer formation of acid-precipitable phosphorus therein, were assumed to be the main factors in reducing the toxicity of 'Sumithion'. M. Decelle reported that carbaryl and 'Sumithion' caused no alteration in cocoa flavour, but that "three or four tasters", out of a total of "22–24", showed slight allergic reactions after tasting the 'Sumithion'-treated samples.

Mr. Youdeowei reported on progress in the development of artificial methods of rearing mirids (particularly *S. singularis*) in Nigeria, work which is of importance not only for the investigation of the genetics of resistance to insecticides, but which could also provide a supply of mirids for laboratory work at times of the year when they are scarce in the field.

Experiments in Ghana, where most of the cocoa is grown beneath the shade of forest trees, have shown that shade removal leads quickly to greatly increased yields[6]. Dr. Gerard presented a paper on the insect pests of unshaded cocoa in Ghana and advised that before widespread shade removal is recommended its side-effects should be investigated on large trial plots.

Mr. W. R. Ingram described the development of injurious infestations of the aphis *Toxoptera aurantii* B. de Fon. on seedlings in nurseries in Uganda, which he thought might be partially attributed to the previous use of DDT to control caterpillars. Menazon, a systemic insecticide, applied at 0·25 per cent, gave excellent control.

Mr. C. O. R. Everard assessed the rodent problem on cocoa in West Africa. Most losses were of pods from the trees rather than during drying or in storage. Under conditions of good husbandry pod losses should not normally exceed 3 per cent. The use of the most efficient traps available would be unlikely to reduce losses appreciably, and probably the best means of protection lies in the use of relatively non-toxic repellents applied directly to the pods.

The working session on the final day of the conference concerned itself mainly with plans for the future, and it was agreed that entomologists at present concerned with experiments in mirid control in West Africa would communicate further with the view of developing standardized systems for evaluating candidate insecticides in the field and, if possible, for sharing some of the labour involved in the conduct of tests designed to provide fermented and dried samples of beans for taint and toxic residue tests. An appeal was made to the manufacturers to develop methods which would allow the use of smaller samples in taint tests, and embody greater accuracy. Some problems could not be dealt with on a joint basis, for example, testing candidate insecticides for their effects on population-levels of other insect pests. Insects normally of only minor importance have been known to increase to pest status on cocoa following applications of insecticides[7]. While several of the species involved occur throughout West Africa, parasite and predator complexes of some of these have been shown to differ regionally. Population increases resulting from the removal of such biological restraints will vary with the effect of insecticides on the particular species complex concerned.

Delegates to the conference visited the Institute's experimental station at Gambari, twelve miles from Ibadan, where spraying machinery and methods in use in Nigeria were demonstrated and local entomological problems illustrated in the field.

The *Proceedings* of this conference will shortly be available from the Secretary, West African Cocoa Research Institute (Nigeria), P.M.B. 5244, Moor Plantation, Ibadan, Nigeria.

P. F. ENTWISTLE

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[2] Anon., *Proc. Cacao Breeding Conf.*, 1956 (1957).

[3] Gerard, B. M., *Nature*, **201**, 353 (1964).

[4] Dunn, J. A., *Nature*, **199**, 1207 (1963).

[5] Wright, H., Theobroma cacao *or Cocoa, its Botany, Cultivation, Chemistry and Diseases* (Colombo, Messrs. Ferguson, 1907).

[6] Cunningham, R. K., and Lamb, J., *Nature*, **182**, 119 (1958).

[7] Entwistle, P. F., Johnson, C. G., and Dunn, E., *Nature*, **184**, 2040 (1959).

SOIL—PLANT—ANIMAL COMPLEX

A MEETING of the Nutrition Society was held in the University of Reading on July 17 under the chairmanship of Prof. R. G. Baskett (National Institute for Research in Dairying, Shinfield). This meeting took the form of a symposium entitled "The Effects of Soils, Fertilizers and Environment on the Yield and Nutrient Content of Plants". In his introductory remarks, the chairman mentioned that the subject of the symposium was something of a departure from the range of subjects normally discussed by the Nutrition Society, but it was clear from the reactions of members and guests throughout the meeting that they considered it entirely appropriate that nutritionists should concern themselves with problems of food production.

The first paper of the day was one by Prof. J. N. Black (Edinburgh) on "The Ultimate Limits of Crop Production". He began by reviewing the major factors limiting the yield of plants which have been emphasized in the past—soil fertility, including nutrient status, temperature, water supply, pests and disease and the genetic make-up of the plant. He continued by asking the question "If it were possible to remove all the factors acting to maintain yields at a low level, what would then determine yield and what might the yield then be?". The significance of this question lies in the fact that, if answered, it would provide a yardstick with which to measure the efficiency of an agricultural system and the rate of progress of improvements in technique. Recent work on the efficiency of the photosynthetic process was then discussed, culminating in the conclusion that, in the absence of other limiting factors, the productivity of crop plants is determined by the amount of solar radiation available. The complex interrelationship between solar energy, leaf area index (the ratio of leaf area to ground area) and crop-growth rate and ultimate yield was emphasized, and the necessity for interpreting the results of field trials, involving fertilizer and other treatments, in the light of these relationships was stressed.

In his paper on irrigation as a factor in increasing food production, Dr. H. Olivier (Sir Alexander Gibb and Partners) surveyed the history of irrigation practices from pre-historic times up to the present day, followed by a critical assessment of the potential increase in food production in selected countries and regions in relation to their land and water resources.

The vital role of increased irrigation in increasing crop production in arid and semi-arid countries was emphasized. Nevertheless, doubt was expressed as to whether present nutritional standards could be maintained in many of these countries if the present-day population forecasts turn out to be correct, even if potentially irrigable land and water supplies are fully exploited. The considerable value of supplemental irrigation for increasing crop production in countries such as the United States and Western European countries, including the United Kingdom, is much less generally recognized and it is a technique which no country in the humid zone can afford to neglect.

Dr. Olivier ended his paper by considering the present outlook for food production and concluded that no reliable estimate of world potential was possible at present owing to a lack of basic information on such vital matters as potential agricultural land and effective rainfall, particularly in areas where the most serious food shortages occur. He stressed the need for the extension of soil surveys, for the reorganization of meteorological observations to make them more meaningful for agricultural purposes and for the compilation of statistical data on crop yields by region and by soil types.

The relationship between plant nutrient content of soil and leaves and crop yield was discussed by Dr. C. Bould (Long Ashton Research Station). He began by summarizing the present state of knowledge concerning the factors affecting the availability to plants of the inorganic ions in the soil and outlined the difficulties inherent in chemical methods of assessing the nutrient status of soils. A biological method, namely leaf analysis, has been shown to provide a more reliable assessment of plant nutrient availability. It is possible to establish for many species a range of nutrient concentrations at specific stages of growth associated with gross deficiency, marginal supply and sufficiency, based on crop yield. However, it is not yet possible to predict how much fertilizer is required to raise the nutrient status of a crop from deficiency to sufficiency, although this may be possible when more precise information relating soil nutrient status to plant nutrient content is available for different soils and crops.

Prof. A. H. Bunting (University of Reading) read a paper on "Effects of Organic Manures on Soils and Crops", and presented strong evidence derived in part from the results of more than 100 field trials conducted from the Rothamsted Experimental Station that the effects on crop yield of organic manures based on straw are for the most part produced by the plant nutrients which the manures contain. On soils which tend to compact, strawy manures, but not sewage sludge, increase the yield of crops to a greater extent than can be explained by their content of plant nutrients. This appears, as work at the National Vegetable Research Station, Wellesbourne, and Rothamsted Experimental Station has shown, to be the consequence of a change in the geometry of the soil which prevents it from compacting and increases the volume of larger pores, and consequently the amount of water which can be held at low tension. In ley fertility experiments, the effects of leys on subsequent crops have proved to be largely due to increases in the supply of plant foods rather than to increases in the proportion of water-stable crumbs. Organic matter *per se* does not appear to have any special properties influencing crop yield except on special soils where the effects are shown only by particular types of organic matter. It is now important to concentrate experimental work on these special soils (which are thought to occupy about one-tenth of the arable land of England), using factorial experiments so designed that the effects of nutrients can be measured separately.

The final paper was one by Dr. R. Waite (Hannah Dairy Research Institute, Ayr) on "The Chemical Composition of Grasses in Relation to Agronomical Practice". He emphasized that modern techniques of grassland management have resulted in greatly increased yields of digestible nutrients and presented data from his own experiments carried out at the Hannah Dairy Research Institute on the changes in composition of leafy herbage from several species of grasses during the growing season. The most significant change was in the fructosan : protein ratio, which was high in the early part of the season and low in the autumn. The former type of herbage would be expected to promote a predominantly propionic acid type of rumen fermentation, which would stimulate the production of non-fatty solids in the milk of cows and promote fattening in beef animals. Herbage low in soluble carbohydrates, on the other hand, would encourage an acetic acid type of fermentation in the rumen and the resulting mixture of volatile fatty acids would be used more efficiently for butter-fat than for solids-not-fat production or for fattening. Herein may well lie the superiority commonly attributed to spring compared with autumn grass for fattening and milking animals.

The symposium ended with a summing-up by Prof. P. W. Arnold (University of Newcastle upon Tyne), who high-lighted the major themes from the various papers and directed attention to some of the important gaps in our knowledge of the way in which plants interact with their environment.

The majority of papers read at this meeting were mainly concerned with the quantitative aspect of the production of plant foods and, indeed, this aspect covers a vast range of problems. The qualitative aspect of the influence of the environment on plants for human and animal consumption is of at least equal interest to nutritionists and might well provide the subject for another symposium.

The re-statement at this meeting of the fundamental dependence of the animal kingdom on the plant was most salutary, and a reminder of the rate at which the total food requirements of the human population of the globe are increasing can never be given too often. It is clear, however, that basic information on the methods by which the production of plant foods can be increased by the use of fertilizers, by growing improved varieties of crops, by irrigation and by better husbandry is freely available. The application of this knowledge is largely a problem of administration, education, finance, power supply, engineering and industrial production, and the avoidance of famine in the latter quarter of this century depends on its solution. The outlook is not wholly depressing, for it has been estimated that by the adoption of improved agricultural techniques, including the more efficient use of water, it would be possible to double the present yield from each acre of land at present under cultivation.

T. G. TAYLOR

SEARCHING FOR CREATIVITY

THE search for creativity—in all its forms—would seem to be commendable and, indeed, of supreme importance for the future of man, yet, reviewing progress so far achieved, P. E. Vernon can rate it no higher than the "latest fashion in American educational psychology"[1]. Those who have interested themselves in the problem have been moved by the impression that the whole educational system fosters conformist attitudes among pupils and students, which gain further support from present-day intelligence and aptitude tests. Prof. Vernon doubts if these strictures necessarily apply to the selective tests of pupils in England and Wales at 11+ and 18+.

He refers to the work of Calvin Taylor, who, on the basis of a number of investigations, including some carried out on industrial and Air Force scientists, maintains that in adult life there is little association between productivity and originality, and previous educational achievement; in fact, the correlations between the results of tests for creativity on one hand, and intelligence on the other, are not greater than 0·2–0·4.

In this context, Prof. Vernon thinks that L. M. Terman's long-term follow-up of a group of children of high intelligence quotient merits attention, both because the test used—Stanford–Binet—was a good one, and because the scores obtained in tests in early youth have been compared with later achievement over the best part of a life-time. As a whole, the group has been very productive in the arts and sciences, although scarcely any member could be characterized as a genius. Here, intelligence is of some relevance, as it is in the findings of Terman and C. M. Cox that, from their biographies, few men in history of outstanding ability had an intelligence quotient below 120; while D. W. Mackinnon's results suggest that above a certain minimum figure, about 120, intelligence quotient plays a relatively minor part in creativity.

J. P. Guilford has carried out a long series of investigations of high-grade adults, and claims to have identified about 60 separate factors concerned with mental ability. Prof. Vernon questions the validity of these factors, partly because they have not received full confirmation from other workers but chiefly because he considers that in itself factorial analysis of mental quality cannot produce the answers unless the factors correspond with what is recognized as 'originality' in daily life.

Another series of tests, similar to Guilford's, has been applied by J. W. Getzels and P. W. Jackson, this time on young high-school pupils. In their case, one group in the top 20 per cent on creativity tests, but not of outstanding intelligence quotient, were compared with another group from the top 20 per cent for intelligence but not of outstanding originality. In scholastic achievement both groups did about equally well; but since, as Vernon points out, the intelligence quotient of the group of high creativity averaged 127, as against 150 for those of higher intelligence quotient, the finding is of limited significance.

In this survey, however, on personality traits and sociometric status, the group of superior intelligence quotient were rated by their teachers higher than the more creative, which leads Vernon to suggest that, in England and Wales, some of the more 'original' 18-year-olds may fail to find places at universities because they were unpopular with their head teachers, and not good mixers.

As C. Burt has pointed out, in the researches by Getzels and Jackson, correlations between separate tests for creativity were about on the same level as they were with intelligence tests, so that in those studies Vernon can find no evidence for a factor for creativity distinct from *g* and *v*. He doubts whether those for creativity cover more ground than the typical battery of tests for verbal intelligence, English and arithmetic already in present-day use. His main criticism, however, is that the tests which Getzel, Guilford and others have devised give no support to the assumption that they are valid for creativity in general: follow-up data are not available, and the gap between tests at about the time of entry to the secondary school and true creativity at the university is much too great.

As indications of originality, Prof. Vernon holds that tests like these scarcely go beyond empirical impressions which indicate that, in general, creative people tend to be rather solitary, independent, non-conformist, with strong drives and motivations on distant goals (Taylor); lone wolves with an overriding sense of the worth of their own ideas (Anne Roe); far more given to intuition than sensation type thinking, with a strong streak of femininity, often having suffered from unhappy childhoods and being rebellious at school and university (Mackinnon); and displaying humour and wit, while being unconventional in their attitudes and aspirations (Getzels and Jackson). Besides, such criteria fail to distinguish really creative people from rebels, delinquents, beatniks, introverts, idealists, etc. Prof. Vernon's opinion is that leisure time interests between 12 and 16, like scientific hobbies and research, poetry and artistic activities, may have some predictive value; but, altogether, efforts may be misdirected if they set out to assess creativity in general, for it may turn out to be strongly individual and specific.

In final conclusion, Prof. Vernon disclaims any desire to disparage research into the assessment of creativity, but in his mind the crucial problem is to devise tests which do not require a long follow-up. He feels that present appraisals by schools and universities are heavily biased in favour of conventional attainment, but research into home, leisure and educational backgrounds might be rewarding, and the influence of an inspiring teacher might be tremendously important.

R. WEATHERALL

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RECORDINGS FROM SATELLITE *Alouette I*

A Very-low-frequency Plasma Resonance

AN unusual band of noise observed in the *Alouette I* satellite has been reported by Barrington and Belrose[1], who noted that the band had a sharp lower frequency cut-off which usually increased in frequency with decreasing latitude of the satellite. Since this lower cut-off frequency varies consistently with the location of the satellite, it is deduced that the observed changes in this frequency arise from spatial rather than temporal effects. Because the lower cut-off frequency is sharp and changes measurably within a few seconds, we may deduce that the horizontal field of view of the satellite for this band is at most a few tens of kilometres.

From observations of triggering of this band by both atmospherics and whistlers, Brice *et al.*[2] concluded that the noise was generated at the same height as the satellite. Thus the observed noise band is generated in the immediate vicinity of the satellite. Other evidence has been obtained suggesting that triggering of this band is enhanced for triggering signals propagating with large angles between the wave normal and the Earth's magnetic field. Furthermore, from examination of simultaneous very-low-frequency recordings made by the satellite and by ground-based stations, it is found that the *Alouette* hiss band is never observed on ground-based recordings.

The observation of spikes in the *Alouette* top-side sounder at the resonance frequencies for the ambient plasma[3] suggests the *Alouette* hiss band arises from a similar plasma resonance at very low frequencies. The only resonance for the frequencies of interest (5–10 kc/s) at the satellite height is the lower hybrid resonance[4] which defines a cut-off frequency for propagation transverse to the Earth's magnetic field. Other features of this resonance support the hypothesis that the lower cut-off frequency of the *Alouette* hiss band is the lower hybrid resonance for the ambient plasma.

In a subsequent paper, it will be shown that from a knowledge of the electron plasma and gyrofrequencies and the lower hybrid resonance frequency, an effective mass for the ions in the ambient plasma may be determined. Thus this hiss band may provide a powerful diagnostic tool for determining the ionic constituents of the plasma surrounding a satellite.

Assuming that the suggestion made above is correct, the observation of the *Alouette* hiss band provides, to the best of our knowledge, the first experimental confirmation of the existence of the lower hybrid resonance.

We thank Mr. John Katsufrakis of Stanford for pointing out a number of the unusual features of the *Alouette* hiss band.

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Triggered Very-low-frequency Emissions

Barrington and Belrose[1] have reported that on occasion the very-low-frequency recordings from *Alouette I* satellite (altitude, 1,000 km) showed bursts of noise triggered by atmospherics which had propagated upwards to the satellite (that is, short fractional-hop whistlers) suggesting that very-low-frequency emissions were triggered at heights of less than 1,000 km.

Since some hypotheses for the generation of very-low-frequency emissions suggested generation in or near the ionospheric *F*-region[2,3], while for other hypotheses generation is more likely near the top of a magnetic field line path[4–6], it might be suggested that triggered emissions observed in *Alouette* could help to identify the mechanism responsible for generation of very-low-frequency emissions observed on ground-based recordings. However, further

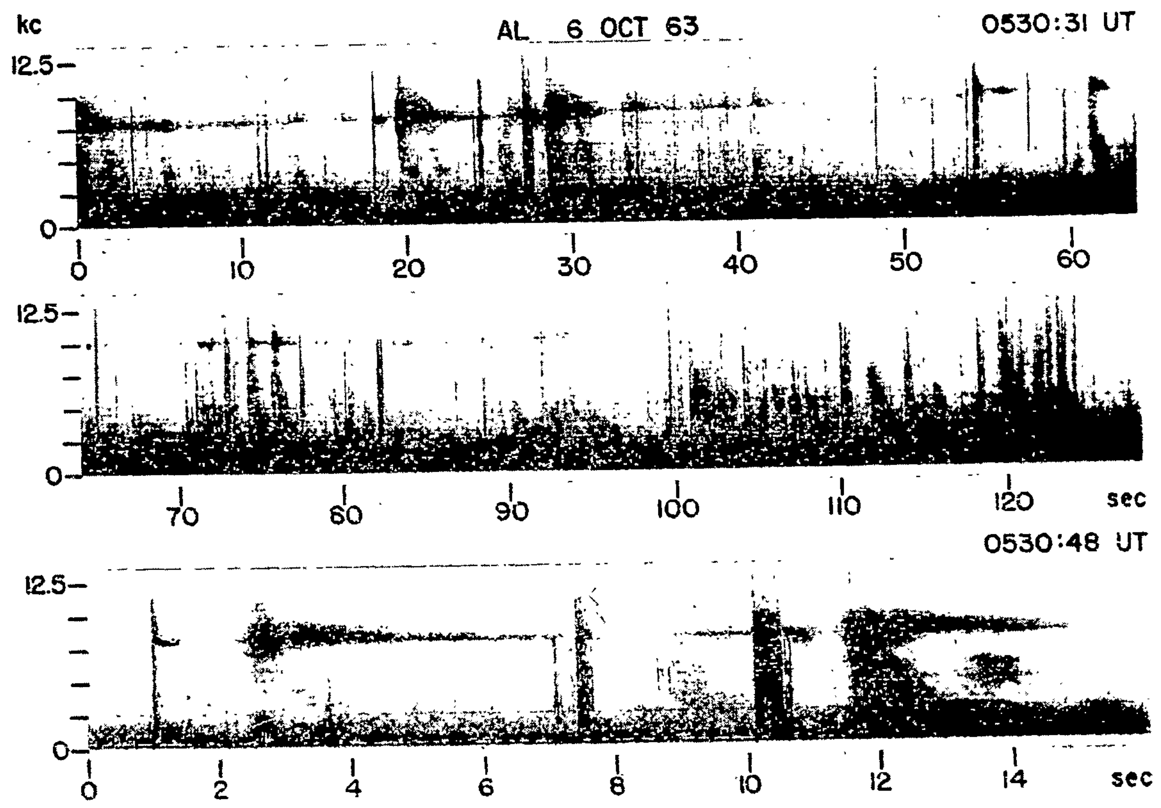

Fig. 1. Very-low-frequency spectra from *Alouette I* showing triggering by atmospherics and whistlers

investigation indicates that emissions triggered by atmospherics observed in *Alouette* are not related to very-low-frequency emissions observed on ground-based recordings[7].

Barrington and Belrose[8] reported an unusual band of noise found in *Alouette* very-low-frequency recordings. This band showed a sharp lower frequency cut-off which increased in frequency as the satellite moved to lower latitudes. This band will be tentatively referred to as the *Alouette* hiss band. Barrington and Belrose[1] noted that the frequency of emissions triggered by atmospherics varied with the satellite location in a manner similar to that of the *Alouette* hiss band already noted here. It has now been found that these phenomena are even more closely related, in that on several occasions the emissions triggered by atmospherics were, in fact, enhancements of the *Alouette* hiss band. Furthermore, this band may also be triggered by whistlers as is illustrated in Fig. 1. The lower record of Fig. 1 is a section of the upper record (17–33 sec) shown with an expanded time-scale. Fig. 1 shows spectra obtained during a north–south pass over Stanford, California, and shows the characteristic increase of frequency with decreasing latitude and the sharp lower frequency cut-off of the hiss band. The range of L values for which the noise is seen in Fig. 1 (3·15–2·59) is consistent with that reported for the *Alouette* hiss band by Barrington *et al.*[9]. The triggering by atmospherics (propagating upwards) indicates that the hiss is generated at or below the satellite (as had been suggested by Barrington and Belrose[1]) and triggering by whistlers (believed to be propagating downwards) suggests generation at or above the satellite. Thus we may deduce that the observed noise band is generated at the same height as the satellite.

Barrington *et al.*[9] reported that examination of very-low-frequency recordings obtained during a pole-to-pole pass showed evidence that the sharp lower-frequency cut-off of the *Alouette* hiss band was very closely related to the magnetic L value and that for a given value of L the lower frequency cut-off appeared to be the same in both hemispheres. Further investigation of other recordings has shown that the close conjugate relationship with L does not always hold. A possible generation mechanism which does not require magnetic conjugacy is discussed in a companion paper by Brice and Smith[7].

Alouette very-low-frequency recordings are made at Stanford from telemetry provided by the Canadian Defence Research Telecommunications Establishment in Ottawa.

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DOES QUANTUM MECHANICS EXCLUDE LIFE?

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A LIVING object and its nutrient environment is a large quantum mechanical system. By supposing such a system to be enclosed in a container impervious to outside influences, the system may be considered to be 'closed', and quantum mechanical concepts such as Hamiltonian, wave function, and the like should be applicable to it. The question arises if, on including in such a system only a nutrient, the spontaneous generation of life is quantum mechanically likely. Secondly, is a system of nutrient together with one living organism quantum mechanically likely to produce a system containing two organisms? This is the problem of self-reproduction.

Both questions are at present beyond anything but the most speculative kind of mathematical consideration. Previous discussion of related questions by physicists exists[1-3], and the present remarks are related to the most recent of these (by Wigner[4]). A review of these questions from a biological point of view has also been published recently[5].

Wigner dealt with the second of the foregoing questions, and found that quantum mechanics leads to practically zero probability for the existence of self-reproducing states. He concluded that either present quantum mechanics cannot furnish an explanation of all that exists, or it should at least be modified to include aspects, such as consciousness, which are not needed when dealing with purely physical problems.

This conclusion makes it desirable to examine if, by broadening the main assumption and the main definition underlying Wigner's argument, it is possible to regard the empirical fact that life exists as not necessarily in contradiction with quantum mechanics. The key assumption in Wigner's paper is that the collision matrix governing the interactions in the system is a random symmetric matrix without special properties. In the present (somewhat different) approach, a weaker statistical assumption is used, and, in a special case, Wigner's conclusion is recovered. This weaker assumption does allow, however, for the possibility that the spontaneous generation of life and the existence of self-reproducing states are compatible with quantum mechanics. The key definition in Wigner's paper is that a state S is a living state if there exists a state of another ('nutrient') system such that the interaction leads with certainty to two systems in state S. If two such states are only occasionally produced (with some probability), then Wigner's argument does not hold[6]. In the present article the important problem of defining a 'living' state quantum mechanically is not faced except in a schematic manner (relations (3), below). While this is a shortcoming, it does enable one to achieve a broader understanding of the problem.

The present approach commences with the observation that the state vector of an isolated system moves with lapse of time through various regions of the appropriate energy shell in phase space. The question arises of whether there are regions in that shell which correspond to the presence of a living organism.

Immediately after death an organism can certainly be treated as a normal physical system which can in principle be fully specified. By arguing back from such a system in what is in principle a definite state, and including with it in an enlarged system, radiation and a nutrient environment, one arrives at the notion of a new physical system S. At the present state of knowledge this must be incompletely specified, but one can imagine its description and its initial state to be given in principle by a large amount of numerical information y. Whereas y is unknown, the lack of precise information about it can be made up by a statistical assumption about y. By then averaging over all y in a certain way, y disappears from the problem. There can be no doubt that this is a very superficial and rough procedure, but at present it is difficult to see how anything more definite is possible. One has, therefore, effectively replaced one system S by a class of systems. The symbol S will be retained for this class.

It is reasonable to suppose that each system (of the class of systems S arrived at in this way) has a region in its energy shell which corresponds to the presence of a living organism. In order for a class S of systems to be discussed together it is supposed here that they all have: (*a*) the same system energy; (*b*) roughly the same 'volume' of permitted states in the corresponding energy shell; (*c*) roughly the same 'volume' in that shell which corresponds to the presence of at least one living organism. In fact, these 'volumes' are replaced by numbers of distinct quantum states of the system (denoted by L_0 and L_1 in section 1).

From what has been said the problem is seen to be related to the ergodic theorem of statistical mechanics. In the more quantitative discussion which follows, results are utilized which were obtained earlier in connexion with this theorem[7]. This article contains additional mathematical information, but the results which are useful in the present context are re-derived here.

(1) Mathematical Formulation

Consider a large and isolated quantum mechanical system in some initial state at time $t = 0$. To define the system S itself and its initial state, values have to be given to a large number of variables. The symbol y will be used to denote them all. For example, the system S may consist of radiation, inorganic and organic matter. When an initial condition y is referred to, it is intended that y includes a full specification of, for example, the amount and nature of the radiation present initially. Thus, when y is given, it is supposed that the system is quantum mechanically specified. The Hilbert space of the system then contains wave-functions belonging to many different total energies. The present interest is confined to a sub-space which contains only those states which are energetically compatible with the initial condition y of the system. Such states are sometimes described as lying in an 'energy shell' in phase space. It will be denoted by the symbol Λ_0. Let its dimensionality be L_0. Each possible state of the system is then represented by a unit vector in Λ_0.

The existence of at least one living organism of any kind at time t is then expressed mathematically by the statement that the wave-function $\Psi(t)$ of the system lies wholly in the appropriate sub-space Λ_1 of Λ_0 having dimensionality L_1 say. Similarly a wave function $\Psi(t)$ lying wholly in a sub-space Λ_2, of dimensionality L_2, implies a state of the system at time t with at least two living organisms. This hierarchy may be continued until a sub-space Λ_r (corresponding to a state of at least r living organisms) is reached such that:

$$L_r \geqslant 1,\ L_{r+1} = 0 \qquad (1)$$

The isolated nutrient at time $t = 0$ can then not give rise to more than r organisms at time t. One must have clearly:

$$L_0 \geqslant L_1 \geqslant L_2 \geqslant \ldots L_{r-1} \geqslant L_r > L_{r+1} = 0 \qquad (2)$$

It is reasonable to suppose that, in fact, each L_{i+1} is very much smaller than the preceding L_i. In particular, the overwhelming majority of the states of the nutrient will correspond to no living organisms being generated:

$$L_0 \gg L_1 \gg L_2 \ldots \gg L_r \gg 1 \qquad (3)$$

Suppose the orthonormal vectors $\omega_1, \omega_2, \ldots \omega_r$ are a basis for Λ_r, and that this set is completed to yield a basis for Λ_{r-1}, then it is again completed to yield a basis for Λ_{r-2}, etc., until one has a basis for Λ_0.

The wave function of the system at time t is given by

$$\Psi(t) = A_t \Psi(0)$$

where A_t is a unitary time evolution operator. $\Psi(0)$ refers to the initial state and so depends on the values of the variables y. The projection of $\Psi(t)$ on a co-ordinate axis ω_j is:

$$a_{t,j}(y) \equiv (\Psi(t) . \omega_j) = (\Psi(0) . A_t^{+} \omega_j)\ (j = 1, 2, \ldots) \qquad (4)$$

and depends on y through $\Psi(0)$. The initial state is a vector in an L_0-dimensional space. The probability of finding the system at time t in a state with at least one living organism is:

$$u_1(t) = \sum_{j=1}^{L_1} |a_{t,j}|^2 \qquad (5)$$

and depends on y.

Consider now all initial conditions y leading to the same system energy, to L_0-values which are all of the same order of magnitude, and to L_1-values which are all of the same order of magnitude. If these initial conditions exist at all, there will be many of them, and they will lead to different interactions in the system. It is desirable to average over the conditions y as explained in the introductory remarks. One finds for the average probability of finding the system at time t in a state with at least one organism.

$$< u_1(t) > = \sum_{j=1}^{L_1} < |a_{t,j}|^2 > \qquad (6)$$

The spread of the individual values of $u_1(t)$ due to different initial conditions y is given by the second moment:

$$Z_1 \equiv \frac{<[u(t) - <u(t)>]^2>}{[<u(t)>]^2} = \frac{<u(t)^2>}{<u(t)>^2} - 1 \qquad (7)$$

Some assumptions must now be made to evaluate equations (6) and (7).

(2) Statistical Assumption

Consider equation (4). Here $\Psi(t)$ depends on the initial conditions y and is a unit vector in the L_0-dimensional space. Its components are complex, so that $\Psi(t)$ is specified by $2\,L_0$ real numbers. The simplest statistical assumption one can make about the initial conditions y is to suppose that they lead to a wave function $\Psi(t)$ such that these $2\,L_0$ numbers are normal variates which are independent (except for normalization). Viewed geometrically, this implies a probability distribution for the end point of $\Psi(t)$ over the surface of a sphere such that the probability of finding it in any area of the surface is proportional to that surface area. This distribution of $\Psi(t)$ will be called the strictly uniform distribution because it makes no *a priori* distinction between the different states of Λ_0. It leads to:

$$< |a_{t,j}|^2 > = 1/L_0,\ < |a_{t,j}|^4 > = 2/L_0(L_0 + 1) \qquad (8)$$

$$< |a_{t,i}\, a_{t,j}|^2 > = 1/L_0(L_0 + 1)\ (i \neq j)$$

There is no need here to prove equations (8), since a procedure will now be described which is more general, and yet simpler. Note that the averages (8) are independent of the co-ordinate on which $\Psi(t)$ is projected. This property will be used to define a weakly uniform distribution of $\Psi(t)$. This name is a reminder of the fact that the states of Λ_0 are no longer treated as exactly on the same footing.

It will be supposed that the distribution of permissible initial conditions y is weakly uniform so that, in generalization of equation (8):

$$< |a_{t,j}|^2 > \equiv P_1,\ < |a_{t,j}|^4 > \equiv Q_1 \qquad (9a)$$

are independent of j and the average:

$$< |a_{t,i}\, a_{t,j}|^2 > \equiv R_1\ (i \neq j) \qquad (9b)$$

is independent of i and j.

The suffix 1 for P, Q and R shows that the average is over only those initial conditions y which lead to L_0-values which are all of the same order of magnitude, and to L_1-values which are all of the same order of magnitude.

The simplest example occurs for vectors with real components in a plane ($L_0 = 2$). $\Psi(t)$ is then a unit vector in a plane and there are only two functions $a_{t,j}$ in equation (4), namely, $a_1 = \cos\alpha$, $a_2 = \sin\alpha$, where any one value of α ($0 \leqslant \alpha < 2\pi$) is the result of the initial conditions y. Suppose that the uncertain initial conditions are such that the probability of finding an angle in the range (α, $\alpha + \mathrm{d}\alpha$) is given by:

$$f(\alpha)\mathrm{d}\alpha = A \sin^b(2\alpha)\mathrm{d}\alpha$$

A is a normalizing constant. Thus for a strictly uniform distribution ($b = 0$):

$$P_1 = \tfrac{1}{2},\ Q_1 = \tfrac{3}{8},\ R_1 = \tfrac{1}{8}\ (b = 0)$$

A weakly uniform distribution is obtained, for example, if $b = 2$, when:

$$P_1 = \tfrac{1}{2},\ Q_1 = \tfrac{5}{16},\ R_1 = \tfrac{3}{16}\ (b = 2)$$

In the second case it is seen that the two states of Λ_0 are not treated as entirely on the same footing. (The equations (8) with $L_0 = 2$ do not give the foregoing result for $b = 0$. This is merely due to the fact that it is presupposed in (8) that all vectors have complex components.)

It is now easily shown by averaging:

$$\sum_{j=1}^{L_0} |a_{t,j}|^2 = \sum_{i=1}^{L_0} \sum_{j=1}^{L_0} |a_{t,i}\, a_{t,j}|^2 = 1$$

that, using equations (9):

$$P_1 = Q_1 + (L_0 - 1)R_1 = 1/L_0 \qquad (10)$$

Thus the distribution (9) has only one unidentified parameter. It is convenient to treat Q_1 as unknown, and work with:

$$X_1 \equiv L_0{}^2 Q_1 - 1 \qquad (11)$$

as a parameter. Then[7]:

$$L_0 - 1 \geqslant X_1 > 0 \tag{12}$$

Note that for a strictly uniform distribution (8) leads to:

$$X_1 = (L_0 - 1)/(L_0 + 1) \sim 1 \tag{13}$$

Using equations (9), (10), (11) in (6) and (7), one finds (see appendix):

$$< u_1(t) > \ = L_1/L_0 \tag{14}$$

$$Z_1 = \frac{L_0 - L_1}{L_0 - 1}\,\frac{X_1}{L_1} \tag{15}$$

Since $< u_1(t) >$ is independent of time, it must be regarded as the probability, averaged over the initial conditions y, of these initial conditions giving rise to at least one living organism at any subsequent time.

One finds from equations (3) and (14) that the initial conditions will on average be exceedingly unfavourable for spontaneous generation of life. But it is exeedingly unfavourable for the overwhelming majority of initial conditions y only if $Z_1 \ll 1$. For this inequality would indicate a strong clustering of the individual $u_1(t)$-values very close to the mean value (14) and this is seen to be very small. If one adopts (3) $Z_1 < X_1/L_1$. If one also uses equation (13), thus assuming a strictly uniform distribution, one does, in fact, find that spontaneous generation has always practically zero probability. There is, however, the crucial question of the size of X_1, in the case of a weakly uniform distribution of the wave function $\Psi(t)$. By equations (2) and (12) $X_1 \sim L_1$ is possible, and would lead to $Z_1 \lesssim 1$. In this event the spread of the unaveraged probability $u_1(t)$ would be sufficient to guarantee the occasional spontaneous generation of life.

(3) Self-duplication

The foregoing considerations may be extended to discuss the question of the existence of a self-reproducing unit. In the text following equation (4) one need merely make the replacements:

$$\text{suffices } 0 \longrightarrow \text{suffices } 1,\ \text{suffices } 1 \longrightarrow \text{suffices } 2 \tag{16}$$

Thus the probability of finding the system at time t in a state with at least two living organisms is:

$$u_2(t) = \sum_{j=1}^{L_2} |a_{t,j}|^2 \tag{17}$$

One now considers only those initial conditions y which, apart from having the same system energy, lead to L_1-values which are all of the same order of magnitude, and which lead also to L_2-values which are all of the same order of magnitude. The averages (9) are over the wave functions $\Psi(t)$ corresponding to these conditions y, and are now given a suffix 2. One finds instead of equations (14) and (15):

$$< u_2(t) > \ = L_2/L_1 \tag{18}$$

$$Z_2 = \frac{L_1 - L_2}{L_1 - 1}\,\frac{X_2}{L_2} \tag{19}$$

This shows again in virtue of equation (3) that the initial conditions will be exceedingly unfavourable to self-duplication. The spread of the probabilities $u_2(t)$ is determined by X_2 which is subject to the analogue of (12):

$$L_1 - 1 \geqslant X_2 > 0 \tag{20}$$

Thus if:

$$X_2 \gtrsim L_2$$

Z_2 is large enough to guarantee the occasional occurrence of initial conditions favourable to self-duplication.

If it is assumed that the distribution of $\Psi(t)$ is strictly uniform, one may use the analogue of (13)

$$X_2 = (L_1 - 1)/(L_1 + 1) \tag{21}$$

This is of order unity, so that $Z_2 \ll 1$. One must conclude that for all initial states considered, the probabilities u_2 lie very close to the exceedingly small mean value (18). Self-reproducing units must then be expected to have practically zero probability. This is essentially Wigner's conclusion which was mentioned in the introductory remarks.

(4) Conclusion

The crucial step in the argument presented here resides in the statistical assumption which has been made. While this assumption, though rough, may be justifiable in the case of equilibrium systems, it is less reasonable in the case of the non-equilibrium situations discussed here. There are, however, two reasons for using it. First, it is known[9] that in some cases non-equilibrium situations may be treated by the methods of equilibrium statistical mechanics. Secondly, some averaging process is clearly needed, and that adopted here appears to be the simplest which retains some degree of flexibility since it contains the parameter X_1. For the most sweeping averaging process $X_1 \sim 1$ (equation (13)), $X_2 \sim 1$ (equation (21)). If this is assumed, the probability of finding either spontaneous generation of life, or a self-reproducing state, appear to be practically zero. This is in agreement with Wigner's result. However, it may be that larger values of X_1 or X_2 are more appropriate. The probabilities are then more favourable and Wigner's contradiction between quantum mechanics and the existence of self-reproducing units can be avoided.

I thank Prof. E. P. Wigner and Dr. I. E. Farquhar for comments on the manuscript.

Appendix: Proof of (15)

By (5) and (9)

$$< u_1^2 > \ = \ < \sum_{j,k=1}^{L_1} |a_{tj} a_{tk}|^2 > \ = \sum_{j=1}^{L_1} < |a_{tj}|^4 > + \sum_{j \neq k}^{L_1} < |a_{tj} a_{tk}|^2 > \ = L_1 Q_1 + L_1(L_1 - 1)R_1$$

Eliminating R_1 by (10),

$$< u_1^2 > \ = \frac{L_1(L_0 - L_1)}{L_0 - 1}\,Q_1 + \frac{L_1(L_1 - 1)}{L_0(L_0 - 1)}$$

By (7),

$$Z_1 + 1 = \frac{< u_1^2 >}{< u_1 >^2} = \frac{L_0^2(L_0 - L_1)Q_1 + L_0(L_1 - 1)}{L_1(L_0 - 1)}$$

Hence one finds (15) in the form

$$Z_1 = \frac{L_0 - L_1}{L_1(L_0 - 1)}\,(L_0^2\,Q_1 - 1).$$

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CHEMICAL POTENTIALS AND ELECTRICAL POTENTIALS IN BIOLOGICAL SYSTEMS

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WHEN an aqueous solution of a protein or another non-dialysable electrolyte is separated by a dialysing membrane from an outer solution, there is an equilibrium between the two solutions in which the distribution of all the diffusible ions is determined by the colloidal charge[1]. The distribution ('Donnan equilibrium') is characterized by the property that the ratios of all ions of the same charge are equal in the two phases. If, for example, the ratio of sodium to potassium is 10 : 1 in the inner solution, it is the same in the outer solution, despite the fact that the inner and outer concentrations may be quite different. A system conforming to these conditions may be said to show the property of perfect or unrestricted mixing of the ions in the two phases; the distribution is perfectly non-selective. In such a system there is a membrane potential which can be calculated from any of the ion distribution ratios by means of an ideal form of the Nernst equation. When allowance is made for the ionic charge, all the ratios are constant. Therefore, there is a simple correlation between the membrane potential and the distribution ratio for any kind of diffusible ion[1]. This is not generally the case in biological structures where insoluble and immiscible phases co-exist and where the ideal equations fail to describe ionic distributions and electrical potentials. A more general treatment is required which takes account of changes in standard chemical potential of all the ions[2,3]. Ideal Donnan and Nernst relations represent a limiting case when these values approach zero.

The most general formulation of heterogeneous equilibrium was given by J. Willard Gibbs[4]. The equations apply to the thermodynamic conditions for stability of a physicochemical system consisting of n components distributed among p co-existent phases. The phases are defined in the most general sense, and may be liquid, gaseous or solids in any possible states of aggregation. In general, there can be no perfect mixing of the diffusible components in heterogeneous systems, and the phases usually differ greatly in composition except in the neighbourhood of certain critical states. It will be shown here that the distribution is determined by the changes of standard chemical potential, $\Delta\mu^0$, of all the ions between any two phases, and that these quantities also account for the deviations of electrical potential differences from the limiting form of the Nernst equation.

Distribution of ions in biological systems. In the derivation of the phase rule, Gibbs[4] showed that for any component, 1, which passes freely across a boundary between any two phases, the equilibrium condition of minimal free energy requires that the chemical potential, μ_1, be constant. If there are p such phases:

$$\mu_{1'} = \mu_{1''} \ldots. = \mu_{1^p} \tag{1}$$

Hence for each such component there are $(p - 1)$ conditions of equilibrium among the p phases. Between any two phases:

$$\Delta\mu_1 = 0 \tag{2}$$

Equations (1) and (2) are thermodynamic and are perfectly general. They do not depend on special properties of any of the phases. The chemical potential of any component is a force function which determines the stability of any distribution involving that component. The change of chemical potential, $\Delta\mu$, of any process measures the driving force and the direction of the corresponding physicochemical change. When $\Delta\mu$ for such a process is zero, the driving force is zero. When all such possible changes of chemical potential of the n components in p phases are zero, the system is in a state of minimal free energy and of maximal stability. In the case that a number of substances are constrained to cross a phase boundary in certain fixed proportions, the equilibrium condition for the two-phase distribution is:

$$a\mu_{1'} + b\mu_{2'} + c\mu_{3'} \ldots = a\mu_{1''} + b\mu_{2''} + c\mu_{3''} \ldots \tag{3}$$

where a, b and c are constants defining the proportionality factors (ref. 4, equation 78). Applied to an electrolyte such as sodium chloride, both a and b are 1. Then the equilibrium condition requires that:

$$\mu_{\mathrm{Na}'} + \mu_{\mathrm{Cl}'} = \mu_{\mathrm{Na}''} + \mu_{\mathrm{Cl}''} \tag{4}$$

and

$$\Delta\mu_{\mathrm{Na}} + \Delta\mu_{\mathrm{Cl}} = 0 \tag{5}$$

For calcium chloride, a is 1 (Ca) and b is 2 (Cl). In general, the distribution of i kinds of mobile ions would be expressed by the following conditions:

$$\frac{\Delta\mu_1}{z_1} = \frac{\Delta\mu_2}{z_2} = \frac{\Delta\mu_3}{z_3} \ldots. = \frac{\Delta\mu_i}{z_i} \tag{6}$$

where z_i is the charge of the ionic species i. It is taken as positive for cations and as negative for anions. For a two-phase system containing Na, K and Cl ions:

$$\Delta\mu_{\mathrm{Na}} = \Delta\mu_{\mathrm{K}} = -\Delta\mu_{\mathrm{Cl}} \tag{7}$$

Equations (5) and (7) state as an equilibrium condition that the tendency of sodium or potassium to pass from phase 1 to phase 2 is equal to that of chloride to pass from phase 2 to phase 1. The tendency of sodium chloride or potassium chloride to cross the phase boundary is therefore zero (equation (4)).

Equations of this form have been applied by us to the description of ionic distributions in biological systems[3]. For the distribution of sodium and potassium in normal cells and tissues the relation takes the form:

$$\Delta\mu_{\mathrm{K}^0} - \Delta\mu_{\mathrm{Na}^0} = -RT \ln \frac{r_{\mathrm{K}}}{r_{\mathrm{Na}}} \tag{8}$$

where $\Delta\mu_{\mathrm{K}^0}$ and $\Delta\mu_{\mathrm{Na}^0}$ denote the changes of standard chemical potential of potassium and sodium, and r_{K}, r_{Na} are the ion concentration ratios:

$$\frac{c_{\mathrm{K}''}}{c_{\mathrm{K}'}} \text{ and } \frac{c_{\mathrm{Na}''}}{c_{\mathrm{Na}'}}$$

The concentrations in a cellular or tissue phase are denoted as $c_{\mathrm{K}''}$ and $c_{\mathrm{Na}''}$; the concentrations in blood serum are $c_{\mathrm{K}'}$ and $c_{\mathrm{Na}'}$. In a system with perfectly non-selective distribution:

$$r_{\mathrm{K}} = r_{\mathrm{Na}}$$

and

$$\Delta\mu_{\mathrm{K}^0} = \Delta\mu_{\mathrm{Na}^0}$$

These are the conditions for the ideal Donnan distribution[1,3]. (The functions $\Delta\mu_{\mathrm{K}^0}$ and $\Delta\mu_{\mathrm{Na}^0}$ are the changes of standard chemical potential between blood serum and the given cell or tissue. According to equation (7), $\Delta\mu_{\mathrm{K}}$ and $\Delta\mu_{\mathrm{Na}}$ are non-specific for the ions. They depend only on the nature of the two phases. The specific terms of the equilibrium are $\Delta\mu_{\mathrm{Na}^0}$ and $\Delta\mu_{\mathrm{K}^0}$. They depend on the nature of the two ions and the nature of the two phases. The terms $RT \ln r_{\text{Na}}$ and $RT \ln r_{\text{K}}$, therefore, depend on the non-specific constants $\Delta\mu_{\text{Na}}$ and $\Delta\mu_{\text{K}}$ and on the two defined specific functions.)

Definition of standard state. In true homogeneous solutions, activity coefficients may be extrapolated to infinite dilutions as a state of reference. This is not possible for cells and tissues, which are more nearly like invariant heterogeneous systems in which ion concentrations cannot be varied continuously or independently of those in contiguous phases. Because biological measurements presuppose a mean state or tendency, the normal physiological resting state is chosen as the standard state and all activity coefficients are assigned the value of 1·0 (ref. 3). Standard values of $\Delta\mu_{\text{K}^0}$ and $\Delta\mu_{\text{Na}^0}$ apply to that state. All phase rule derivations assume the equilibrium condition that the free-energy change, ΔG, is zero. For cells and tissues in their most stable resting states the standard free energy of sodium–potassium distribution, $\Delta G^0{}_{(\text{K,Na})}$, may be calculated from the observed distribution ratios[3]. When defined as:

$$\Delta G^0{}_{(\text{K,Na})} = -RT \ln \frac{r_{\text{K}}}{r_{\text{Na}}} \tag{9}$$

it may be determined from the normal invariant ratios, r_{K} and r_{Na}. As calculated for a number of cells and tissues in solid or semi-solid states of aggregation, the value generally falls between $-2{\cdot}1$ and $-3{\cdot}2$ kcal (Table 1). The value $-2{\cdot}1$ kcal corresponds to an approximate equality between intracellular sodium and potassium, while the value $-3{\cdot}2$ kcal is found when the intracellular potassium exceeds the sodium by a factor of about 5. All distribution ratios are referred to the normal blood serum of the species.

Table 1. STANDARD FREE ENERGIES OF SODIUM–POTASSIUM DISTRIBUTION (values in kcal)

Cell or tissue	Species Values of ($-\Delta G^0{}_{\text{K,Na}}$) Man	Cattle	Rabbit	Rat	Mouse	Frog	Ref.
Erythrocyte	3·1	1·2	3·2	3·0	—	—	(5)
Whole brain	2·1	2·4	2·5	2·5	2·6	—	(6)
Skeletal muscle	2·7	2·6	3·2	3·0	—	2·9	(6)
Cardiac muscle	2·4	2·4	2·2	2·1	—	—	(6)
Lens	2·2	2·4–3·2	3·2	2·5	—	—	(7)
Epidermis	2·4	—	—	—	2·4	2·5	(8, 9)

* Calculated by means of equation 9 from values of r_{Na} and r_{K}. Analytical data obtained from refs. 5–9, using mean values.

In many body fluids the value of r_{K} approaches that of r_{Na}, and $\Delta G^0{}_{(\text{K,Na})}$ approaches zero[3]. This conforms to the condition for the ideal Donnan distribution, in which all the ion ratios (univalent) are equal. It is not true of coexistent phases in general, where the changes of standard chemical potential are not zero. Equations 1–9, inclusively, are more general and apply to all possible states of aggregation and to all degrees of mixing within any phase.

Normal resting potentials of cells and tissues. When electrical differences of potential are measured between an intracellular phase and the external milieu they are often found to be of a magnitude of -50 to -100 mV, with the negative sign referring to the intracellular region. When the electrical circuit is closed, chloride and other anions transport current from the external fluid into the cell, while potassium and sodium flow in the opposite direction. The free-energy change of the process is negative, and each kind of ion flows from a higher to a lower chemical potential. In a system with perfectly unrestricted mixing of the diffusible ions in each phase, $\Delta\mu^0$ is zero for all the ions. Accordingly, for any kind of anion or cation:

$$\Delta\mu_i = RT \ln \frac{c_i''}{c_i'} \tag{10}$$

The difference of electrical potential in the limiting case is expressed by the ideal form of the Nernst equation, as applied by Donnan[1]:

$$\Delta\mu_i = -96{,}500\, z_i E \text{ (joules)} \tag{11}$$

where the value of the Faraday constant is 96,500 coulombs. Also:

$$\Delta\mu_i = -23{,}060\, z_i E \text{ (calories)} \tag{12}$$

Since the ratio of $\Delta\mu_i$ to z_i is constant for all diffusible ions:

$$E_{i,\text{ideal}} = -\frac{2{\cdot}303\, RT}{96{,}500\, z_i} \log_{10} \frac{c_i''}{c_i'} \tag{13}$$

In the more general case:

$$\Delta\mu_i = \Delta\mu_{i^0} + RT \ln \frac{c_i''}{c_i'} \tag{14}$$

(The change of standard chemical potential $\Delta\mu_{i^0}$ is defined by equation (14). Its value is determined by the difference between $\Delta\mu_{i,\text{real}}$ and $\Delta\mu_{i,\text{ideal}}$. In any biological phase the absolute value of the standard chemical potential of any kind of ion is unknown and indeterminate. The measurable quantities are the real and ideal changes of chemical potential. It is usually true in thermodynamic calculations that the absolute values of the functions are unknown. For example, the standard electrode potential of sodium is defined in relation to that of hydrogen, which is assigned the value of zero volts. The measurable quantities are the changes of the functions between any two states: changes of free energy and chemical potential, for example. In cells and tissues any kind of ion occurs at different concentrations in all the phases. Each phase in the normal state is characterized by the value of μ_{i^0} for the particular kind of ion in that phase; this is a factor in the distribution. The measurable quantity is $\Delta\mu_{i^0}$ rather than μ_{i^0}.)

The electromotive force, E, is then calculated from equations 11 and 14:

$$E = -\frac{\Delta\mu_{i^0}}{96{,}500\, z_i} - \frac{2{\cdot}303\, RT}{96{,}500\, z_i} \log_{10} \frac{c_i''}{c_i'} \tag{15}$$

or

$$E_{\text{real}} = E_{i,\text{excess}} + E_{i,\text{ideal}} \tag{16}$$

Correlation of distribution ratios and electrical potentials. In the general case the observed electromotive force may be expressed as the sum of an ideal term, given by the distribution ratio of any ion, and an excess term, proportional to $\Delta\mu^0$ for that ion. The excess term may be measured as the difference between the observed electromotive force and the ideal electromotive force estimated from the distribution ratio for the given kind of ion. The change of standard chemical potential between the two phases is $\Delta\mu_{i^0}$; according to equation (15), it may be estimated from the excess electrical potential difference. Thus:

$$\Delta\mu_{i^0} = -23{,}060\, z_i E_{i,\text{excess}} \text{ (calories)} \tag{17}$$

By the use of the conversion factor, 100 mV are equivalent to 2·306 kcal. Observed resting potentials for a number of biological structures are given in Table 2. The values range from about -50 to -100 mV. In each case the values of r_{K} and r_{Na} are also given, and from

Table 2. EXCESS ELECTRICAL AND CHEMICAL POTENTIALS (100 mV = 2·3 kcal)

	Skeletal muscle (frog)	Skin (frog)	Lens (rabbit)	Uterus (cat)	Giant axon (squid)
Reference	(10)	(11)	(12)	(13)	(14, 15)
$E_{\text{obs.}}$ (mV)	−100	−80	−70	−50	−55
r_{K}	50	32	55	20	32·3
r_{Na}	0·4	0·5	0·25	0·61	0·1
$E_{\text{K,ideal}}$ (mV)*	−100	−90	−104	−78	−90
$E_{\text{Na,ideal}}$*	24	18	36	13	59
$E_{\text{K,excess}}$†	0	10	34	28	35
$E_{\text{Na,excess}}$†	−124	−98	−106	−63	−114
$\Delta\mu_{\text{K}^0}$(kcal)‡	0	−0·2	−0·8	−0·6	−0·8
$\Delta\mu_{\text{Na}^0}$(kcal)‡	2·9	2·3	2·4	1·5	2·6
$-\Delta G^0{}_{(\text{K,Na})}$(kcal)	2·9	2·5	3·2	2·1	3·4

* Equation (13). † Equation (16). ‡ Equation (17).

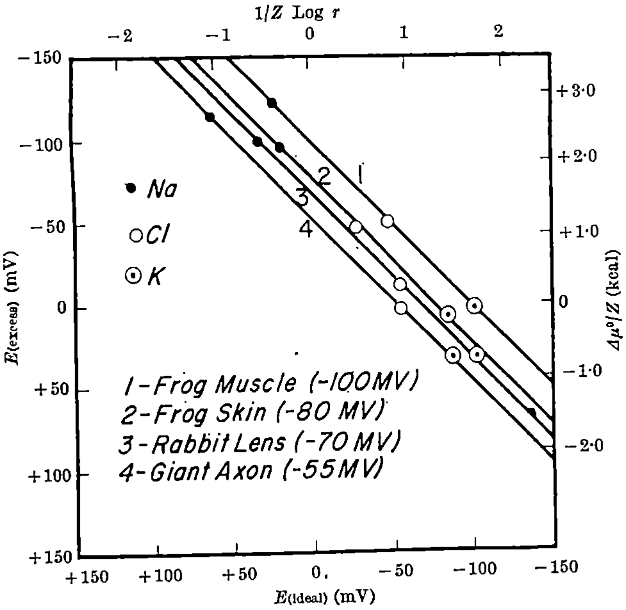

Fig. 1. Distribution of sodium, potassium and chloride ions in relation to resting electrical potentials. Family of parallel lines represents resting potentials of four different tissues. Ordinates, excess electrical potentials; excess chemical potentials. Abscissæ, ideal electrical potentials and ion distribution ratios on logarithmic scale

these the values of $E_{\mathrm{K,ideal}}$ and $E_{\mathrm{Na,ideal}}$ are estimated by means of equation (13). The excess electrical potential for each ion is estimated from equation (16); from these values, $\Delta\mu_{\mathrm{K}^0}$ and $\Delta\mu_{\mathrm{Na}^0}$ are obtained in calories (equation (17)). The values of $\Delta\mu_{\mathrm{K}^0}$ range from 0 to about −0·8 kcal (35 mV); those of $\Delta\mu_{\mathrm{Na}^0}$ are typically of the order of magnitude of 2·3 kcal (−100 mV). Table 2 also includes values of $\Delta G^0_{(\mathrm{K,Na})}$, the standard free energy of sodium and potassium distribution. These values range from −2·1 to −3·4 kcal. It is evident that the magnitudes are largely determined by the high positive values of μ_{Na^0}, the standard chemical potential of sodium.

Results are shown graphically in Fig. 1, which also includes values for chloride. The family of parallel diagonal lines represents the observed resting potentials, each of which is the sum of an ideal term (abscissa) and an excess term (ordinate). The ion concentration ratios are expressed by a logarithmic scale parallel to the abscissa. The changes of standard chemical potential of each kind of ion are shown on the vertical scale at the right. Potassium and chloride ions show the smallest deviations from the ideal Nernst equation and the smallest changes of standard chemical potential. Fig. 1 illustrates the relation between ion distributions, excess electrical potentials and changes of standard chemical potential for each kind of ion.

Distribution of ions and electrical potentials at equilibrium is based not on equality of concentration ratios but on equality of chemical potentials. Depending on the nature of the phases, biological systems may show changes of standard chemical potential which are close to zero, in which case they would conform to the Donnan distribution and to the ideal Nernst equation. Examples of this are the blood serum and contiguous body fluids. On the other hand, cells and tissues are highly ordered structures, made up of insoluble and soluble charged macromolecular components, where the criterion for equilibrium is that the changes of chemical potential be constant for all ions of the same charge. Then both distribution ratios and resting electrical potentials are determined by the standard chemical potential of each kind of ion in each of the phases.

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EARLY FOLDS AND THE *a*-LINEATION AT TINTAGEL, CORNWALL

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The small-scale tectonic structures imprinted on the Upper Devonian slates and volcanic rocks of Tintagel, on the north coast of Cornwall, were used by Dr. Gilbert Wilson[1] to demonstrate that a general shearing movement had taken place throughout groups of beds and on separating thrust planes. Upper beds had moved north-north-westerly relative to those below. Earlier, Henry Dewey[2] had shown that the structure of the area was apparently a simple, conformable pile of gently dipping strata sliced locally by flat-lying thrusts. The later work confirmed this view but corrected Dewey's interpretation that thrust-bounded slices had ridden up the thrust planes in a general easterly direction, and thus rehabilitated the area into the conventional Hercynian tectonic pattern of Cornwall.

In accepting the general congruity of the structures, the observed linear structural elements were grouped by Dr. Wilson into those indicative of the *b*-tectonic direction and others at right-angles, which had perforce to be labelled as structures parallel to the direction of tectonic transport, namely, the *a*-tectonic direction. The *b*-lineations were the axes of drag folds, the intersection of fracture-cleavage with bedding, and the long axes of boudins; *a*-lineations included certain bedding plane lineations, elongated mineral spots, and the long axes of stretched pillows in the interbedded lavas.

One anomalous feature remained, however, and that was the general absence of folds other than drag folds in the tectonic pattern of the area. Indeed, De Sitter[3], in discussing the boudins which are so well displayed at

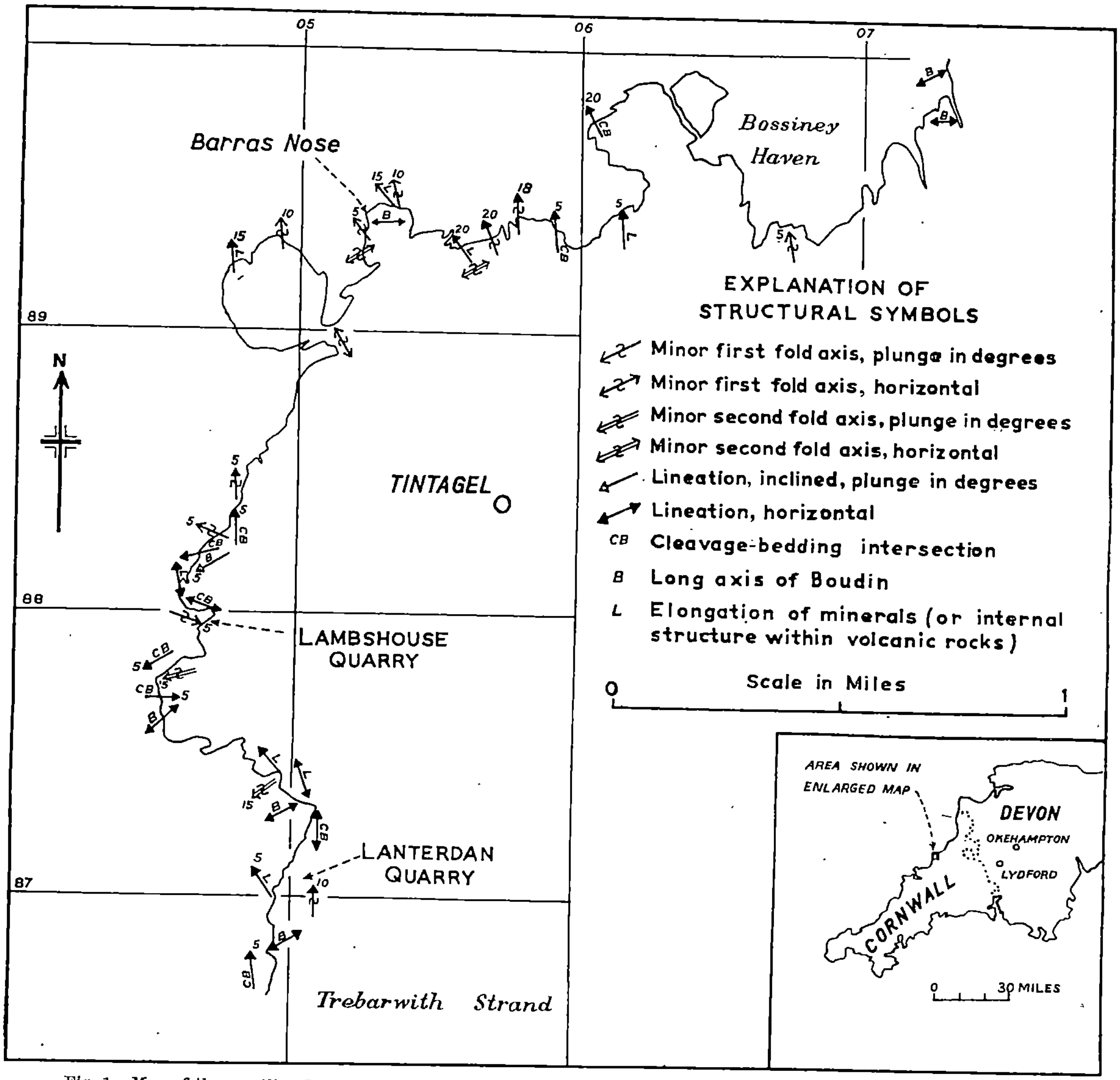
05
06
07
89
88
87
N
Barras Nose
Bossiney Haven
TINTAGEL
LAMBSHOUSE QUARRY
LANTERDAN QUARRY
Trebarwith Strand
EXPLANATION OF STRUCTURAL SYMBOLS
Minor first fold axis, plunge in degrees
Minor first fold axis, horizontal
Minor second fold axis, plunge in degrees
Minor second fold axis, horizontal
Lineation, inclined, plunge in degrees
Lineation, horizontal
CB Cleavage-bedding intersection
B Long axis of Boudin
L Elongation of minerals (or internal structure within volcanic rocks)
Scale in Miles
0
1
AREA SHOWN IN ENLARGED MAP
DEVON
OKEHAMPTON
LYDFORD
CORNWALL
0 30 MILES

Fig. 1. Map of the coastline from Trebarwith Strand to Bossiney Haven showing the directions of F_1 and F_2 lineations

Tintagel, remarked that the parallelism of nearly horizontal bedding and slaty-cleavage implied the presence of recumbent folds.

Meanwhile, investigations in neighbouring areas in Cornwall and Devon[4] have revealed a consistent pattern of superimposed structures in Devonian and lowest Carboniferous slates. Particularly relevant to the present discussion are the minor structures at Lydford in Devon (inset map, Fig. 1) on the north-west border of Dartmoor. Here the River Lyd Slates-with-lenticles Group is the lateral equivalent of at least part of the Tintagel succession[5-7]. Early folds in the slates are very tight to isoclinal with an axial-plane slaty-cleavage and often a well-marked bedding-cleavage lineation. The axial trend of these early, or F_1, folds varies from south-west to north-west in areas where the cleavage is flat-lying. F_1 folds are folded by later, more open, F_2 folds with a gentle north-easterly plunge, an approximately axial plane strain-slip cleavage, and a strong lineation resulting from the intersection of bedding or slaty-cleavage and the strain-slip cleavage. These F_2 folds are overturned to the north.

Such rectangular combinations of structures, here and at other localities in Cornwall, are strong evidence for assuming that similar structures occur at Tintagel (Fig. 1). In considering this possibility, it was thought that the recorded presence of more gritty quartzitic bands in the black carbonaceous slates comprising the Barras Nose Beds[1] would serve to make F_1 folds, if present, easily visible and would at least provide an obvious bedding-slaty-cleavage lineation. The initial enquiry was therefore concentrated on Barras Nose (Grid ref. *SX* 05358944). Recumbent, very tight folds with axial-plane slaty cleavage (S_1) (Fig. 2) and gentle north-north-westerly plunge of axes occur on both sides of the headland on easily accessible ledges. The folds have limbs of unequal length, the short limbs being about 1 ft. long, while the others exceed 5 ft. Cross-lamination in the quartzitic bands shows that the short limbs are inverted and the early folds thus face west-south-west on the axial plane cleavage. Later F_2 folds (the local drags in *b*) deform the S_1 cleavage and have associated with them an axial-plane strain-slip cleavage (S_2); the latter cleavage gives rise to a second lineation trending west to west-south-west on the S_1 slaty cleavage.

Another locality investigated was the Lanterdan Quarry (Grid ref. *SX* 05108709) (Fig. 1) in the cliffs above Hole Beach, Trebarwith Strand. Greyish green slates, with thin calcareous lenticles and 2-in. thick beds of crinoidal limestone, are exposed in many easily accessible quarry faces. A small quarry (Grid. ref. *SX* 05138706) on the landward side of the cliff-top path above the main quarry provided an example of a complete fold hinge (F_1) picked out by a thin irregular band of friable weathered lime-

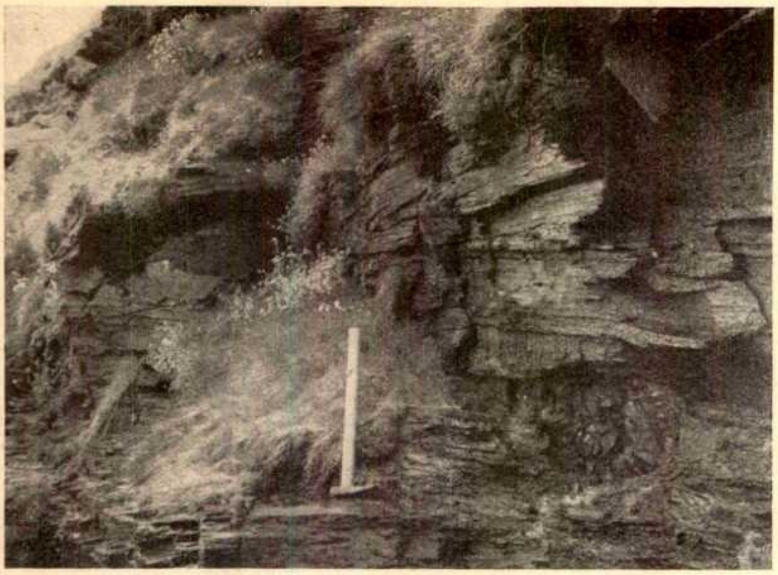

Fig. 2. Recumbent F_1 folds at Barras Nose, Tintagel

stone. The fold axis plunges gently to the north-north-west. Other F_1 folds were seen within the main Lanterdan Quarry, and wherever there are lenticles it is possible to demonstrate that the lineation on the slaty cleavage is parallel to the intersection of bedding with cleavage. Moreover, the flattened chloritic impressions of *Spirifer verneuili*, so abundant at certain levels in this quarry, are stretched out in the same direction and are affected by the later strain-slip cleavage lineation.

Other F_1 folds, with axes plunging to west-north-west and east-south-east, that is, at 60° to the north-north-west trend described, are seen at Lambshouse Quarry (Fig. 1: Grid ref. *SX* 04698795) and in the surrounding cliff faces. Northward-directed lineations are rare at this locality and may be attributed to F_2 movements.

It therefore appears that the accepted *a*-lineation at Tintagel is a *b*-lineation related to an earlier phase of recumbent isoclinal folding. Stretch structures and north-north-west directed lineations are clearly products of the first, F_1, phase of folding; the drag-folds, boudins and strain-slip lineations belong to a second, F_2, phase of folding. The dominance of north-north-west lineations implies a constant orientation of F_1 folds over most of the area from Trebarwith to Boscastle with the early folds facing west-south-west. This orientation, and the facing direction, must eventually be reconciled with the continuity of the outcrop of these strata from the coast at Boscastle to the western margin of Dartmoor, and perhaps even as far east as Okehampton.

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1-^{14}C-GLUCOSE UTILIZATION OF INTACT NUCLEATED RED BLOOD CELLS OF SELECTED SPECIES

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INTACT normal human red blood cells metabolize 1-^{14}C-glucose via the pentose phosphate pathway (hexomonophosphate shunt) to produce $^{14}CO_2$, and this production can be stimulated by pyridine nucleotides, especially triphosphopyridine nucleotide (TPN)[1]. Since human red blood cells are non-nucleated, they are incapable of protein synthesis according to present theory. This raised the question as to whether nucleated red blood cells, presumably capable of protein synthesis, would utilize 1-^{14}C-glucose similarly to human red blood cells via the pentose phosphate pathway. We wondered whether nucleated red blood cells would metabolize 1-^{14}C-glucose more actively than non-nucleated red blood cells. Therefore the 1-^{14}C-glucose utilization of nucleated red blood cells of selected species was investigated. In particular, we examined the nucleated red blood cells of chickens (*Gallus gallus*), geese (*Anser domesticus*), ducks (*Anas boscas*), and frogs (*Rana pipiens*). Intact red blood cells were examined in order to approximate more closely their usual physiological state. In addition, total hæmolysates were also examined. Red blood cells of normal human subjects were used as controls.

Blood was obtained by venipuncture from man and by cardiac puncture from birds and frogs. The $^{14}CO_2$ production from 1-^{14}C-glucose was measured as previously described[1]. Each flask contained approximately 22,200 d.p.m. of 1-^{14}C-glucose (specific activity 1·79 mc./mmole). Avian and frog red blood cells were centrifuged at 1,800 r.p.m. Washed packed red blood cells from each individual animal or pool of blood from several animals were incubated in duplicate flasks using 0·2 ml. of cells in a total volume of 2·5 ml. with and without TPN at a concentration of 2 mM. Total hæmolysates were prepared by substituting water for the Krebs–Ringer phosphate buffer used as the incubation medium. Blood samples from each of five geese and four ducks were used, while pools of blood from 12 chickens and 23 frogs were used to constitute five separate samples of blood from these latter species. Incubation conditions were as previously described[1]. Values were expressed as the percentage of initial radioactivity counted as $^{14}CO_2$.

The results are given in Table 1. It can be seen that the intact red blood cells from man and the frog were stimulated significantly by 2 mM TPN, whereas no such stimulation occurred for the chickens, geese and ducks. When the data from each species were compared with the corresponding data for man, it was found that the avian and frog red blood cells differed significantly from the red blood cells of man. This comparison is presented in Table 2. Surprisingly, the intact avian red blood cells produced very little $^{14}CO_2$ from 1-^{14}C-glucose and the addition of TPN produced only a slight increase, if any. The frog red blood cells were intermediate between human and avian red blood cells in the amount of $^{14}CO_2$ produced and were significantly stimulated by TPN, but to a degree less than the human red blood cells.

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Table 1. $^{14}CO_2$ PRODUCTION FROM 1-^{14}C-GLUCOSE BY INTACT RED BLOOD CELLS OF VARIOUS SPECIES WITH AND WITHOUT TPN

The data are given as mean values expressed as the per cent of initial radioactivity recovered as $^{14}CO_2$. Values in parentheses give the range. Statistical comparison was made using Student's t test for paired data (ref. 2)

Species	No. of subjects	Additions		Statistical comparison	
		None	TPN	t	P
Man	5	11·3 (4·6–22·6)	21·8 (11·7–41·7)	4·60	0·01
Chicken	12 animals to give 5 pools of cells	0·6 (0·1–1·2)	0·7 (0·1–1·3)	1·83	> 0·05 (n.s.)
Goose	5	0·1 (0·02–0·4)	0·7 (0·2–1·3)	2·45	> 0·05 (n.s.)
Duck	4	1·1 (0·2–2·6)	2·0 (0·3–3·7)	1·64	> 0·05 (n.s.)
Frog	23 animals to give 5 pools of cells	2·2 (1·5–3·0)	3·1 (2·3–4·6)	4·66	0·01

Table 2. Statistical comparison between intact nucleated red blood cells (RBC) of selected species and intact human red blood cells in $^{14}CO_2$ production from 1-^{14}C-glucose with and without TPN, using Student's t test for unpaired data (ref. 2)

Species RBC	No TPN		Added TPN	
	t	P	t	P
Chicken	3·12	0·02	3·74	0·008
Goose	3·18	0·02	3·75	0·008
Duck	2·94	0·015	3·48	0·01
Frog	2·64	0·04	3·31	0·01

Since the intact red blood cells produced much less CO_2 than was anticipated, total hæmolysates were examined to help determine the reason for this decreased output. Table 3 gives the results for various chicken and goose red blood cells. The chicken red blood cell total hæmolysates showed much greater production of carbon dioxide than the corresponding intact red cell preparation. The goose red blood cell total hæmolysate also produced more carbon dioxide than its intact cells but not so much as the chicken hæmolysates. TPN was greatly stimulatory in both hæmolysates.

Thus, the intact nucleated erythrocytes were far less active with regard to production of carbon dioxide from 1-^{14}C-glucose, contrary to our expectation. Several possibilities exist to account for the low production of carbon dioxide. $^{14}CO_2$ is produced from 1-^{14}C-glucose via the pentose phosphate pathway and the Krebs's tricarboxylic acid cycle (TCA cycle). Carbon dioxide would not be produced if these pathways were not present. Certainly the pentose phosphate pathway enzymes, glucose-6-phosphate dehydrogenase (G-6-PD) and 6-phosphogluconic acid dehydrogenase (6-PGAD), exist in avian red cells. Salvidio *et al.*[3] measured these in pigeon red blood cells. We have also measured these enzymes in chicken red blood cells by the method of Kornberg and Horecker[4] and found these values: G-6-PD, 15,330 units; 6-PGAD, 1,330 units (1 unit is the change of optical density of 0·001 at 340 mμ/g hæmoglobin at room temperature using a 1-cm light path). Furthermore, carbon dioxide production in the total hæmolysates was appreciable and was stimulated by TPN, which is good evidence for the presence of an active pentose phosphate pathway.

Table 3. THE $^{14}CO_2$ PRODUCTION OF INTACT RED BLOOD CELLS AND TOTAL HÆMOLYSATES FROM CHICKENS AND GEESE

The data are expressed as in Table 1

Species	No. of animals	Intact cells		Total hæmolysate	
		No additions	TPN	No additions	TPN
Chicken	Pool of 3	0·2	0·4	15·2	40·5
Chicken	Pool of 5	0·2	0·5	19·2	32·0
Goose	1	< 0·1	0·2	0·9	39·5
Goose	1	0·4	0·7	3·9	51·3
Goose	1	< 0·1	0·6	1·3	39·5
Goose	1	0·1	0·3	3·2	37·2

Dajani and Orten[5] have shown that labelled acetate is incorporated into the TCA cycle intermediates in chicken red blood cells, from which it was concluded that an active TCA cycle exists in chicken red blood cells. Thus, the presence of the pentose phosphate pathway and a presumably functional TCA cycle would be expected, if anything, to lead to greater production of carbon dioxide than in human red blood cells where a TCA cycle is lacking. Therefore, the low production of carbon dioxide by intact chicken red blood cannot be due to an absence of these pathways.

Since chicken red blood cells can utilize exogenous glucose via the pentose phosphate pathway in total hæmolysates but only to a very limited extent in the intact cell, there is the implication that there is some sort of impairment of glucose movement into the intact chicken red blood cell. The results are less striking in the goose red blood cell, though the hæmolysates still produced more carbon dioxide than the corresponding intact red blood cells.

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ELECTRONIC EFFECTS AND L-AMINO-ACID OXIDASE SPECIFICITY

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QUANTITATIVE investigations of substituent effects on organic reactivities yielded linear free-energy relationships[1] which not only emphasized regularities in reactivities but also brought to attention detailed aspects of mechanisms when the linear Hammett equation was not strictly followed[2,3]. Little is known, however, about the importance of electronic effects in determining enzymatic reactivities[4,5]. The present article, as part of our work on the mechanism of reactions involving flavins and flavoproteins[6,7], deals with an investigation of substituent effects on the oxidation rates of amino-acids catalysed by L-amino-acid oxidase (LAO) found in snake venoms.

The enzyme purified from the venom of the Malayan pit-viper (*Agkistrodon rhodastoma*), not previously described, was compared with that obtained from *Crotalus adamanteus* (Sigma, Type IV)[8]. The former enzyme is very similar in physical and spectral properties to those obtained from other venoms. It has a sedimentation coefficient of 6·3 S at 0·1 per cent protein concentration, maximum absorption at 280, 390 and 460 mμ with a ratio of absorbancy 280/460 = 9·3. The method of purification and other physical properties of the enzyme will be described elsewhere.

The substrates examined were α-amino phenylacetic acid (phenylglycine) and its derivatives substituted in the aromatic nucleus. This choice was governed by the consideration that substituents should have a more significant effect when the reaction centre is conjugated with the aromatic ring[2,3]. The rates of oxidation—measured by manometric methods—catalysed by the two oxidases under identical conditions are shown in Table 1. They were calculated from the linear part of the rate curves during the first 10 per cent reaction. In the reactions when oxygen uptake was observed the products were shown to be ketones (in the presence of catalase).

Table 1. RATES OF OXIDATIONS OF AROMATIC AMINO-ACIDS BY L-AMINO-ACID OXIDASE

Substrate	Rate μl. O_2/min/mg enzyme from *Agk. rh.*	Rate μl. O_2/min/mg enzyme from *Crot. ad.*
DL-Phenylglycine	1·71	1·7
D-Phenylglycine	0	—
DL-*p*-Methyl-phenylglycine	1·46	~2·0
DL-*p*-Methoxy-phenylglycine	0·86	0·83
DL-*m*-Amino-phenylglycine	1·43	1·53
DL-*p*-Fluoro-phenylglycine	4·48	4·3
DL-*p*-Chloro-phenylglycine	5·56	5·65
DL-*m*-Fluoro-phenylglycine	0·27	~0·5
DL-*m*-Chloro-phenylglycine	0·31	~0·5
DL-*m*-Nitro-phenylglycine	0·05	v. slow
DL-*o*-Fluoro-phenylglycine	0·15	0·3
DL-*o*-Chloro-phenylglycine	0·1	—

Solutions contained: 1 mg enzyme, 30 μmoles substrate in 3 ml. buffer (0·2 M *tris*, *p*H 7·2). Rates were measured at 38° C with air in the gas phase.

The rates of oxidation of some natural amino-acids under identical conditions were also measured using LAO of *Agkistrodon rhodastoma* venom (Table 2). Taking the rate of oxidation of L-leucine as 100, the specificity of this enzyme can be compared with that obtained from *Crotalus adamanteus*[9].

Table 2. RATES OF OXIDATION OF NATURAL AMINO-ACIDS CATALYSED BY LAO from *Agkistrodon rhodastoma* VENOM

Substrate	Rates μl. O_2/min/mg enzyme	Rates rel. to leucine	Rates rel. to leucine with LAO of *Crot. ad.**
L-Leucine	24·4	100	100
DL-Leucine	25·2	103	—
DL-Tyrosine	14·2	58	82
L-Tryptophan	19·4	79	88
DL-Phenylalanine	21·2	87	82
DL-Alanine	1·2	5	0·4

Solutions as in Table 1 except that 15 μmoles of substrate were used with pure optical isomers.

* Data from ref. 9.

It is clear that the properties of the two enzymes are very similar, if not identical. The fact that phenylglycine and its derivatives are oxidized supports the hypothesis that the reaction proceeds via the α-imino-acid rather than through an αβ-unsaturated compound[10]. The mechanism of oxidation, then, probably involves the loss of the α-hydrogen (either as a hydrogen atom or as a hydride ion) to the flavin and of a proton to the solvent. This is followed by the hydrolysis of the α-imino-acid and the dissociation of the substrate from the enzyme. If the loss of the α-hydrogen is the rate-limiting step, one would expect electron-donating substituents (for example, *p*-OMe, *p*-Me, *m*-NH_2) to accelerate the reaction, and electron-withdrawing groups (for example, F- and Cl-) to retard it. The rates are affected in the opposite direction by the groups examined, with the exception of the three most strongly withdrawing meta-substituents. More significantly, when Hammett σ-values are plotted against log k/k_0 (where k_0 = rate of oxidation of unsubstituted compound; k is that of the substituted compound), a reasonably good linear correlation is obtained for the first six substituents with a ρ-value of 1·8. The three most electron-withdrawing substituents (*m*-Cl, *m*-F, *m*-NO_2), on the other hand, fall on a line with a negative slope. (The exact slope of this line is subject to large errors because of the inaccuracies in following the rates of very slow reactions.) These observations suggest that the rate-determining step is normally not the loss of the α-hydrogen but one of the two subsequent processes. The fact that tyrosine reacts slower than phenylalanine is consistent with this. In cases when the substituent would slow down the first step considerably (that is, groups with large positive σ-values) the loss of the α-hydrogen may become rate limiting, hence the anomaly in the Hammett plot. Similar breaks in Hammett plots have been reported for two other enzymatic reactions[4,5], with the difference that the change-over occurred at the unsubstituted compound.

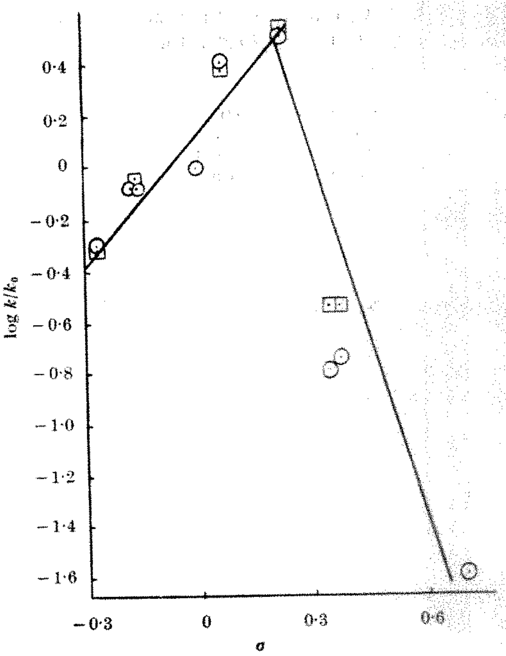

Fig. 1. Hammett plot for rates of oxidation of aromatic amino-acids. ○, by LAO from *Agkistrodon rhodastoma*; □, by LAO from *Crotalus adamanteus*

The importance of steric factors and the 'goodness of fit' of the substrate on the enzyme surface cannot, of course, be overlooked. Unfavourable steric effects may be responsible for the very low rates of oxidation of the two ortho-substituted phenylglycines. They may also effect to some extent the oxidations of the meta-substituted compounds and therefore cause the break in the Hammett plot. If this is the case, however, it is difficult to reconcile the fact that the *m*-fluoro substituent has an equal effect to the *m*-chloro group, or that the *m*-amino group has such a weak effect, for the steric requirements of these groups are considerably different, the amino group being the largest. For this reason the first interpretation for the unusual Hammett plot seems more plausible.

In considering the susceptibility of the reaction to electronic variations (this is measured by the ρ-value) it is tempting to draw comparisons with non-enzymatic reactions. The ρ-value of 1·8 is close to those observed for base-catalysed hydrolyses of esters of benzoic acid derivatives[1]. On the other hand, it is considerably larger than values for reactions or acidities involving phenylacetic acid derivatives[2]. It is therefore a reasonable working-hypothesis that the rate-determining step of the enzymatic reaction is the hydrolysis of the α-imino-acid (which is similar in mechanism to the base-catalysed hydrolysis of ethylbenzoate) rather than the dissociation of the product from the enzyme, which is likely to involve the carboxyl group insulated from the aromatic nucleus by an intervening carbon atom, as in phenylacetic acid.

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EFFECTS OF LUMBAR SYMPATHECTOMY ON THE UTERINE UPTAKE OF CATECHOLAMINES

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TRITIATED *nor*epinephrine (NE-^{3}H) is actively taken up from the circulation and bound in sympathetic nerve endings in many tissues, including heart, muscle and salivary gland[1]. Electron microscopic examinations have shown that the tritiated catecholamine is stored in dense-core vesicles[2]. When heart[3] or salivary gland[4] are subjected to chronic sympathetic denervation, their capacities to take up circulating tritiated catecholamine are markedly reduced.

The catecholamine stores of rat uterus differ from those of most other tissues in that the capacity of the uterus to bind circulating epinephrine is endocrine-sensitive, rising four-fold between diœstrus and œstrus[5]. In uterine homogenates, tritiated epinephrine is present largely in the 'supernatant' and not the 'microsomal' fraction[7] as in other tissues[3]. The uptake of catecholamines in the uterus is not blocked by pre-treatment with cocaine, and their release is only slightly enhanced by tyramine[7]. On the basis of these observations it has been suggested that a major fraction of the uterine catecholamines may be present in a non-neuronal store[7].

In order to obtain further information about the cellular locus of uterine epinephrine and *nor*epinephrine, estimates were made of the ability of this organ to take up and retain circulating tritiated catecholamines several weeks after bilateral lumbar sympathectomy. The effects of denervation on catecholamine uptake of gastrocnemius muscle were also determined, since this muscle is also innervated by the lumbar sympathetic chain.

Catecholamines are cleared rapidly from the circulation; thus one of the determinants of the amount of administered neurohumours bound in an organ is the proportion of cardiac output it received[8]. Sympathetic denervation could affect regional perfusion, thereby changing the proportion of tritiated catecholamine which is made available to a given organ. The fractions of the cardiac output delivered to the uterus and gastrocnemius muscle were therefore measured in denervated and sham-operated animals[9].

Female Sprague–Dawley rats weighing 170–200 g were anæsthetized with ether, and both lumbar and sympathetic chains were removed from the level of the left renal artery to below the bifurcation of the aorta. All visible ramicommunicans were extirpated and the sympathetic nerves were stripped off the iliac arteries as well. It was believed that most or all of the hypogastric plexus was destroyed. Sham-operated rats served as controls.

Two weeks after operation, each animal received 30 μc. of *d,l*-*nor*epinephrine-7-^{3}H (6·0 mc./μmole, New England Nuclear Corp., Boston, Mass.) or 30 μc. of *d,l*-epinephrine-7-^{3}H (5·0 mc./μmole) intravenously. One hour later, 1 μc. (0·2 ml. final dilution) of potassium-42 as potassium-chloride (4 m.equiv./l.) was injected intravenously. Animals were killed by decapitation 20 sec later. Uterus and gastrocnemius were rapidly removed, weighed and assayed for potassium-42 in a γ well counter. A potassium-42 standard, representing 1 per cent of the injected dose, was assayed periodically to correct for potassium-42 decay. It was assumed that uptake of potassium-42 was proportional to the percentage of cardiac output received[9]. Tissues were then homogenized in cold 0·4 N perchloric acid and the radioactive catecholamines assayed as previously described[1].

Following lumbar sympathectomy, the gastrocnemius muscles took up considerably less tritiated-epinephrine and tritiated-*nor*epinephrine (Table 1), even though they received a somewhat larger share of the cardiac output (Table 2). Denervation increased the blood flow to the uterus as well (Table 2), but uterine uptake of tritiated *nor*epinephrine and tritiated epinephrine was not changed (Table 1).

Table 1. Fractional Blood Flow to Uterus and Gastrocnemius Muscle in Bilaterally Lumbar Sympathectomized and Sham-operated Animals

Results are expressed as per cent cardiac output/g ± *S.E.M.* for groups of 12 animals

	Uterus	Muscle
Sham-operated	1·82 ± 0·16	0·213 ± 0·014
Lumbar sympathectomized	2·46 ± 0·14*	0·258 ± 0·023

*$P < 0·01$.

Table 2. Effect of Bilateral Lumbar Sympathectomy on Uptake of Tritiated *nor*epinephrine and Tritiated Epinephrine in Uterus and Gastrocnemius Muscle

Results are expressed as mμc. of tritiated catecholamine per gram of tissue ± *S.E.M.* Each figure represents the mean for 12 animals

	Tritiated *nor*epinephrine	
	Uterus	Muscle
Sham-operated	4·69 ± 0·39	9·16 ± 0·62
Lumbar sympathectomized	4·83 ± 0·35	4·50 ± 0·14*
	Tritiated epinephrine	
Sham-operated	3·45 ± 0·30	6·38 ± 0·33
Lumbar sympathectomized	4·14 ± 0·73	3·46 ± 0·11*

*$P < 0·001$.

It has been shown that circulating tritiated catecholamines are bound by sympathetic nerves in many tissues[3], and that the capacity of these tissues to bind epinephrine and *nor*epinephrine is diminished following sympathetic denervations[3,4,10]. Unimpaired uptake following chronic sympathetic denervation would suggest either a failure in postganglionic denervation or the presence of an extraneuronal catecholamine store.

The uterus of the rat receives its sympathetic nerve supply from the lumbar sympathetic chain via the hypogastric plexus[11]. It is not known what fraction of the uterine sympathetic nerves have their cell bodies within neither the ganglia of the lumbar sympathetic trunks nor the hypogastric plexus. Some sympathetic ganglion cells may be present on the surface of the uterus, or within the uterine muscle; such has been shown to be the case in the vas deferens[12]. Hence the 'denervation' procedure used here may represent a total decentralization of the uterus but only a partial postganglionic sympathetic denervation. Presumably a major portion of the uterine postganglionic sympathetic nerves was destroyed, yet, unlike the gastrocnemius muscle, there was no decrease at all in the catecholamine-binding capacity of the uterus. It thus appears that either the usual correspondence between sympathetic innervation and catecholamine storage does not hold for the uterus, or that most postganglionic fibres to the uterus arise distal to the areas of dissection.

The 'extraneuronal' pool of *nor*epinephrine present in denervated salivary gland is reserpine resistant[4]. Reserpine, however, depletes the uterus of *nor*epinephrine[7]. Since reserpine blocks the uptake of tritiated *nor*epinephrine to sympathetic ganglia[13], the data are consistent with the hypothesis that a fraction of uterine catecholamines is present in ganglion cells in the uterus.

If the catecholamines in the uterus are 'extraneuronal' their binding site is pharmacologically different from that described in all other organs examined thus far. The choice of the correct alternative requires the accumulation of more information on the anatomy of the uterine sympathetic nerves.

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SPECIFICITIES OF SEVERAL ACID PHOSPHATASES FROM PLANT SOURCES

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UNLIKE the phosphatases of animal origin[1], those from plant sources have so far been examined only to a limited extent and some of the reported results are incompatible with each other. The possible identity of phosphomonoesterase and pyrophosphatase in higher plants has been suggested by some workers[2,3], whereas the independent nature of these enzymes in yeast has been claimed by others[4,5]. On the other hand, the relation of these phosphatases to phytase, which has often been believed to be a distinct enzyme[3,6], has recently been criticized[7]. Meanwhile, the presence of isodynamic enzymes or isozymes was reported for the plant phosphatases[8,9].

In the present work the crude preparations of phosphomonoesterase from the ears of rice plant, mycelia of *Aspergillus niger* and kernels of apricot were fractionated and the substrate specificity of each fraction was examined. As a result of these investigations, the existence in these plant materials of isodynamic phosphatases was confirmed and their specificities were investigated.

The precipitate (125 mg protein), obtained from an aqueous extract of unripened rice-ear with ammonium sulphate between 25 and 70 per cent saturation, was dissolved in 0·01 M *tris*-buffer of *p*H 7·6, applied on a DEAE-cellulose column (1·5 × 14 cm with 6 g of DEAE cellulose) and eluted with gradient concentrations of 0–0·42 M sodium chloride solution (180 ml.) at a rate of 8 ml. effluent/h.

Dried mycelia of *A. niger* were extracted with 0·2 per cent orthophosphate. The precipitate (120 mg protein) formed with 70 per cent acetone was dissolved in *tris*-buffer of *p*H 7·6 and subjected to DEAE-cellulose column chromatography (1·0 × 12 cm with 3·5 g DEAE-cellulose) in a similar way to the foregoing with a flow rate of 2·5 ml./h.

An enzyme extract (42 mg protein), obtained from the acetone powder of apricot kernels, was fractionated by DEAE-cellulose column chromatography under the same conditions as with *A. niger*, except that the flow rate was 4 ml./h.

The phosphatase preparation from rice-ears was resolved into three fractions (I–III) as shown in Fig. 1. Phosphatase III (Phos III) showed on zone electrophoresis a single component which was active for all substrates tested. The electrophoretic conditions were: $\mu = 0{\cdot}05$, 250 V, 16–18 m.amp, 26 h. The other two fractions (phosphatases I and II) were not examined electrophoretically. However, on heating at 50° C for 15 and 30 min, phosphatase I uniformly lost its activities by about 58 per cent and 65 per cent, respectively, towards various substrates, and phosphatase II also uniformly lost about 30 per cent and 35 per cent of its activities, respectively, under the same conditions. This finding may suggest the homogeneous nature of the two fractions. The activities of these fractions towards a variety of substrates are summarized in Table 1. In contrast to phosphatase II, the specificities of phosphatases I and III appear to be broad, as revealed by their activities on pyrophosphate and phytin besides phosphomonoesters. Particularly high activities of phosphatases II and III towards arylphosphate are to be noticed.

The maximal activity of phosphatase I was found at *p*H 4·5 for *p*-nitrophenyl phosphate, β-D-glucose-1-phosphate, pyrophosphate and phytin, and that of phosphatases II and III at *p*H 5·0 for respective substrates. Mg^{++} and Mn^{++} in 10^{-3} M showed no effect. However, the inhibition by F^- (2×10^{-2} M), Mo^{++} (2×10^{-3} M) and Cu^{++} (10^{-3} M) was marked for phosphatases I and III, but moderate for phosphatase II. The hydrolysis of

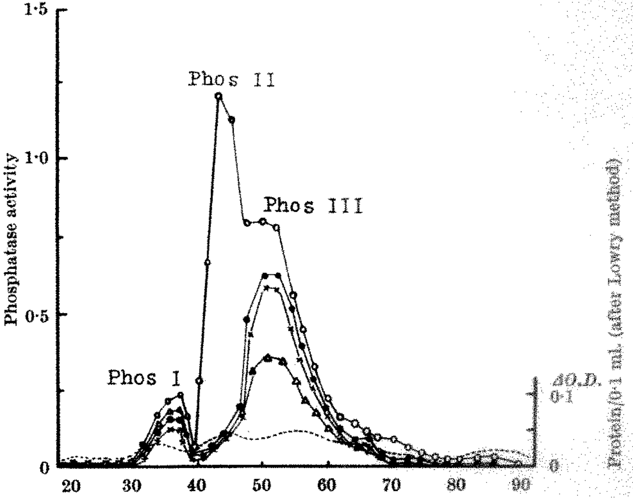

Fig. 1. DEAE-cellulose column chromatography of acid phosphatase in rice-ear extract. 2·0 ml. fractions were obtained by gradient elution with 0–0·42 M NaCl. ○, *p*-Nitrophenylphosphate; ●, β-glucose-1-phosphate; ×, pyrophosphate; △, phytin; ---, protein

Table 1. SUBSTRATE SPECIFICITIES OF SOME PLANT ACID PHOSPHATASE FRACTIONS OBTAINED BY DEAE-CELLULOSE CHROMATOGRAPHY

	Phosphatase activity (μmoles of phosphate liberated)*					
	Rice-ear			*Aspergillus niger*		Apricot kernel
	Phos I	Phos II	Phos III	Phos I′	Phos II′	Phos I″
p-Nitrophenylphosphate	0·65	2·94	2·28	0·16	0·68	2·16
Phenylphosphate	0·42	2·98	2·05	—	—	—
α-D-Glycerophosphate	0·32	1·14	0·72	0·02	0·35	0·40
β-D-Glycerophosphate	0·20	0·58	0·55	0	0·37	0·36
α-D-Glucose-1-phosphate	0·18	0·15	0·08	0	0·42	0·03
β-D-Glucose-1-phosphate	0·30	0·19	0·99	0·45	0·52	1·82
α-D-Galactose-1-phosphate	0·30	0·17	0·78	1·17	0·39	1·62
β-D-Galactose-1-phosphate	0·21	0·29	1·12	0·31	0·42	1·72
D-Glucose-6-phosphate	0·15	0·25	0·41	0·04	0·20	0·74
D-Fructose-6-phosphate	—	—	—	0	0·50	0·30
D-Fructose-1,6-diphosphate	0·15	0·37	0·34	—	—	—
Riboflavin-5′-monophosphate	0·10	0·71	0·67	—	—	—
Adenosine-5′-monophosphate	0·06	0·11	0·10	—	—	—
Adenosine-diphosphate	0·21	0·35	0·66	0	0·31	1·73
Adenosine-triphosphate	0·07	0·28	0·57	0	0·39	1·38
Pyrophosphate	0·26	0·10	0·80	0	0·48	1·04
Phytin	0·45	0·10	0·60	—	—	2·13

* Assay conditions: 0·5 ml. of the reaction mixture contained 5 μmoles of substrate (with rice-ear and apricot) or 2·5 μmoles (with *Aspergillus*), 40 μmoles of acetate buffer and enzyme solution; *p*H of reaction mixture was 4·5 for Phos I, 5·0 for Phos II, III and Phos I″ and 3·2 for Phos I′ and II′. Reaction time; 30 min. Reaction temperature: 30° C.

p-nitrophenylphosphate by phosphatase II was inhibited by F^- in competitive fashion, while that by phosphatase III was inhibited in a non-competitive manner. The Michaelis constants of these inhibitions were $7{\cdot}4 \times 10^{-3}$ M and $3{\cdot}0 \times 10^{-4}$ M, respectively.

The phosphatase components (phosphatases II and III) showed a transphosphorylase activity of somewhat narrow acceptor specificity. Of the acceptors tested, primary alcohols were effective with phosphatase III. Inositol, adenosine and glucose did not serve as acceptors with any of the phosphatase fractions.

The phosphatase preparation from *A. niger* was resolved into one minor component (phosphatase I′) and one major component (phosphatase II′). Phosphatase I′ seems to have limited substrate specificity. Thus, only 4 out of 12 substrates tested were attacked with measurable rapidity. The preferential high activity towards α-galactose-1-phosphate as contrasted with the inertness for α-glucose-1-phosphate appears worthy of note (Table 1). In contrast, phosphatase II′ showed broader specificity, all the substrates tested being hydrolysed more or less rapidly. The *p*H optima for phosphatases I′ and II′ were 3·2. Both these activities were abolished by Mo^{++} (2×10^{-3} M), but not or scarcely inhibited by other divalent cations such as Mg^{++}, Mn^{++} and Cu^{++} (each of 10^{-3} M). Even a very low concentration of Hg^{++} (2×10^{-5} M) inactivated phosphatase I′ but not phosphatase II′.

The phosphatase preparation from apricot kernels could not be sharply resolved under the present experimental conditions. Although the homogeneity of this phosphatase I″ is questionable, it easily hydrolysed phytin, arylphosphates and all the hexosemonophosphates except α-glucose-1-phosphate, which is regarded as physiologically important in higher plants. In this respect, this phosphatase is similar to phosphatase III of rice and phosphatase I′ of *A. niger*. The fact that the sugar phosphates of β-D-series were more rapidly hydrolysed than α-glucose-1-phosphate has recently been reported also for a highly purified phosphatase of *Phaseolus aureus*[10]. Phosphatase II′, in contrast to other phosphatases tested in this investigation, acts on α-glucose-1-phosphate as easily as on other hexosemonophosphates, its activity being scarcely affected by the differences in the structure of substrates.

From results thus obtained, it may be concluded that the plant phosphatase from each source is, in general, composed of more than one isozyme of different range of specificity. Thus, one of them is capable of attacking a wide range of esterphosphates including arylphosphates, phytin, and other phosphatemonoesters, while in the other the activity is limited to only few phosphates.

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ETHOLOGICAL CHANGES IN *Anopheles pseudopunctipennis* IN MEXICO AFTER PROLONGED USE OF DDT

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THE progress of the malaria-eradication campaign in Mexico during recent years has revealed that in certain areas malaria transmission has persisted despite adequate DDT spraying and in the absence of physiological resistance to this insecticide among the vectors. Investigations carried out in these problem areas, particularly in the state of Oaxaca[1], showed that the main cause accounting for the persistence of transmission was the behaviour in relation to DDT of the two local vectors —*A. albimanus* and *A. pseudopunctipennis*. The females of both species were able to penetrate the DDT-sprayed houses, bite the inhabitants inside them and escape without having picked up a lethal dose of insecticide.

When it became clear that behaviour of mosquitoes was an important factor in the persistence of transmission, the question arose as to whether the behaviour observed had existed before the introduction of DDT or was a development brought about by the prolonged use of the insecticide. In a country-wide campaign, such as that carried out in Mexico, all human dwellings in the malarious territory are regularly sprayed. This means in practice that the finding of an area untouched by insecticides where the original behaviour of vectors can be examined is virtually impossible. What can be done, and in fact was done in the present case, was to compare existing behaviour patterns with previous observations on the subject.

Downs and Bordas[2,3] examined the behaviour of *A. pseudopunctipennis* in sprayed and unsprayed huts in Acatlipa, a village of the State of Morelos considered as representative of conditions in the Mexican plateau. Experimental huts of mud walls and thatch roofs, similar to those described by Muirhead-Thomson[4] in Africa, were used in this work. Preliminary work had indicated that the use of baffles or louvres easily deterred the approaching *A. pseudopunctipennis* from entering the huts but that an L-shaped tunnel permitted their entry and at the same time prevented their escape. The L-shaped tunnel proved very useful for examining the entry pattern of *A. pseudopunctipennis*, and the egress was investigated by means of an exit trap fixed to the door of each hut. Observations on behaviour in untreated huts were made in 1949[2] and on the effect of DDT and BHC in 1950[3]. Eleven years later huts Nos. 1 (DDT) and 2 (control) of Downs and Bordas were still standing and it was decided to repeat the original observations to determine whether the prolonged use of DDT (Acatlipa had been sprayed since 1944) had produced any ethological changes in the local *A. pseudopunctipennis*.

The two huts had their mud walls replastered and their thatch roofs renewed and were fitted with L-shaped entry tunnels and exit traps identical to those used in the investigations of 1949–50. Throughout the present investigations the huts were operated 4 nights every week from dawn to dusk—weeks of entry captures alternating with weeks of exit collections. In the former the mosquitoes were caught at the time they reached the entry tunnel (as had been done in the earlier investigations) and in the latter they were removed every hour from the exit trap. In both types of captures counts were made of mosquitoes found dead on the floor.

Observations were made for a period of six weeks before spraying hut No. 1, using DDT water-dispersible powder at the dosage of 2 g/m^2 as had been done in the 1949–50 investigations. Hut No. 2, which had served then as control, was again left unsprayed and served once more as comparison hut. Collections were made for another period of six weeks, after which hut No. 1 was replastered and its thatch roof was changed before it was sprayed again using the same DDT formulation and dosage as in the first spraying. Eight months later a new series of captures of incoming and outgoing mosquitoes was carried out for another period of six weeks. Conditions in these last captures produced the same results as Downs and Bordas's investigations[3] regarding season and time of spraying. According to their published records[3], only exit captures were made after spraying, and for this reason in the comparison of Downs and Bordas's and our own results given in Table 1 only results from exit captures are given.

Table 1. RESULTS OF CAPTURES OF *A. pseudopunctipennis* IN EXPERIMENTAL HUTS AT ACATLIPA (MORELOS)
(Exit trap and floor collections)

		Hut No. 1 (DDT)		Hut No. 2 (control)	
Time after spraying	No. of captures	No. of males	No. of females	No. of males	No. of females
Original experiments of Downs and Bordas, April–July 1950					
8–10 months	21	670	510	1,185	907
Present observations, July 1962–*August* 1963					
Before spraying	12	444	438	367	412
2–35 days	12	425	374	315	256
8–10 months	12	353	245	338	252

As can be seen from these results, the number of mosquitoes caught in huts Nos. 1 and 2 before DDT spraying was very similar, and this, it should be added, is in agreement with what had been observed by Bordas and Downs[2]. After the introduction of DDT, however, an important difference is noticeable between the early investigations and the present ones. In the Downs and Bordas[3] observations the number of males and females of *A. pseudopunctipennis* caught after spraying in hut No. 1 was considerably lower than in hut No. 2. The treated hut caught only 65·7 per cent of the males and 56·2 per cent of the females caught in the control hut. In the present observations there was no reduction in the number of *A. pseudopunctipennis* caught in the DDT-treated hut. As Table 1 shows, there was in fact an increase in the number of males and females caught in the treated hut immediately after spraying. A slight reduction, it is true, was observed 8–10 months after treatment, but if the total numbers caught in each hut are added up it will be seen that the treated hut caught slightly more mosquitoes than the unsprayed hut. This, as pointed out before, is in strong contrast with the observations of Downs and Bordas[3].

Although ingress captures do not appear to have been made by these investigators after spraying the huts, they were made in the present studies both before and after spraying. Their results confirm the lack of a reduced entry in hut No. 1 after spraying. Ingress captures in hut No. 1 yielded 258 males and 276 females immediately after spraying and 212 and 256 males and females respectively, 8–10 months after. Corresponding figures for hut No. 2 (control) were 317 and 307; 168 and 201. There seems to be a slight reduction in numbers in hut No. 1 immediately after spraying; but this is compensated by a small increase 8–10 months after spraying. Altogether more mosquitoes were caught in the treated hut than in the control, as was the case with the exit trap captures. Here it should be pointed out that in the ingress captures mosquitoes are caught (by the men in charge of the captures) at the very moment they reach the ingress tunnel so that an unrecorded escape from the huts (a possibility in the exit captures) is ruled out here.

The complete lack of deterrency, a term proposed by Zulueta and Cullen[5] to embrace all possible causes that prevent mosquitoes from entering sprayed structures, is a remarkable finding, particularly when it is considered that it was observed among mosquitoes which for the most part were seeking shelter and not a blood meal (for example, males and engorged females). A behaviour like this suggests immediately a development of physiological resistance, but periodical checks on the susceptibility of the local *A. pseudopunctipennis* gave results which indicated normal susceptibility of the species to DDT. If the susceptibility under test conditions seemed to be unchanged the same cannot be said of the lethal effect produced by DDT in the treated hut. Immediate mortality in hut No. 1, based on numbers of mosquitoes dead at the time of collection (window trap and floor collections), was in the Downs and Bordas[3] observations 49 per cent in males and 85·9 per cent in females. Corresponding figures in the present observations were 45·6 per cent and 59·6 per cent soon after spraying and 34·3 per cent and 44·1 per cent 8–9 months after spraying. These results seem to indicate that, in spite of unchanged susceptibility, *A. pseudopunctipennis* avoided better the lethal effect of DDT in 1962 and 1963 than it did in 1950. As in the case of deterrency, a change in behaviour seems to have taken place here after eleven years use of DDT.

The changes observed in Acatlipa are very similar to those noted by Trapido[6,7] in the Chagres River of Panama, where after six years use of DDT, and in the absence of physiological resistance, there was an increase in the number of *A. albimanus* entering treated houses and in their survival inside them. The similarity of these findings with those of Acatlipa makes it likely that the same situation may exist in other countries where, in spite of normal susceptibility of the vectors, the effectiveness of DDT seems to have been blunted after prolonged use of the insecticide. Their changes of behaviour may provide the clue to difficulties and failures.

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EFFECT OF SOIL/SOLUTION RATIO ON DETERMINING THE CHEMICAL POTENTIALS OF PHOSPHATE IONS IN SOIL SOLUTIONS

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SCHOFIELD[1] suggested that an index of soil phosphate availability could be given by the negative chemical potential of monocalcium phosphate—the 'phosphate potential'—defined as $(\frac{1}{2}p\mathrm{Ca}+p\mathrm{H_2PO_4})$ and determined in a 0·01 M calcium chloride extract. Aslyng[2] found, however, that this potential varied markedly with the soil/solution ratio and he attempted to correct for this 'dilution effect' by extrapolation of his experimental results to 'zero dilution'; others[3,4] have adopted this procedure. However, this is not a justifiable correction because, as shown by Aslyng's own results and those of others[5,6], there is no approach to a limiting value towards zero dilution.

Larsen and Court[6] suggested a number of possible explanations for the effect of soil/solution ratio on the phosphate solubility but were unable to decide which was operative. Olsen *et al.*[7], using soils comparatively rich in soluble phosphate (about 100 times more than in most other soils), found that the negative dicalcium phosphate potential ($p\mathrm{CaHPO_4}$) of the 0·01 M calcium chloride extracts decreased with the amount of soil in the suspension. They equilibrated the soils with calcium chloride for 4 days at a constant carbon dioxide pressure and measured the concentration of bicarbonate ions in the extracts. This was found to increase with the amount of soil and they attributed the increase in phosphate concentration (decrease in negative phosphate potential) to the ability of carbonate ions to displace $\mathrm{HPO_4}$ ions from surfaces containing phosphate. When the chemical potential of monocalcium phosphate is calculated from the experimental results of Olsen *et al.*[7] it is independent of the soil/solution ratio (Table 1). This suggests that the observed effect of soil/solution ratio on the phosphate potential $(\frac{1}{2}p\mathrm{Ca}+p\mathrm{H_2PO_4})$ may be due to accumulation of carbon dioxide produced by microbial activity during equilibration with the calcium chloride solution, since this usually occurs in stoppered bottles mounted on an end-over-end shaker.

To test this assumption, varying aliquots of soil 5 from the experiment of Larsen and Court[6] were equilibrated for 16 h with 0·01 M calcium chloride under three contrasting conditions: (1) in stoppered bottles placed in an end-over-end shaker; (2) open bottles on a roller shaker; (3) bubbled with atmospheric air, previously moisture saturated by passing through a wash bottle containing 0·01 M calcium chloride; wrist action shaking.

From the results (Table 2) it can be seen that when stoppered bottles were used there was a reduction in pH, increase in phosphate concentration and decrease in phosphate potential with increasing weight of soil in the suspension. When the bottles were left open the same trends were observed but to a smaller degree. However, when atmospheric air was passed through while shaking, all three values became independent of the amount of soil in the suspension. Similar results were obtained with other soils.

Table 1. EFFECT OF SOIL/SOLUTION RATIO ON SOIL PHOSPHORUS SOLUBILITY *

g soil/ 50 ml.	pH			$C_P \times 10^6$			$\frac{1}{2}p\mathrm{Ca} + p\mathrm{H_2PO_4}$†		
	1	2	3	1	2	3	1	2	3
5	6·75	6·70	6·46	474	103	224	5·76	5·39	4·99
10	6·82	6·75	6·52	455	136	331	5·79	5·29	4·83
20	6·92	6·84	6·62	593	149	403	5·75	5·29	4·79
40	7·02	6·95	6·73	600	149	406	5·78	5·35	4·82

Soil 1, Pierre clay
Soil 2, Apisha silt clay loam
Soil 3, Rocky fine sandy loam
} equilibrated 4 days in 0·01 M $\mathrm{CaCl_2}$ at $-\log P_{\mathrm{CO_2}} = 1·42$

* Data of Olsen, Watanabe and Cole[7].

† Calculated by the equation: $\frac{1}{2}p\mathrm{Ca} + p\mathrm{H_2PO_4} = p\mathrm{CaHPO_4} - pK_2 + (p\mathrm{H} - \frac{1}{2}p\mathrm{Ca})$, where pK_2 is the 2nd dissociation constant of phosphoric acid and equals 7·20.

Table 2. EFFECT OF SOIL/SOLUTION RATIO AND CARBON DIOXIDE EQUILIBRATION ON SOIL PHOSPHORUS SOLUBILITY *

g soil/ 50 ml.	pH			$C_P \times 10^6$			$\frac{1}{2}p\mathrm{Ca} + p\mathrm{H_2PO_4}$		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
1	7·23	7·31	7·36	0·71	1·04	1·11	7·79	7·68	7·68
2	7·16	7·32	7·41	0·93	1·16	1·13	7·63	7·65	7·72
4	7·04	7·31	7·43	1·16	1·25	1·16	7·47	7·60	7·73
8	6·98	7·25	7·42	1·35	1·42	1·19	7·37	7·51	7·70
16	6·94	7·20	7·40	1·53	1·59	1·07	7·30	7·42	7·74

* Soil 5 of Larsen and Court (ref. 6).

a, Stoppered bottles, end-over-end shaking for 16 h.

b, Open bottles, roller shaking for 16 h.

c, Bubbled with atmospheric air, previously moisture saturated by passing through a wash bottle containing 0·01 M calcium chloride; wrist action shaking.

In a separate experiment the amount of carbon dioxide produced by the soil was measured by absorption in sodium hydroxide using the apparatus described by Kent-Jones and Taylor[8]. The carbon dioxide pressure was calculated from the solubility of carbon dioxide in 0·01 M calcium chloride at room temperature[9], and the volume of the bottles. The results (Table 3) show that the amount of carbon dioxide produced was proportional to the amount of soil in the suspension. This suggests that the production of carbon dioxide at the individual soil/solution ratios fully explains the variation of pH with the soil/solution ratio.

Table 3. EFFECT OF CARBON DIOXIDE ON SOIL pH

g soil/ 50 ml. 0·01 M $\mathrm{CaCl_2}$	$P_{\mathrm{CO_2}} \times 10^3$	$p\mathrm{H}\alpha$	$p\mathrm{H}\beta$
1–16	(0·3)	(7·43)	(7·55)
1	2·6	7·32	7·42
2	3·2	7·22	7·32
4	5·5	7·14	7·17
8	11·8	7·01	7·07
16	21·2	6·94	7·00

$p\mathrm{H}\alpha$ measured after shaking for 16 h in stoppered bottles.

$p\mathrm{H}\beta$ measured when soil suspensions (10 g soil/50 ml. solution) were equilibrated in the presence of carbon dioxide buffers.

Figures in brackets obtained when the suspensions were equilibrated with atmospheric air.

Using the method of Pardee[10], carbon dioxide buffers were made up to give carbon dioxide pressures corresponding to those calculated when the soil suspensions were shaken in stoppered bottles. 10-g aliquots of the soil suspended in 50 ml. 0·01 M calcium chloride were then shaken overnight with an atmosphere that was in equilibrium with these buffers. The values of the soil suspensions (Table 3) compare closely with those obtained when varying soil/solution ratios were shaken in stoppered bottles without carbon dioxide absorption.

These findings have a bearing on the interpretation of lime and phosphate potentials, for the carbon dioxide content of soil air varies greatly in the field[11] because of variation in biological activity and soil aeration.

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SCIENCE AND BEHAVIOUR

By The Right Hon. Lord BRAIN, F.R.S.

President of the British Association

Introduction

IT is with great pleasure that the British Association comes back to enjoy once again the hospitality of Southampton. Since we were last here in 1925, the University College has become the University and the town a city, and Southampton to-day looks ahead to new and great challenges to its civic and educational life.

It is interesting to look back at former meetings of the Association which have been held here. We hear a good deal to-day about the accelerating acceleration of scientific knowledge and development; but a review of the Association's past would suggest that there is also a factor of inertia to be taken into account. In 1882 when the 52nd meeting was held in Southampton, the report says that "this year the Channel Tunnel Company sought to obtain powers from Parliament to enable it to make a tunnel to connect this country with France by a railway beneath the channel", and one speaker advocated both metric measurements and decimal coinage. Going even further back, to the 1846 meeting, we find Dr. Laycock directing attention to "some diseases resulting from the immoderate use of tobacco", and mentioning particularly "congestive inflammation of the . . . bronchial lining of the lungs". In the same year, there was a notable communication by Dr. Carpenter "on the physiology of the encephalon" which is relevant to my subject. Carpenter drew from comparative anatomy some conclusions about the role of what he called the sensory ganglia, situated in the base of the brain, in both sensation and emotion—ideas which were not to find full confirmation until the present century.

In choosing to talk about "Science and Behaviour" I have several objects in view. The primary one of course is to fulfil the aim of the British Association to bring some scientific facts before a wider public than would normally hear about them. But this at once raises a problem of scientific communication. As science becomes increasingly specialized, it seems to me that two kinds of scientific communication are required. First, there is the obvious need to tell people about what is going on in particular sciences, but there is also, surely, an equal need to show how researches in many different sciences may be related to one another. The difficulty is that such an enterprise does not fall within the scope of any individual specialist, and we lack specialists in generalization. To try to give such a satellite view is to court attack from those over whose territory one flies in one's survey. Nevertheless, I propose to make the attempt.

By behaviour I mean the reactions of living animal organisms to their environment. As human beings, we are increasingly occupied with problems arising out of our own behaviour: one need look no further than the addresses of my presidential predecessors during the past decade to illustrate this. Not unnaturally, perhaps, we tend to interpret human behaviour exclusively in human terms, and there is no lack of such interpretations. I believe, however, that what man has in common with other animals may prove to be just as important for our understanding of his behaviour as are his distinctively human characteristics. Indeed, I think it will illuminate our problems. I propose, therefore, to try to present a biological view of behaviour, including our own. This should not be a narrow view, if, as I hope to show, it embraces all aspects of life.

Behaviour as Response to Environment

The study of the behaviour of sub-human mammals, birds, fishes, and insects, has one great advantage over the study of human behaviour. Organisms with comparatively simple nervous systems may exhibit quite complex behaviour, but in studying such creatures it is much easier than in the case of man to isolate the factors involved. At the same time there are cardinal differences between the environment of man and that of all other living creatures. The environment to which any sub-human living organism reacts is a much simpler one, because it is a more narrowly selected environment—selected by the innate structure of its sense organs and, still more, its nervous system. Each living organism, through natural selection, has become equipped to respond to those environmental stimuli which are biologically important to it. For example, the study of sex-attractant odours in insects[1] has shown that these may be so highly specific that a very slight change in their chemical structure may make them less effective or even quite ineffective. So sensitive is the insect's sense of smell that it will respond to a few molecules of the substance. Bats use ultrasonic vibrations like radar to detect the insects on which they prey; but there are noctuid moths which have evolved so that they react selectively to the ultrasonic cries of the bats[2].

Since these moths have only two nerve fibres connecting their hearing organ with their nervous system, it has been possible to make a detailed study of their nervous responses to the bat's cry, and to show how they receive information about the distance of the bat, and the direction of its flight; and that their movement of response is modified in accordance with the information they receive. Much work done by ethologists throws light on the perceptual element in the behaviour of a large range of living creatures.

But this highly selective response to particular features of the environment is far more than a simple reflex reaction to one sensory modality. Even those organisms with a comparatively elementary nervous system exhibit very complex behaviour. Thus it has been shown that the fruit-fly, when using its sense of smell to find its way to a substance on which it feeds, can do this only with the aid of visual observation of the ground, without which it cannot 'know' from which direction the wind is blowing and so cannot fly up-wind in the direction from which the attractive scent is coming[1].

Perhaps the most notable examples of the complexity of the factors influencing behaviour are to be found in the way in which honey-bees orientate themselves in relation to a source of honey, and communicate information about this to their fellow workers; and also in the direction-finding methods of migrating birds. Dr. W. H. Thorpe[3] says on this subject: "In both there is presumably an innate appreciation of direction and speed of movement of the sun, together with an accurate internal clock. In both there is the innate ability to relate a 'desired' direction to the spacetime standards innately provided, to extrapolate from the observed situation and to relate to it the knowledge of general topography and special landmarks gained from individual experience. In both there is an 'appreciation' of the relation between '*direction*' of a light source and the '*direction*' of a movement (foraging flight, migration flight, homing flight). But the honey-bee does better than the bird in two respects. Firstly, it can and must 'transpose' between sun direction and gravity direction, and secondly, it must be able to transmit information by transposition between foraging flight and a straight run of the dance"—which is the specially directed movement which the bee carries out on its return to the hive to convey the necessary information to its fellows. As Thorpe says, "what are we to make of the bee's brain of 2·5 mgm in view of its astounding performance?". Recent work suggests that the bee's signalling is even more complex, sound also being used to convey information about distance[4,5].

Thus the perceiving organs and the nervous system of the bees and the birds are able to obtain from their environment the kind of information which the navigator gets by using his sextant, compass and chronometer, and to process this information, and turn it into motor programmes, which result in flight in the right direction and for the right length of time.

Organization of Behaviour in the Nervous System

Now I must say something about how the animal's behaviour is organized in the nervous system. In a sense that would involve describing everything which the nervous system does, for it exists only to organize behaviour.

In outline, we begin with the sense organs, and the sensory nerves conveying information from them. Recent physiological work shows how the discrimination of different features in the environment begins with the structure and activities of the sense organs. The retina of the frog's eye, for example, can 'distinguish' a curved edge from a sharp one[6] and the cat's retina is so organized as to react differently to movement in one direction and movement in another, bringing different nerve fibres into action in each case[7]. From the vast number of nerve impulses streaming into it from all the sensory channels, the brain makes possible the recognition of patterns, and hence of objects. If you teach animals that, if they react to a certain visual pattern, they will get something they like, you can test them with different patterns, and find out to what elements in a complex pattern they respond, and why[8].

How an animal responds to an object depends on its instinctive drives, and these are to a large extent mediated by the activity of the nerve centres situated at the base of the brain in what is in evolution its oldest part. There, in many mammals, are centres for rage or aggression, and when these are stimulated electrically or cut off from higher control, the animal exhibits all the manifestations of rage, while if they are destroyed, or cut off from communication with the rest of the nervous system, it becomes abnormally docile[9]. Fear and avoidance, feeding, and sexual and maternal activity are similarly represented in this region. So we must picture sign-stimuli being received, and the resulting information, reinforced by previous experience through memory, being brought to this part of the brain, where it rouses into action the right organizing centre to call forth the appropriate response. These centres are influenced also by internal factors. Chemical substances, such as the products of the endocrine glands, play on them and prime them; their activities are also modified by a complex system of nerve fibres which regulates alertness, attention, sleep and waking, and so consciousness itself.

Thus animals react to situations in a complex fashion, often using more than one sense. But even in the highest sub-human animals, the extent of their environment to which they react, and the range of their reactions to it, are very limited. We think in terms of sign-stimuli, such as a particular colour, shape or movement, as eliciting a behavioural response, because the scientific method seeks to isolate individual variable factors; but the sign-stimulus we isolate is part of an environmental situation, and the response is that of the animal as a whole. But we must not become too anthropomorphic, and picture birds and animals as regarding their mates or their young or their prey as though these were as coherently and enduringly objectified to them as they are to us. At present, in any event, we cannot imagine what an animal perceives; but we can avoid thinking that this is like our own ideas or experiences.

The Uniqueness of Man

This leads me to ask in what respects—important for our present purpose—human beings as animals differ from other animals.

The evolution of man has been a good deal discussed in the light of recent research, particularly in Africa. We need not now concern ourselves with the details. It seems established that at some time from half a million to a million years ago, our hominid ancestors evolved by descent from a kind of anthropoid ape not represented by any living species. A number of factors were involved in this transition, notably changes in the shape of the hand, the pelvis, the legs, and the feet. But changes in the brain were of fundamental importance, and already we can see in the brain of the anthropoid ape developments which are an important move in the direction of the human brain. In the primate brain, the occipital lobe at the back becomes enlarged to accommodate the expanding visual area, and there is also a rapid development of the temporal and frontal lobes—higher centres concerned with bringing perception, memory and the instinctive drives into relation with one another[10]. These areas are further developed in man both relatively and absolutely, with the significant expansion also of an area in the lower part of the parietal lobe, which plays an important part in speech, and the use of symbols generally[11-13].

We find, then, that the basic structure of the human brain is similar to that of other animals. We have inherited from our early ancestors the nervous mechanisms which underlie much emotional behaviour. What is distinctively human is not only the increase in the size of the brain, but also the selective development of particular areas relative to the rest. What does this mean in terms of function?

I have already shown what the honey-bee can do with its brain of 2·5 mg. The average human brain weighs 1,500 g—half a million times as much. Clearly a very small nervous system does some things very successfully. It does them well because it is the product of evolution, and in evolution nothing leaves so many successors as success. But creatures with small brains exhibit only a small number of behaviour patterns. Since each nerve cell is capable of receiving information from hundreds of others, even a small increase in the number of nerve cells in the brain greatly adds to the complexity of the interrelationships of the total number. The increase in the size of the brain and the particular development between the anthropoid apes and man are associated with a change of behaviour which is qualitatively so great that the gap between man and his immediate pre-human ancestors is greater than that between those and the lowest mammals; and it is the human brain which is responsible for this difference for, however important in human evolution may have been the development of binocular vision, the assumption of the erect posture, and changes in the structure of the hand, it was the development of the nervous system which enabled man to put these structural changes to new uses.

Beginning on the receptive side, we find in man an enormously increased visual discrimination, which is not due to better eyes, but to the better use made of them by the brain. Added to this is the greatly increased capacity for learning and memory. But the new ability, which above all others transformed an animal into a man, was the use of symbols in language. I will not stop now to discuss the much-debated question of the relationship between language and thought. The two are certainly intimately related, and without language thought cannot get very far. By means of symbols, which now include special languages and meta-languages, such as those of mathematics and logic, the mind can represent things, and the relations between them, in a world of its own, and in the absence of the things for which the symbols stand, and it can then go back to the outer world and test the results of its activities.

Just as brain development has greatly increased the range and scope of perception (that is, the receptive side of its activities), so it has enhanced the range and power of man's control over his environment. Manipulation, which literally means handling, has been extended from the simple things an ape can do with its hand to the construction of tools, which, beginning with flaked bones and chipped flints, have developed within a few millennia into the electron microscope, the radiotelescope, and the vast and powerful machines which are used to split matter into its minutest components: and this, in modern parlance, is a feed-back mechanism, for manipulation enhances perception and conversely, and symbolic languages make the whole process possible, so that man's range of action now extends from the omega particle to the planets, and his perception millions of light years further.

But this is still only half the story of human distinctiveness. Man is a social animal in a special sense. Many types of animal society exist, some, among insects, quite complex; but man alone has built up his society on the basis of language, and it is language which has made possible the unique complexity of human social relationships, out of which have sprung, among other things, the scientific achievements of which I have just been speaking: and, of course, the development of written language and printing has endowed man with a new mode of social inheritance through which ideas can survive those who produce them, and influence succeeding generations. Moreover, broadcasting has greatly multiplied the numbers to whom speech can be directly transmitted.

We see then how each expansion of one aspect of behaviour in the development of man from ape has stimulated and aided the growth of others. This is well illustrated by human emotional life. Man's increased powers of perceptual discrimination and memory unite with his capacity for symbolism to throw his feelings open not only to a vast range of experiences of things and events in the outside world, but also to his own ideas, and to both expressed and transformed in literature and art: and so man becomes self-conscious. Whether any higher animals other than man are self-conscious is a matter for speculation; but it is safe to say that man alone has an idea of himself, which plays an important part in his behaviour. In a special sense, then, man possesses two environments. There is the external environment which we all share, and an inner world, the world of the mind, private to each one of us, in which dwell our memories, thoughts, and feelings, and the outer world reflected in them. "Thus is man", as Sir Thomas Browne[14] says, "that great and true Amphibium, whose nature is disposed to live not onely like other creatures in divers elements, but in divided and distinguished worlds"; and with man's self-consciousness comes the impulse to understand himself. Can man explain himself? Is there a science of human behaviour?

Science and the Mind

Science deals chiefly with what is measurable. It observes, constructs hypotheses, and goes back to the facts, to see if it can verify them by further observations, which include experiments. How are we to measure factors in the complexity of the human mind ? Sometimes this is comparatively easy. Experimental psychology, for example, devises tests to measure human learning and memory, attention and vigilance, decision-making and skill: in his laboratory the psychologist can vary the conditions of perception, and also study how perceptual activities break down as a result of brain disease. Language and its disorders offer a particularly valuable field for psychological investigation, and here psychology joins hands with linguistics. Word-frequency studies and observations of the choice of words, when speech is disordered, may be expected to throw light on the way in which the physiological processes underlying language are organized in the brain.

But the difficulties inherent in psychological measurement are well illustrated by the problems to which intelligence tests have given rise. The intelligence tester gives the subject various tasks to perform (some involving the use of words, and some not), allots a score of marks, and compares the results with standard scores, corresponding to percentiles in a representative population. The difficulties begin when we ask what these tests are measuring, and what we are doing when we subject the results to factorial analysis. One may of course evade these questions by merely saying that the tests do no more than measure individual responses to particular problems, and render possible predictions, which experience shows to be on the whole valid. But this is to neglect the scientific value of hypothesis.

Prof. M. D. Vernon[15] directs attention to the essentially cumulative nature of mental development, which, he says, "implies that those who, in early life, acquire a richer stock of perceptual schemata and verbal labels, are better able to build up the more complex and more flexible schemata necessary for conceptual thinking". Moreover, a stimulating environment and the absence of emotional stresses during childhood are factors favourable to the development of schemata, while children who have been emotionally starved, or frustrated, have schemata which are in various ways inadequate, and fail to develop to the fullest possible extent.

But it is in the realm of human emotion and man's instinctive drives that we encounter the greatest difficulties for measurement and classification. The idea that human beings can be classified as belonging to one or other of certain psychological types goes back at least as far as the Greeks, and in such classifications stress has always been laid on their emotional reactions. But if it is hard to know exactly what we are testing, when we carry out intelligence tests, tests of emotion would seem to be even more difficult to interpret.

The analysis of human emotions seems itself to be strongly evocative of emotion, judging by the vigour with which the elaborate theoretical systems of human behaviour, which have come into existence, are attacked and defended. Since the beginnings of civilization there have been systems of belief about human nature which are unscientific, because they are unverifiable, there being no tests by which we can determine whether they are true or not. Prof. K. R. Popper[16] has pointed out, in connexion with psychoanalysis, that theories which explain everything are unverifiable. There is no means of testing them: there is no conceivable human behaviour which could contradict them. But though such theories may be unscientific, they are not necessarily valueless. The contributions which science can make to the interpretation of something as complex as human nature are at present limited, and in unscientific theories, as in art, there may be insights, intuitions and illuminations, which are of value for practice as well as for theory. But in no pejorative sense of the word they belong to the sphere of myth rather than science.

There are two aspects of the human mind which add to the difficulty of a scientific interpretation of emotions. There is in psychology a principle of indeterminacy which is of much the same kind as Heisenberg's in physics, for in an enquiry into a person's emotions, the mere fact that the enquiry is made creates an emotional reaction, so that the intervention of the observer modifies the effects of his observation.

Secondly, as has now long been recognized, the explanation which a person gives of his behaviour and his motives, though it appears to him to be both true and adequate, may omit relevant factors because he is unconscious of them. The existence of what is called the unconscious mind was recognized long before the present century, but it was Freud who chiefly brought the idea into prominence by using it to introduce into the explanation of behaviour emotional factors of which the subject is unaware. Freud himself and other psychologists have attributed to the unconscious a complex structure and dynamic functions; but these, as I have said, are not scientific concepts.

There are ways of looking at the unconscious mind which may provide a bridge between psychology and physiology, and which were to some extent envisaged by both Freud and Jung. If, as we saw earlier, emotion is to be regarded as an intrinsic part of the behaviour of the organism under the influence of its instinctive drives, it should not surprise us if feelings and impulses which are mediated by the most primitive part of the brain can sometimes move us without our being conscious of any rational explanation for them. Some psychologists believe that, by a process which is called conditioning, past experience may have operated to link together certain objects and certain feelings, although we can no longer remember the origin of their association, which may therefore now be described as unconscious. Man's very self-consciousness may entrap him when he seeks to explain his own or other people's behaviour if it encourages him to look for complete explanations in the conscious self.

Mind and the Social Environment

One of the characteristics of Western culture for many centuries has been the stress laid on the individual man. Whether the emphasis has been on human depravity, responsibility or perfectibility, or all three, the moulding influence of man's social environment on him has, until recently, been on the whole neglected. But if we neglect the individual's relationship with society, we shall not only fail to understand much of his behaviour, but also lose opportunities of influencing it. Only recently has man in society been studied scientifically by the social sciences; and what I have already said about the complexity of human behaviour will have shown how difficult this task is.

It has been pointed out[17] that we tend to enquire about causes only when we are confronted by some breach of routine. Thus we ask why a person is ill, and not why he is well. This tendency is exemplified in our attitude to social psychology, for it is when people behave in an abnormal manner that we call on sociologists to explain their behaviour, while we take for granted the behaviour of the large majority which presents us with no problem though this is of equal importance to the social psychologist and social anthropologist. Even a slight acquaintance with the literature of what has been called "social pathology" is enough to show the difficulties encountered by those who work in this field. Baroness Wootton[18] in her recent comprehensive monograph lists twelve hypotheses of separate factors believed to influence crime and delinquency. As she also points out, the relationship between anti-social behaviour and mental disorder, and indeed the concept of mental health itself, are the subjects of much confused thinking. For example, it is very difficult to define a psychopath, and to apply medical criteria to the legal concept of criminal responsibility.

As we have seen, the individual personality is the product of a number of factors: genetic ones may largely determine his intelligence and influence his psychological type, and he is exposed to a very complex and variable environment. How far are crime and delinquency determined by genetic factors? We must first bear in mind that, as Prof. L. S. Penrose[19] says, "crime is a relative phenomenon and it is measured by the standards of behaviour appropriate to a particular social system at a given time and place". In other words, it has meaning only in relation to the cultural environment. Penrose concludes that criminality is not a suitable trait for genetic studies except when it is associated with a clearly defined pathological condition. Genetic influences are probably important, but very little can be learned about their nature. We may conclude, therefore, that even when they exist, the most hopeful way to deal with them at present may be to discover what environmental factors activate them. A great deal of work is being done in the attempt to discover these, and there seems no doubt about the importance of family deprivations and social disturbances in the early years of childhood, but as the Gluecks[20], who have been working in this field in the United States for many years, say, "whether one looks at neighbourhoods or classes or cultures or sub-cultures, only certain individuals are chosen or choose themselves for careers of crime". Delinquent behaviour cannot therefore be put down solely to unwholesome environmental influences, which affect many people: it must also be attributed to individual lack of immunity to these influences. Both factors need to be taken into account. This is on the whole a hopeful view. "In delinquency", to quote the Gluecks again, "one is dealing not with destiny, but with destination, and destination can (hopefully) be altered." We clearly need much more knowledge, not only about individual reactions to environmental stresses, but also, more generally, about the normal emotional bonds between the individual and society. The social psychologist and anthropologist can help by comparing the features of very stable societies with those of changing cultures, and especially perhaps those in which, as in the developing countries, a previously stable society is rapidly exposed to new systems of ideas. It may well be relevant to consider not only the individual's emotional bonds with society but also the effect on him of the present view or conflict of views about the nature and purpose of life. We need, then, many more scientific observations over the whole range of human behaviour, normal and abnormal; but in science hypotheses too play an important part and in the social sciences we need fruitful hypotheses just as much.

Need for Extrapolation

Until man came on the scene, it was always the environment to which organisms were currently exposed that was important for them. Survival has never in the past depended on the conscious prediction of changes which had not yet occurred. Indeed, until man appeared, no animal possessed the power to do this, and even though man has profoundly modified his environment, and, incidentally, that of many other living creatures, his ability to predict the consequences of the changes he is producing has until recently been small. Even now, are we trying hard enough to foresee and prepare? As Prof. A. N. Whitehead[21] once put it, "a muddled state of mind is prevalent. The increased plasticity of the environment for mankind, resulting from the advances in scientific technology, is being construed in terms of habits of thought which find their justification in the theory of a fixed environment".

This truth has been overwhelmingly illustrated by the history of the thirty years which have passed since Whitehead wrote these words. The potentialities of science and technology for the benefit of mankind as a whole are almost inconceivably great; but the preparations which we are making for their use and development are pitiably small.

One of the outstanding examples of our failure to predict and prepare for the consequences of scientific development has been population growth. Now, thanks to the energy of a few people, chiefly in Great Britain and the United States, it is fairly generally recognized that there is a population problem, and that there is every prospect that the world population will have doubled from three thousand million to six thousand million by the year 2000. This of course is partly the result of widespread improvements in medicine and hygiene, and their life-saving consequences. The Food and Agriculture Organization's Third World Survey[22] shows that 10–15 per cent of the world's population are now living on a diet with insufficient calories, and 50 per cent are either undernourished or malnourished, or both. The task, not only of raising the standard of living for them, but also of providing such an improved standard of living for twice the present numbers in forty years, is the challenge which now faces us, and we must not forget that people need not only food, but also water, roads, industrial plant, education, and indeed all the means to a full life.

This is not a simple problem, nor is there any single solution. In some countries a larger population may be desirable: others will not be able to attain to a reasonable standard of living until they control their population growth. What is disturbing is that until now the human race has left its future numbers to chance. The average animal breeder has shown more concern about the consequences of his acts than the ordinary human being about what will happen to his own descendants. The animal breeder looks ten generations or more ahead; the human breeder not more than two, if that.

Great Britain has no population policy. The present rate of population growth took us by surprise, and we have not yet given thought to its implications. To take one instance from my own profession, it is clear that we shall need many more doctors in thirty years' time. This means more medical schools. It takes a minimum of 15 years to turn a first-year medical student into a consultant. Allowing ten years to build and equip a new medical school, we ought soon to begin to build the medical schools necessary to produce the consultants we shall need in the year 2000. Similar needs will arise in connexion with education, housing, roads and other necessities, and our existing road problems show how little we have prepared for what could have been foreseen. This is not a matter of obtaining more knowledge, but of persuading the Government to use the knowledge we have.

There is another aspect of the proper use of our resources which also urgently needs consideration. It is predicted that the progress of automation will leave many people with much more leisure. But automation will make little difference to doctors, midwives, nurses or school teachers. At present we have too few of all of these, and as the population increases, so will the shortage. Are we then to envisage a state of affairs in which the industrial part of our population enjoys compulsory leisure, while the professional part continues to be overworked? All that automation means is that fewer people are needed to do certain jobs. Should we not now be preparing for this by expanding the opportunities and incentives in medicine, nursing and teaching, to encourage more people to work in these fields? The benefits of automation would then be more evenly spread among those who work, and the community as a whole would benefit.

But world population growth cannot be dealt with adequately at national levels. We need an international body, sponsored by the United Nations Organization, to collect all available demographic information (and promote the search for it when it is not available), take into account present and prospective supplies of food and water, and the economic and cultural needs of growing populations, and review all present scientific work on population control. Every nation would then have the information on which to base its own population policy, and all would know what each was doing in a matter which collectively concerns us all.

Our greatest need to-day, then, is to acquire the power of looking ahead, forecasting, and preparing for the consequences of the accelerating developments in science and technology. Many years ago, H. G. Wells pointed out the need for what he called a "world brain" to correlate information and extrapolate it on the world scale, and I hope that one day we shall achieve something like that. But I can see only one main preliminary step towards all this essential foresight—education, and by education I mean education at all levels of society.

To my mind, the important division between people is not between the classical and scientific outlook on life, but between those who have been educated to see the world in terms of the rapidly changing environment, which science is creating, with all its potentialities, and those who see it in terms of the static environment, and frozen emotional attitudes of the past. The first group have to educate the second, which includes many of our rulers, as quickly as possible, and much encouraging work is being done on these lines, especially by international groups of scientists. But that, of course, is not enough. So far as we can, we must prepare all our people for a new world which will be quite unlike the old. This will mean spending more money and thought on education than we do at present, remembering that the best educational results are unlikely where home conditions are bad[23].

Education to-day has two tasks. First, it must concern itself with the whole individual, and therefore with behaviour. But here it must largely reflect the views and standards of society; and one of our needs as a society, now predominantly secular, is to develop a code of social responsibility which can form an integral part of education. Secondly, it must prepare people for leisure. Again taken by surprise, we find ourselves in a world in which many people have more leisure than ever before, and are at a loss to know what to do with it; and in a few years' time this problem will be much more acute. This is not only an educational problem, because the proper enjoyment of leisure, involving the re-creation of body and mind, calls for social facilities for which the State must largely be responsible.

Faced with these difficult educational tasks, we have one great new asset, which science has produced—broadcasting. Here is a means through which all who are willing can continue their education, and enjoy the arts throughout life, and which can bring to the attention of millions the issues which confront us. Broadcasting has re-created for the great modern democracies one of the conditions of the Greek city state: all citizens can hear and see their leaders, and a man need not even go into the market-place to hear the debates of the sophists: he can listen to them at home in his armchair.

I have left to the end the most difficult question of all. I asked earlier how far science can explain man? Many will say that science is a mode of knowledge which is concerned only with what is measurable, and that much of importance for mankind, including values, lies outside its scope. Science, it will be said, is ethically neutral, and the uses we make of it must be judged by standards of value arrived at independently. Others, however, will reply that human values themselves are the product of evolution, and survive only as fulfilling social and biological needs. Some difficult scientific and philosophical questions arise. Perhaps the most fundamental is this. Science uses symbols to denote events, and the relations between them. If there are events or relationships, human or otherwise, which cannot be adequately represented by scientific symbols, must not some aspects of them fall outside the scope of science? One of the difficulties of living in a time of rapid change like our own is that knowledge outstrips thought about it, but I feel sure that not all our present antinomies are permanent. We are learning every year more about the nature of matter, the relationships between events and the observer, between the brain and the mind, and between the living organism and its environment; and information theory may provide unifying concepts between the gene and the mind.

I started with the nervous system. Let me end by using it as an analogy. As individuals, we are all receptors, capable of supplying the higher centres with information. What information they get, therefore, depends on us. We are also the motor nerves, and what society does is done by us. But we are again, collectively, ourselves the higher centres, the forebrain, which mediates for the social mind the difficult task of receiving the information, learning from past experience, reacting to it emotionally, yet controlling its emotions; and, above all, looking to the future.

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SUMMARIES OF ADDRESSES OF PRESIDENTS OF SECTIONS

THE SUN AND THE IONOSPHERE

THE subject of Mr. J. A. Ratcliffe's presidential address to Section A (Mathematics and Physics) is "The Sun and the Ionosphere". He directs attention to the fact that in recent years it has been possible to explore the upper portion of the ionosphere, above the peak of the *F* layer. This part has been called the 'topside' of the ionosphere. One of the most productive experiments makes use of an ionospheric sounder installed in an artificial satellite so that it sounds the ionosphere, downwards, from above. The principle is the same as that which is used in sounding from the ground; it consists in sending out short pulses of radio waves and measuring the time-interval before they return to the sender after reflexion at the ionosphere. The results are sent to the ground by telemetry. A very successful topside sounder of this kind has been made by workers in Canada.

In another experimental method, powerful waves are sent through the ionosphere on a frequency which will penetrate it completely, and the intensity, and spectral distribution, of waves scattered incoherently from it at different heights are measured. The electron distribution at still greater heights, out to distances of about five Earth radii, have been deduced from observations of a particular type of atmospheric called a 'whistler'. This results from the dispersion of the electro-magnetic pulse radiated by a lightning flash as it follows the lines of force of the Earth's magnetic field out to great distances.

The most distant parts of the ionosphere and the way in which it merges into the solar corona have been investigated by means of a space probe carrying a magnetometer. It has been found that the Earth's ionosphere merges into the Sun's ionosphere (the corona) at a distance of about ten to fourteen Earth radi[1]. Experiments of a different kind have been made in the first Anglo-American satellite, *Ariel I*, which carried experiments designed by workers in England. These experiments were designed to measure a series of quantities in the neighbourhood of the satellite. The concentration of the electrons, their temperature, and the masses of the positive ions which accompany them, were the quantities important for the present discussion. The density of the neutral atmosphere at these great heights has been deduced by observing how the orbits of satellites change as a result of the air resistance they experience in their motion.

The results from all these experiments provide information about the distribution in the outer ionosphere of the constituent gases, atomic oxygen, atomic hydrogen, and helium, and about the temperatures of the electrons, the ions, and the neutral gas. Theoretical explanations of the results involve consideration of how the energy of the photo electrons is transferred, first to the ions, and then from the ions to the neutral particles. Consideration of the rate of production of electrons, and of the rate of energy input, leads to suggestions about the incidence of energetic particles on the Earth's outer atmosphere.

CONTRIBUTION OF NEW INSTRUMENTS TO PROGRESS IN CHEMISTRY

DURING the past twenty years, the progress of chemistry has been dominated by the demand for, and the evaluation of, new techniques. This trend has been based to a very large extent on the development of new physical instruments and, if the present progress in this field is maintained, there are an infinite number of problems in chemistry which will be solved. Sir Harry Melville's presidential address to Section B (Chemistry) deals with the wider implications of instrument development and shows how progress in certain areas would have been impossible without the introduction of these new methods.

The often tedious chemical approach to the problems of measuring molecular weights has been completely superseded by the use of physical instruments. High-resolution mass spectrometry has enabled accurate determinations to be carried out in a matter of hours. The high-resolution electron microscope, for example, can be used to determine directly the weight of completely coiled high polymer molecules by measuring their diameter. Radioactive labelling is a method of micro-chemistry entirely dependent on instrumental techniques. In the field of polymer chemistry, where the weight of material being manipulated may be less than 10^{-7} g, it has put an end to many speculations about the course of chemical reactions occurring to minute amounts in high polymer systems.

The interaction of gases with solids has been investigated for years, but the preparation of uncontaminated surfaces has been a barrier to progress. The great improvement in vacuum pumps over the past few years has enabled this barrier to be surmounted, and clean uncontaminated surfaces can now be prepared and examined at leisure, using sensitive techniques such as mass spectrometry, gas/liquid chromatography, and electron field emission and ion emission techniques. The mechanism of homogeneous chemical reactions has yielded in many cases to complete analysis as the result of the development of new techniques. However, reactions which involve activated molecules, or which go through ionic or ion-pair mechanisms, have not yet yielded to these techniques, and here there is an enormous field for study.

Methods for tackling these problems are described. One involves the application of external stimuli to the system and observing the way it returns to the steady state. The problem is to devise equipment sufficiently sensitive to measure the small amounts of chemical change during the recovery period. The second method involves the application of electron spin resonance—here a considerable improvement in the sensitivity of present equipment is desirable. A third method involves the investigation of energy transfer in given systems. This involves the design of shock tubes and plasma-discharge apparatus, and the instrumentation to go with them.

These problems illustrate why a modern chemical laboratory is equipped in a very different way from ten or more years ago. The important point is that all the basic requirements of chemistry can now be carried out by more direct and sensitive methods. Increased sensitivity and greater resolving power of instruments are still very necessary and these depend on still further advances in physical techniques. To be effective, a modern laboratory must have this physical equipment, but the equipment must not be allowed to dominate the scene—the purpose of the equipment is to help the research worker to get on more quickly and precisely with his basic chemistry.

SOME ASPECTS OF EAST AFRICAN VULCANOLOGY

IN his presidential address to Section C (Geology), Mr. Wilcockson chooses to describe the Eastern, or Gregory, Rift Valley. This is a downfaulted trough extending from northern Kenya down to the latitude of Dodoma in Tanganyika. It is associated with minor structures on both sides. In the latitude of Nairobi, on the west side is the rift valley containing the Kavirondo Gulf of Lake Victoria and on the east, nearly opposite, a general fracture zone in which is situated the volcano Mt. Kenya. In northern Tanganyika, just south of the Kenya border, there is a similar pair of structures, on the west side the Eyasi Rift and the east side a fracture zone along which is a line of volcanoes culminating in Kilimanjaro. These crossings, as they may be termed, are the loci of intense volcanic activity.

On the west side of the southern crossing, the Eyasi Rift is filled with the eruptive products of the 'Giant Craters', a group of volcanoes mainly composed of alkaline lavas with little fragmental material and including the great Ngorongoro crater. In the nearby section of the rift valley are volcanoes, relatively recent in date, among which is the still active Oldonyo L'Engai. Among their outpourings are highly alkaline rocks associated with carbonatites, a type which has been reported recently as lava flows from Oldonyo L'Engai.

On the east side, Kilimanjaro has produced mainly lavas, but also some pyroclastics. Its early history is, of course, buried under later products, but the considerable proportion of the succession that can be seen shows a general change from trachybasaltic lavas to highly alkaline nepheline phonolites; the other volcanoes in this group contain similar rock types. One striking late feature is the presence of a very large number of parasitic cones which often show a reversal to basaltic types and ultra-basic crystal accumulates.

At the northern crossing the general course of events has been similar, though differing in detail. The volcanoes of the Kavirondo Gulf are alkaline in character and are associated with the intrusion of carbonatites. Mt. Kenya on the east contains phonolitic rocks, including the curious type kenyte, associated with basalts and an intrusive mass of nepheline syenite, while further to the east the Nyambeni Range is largely basaltic. Again, as on Kilimanjaro, the main activity is followed by a widespread parasitic episode.

In this latitude volcanoes associated with the rift-valley faulting poured out great floods of basaltic and phonolitic plateau lavas with some pyroclastics which cover enormous areas in the Aberdare Mountains and west of Nairobi. It is difficult to give any general succession of rock types for the whole region, but the early stages of the vulcanicity often seem to have been associated with the eruption of basaltic rocks which later gave place to more alkaline types, sometimes associated with carbonatites. In the rift valley west of Nairobi and elsewhere an acid trend developed resulting in quartz trachytes or even rhyolites.

The age of the vulcanicity has been the subject of much controversy. It is fairly certain that round the northern crossing the eruptions began in the lower Miocene while the outpouring of Kilimanjaro began on the end-Tertiary peneplain and much of its activity and that of the "Giant Craters" was of Pleistocene age.

ASPECTS OF MARINE FISHERIES RESEARCH

LARGELY owing to Government support, marine fisheries research has developed greatly since it was first a topic for an address to Section D (Zoology); hence it is appropriate that Dr. C. E. Lucas should review the present state of the subject in his presidential address to that Section at Southampton. Numbers and stock censuses are the very foundation of fisheries research and, during this time, their investigation has developed sufficiently to provide a theory of fish populations which is not only a basis for conservation and prediction but is also a major contribution to ecology. Yet, despite this and other developments, the seas and oceans are so vast, fish stocks extend and migrate over such distances, and marine processes (from water movements to productivity) are so complex that problems may increase as appreciation of them grows.

First, if the potential of the World's marine resources is to be exploited adequately, much greater knowledge of environmental influences is necessary. This has long been recognized but only recently has it become appreciated that equally necessary is a corresponding understanding of the behaviour and physiology of fishes, of their reactions to both natural and artificial stimuli. Nor are we here concerned only with the natural stocks and the natural conditions in which they come and go, and wax and wane. Such knowledge is, if possible, even more essential if the urge to cultivate the seas is to result in anything more than ill-considered, and consequently uneconomic, attempts to interfere with an exceedingly complex network of ecological interrelationships.

From the practical point of view, the problems facing marine scientists are not only fascinating and exciting, but also vital in terms of their potential contribution to the solution of increasing world food needs. Considerations such as these led, sixty years ago, to the foundation of the International Council for the Exploration of the Sea, under which the marine research resources of a number of nations have been increasingly co-ordinated over the years, to undertake investigations in the northern Atlantic which none could hope to solve alone. Its example has been followed by other groups, outside or within one or other of the fishery commissions which were later established to conserve marine resources; already various international projects have shown the inestimable value of collaboration in a field where events in one place may influence others more than a thousand miles away, and thus at one time may influence others much later in a complex space-time continuum.

In such a broad field, co-operation is essential at all levels, and especially between the 'pure' worker and the so-called 'applied' worker. Whenever a practical problem is anticipated the question is not 'how much time', or 'what resources' are necessary, but whether the essentially basic research has been done to be able to formulate the problem and its solution correctly. Thus a fair proportion of basic research is essential for a successful programme in a fisheries laboratory—and not only for this reason but also in order to attract to it workers of adequate calibre, who will never be satisfied solely with applying fundamental knowledge gained by others to the solution of practical problems which may lose their interest as soon as they are solved.

For such reasons, fundamental problems cannot be left to the academic laboratories alone. Not only does their specialization sometimes tend to isolate them from practical matters, but the broadly undirected nature of their work renders it chancy that they should contribute substantially to any immediate problems. Yet the resources of the fisheries laboratories alone are insufficient, despite the various special disciplines they now utilize, when faced with the vastness of natural processes on such an ever-varying scale. An important question, here as elsewhere, is how to encourage academic workers to devote rather more attention to those aspects of their investigations which could contribute to the solution of practical issues, without restricting unduly the freedom which is recognized as essential to the pursuit of understanding. The answer, here as elsewhere, seems to lie in an even closer association between the pure and the applied workers and laboratories. Applied research which is divorced from pure research is sterile indeed; perhaps pure research in its turn has some valuable stimulus to receive from the processes involved in applying research.

PLANNING AND THE GEOGRAPHY OF GREAT BRITAIN

THE subject of Mr. A. A. L. Caesar's presidential address to Section E (Geography) is "Planning and Geography in Great Britain". The need for planning on the city scale is now generally appreciated. There is also a gradually increasing awareness of the significance of wider relationships and the need to plan on the regional scale; but there is still very little appreciation of relationships on the national scale and of the profound changes in values, with changing techniques, inherent in the geography of Great Britain as a whole. Changes in techniques since 1918 have been many and varied, two main groupings being dominant—changes in the industrial and occupational structures and distributions, and the 'revolution' in transport.

The First World War produced marked and sudden changes in the economy of Great Britain. Consequential changes in the distribution of industry generally between the Wars were frequently described as 'the drift to the South', but this is an inaccurate description. The overall problem was not a contest between North and South, but between a declining periphery and a growing centre.

The effects of new developments in transport for both passengers and freight, and particularly the coming of modern road transport, did much to enhance the contrasts between the periphery and the centre. In absolute terms, the periphery is much more accessible than it was, but in relative terms it is less so and it is the relative relationships which are so important.

Since the Second World War, Great Britain has experienced near 'full employment' in marked contrast to the heavy unemployment of the inter-war years. Nevertheless, problem areas have presented themselves, and all the Development Areas or 'Development Areas Treasury Advisory Committee' areas are on the periphery. National circumstances have changed markedly, but the contrasts between the periphery and the centre persist. They have

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been further emphasized by many developments of the past twenty years. Analysis of the likely effects of the new road bridges and tunnels of the major estuaries, of the layout of the 275-kV grid, of the proposals of the Beeching Report, of access to commercial services and many other features, all show that the periphery is at an ever-greater comparative disadvantage.

Great Britain's problem is the concentration of urban functions and much economic activity. This problem is common to many parts of the world, but it presents particular difficulties and greater urgency in this small, irregular and densely-peopled island. To think of present planning problems simply as the rapid relative growth of the South-east at the expense of other regions is to think in the wrong terms. Great Britain must face the coming of 'Megalopolis'—the vast diffused city which will occupy much of the Midlands of England with dormitories on the nearer coastal areas particularly in the South-east. The days of planning separate cities and towns, and possibly of local government areas as now known, have gone. The planning of 'Megalopolis' requires the biggest minds and a carefully controlled imagination—personnel will be difficult to find. One thing at least is clear—it must be planned with a full appreciation of the profound and continuing changes in geographical values. Given the changes in spatial relationships of recent years thinking even on a regional scale is not big enough, and we must take cognizance of the geography of Great Britain as a whole.

ENGINEERS AT THE CROSS-ROADS

TO-DAY engineers in Britain are facing a new dilemma —the dilemma of professionalism. The problem as Mr. H. G. Conway sees it in his presidential address to Section G (Engineering) is: do engineers want to become 'respectable', raising the title 'engineer' above the level of a garage mechanic? Moreover, what is Britain's greatest need: for scientists to advance the boundaries of technology, or for engineers to apply its fruits? In many fields, remarkable engineers are found who have had little or no technical education. These men, the Henry Royces of to-day, succeed because an instinctive ability, particularly in design, cannot be held back by a lack of formal qualifications. In science, however, academic training is more necessary, and there is perhaps less scope for intuitive genius without academic embellishment. Many professions, such as medicine and the law, require strict compliance with the rules of the profession if success is to be achieved.

While comparisons between engineers and scientists can be made (but tend to be very inaccurate), one common factor is that the successful individual in either discipline tends to have an intelligence well above the average of the community. That there are certain essential differences within each group can broadly be related to an interest in the practical or in the abstract. It is essential that these differences are kept in mind when educational patterns are directed towards the future needs of technology. Obviously too rigid an educational system might hamper the progress of some men, and this is particularly true of the visually perceptive and mechanically apt men who may not be so successful in passing through the normal university degree course.

Intelligence is a quality that many men have. However, the knack of making use of it is another quality. Psychologists in the United States, investigating the difference between creativity and intelligence, have shown that the conventional intelligence quotient rating system leaves much to be desired in assessing those qualities which comprise creativity. Indeed they give some evidence indicating that young people of high intelligence (by *I.Q.* rating) are generally low in creativity. Perhaps here is the essence of the differences between the scientist and the engineer. While the average scientist would probably be proud to have a high *I.Q.* rating, and certainly gifted, highly intelligent children are most likely to be able to follow the educational stream which produces scientists, in some branches of technology, virtually throughout engineering, creativity is highly important. It is common experience that those who do best have some innate ability which is not necessarily nurtured by a university curriculum.

Traditionally, the ambition of the British parent is for his child to go into a 'respectable' profession, such as medicine, law or finance. It is only recently that science and technology have become recognized as 'respectable' professions. It can be questioned whether steps are being deliberately taken to raise the professional status of engineers, so that they have the same public standing as doctors, dentists, accountants, etc. A generation ago the engineer would join a learned society because he was interested in his new profession and wanted to keep up to date, 'in the swim', by means of its journal. Meetings were only attended occasionally, branches outside London being few. There were no ties to wear symbolically! The young engineer of to-day is much the same as his father, except that competition is far keener and technology vastly more complex. Specialists have brought about the setting up of artificial barriers within this creative discipline, and now it is necessary to distinguish between electrical and mechanical engineering, etc. It is clear, however, that a method must be found for rating qualified engineers in such a way that the general public will know them for what they are.

A step forward in this endeavour was made in 1963 when the Engineering Joint Council was formed. Hereby a means was established for thirteen learned societies to set common standards which will enable the 'professional engineer' to be given a good standard without reference to whether he is electrical, mechanical, chemical, etc. The problems of standard are immense, and in no circumstances can a lowering of any standard be tolerated. Certainly the Joint Council must set a standard which will be equivalent to a university degree, and it must lay down the standards of professional experience which must also be achieved before the young member of a constituent institution can be allowed to call himself a 'professional engineer'.

HISTORY AND ARCHÆOLOGY

THE rapid expansion of British archæology in the late nineteenth and early twentieth centuries was largely concerned with prehistory and was carried out in close association with the natural sciences. As a consequence, the link with historical studies, which had always been realized in the field of Roman Britain, became dangerously weakened in other branches of British archæology. The necessity for re-examining the relations between historical and archæological studies has become increasingly evident as medieval and post-medieval archæology have grown in stature in the past generation. It is thus fitting that Dr. C. A. Ralegh Radford should choose

"History and Archæology" for the subject of his presidential address to Section H (Anthropology).

Until the margin of error in scientific dating by carbon-14 or other methods becomes substantially less than at present, the traditional archæological methods of dating by association will continue to form the basic element in establishing the chronology of later prehistoric ages. These methods rest ultimately on historical data, and their successful application demands an understanding of these data. For the same ages the written record provides isolated pieces of information, which, rightly used, enable the student to fill out and bring to life the picture built up by the scientific analysis of data afforded by archæological investigation, which must continue to form the basis of any reconstruction. An example from British and Gaulish protohistory is provided by Cæsar's account of the equities. This dominant secular class in Gaulish society should certainly be associated with the rich *La Tène* burials of Britain and the Continent and probably with the great contemporary farmhouses of the Little Woodbury type. The fortunes of this class can be followed into the Roman period, when a connexion between these farmhouses and the villas is beginning to emerge.

In the later prehistoric and Roman ages the written record concerned with Britain consists of isolated references by distant writers, whose main interests lay elsewhere. With the coming of Christianity in the seventh century, the evidence of native writers and documents becomes increasingly available and historical studies gradually become dominant.

Instances where the written record of a particular site or building can be directly tested by archæological methods are rare. Hexham, which might have become a text-book example, is rendered difficult by the obscurities of the texts and the inadequacy of the excavations. Winchester, where a large-scale investigation is in progress, may afford more useful results.

But real collaboration between historical and archæological research must rest on a wider basis. The picture obtained by the analysis of the whole range of historical material must be tested by field research and by the excavation of key sites. These sites must be regarded as historical and sociological documents. In pre-Conquest England the towns—especially the late Saxon burghs—and the dwelling-houses provide suitable fields for investigation.

BIOCHEMISTRY OF THE ENDOCRINE SYSTEM

THE biochemistry of the endocrine system forms the subject of Prof. F. G. Young's presidential address to Section I (Physiology). Although the idea that certain glands may provide internal secretions, liberated directly into the blood stream, is sometimes ascribed to Claude Bernard, the belief that glands which do not possess a secretory duct may discharge useful material directly into the blood seems to have been generally current during the 1840's. The important observations of Thomas Addison about the effects of disease of the adrenal glands (1849, 1855), and of A. A. Berthold (1849) concerning the endocrine activity of the testes, may well have been influenced by general contemporary ideas about internal secretion before Bernard's observations concerning the secretion of glucose by the liver (1853).

Many hormones are concerned in the control of the movement of materials across barriers. In many instances endocrine glands are themselves derived from tissues which, at one stage in evolutionary and embryological development, constituted a part of a barrier tissue between the external and internal environments of an animal. This may be significant with respect to the evolutionary development of what are now called 'hormones'. Hormones may be metabolites to which responsive tissues concerned with transport have been evolved and the production of which became largely, though not entirely, confined to one tissue.

In some instances two hormones may act on the same process in such a manner that under the influence of one hormone the process is intensified while under the influence of the other the process is depressed. Such an antagonism between the action of hormones can be of particular physiological significance, and is well illustrated by the actions of insulin and of pituitary growth hormone. Under the influence of growth hormone there is a tendency for the blood sugar to rise, and for the amount of albumin-bound fatty acid in the plasma to rise also. These two actions are the converse of those of insulin. The action of growth hormone probably prevents the development of post-prandial hypoglycæmia and an excessive fall of plasma-free fatty acids, and conserves amino-acids, while insulin provides a means of preventing an excessive rise of these constituents of the blood after a mixed meal. In simple terms, insulin is the hormone of plethora while growth hormone is that of scarcity. Antagonism between their actions can provide for balanced metabolic control. Growth may result when the metabolic pattern normally associated with scarcity is imposed on conditions of plethora.

BIOLOGICAL BASIS OF CRIMINAL BEHAVIOUR

PROF. H. J. EYSENCK reminds us at the opening of his presidential address to Section J (Psychology) that constitutional views about the causation of crime were widely accepted at the turn of the century; they have been superseded by views stressing environmental causes. It would seem that the pendulum has swung too far over in this direction; man is a biological organism as well as a social one, and it would seem that the time has come for a re-awakening of interest in the biological causes of criminality. The facts certainly leave little doubt about the importance of constitutional factors; following the work of Lange, many other investigators have demonstrated that when criminals with identical sex brothers or sisters are singled out in prison the concordance rate of their twins is more than twice as much when they are identical than when they are fraternal. Along similar lines it has been shown that the personality make-up of criminals combines high emotionality with a high degree of extraversion, as contrasted, for example, with neurotics who tend to combine high emotionality with high degrees of introversion. Both emotionality and extraversion/introversion have been shown in many different studies to be markedly influenced by hereditary factors. Most of these facts have been known for a long time; the main reason for disregarding them has usually been the difficulty of seeing how biological factors could influence conduct classified on ethical or social grounds. What is inherited can only be structure, not behaviour, and no clear theory existed relating the one to the other.

In recent years it has been suggested that there is a close relationship between extraversion/introversion on one hand, and cortical excitation as opposed to cortical inhibition on the other. These terms refer to facilitation or its opposite in relation to cortical activity, and it is likely that there is a close relation between excitation and the activating part of the reticular formation, and inhibition and the recruiting or synchronizing part. It may be deduced from this hypothesis that introverts form conditioned reflexes better than extraverts, and much evidence exists to show that this is indeed so. Given then that extraverts and introverts differ with respect to the ease with which conditioned reflexes are formed, we may here have the necessary link between structure and behaviour. More specifically, Prof. Eysenck suggests: (1) that neurotic reactions are conditioned autonomic reflexes, or the skeletal or hormonal reactions thereto; (2) that criminal activity is facilitated or brought about by the absence of conditioned autonomic responses to the contemplation and execution of socially undesirable actions which are subjectively experienced as 'conscience'. According to this theory, the new-born child's conduct is completely asocial or 'criminal'; in order to learn a socialized mode of behaviour, a lengthy process of conditioning is necessary in which certain types of activity labelled 'bad', 'naughty' or 'wicked' are immediately followed by some form of punishment. This punishment acts as the unconditioned stimulus, and by virtue of its contiguity becomes associated with the conditioned stimulus, that is, the contemplation or carrying out of the forbidden activity. Gradually the conditioned stimulus will acquire the power of producing the same response as the unconditioned stimulus, that is to say, sympathetic arousal, anxiety, fear, etc., thus acting as a deterrent from any asocial activity. The whole process is aided very much by the labelling of all such activities by parents, teachers and peers; in this way stimulus generalization occurs, and unifies and extends the whole field of forbidden activity.

It will be clear that in this process extraverts, who condition poorly, are handicapped and will acquire a well-established 'conscience' only with difficulty, whereas introverts will condition only too well and be plagued by guilt feelings, even where the average person would consider such guilt feeling inappropriate. These genetically determined factors interact with the environment, which provides the conditions of reinforcement, and variations in these prevent the correlation between conditioning and criminality from being perfect. It is suggested that from the point of view of prophylaxis and treatment, individual differences in rate of conditioning must be taken into account and that ways and means must be found to make use of the accumulated knowledge of psychologists regarding the establishment and extinction of conditioned responses. It is possible, for example, to increase the conditionability of a person by the administration of stimulant drugs; it is possible to prevent extinction to a large extent by partial reinforcement, and there are many other ways in which behaviour can be manipulated so as to avoid criminal behaviour or reclaim the criminal. All such activities to be successful must, however, rest on a proper scientific analysis of the problem and a recognition of the importance of innate individual differences, particularly in the rate of conditioning.

FUNGAL FRIENDS AND FOES

"FUNGAL FRIENDS AND FOES" is the title of Prof. C. G. C. Chesters's presidential address to Section K (Botany). Fungi are part of man's normal environment and are vital for the circulation of carbon, nitrogen and minerals in Nature. They become important in man's economy because they depend on organized sources of carbon for their own synthetic processes and energy supplies. Throughout time man has engaged in the monoculture of food crops and, in so doing, has provided phytopathogenic fungi with an assemblage of hosts which they have continually exploited. The ability of such pathogens to develop new strains and the wide distribution of mixed populations of pathogenic strains requires the continual vigilance of mankind, and a never-ceasing attempt to find more effective means of prevention and control.

Animals, as well as plants, represent food sources for fungi, and many of them cause serious diseases in animals. Some depend on animals for their dispersal and others are closely knit with the life-cycles of insects. This is a feature of ambrosia beetles. In certain insects, digestion of their food and perhaps their vitamin requirements depend on fungi and bacteria located in their digestive tracts.

While pathogenic fungi continually menace food crops, saprophytic fungi cause spoilage of many of the essentials of man's livelihood. Heart-rot of standing trees only differs in degree from moulding of stored food products and textiles. The natural versatility of fungi, their wide battery of enzymes, the profusion of spore production, and the efficiency of spore dispersal are factors which enhance their nuisance value.

However, these same characteristics make them useful to mankind when they are cultivated on sterile media. The vast array of syntheses of which they are capable and the ingenuity of the chemical engineer have placed at man's disposal not only antibiotics, but also a wide range of organic acids, enzymes, vitamins, proteins, amino-acids, and other carbon compounds. The exploitation of fungi is only limited by the costs of operations, but the combined skills of mycologists, chemists, and engineers should open wider fields of usefulness, particularly in a world facing the necessity of finding new sources of food materials.

Underlying all their destructive propensities, and their usefulness to mankind, lies one very simple feature: fungi lack chlorophyll and are entirely dependent on organized sources of carbon for their existence.

NEED FOR FORESTS IN AN INDUSTRIAL SOCIETY

EVER since early man began to develop a communal existence he has tended to slaughter his forests to make room for other land uses or to safeguard himself from enemies sheltering therein. In Britain this process had advanced far at the period when she began to take possession of overseas colonies, whereupon her rapid industrialization made tremendous calls on the newly discovered timber reserves overseas. Belatedly, skilled forest conservation has endeavoured to ensure the safety of future supplies.

To-day Western Europe as a whole is a gross importer of timber and timber products, and Britain is dependent on overseas supplies to the extent of 90 per cent of her needs. Only recently have most countries begun scientifically to look ahead to their long-term industrial, social and domestic planning; in doing so it begins to be possible to foresee how timber resources will need to be conserved or increased in relation to the known world availability and world requirements. The picture is far from rosy, but with increased knowledge of the cultivation and use of fast-growing species immediate action can be expected to safeguard the situation. The aim of Mr. G. B. Ryle's presidential address to Section K* (Forestry) is to express some general thoughts as a challenge to our sense of urgency.

The British forest policy, hitherto based largely on the need to build up a reserve of growing timber as a safeguard in the event of another war, has now moved towards the need to maintain and increase forest production for economic and social reasons, to ensure the improvement of conditions in the poorer upland areas where grazing husbandry is failing to support a modern educated population and to improve the broad use of rural land also for the recreational benefit of the people as a whole.

The development of high-production forestry since 1919 is already creating an intense interest among industrialists, and as more supplies of the raw material become available so the building of new wood pulp factories, particle board mills and sawmills is following. As Mr. Ryle points out, the forest has much to offer to an industrial society in addition to its direct provision of an industrial product. By a close collaboration with the National Parks Commission and the Nature Conservancy so the forest is providing additional recreational facilities and opportunities for biological study equal to their national parks and their nature reserves.

The forest in a highly populated country must not be managed simply as a timber-producing factory. It is a storehouse of industrial wealth, of animal and plant communities and a place for recreation for the townsman. It is hoped that the townsman will collaborate to preserve this growing heritage.

RESPONSIBILITY AND EDUCATION

THIS year, half-way between the optimism of the Education Act of 1944 and the pessimism of Orwell's "1984", the Education Section (L) of the British Association has resolved to pass in review some of the major issues of responsibility in education. In his presidential address to this Section, Dr. F. Lincoln Ralphs attempts to indicate some of the broad issues involved and to set the stage for subsequent papers on the responsibility in education of the State, the Press, teachers, parents, etc.

Education and responsibility are held to be as inseparable as the obverse and reverse faces of a coin. By them *Homo sapiens* buys his right to a status unique among the animals. The educative potential of the totality of human environment must be recognized and our failure to accept responsibility in this context leads to a confusion of education with schooling and with erudition. It has led to a dichotomy between the standards advocated in school and accepted by Society. It may be there is a present preoccupation with truth at the expense of some neglect of beauty and goodness, a neglect that is excused as a revolt against hypocrisy.

Against this background the religious clauses of the 1944 Act reflect not only an attempt to reconcile the education provisions of the State and the Church but also the manifestation of war-time faith and fears in marked contrast to the present climate of thinking. There is at the present time an emphasis, even an over-emphasis, on scientific method and measurement as the only acceptable form of enquiry. Dr. Lincoln Ralphs considers that this produces a quantitative rather than a qualitative concept and is reflected in a change of emphasis from excellence and perfection to the average and the normal.

The present rapidly changing pattern of education contrasts markedly with the broad philosophy of education which had developed in the nineteenth century. "To the needs of this tranquil, stable but steadily evolving society the education and responsibility of the three R's ministered with servile conformity. Parents were as much concerned to enquire about their children's conduct as their competence. Integrity was rated as high as intellect. Manners still made man. Then, in 1914, the smouldering embers of the Balkans caught fire at Sarajevo and the world was at war. Solid structures of the past softened and melted in the new, accelerated way of life. The stage was being set for a new standard of values." The privileges of a minority in the nineteenth century became demanded as the rights of all in the twentieth, but this standardization, rationalization and certification are not necessarily the best guarantees for educational advance. "Large classes and increasing emphasis on factual knowledge which examinations tend to encourage are not calculated to inspire pupils to accept responsibility either for themselves or for others."

The responsibility of parents is basic. Their training to meet the responsibility is minimal. If the education problem begins in the home its ultimate extension is world-wide and its responsibility cannot be circumscribed in any parochial fashion. "A brain drain may be necessary if we are to deal with the effluent of ignorance that a divided world has left to poison society with prejudice and violence." In a final analysis of responsibility and education it is impossible to escape the conclusion that the central theme depends on the concept of the nature of man. "Our origins may be seen from the stern of the *Beagle*. Our destiny is not decided in its wake but in our own consequent involvement in the conflict of good and evil and our growing recognition of what is true and what is not true. . . . If we offer human nature as an excuse for what we no longer hope to perfect we return to the dust."

THE AGE OF ADVICE

PROBABLY the earliest record of science being called to the aid of agriculture is contained in the writings of John Milton the poet to Samuel Hartlib in 1651. The recommendations of other writers in the seventeenth and eighteenth centuries and the activities of the Brecknockshire and other agricultural societies from 1755 onwards undoubtedly led informed public opinion to support the private endowment of the first chair of agriculture in the University of Edinburgh in 1790.

Subsequent development, as Mr. Eric Rea points out in his presidential address to Section M (Agriculture), was slow and spasmodic with a pronounced active period in the decade around 1840 which saw the inauguration of the Royal Agricultural Society of England, Rothamsted, the agricultural colleges at Cirencester and Glasnevin, and further chairs of agriculture at Oxford, Aberdeen and Belfast. The last thirty years of the nineteenth century saw the foundation of a number of small colleges and teaching institutes—many of them now defunct—and a trio of significant events in 1889; namely, the County Councils Act; the Technical Instruction Act and the founding of the Board of Agriculture. By this means, Government funds and support were available on a modest scale and for the first time.

The Development Act of 1910 provided the next stimulus to county councils and universities, who co-operated and laid the foundations of the nation-wide system of agricultural education and advice developed between the two World Wars. The interruption of the Second World War provided the opportunity for a systematic review by the Luxmoore Committee, whose report led to the setting up of the present National Agricultural Advisory Service within the Ministry of Agriculture. Farm institutes and extra-mural activities were left with the county councils and the Ministry of Education.

The new unified service offered equal farmer facilities in all parts of England and Wales and improved service conditions and career prospects. Formed in 1946 when siege conditions had barely eased, the new service had a difficult start, but within ten years the developed world—aided by an unparalleled scientific revolution—was moving into an era of plenty. Scarcity gave way to plenty—even to food surpluses—and to keen international competition. Scientists produced new concentrated, friable fertilizers to exploit the potential of high-yielding stiff-strawed cereal varieties, selective herbicides to increase yields and reduce cultivation costs and a profusion of mechanical equipment to supply cheaper power and reduce labour requirements.

Livestock numbers and production efficiency show marked increase and farm output has nearly doubled compared with pre-war despite a decline of more than 20 per cent in farm workers. The total cereal acreage in South-east England has doubled since 1939. Wheat is a little above pre-war, oats show a sharp decline and there has been a nearly universal swing to barley in defiance of rotations and of the previously accepted husbandry code.

The effects of economic pressure have been felt most severely on small farms which lack the advantage of scale and rely heavily on livestock, which provides 70 per cent of the national farm income. This situation has been countered by the advisory service developing widespread economic or farm business management advice with the co-operation of the university departments. Simple enterprise costings have tackled feed costs, which loom large in livestock production, and more sophisticated whole-farm business analysis has been developed. Thus the major factor in doubling farm output and in lowering production costs has been the impact of the advancement of science brought to the farmer by Government-sponsored research and advice.

Changes in consumer demand in an affluent society are reflected in modified streamlined production patterns and simpler farming systems. Important developments in marketing are in progress. In the future, development will continue, but whatever form it takes there is every indication that farmers will depend heavily on unbiased scientific guidance in this, the "Age of Advice".

HOW SMALL-SCALE SOCIETIES CHANGE

IN her presidential address to Section N (Sociology), Dr. Lucy Mair suggests that when problems of social change are discussed it is often assumed that change in itself needs to be accounted for. Analyses of society in terms of structure and of socially approved behaviour in terms of roles have been called deterministic. Studies of the relation between culture and personality can result in a picture of society in which everyone's behaviour has been fixed by his or her upbringing in so rigid a mould that any change from established patterns calls for an explanatory theory.

Our nineteenth-century predecessors would have been astonished at the idea that social change as such presented a problem. In fact, it has become the subject of interest in the special form that it takes in this century, where the technical inventions of the Western World are being introduced to societies the indigenous technology of which is very simple.

It is suggested that the social changes resulting from this can be best understood if we recognize that a field of free choice is open to the members of any society, although the field is narrower where the level of technique is lower and the total field of social relationships narrower. The choices that men make are directed to comparable ends in all societies. They pursue wealth, power and the esteem of their fellows. These ends may be incompatible; people may have to choose between material advantage, increase of power, and the esteem given to those who respect their obligations.

In social terms, the entry of the newer States into the world economy has widened the range of opportunities open to individuals. They choose whether to enter into new types of relationship by balancing the advantages that these offer against the cost in loss of esteem through their neglect of pre-existing obligations. The conservatives are either the unimaginative or those who have more to gain by keeping to the *status quo*.

With the attainment of independence, these countries have rejected the claim of Europeans to tell them how they should take on the roles required by the social structure of a modern nation. In Africa they say that such roles must be so interpreted as to express the 'African personality'. This applies particularly to the field of politics, where the characteristic departures from Western ideals of democracy are explained in terms of traditional African customs and values. But a preferable explanation is that persons taking on political roles in the new states find themselves in an open situation in which they can themselves create their roles. These men are drawn from a different stratum of society from the traditional rulers, and are not seeking to follow tradition, but to work out for themselves the means of attaining and holding power. They interpret the new political roles in the manner which seems to them most apt for these aims, and there can be no effective challenge to this interpretation in the present social structure of their societies.

Contemporary social changes, like social change in the past, is produced by the manipulation of whatever areas of free choice there may be by people who are able to calculate where their advantage lies.

RESURGENCE OF INTEREST IN THE OBSERVATIONAL SCIENCES

IN his address to Section X (General), Sir Graham Sutton contrasts the progress in the observational (or environmental) physical disciplines with that in the experimental (or laboratory) physical sciences. In the observational sciences, naturally occurring uncontrolled events are investigated, usually on a synoptic basis, whereas in experimental physics controlled events are created and investigated, so far as possible, in isolation from other events. The two branches have a common foundation in the universal laws and principles of theoretical physics.

Much of early Western science was observational, usually with astronomical events as its basis, for at that time there was an almost complete lack of reliable laboratory data. Newtonian mechanics is often represented as a breakaway from the teleological science of Aristotle; but Newton himself seems to have been influenced by a desire to explain natural events as part of a universal order instituted by the Creator. It was not until some hundreds of years later that the growing skill of the instrument-maker and the emergence of the modern laboratory made it possible to extend the study of the universe by man-made events and laboratory artefacts.

The handicaps suffered by the environmental physical disciplines are well illustrated by meteorology, which for centuries could not advance significantly until the telegraph provided a rapid and certain means of building up the synoptic picture and ways had been found to explore the upper atmosphere. Modern meteorology began to take shape when advances in electronics produced the radiosonde, the radar wind-finder and finally the high-speed computer.

To-day the environmental physical sciences have been rejuvenated by technical developments. They demand a wide-ranging knowledge of physics, as exemplified by modern geomagnetism, which began in the seventeenth century with a study of variations in magnetic compass directions and now extends from the inner parts of the Earth to the fringes of its outer atmosphere, involving subjects as remote as the study of convection currents in the molten interior of the Earth to the physics of charged particles emanating from the Sun. It has also led to the examination of conditions in the remote past, with links with the drift of continents and palæoclimates.

The most exciting possibility in the Earth sciences to-day is that of modifying or even controlling weather. Examination of the orders of magnitudes suggests that little can be achieved by a direct contest with Nature, but that something might be possible by more subtle means. This requires, however, a much more detailed knowledge of large-scale atmospheric processes than is available now, and the conclusion is reached that the most that should be attempted at present is the investigation of model atmospheres with the help of computers. Any large-scale effort in the real atmosphere might have disastrous consequences.

THE SOUTHAMPTON REGION

A Survey of Southampton and Its Region

Edited by Prof. F. J. Monkhouse. Pp. xviii + 349 + 56 plates. (Southampton : The University Press, 1964. Published for the Southampton Local General Committee of the British Association.) 35*s*.; 5 dollars.

THE first impression gained of *A Survey of Southampton and Its Region* is that in the area they—the inhabitants—obviously take things rather seriously, especially their own extremely beautiful 'Wessex'. The book has all the appearances of being rather academic, with many detailed tables, maps, intricate graphs and diagrams. Is this well-illustrated book meant for the general visitor to the Southampton meeting of the British Association for the Advancement of Science, or will it apply itself mainly to those whose minds can take in such detailed information and, so to speak, tell the tune from a glance at the musical score?

The present survey is a far cry from the 1882 *Guide to Southampton and Neighbourhood* ". . . prepared by Mr. Thomas W. Shore, of the Hartley Institution in that town [now city], in view of the approaching visit of the 'British Association'. A few pages are devoted to notes on climate, vital statistics, geology, botany, zoology, etc." (*Nature*, 26, 352; 1882). Prof. F. J. Monkhouse, professor of geography in the University of Southampton, who has been responsible for the editing of the present *Survey*, has guided his team of fifty writers well, and they have together prepared a comprehensive account of the region. In planning it they have divided the *Survey* into three parts. First, "The Character of the Region" (151 pp.), which deals with the geology, geomorphology, hydrography, soils, weather, hydrology and water supply, botany, zoology, land-use and the Hampshire Development Plan. Secondly, there is "The Historical Background" (68 pp.), and, finally, "The Character of the Town of Southampton" (111 pp.).

With a *Survey* of this nature, where each chapter deals with a specific aspect of a region and is packed with detail and facts, it is not possible in a limited space to review it as a whole. Instead, one can but take examples here and there to show just what can be found and what the area offers. In what follows here only Part 1 will be dealt with in detail, while Parts 2 and 3 will be treated briefly.

The introduction is a jointly written overture by Prof. F. J. Monkhouse and R. Douch. This deals with the region in very general terms and in an ideal racy style—sufficient to encourage the reader to probe on if not read on. One is promised excursions into the Hampshire mainland, Wiltshire, Dorset and the Isle of Wight, not only by land or sea, but also in time. The clock is set to start at the Cretaceous geological period and is wound to run until plans have been reached for the last quarter of the twentieth century.

The area which lies within the confines of the Hampshire Basin is of special interest to geologists, whether professional or amateur, especially for its wide range of Mesozoic and Tertiary formations accessible for detailed study along extensive coast line. These offer text-book exposures which, due to extensive coastal erosion, vary from year to year. Sections of beds ranging from the Lower Cretaceous Wealden Marls up to the highest British Oligocene strata are revealed for investigations with varying degrees of completeness; notable features are the Isle of Wight monocline, and the Winchester, Portsdown and Purbeck anticlines. Within the Cretaceous system are represented the Wealden Series, the Lower Greensand (Aptian and Albian), the Gault, the Upper Greensands and the Chalk. These are succeeded unconformably by rocks of the Eocene system including the Reading Beds, London Clay, Bagshot Beds, Bracklesham Beds, Barton Series and Lower Headon Beds. The Oligocene system present in the area incorporates the higher Headon Beds, Osborne Beds, Bembridge Series and Hampstead Beds. Superimposed on all these the geomorphologist will find particular interest in the chalklands, where smoothly curving slopes lead down to winding, dry valleys. Occasionally the land is divided into separate blocks by deep trenches through which the Rivers Stour, Avon, Test and Itchen flow. But, for the most, gentleness of slope is the order. In stark contrast the Tertiary rocks around Southampton itself give rise to dull featureless lowlands. However, the magnificent coastline elsewhere in the region owes much to the structures produced by the Alpine folding, and notable features are to be seen around the shores of the Isle of Wight, Portland Bill, Lulworth Cove, etc. In fact, the very forces of Nature responsible for these features are responsible for the wealth of the area—Southampton Water. Their results have had profound effect on the unusually providential tidal phenomena in the Solent which have made Southampton a unique port.

The creation of the English Channel had two main hydrological consequences. First, the progressive introduction of the semi-diurnal tidal pulse of the North Atlantic Ocean right into the Straits of Dover. Secondly, the opening of this converging gulf to the wave-action of the ocean with an immense fetch, along which the prevailing westerly gales can raise wave amplitudes of 30–40 ft. with pressures of up to 5 tons/ft.^{2}. The chapter on "Hydrography of the Solent and Southampton Water" deals with these tidal phenomena most effectively, along with such topics as, 'double' or prolonged high tides, synchronous tides, the Solent tidal stream, etc. The fortuitous circumstances which led to the formation of Southampton Water have endowed it with many favourable qualities: to name but a few, the unique tidal region, where for 8·75 h the water-level is either rising or standing and the ebb lasts only 3·75 h; the protecting and sheltering barrier of the Isle of Wight; excellent anchorage for the largest ships in the world, etc. The chapter is completed by sections dealing with hydrographic surveys and modern developments in Southampton Water.

From the point of view of soils, the region can be divided into those of the Tertiary Basin and those of the Chalk rim. Most of the soils developed in the Hampshire Basin can be assigned to three major groups: the podzolic soils, the brown earths and the gleys. Typical podzol profiles are to be found in the New Forest and Dorset heathlands. Gleys vary from those associated with brown earth to ground-water gleys, and, in the wettest gleys, peaty *A* horizons are to be found—these occur in most New Forest bogs. The Chalk outcrops over about 60 per cent of the region and provides a homogeneous parent material over considerable areas. Varieties of soil from these areas are the shallow rendzina deposits, the brown calcareous soils, clays and clays-with-flints—the last-mentioned originated under peri-glacial conditions and cap some of the Chalk ridges.

The coastal districts of central south England are noted for their warm, sunny summers and generally mild winters. These characteristics add together to make the region one of the most popular parts of Britain for summer holidays, and also for retirement. The chapter on weather and climate, after the short introduction, deals first with synoptic climatology in January and July. A section follows which treats the climatic elements throughout the year, including precipitation, snow, thunderstorms, temperature, ground frost, winds and fog. It is well illustrated with a map concerning annual precipitation 1916–50, and graphs of daily temperatures at Southampton in winter, monthly precipitation, droughts at Ventnor, Isle of Wight, temperatures at Southampton 1921–50, and diurnal variation of fog throughout the year at Calshot. Seven tables detail daily precipitation, temperature, average rainfall, snow or sleet, and ground fog.

In view of the ever-increasing demands of its expanding population and growing industries, Hampshire is fortunately 'stocked' from the point of view of water resources. While the average rainfall is only about 30 in., the Chalk forms a favourable factor with its porosity, fissures, well-defined bedding planes, etc. The minor folding has a general southward dip, and this is the broad direction of the surface drainage. The overlying Tertiary deposits in the south of the county are relatively impermeable. The chapter on hydrology and water-supply details flow measurement and ground-water observations, and the water supply of Southampton and the neighbouring district. It ends on an interesting historical note in the form of an appendix, "Hydrology in the Past", which discusses Saxon charter evidence, the anomalous Alre and water mills.

Several large tracts of natural and semi-natural vegetation have survived the encroachments of man in the region around Southampton. Among these, the New Forest, with its woodlands, heaths and bogs on Tertiary and Quaternary deposits, is outstanding. Inland, valleys of large rivers flowing off the Chalk provide examples of freshwater vegetation, marsh and water meadow. Much of the woodlands of the region consist of oak and beech, occasionally pine, birch, ash or alder carr. However, not only has much of the woodland been reduced in recent years, but also much of the grassland. Reduction in the rabbit population on Chalk downlands has led to reductions in the low-growing herbs due to vigorous growth of grasses, but seedlings of hawthorn and juniper are becoming established. In the New Forest excellent examples of heath and bog vegetation exist, while a notable series of valley bogs occur in the Matley and Denny Forest Nature Reserve incorporating bog-moss, cotton grass, bog myrtle and alder carrs. The large rivers flowing southwards from the Chalk into Southampton Water support, on the more waterlogged marshes of their alluvial flats, sweet-grass, reed, large sedges, etc. Without doubt the most important coastal species is *Spartina townsendii*. This vigorous maritime grass—only introduced into the area about a century ago—forms virtually monospecific swards over great stretches of mud bordering the coastline almost continuously from Hurst Castle to Chichester. More traditional salt marshes are represented only locally around the coast, ranging from *Aster tripolium* swards to mixed saltmarsh vegetation. The chapter on the botany of the region ends with a section in which the flora is described in detail under headings of: vascular plants; mosses and liverworts; fungi; marine algae; freshwater algae. Certainly the botanist, whether amateur or professional, cannot fail to find something of interest in the region.

As would be expected from such a diversity of habitat, the region has a rich and varied fauna. Worthy of special attention is the marine fauna of Southampton Water with extensive beds of *Mytilus* and the interesting American hard clam, *Venus mercenaria*. Wood-boring marine invertebrates are plentiful, especially *Limnoria* and *Teredo*. Demersal fishes include gobies, blennies, and, from time to time, sprats and herring. *Acartia* is dominant among the copepod species, while in deeper waters carnivorous mysids are to be found. The Chalk-streams provide a good habitat for trout, and of special interest to the freshwater biologist is the River Avon, which has been the focus of many special investigations by the University of Southampton from the point of view of salmon. Throughout the region invertebrates are plentiful, and recently three groups of nematocerous Diptera have aroused special interest: Chironomidae, Simuliidae and Tipulidae. Special mention should be made of the medicinal leech, *Hirudo medicinalis*, which, rare in Britain, is to be found in some New Forest ponds. Branchiopod crustaceans of interest are the rare *Triops cancriformis*, and the fairy shrimp, *Chirocephalus diaphanus*. In an average year it is claimed that Hampshire sees the recording of some 230 species of birds and, to mention but a few, these have included honey buzzards, kestrels, wood-larks, stone curlews, godwits, egrets, white stork, nightjar, wintering white-fronted geese, various buntings and goshawks. The British mammal fauna is well represented in the region and includes fallow, red, roe, sika and a few escaped chinese water deer, fox, badger, otter, red and grey squirrel (the former ousted from the mainland region by the latter, but still to be found on the Isle of Wight), rabbit, vole, hare, etc. Of special interest are the representatives of Chiroptera, as the region is one of the richest areas in the British Isles for bats. Of marine mammals, porpoises and dolphins have been found. All six British species of reptiles are present.

The chapter on "Land Use, Agriculture, Forestry and Conservation" opens on a historical note. Later sections deal with modern agricultural trends, the present agricultural pattern, land classification, land use and sylviculture in the New Forest, and conservation. Of special importance is the last mentioned as much attention has been directed to improving the deteriorating conditions of the New Forest, the Chalk grasslands, and the tidal and fresh-water marshes along the coast. The chapter is well illustrated, with five graphs dealing with holdings, crop and livestock returns, crop acreages, and consumption of plant nutrients. Two maps show land classification in Hampshire and the New Forest.

"The Character of the Region" is rounded off with a descriptive chapter on the Hampshire Development Plan. The background to the Plan is described and sections are included on population and housing, industry and employment, road developments, social facilities, areas of comprehensive development, and areas of special interest.

Part 2, "The Historical Background", opens with a chapter in which an endeavour is made to define the mysterious region of Wessex. Theories are put forward as to how far the boundaries of Wessex spread, and the part played by Hampshire and Southampton. Wessex for many centuries lost its regional significance, but owes much of its revived popularity to Thomas Hardy. "Place Names of Hampshire" is a delight for all to read. The central position of Hampshire on the south coast of England has had a striking effect on its place-name structure. To the west of the Hampshire Avon, Celtic place-names become increasingly more frequent, while to the east of the county few pre-English names have survived. Sections deal with the earliest names, earthworks, Germanic names, Scandinavian names, Norman and French names, the New Forest and its place names in Domesday Book. The third chapter considers "Settlement in Prehistoric and Roman Times". This treats, in succession, the palæolithic, mesolithic and neolithic periods, the Copper and Bronze Ages, the Iron Age, and finally the Roman period. A thirty-two page section on the history of Southampton rounds off the historical background. This commences with pre-conquest Southampton, medieval Southampton and aspects of its industry and power, the civic authority, trade and communications, Southampton in the sixteenth and seventeenth centuries with the collapse of commerce and later the revival of the Town's trade, and the effect of war with Spain. This chapter ends with an account of Southampton in the eighteenth and nineteenth centuries.

It was fortunate that the editor of the *Survey* was able to include in his preface the fact that "Her Majesty the Queen, on the recommendation of the Home Secretary, was graciously pleased to raise by letters patent the Town and County of Southampton to the title and dignity of a City on 11 February 1964". Unfortunately, the text of the volume was in the press at that time, and hence it was not possible to change the title of the third part, "The Character of the Town of Southampton". Essentially, the chapters within this part of the *Survey* deal with the social pattern, economic activity, the port of Southampton, communications, aspects of the civic scene, the Ordinance Survey, the University and the planning of Southampton. Under "Social Patterns" sections are included on the past and present activities of the city, the growth of the residential area, population growth, social ecology, industrial development, employment, etc. The chapter on the Port of Southampton describes the functions and activities of the Harbour Board, comparative port traffic, the docks, and the effects on Southampton if the recommendations of the Rochdale Committee's report, *The Major Ports of Great Britain*, are implemented.

It is inevitable that as each chapter has been written by separate authors there should be a certain amount of overlap. Prof. Monkhouse is to be congratulated on keeping this to a minimum and for encouraging bibliographies with almost all chapters. The absence of a general index is to be deprecated; no doubt its absence was necessary for the sake of expediency of publication. However, it is greatly to be hoped that one will be issued as a supplement in due course.

PRESIDENT FOR 1965 OF THE BRITISH ASSOCIATION

SIR CYRIL HINSHELWOOD, O.M., F.R.S.

SIR CYRIL HINSHELWOOD, former Dr. Lee's professor of chemistry in the University of Oxford, has been elected president for 1965 of the British Association for the Advancement of Science. Sir Cyril will deliver his presidential address at the next annual meeting in Cambridge.

Sir Cyril is retiring from his professorship in Oxford and has been succeeded by Dr. R. E. Richards, F.R.S., Oxford University reader in physical chemistry (see p. 917 of this issue).

He was appointed to the chair in 1937, and he first organized the transfer of the Department from the historic cellars and converted bathrooms of Balliol and Trinity Colleges to the first laboratory for physical chemistry at Oxford, built with the support of a benefaction from Lord Nuffield. Here he strengthened and enlarged his school of chemical kinetics on a broad front, to include physical and biological systems; and during his tenure of office he was able to see the development by members of his staff of distinguished groups in numerous branches of physical and inorganic chemistry. Now he has left the Oxford Department, his plans for the further expansion of the physical chemistry laboratory are still continuing.

Sir Cyril's distinction has been recognized far and wide, by awards from many universities, from the Royal Society, and from scientific academies in many countries. He is a Nobel prize-winner and holder of the Order of Merit. He can claim the unique record of having been at the same time president of the Royal Society and of the Classical Association. The tercentenary celebrations of the Royal Society in which he played the leading part will long be remembered.

Not only has Sir Cyril been awarded the Copley Medal of the Royal Society (*Nature*, 196, 1025; 1962), but he was also the first recipient of the Leverhulme Tercentenary Medal (*Nature*, 192, 907; 1961) and was awarded the Medal for 1961 of the Society of Chemical Industry. This Medal, a senior award of the Society, is presented every two years for conspicuous services to applied chemistry by research, discovery, invention or improvements (*Nature*, 189, 22; 1961).

Sir Cyril is now retiring to his native London, but his future will not be a period of inactivity. By his election to a senior research fellowship at the Imperial College of Science and Technology he will be able to continue scientific work, and his counsel will also be valued by the many organizations with which he is associated.

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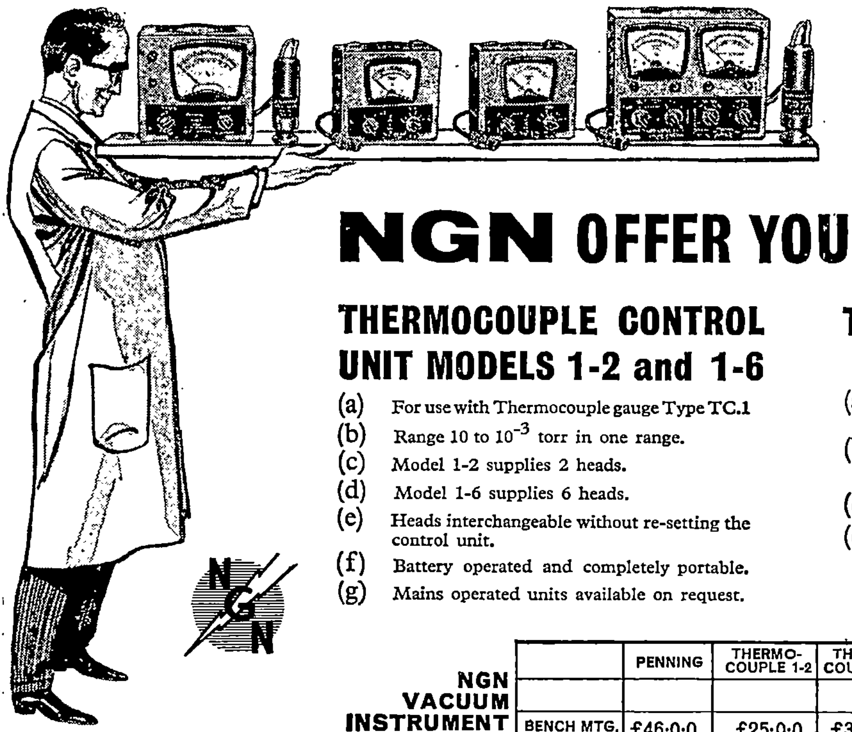

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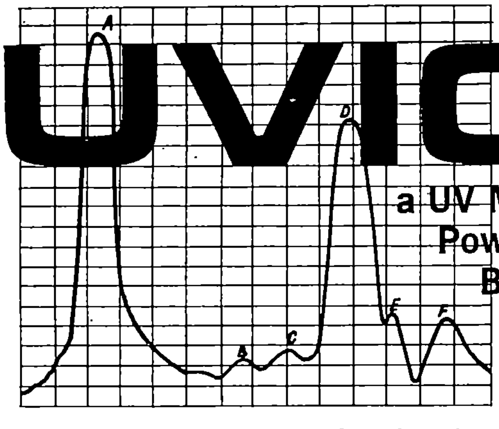

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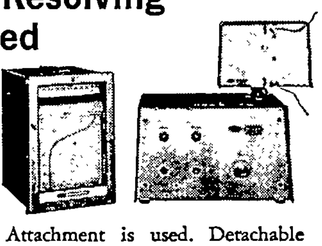

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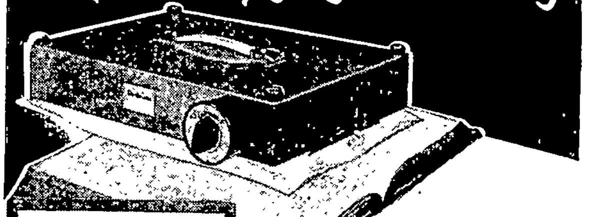

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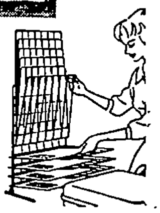

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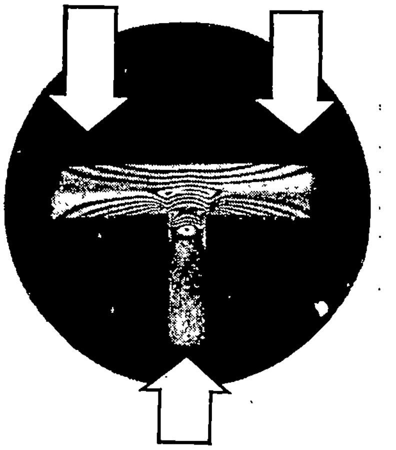

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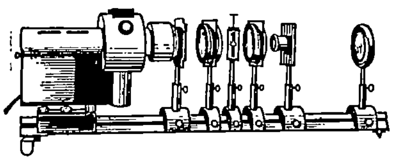

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LETTERS TO THE EDITOR

SPACE SCIENCE

Variations in Upper-atmosphere Density between Sunspot Maximum and Minimum

The atmospheric density at heights of 200–800 km has now been quite thoroughly charted during a complete half-cycle of solar activity from the intense sunspot maximum in 1957–58 to the solar minimum in 1964. Nearly all the information has come from investigations of the changes in satellite orbits caused by air drag, from which the air density at heights a little above perigee height can be deduced[1]. These investigations have shown that at heights of 200–800 km the atmospheric density and temperature are strongly under solar influence: they depend on the presence or absence of solar radiation (the day-to-night effect), and also respond vigorously to changes in solar activity.

Day-to-night effect. At any given height between 250 and 800 km, the air density increases during the morning and reaches a fairly sharp maximum at about 14 h local time. It then declines until midnight, reaches a flat minimum between midnight and dawn, and begins its morning increase again[2–7]. Although the day-to-night changes are most conveniently described in this way, in terms of local time, the description is slightly misleading, since the density and temperature depend primarily on the elevation of the Sun above the horizon, showing a roughly circular symmetry about a point on the Earth which is vertically beneath a 'mock Sun' that lags some 2 h (30°) behind the real Sun[8]. Thus the Sun, as it were, draws up the atmosphere into a bulge centred at a point on the Earth almost underneath the Sun. At any given latitude and height, the density does show the daily variation described, with a maximum at 14 h; but the maximum density is only reached in near-equatorial regions, and in the darkness of the polar midwinter the density will exhibit a night-time value, even though the local time may be noon.

The day-to-night variation is small at a height of 200 km, but at greater heights becomes extremely large. Fig. 1 shows the variation with height of both the maximum day-time density (full lines) and minimum night-time density (dot-dash lines) in various years from 1958 until 1964, as determined[9] from the orbits of the 45 satellites found to be suitable for the purpose. The day-time values for 1963 and 1964 were not distinguishable and are given as one curve in Fig. 1; similarly for the 1962–64 night-time values. Some curves have also been truncated to improve clarity. If the ratio (maximum day-time density) ÷ (minimum night-time density) is denoted by f, then, for 1959 when solar activity was high, the value of f increased from about 1·1 at a height of 200 km, to 1·6 at 400 km and 5·5 at 600 km[10,11]. As solar activity declined, the-day-to-night effect began to extend down to lower altitudes: thus in 1964, f exceeds 2 at a height of 250 km, increases to 2·8 at 400 km, and then to a maximum of 4 at 500 km, before decreasing slowly at greater heights[3,9,12].

Response to solar activity. Although the day-to-night variation in air density is large, the variations due to solar activity can be even larger. As Fig. 1 shows, the upper-atmosphere density has greatly decreased as solar activity declined from its intense maximum in 1957–58 to its minimum in 1964. At a height of 200 km the maximum day-time density was 1·6 times lower in 1964 than in 1958; at 400 km, 6·7 times lower; and at 600 km, 32 times lower.

Quite apart from these large changes over the long time-scale of the solar cycle, the temperature and density also respond to the shorter-term variations in solar activity, exhibiting the 27-day recurrence tendency which is characteristic of solar activity, and showing a striking correlation from day to day with various indices of solar activity, such as the planetary geomagnetic index and the solar radiation on 10·7-cm wave-length[2,4,8,13–16]. During the greatest magnetic storms, the density increases for a few hours by a factor of 6 at heights near 600 km and the temperature increases by about 500° K; but even minor changes in solar activity are faithfully reflected in the density and temperature of the upper atmosphere[14,15].

Mechanism of solar control. It appears that at heights above about 300 km the atmosphere may, despite its wide variations, be regarded as in a state of diffusive equilibrium, with temperature almost independent of height[17]. The temperature above 300 km is, however, strongly dependent on the level of solar activity and the time of day: the maximum day-time temperature varies between 2,000° K in a year of very high solar activity (for example, 1958) and 900° K at solar minimum. The minimum night-time temperatures are 25 per cent lower[8,15].

The solar influence over upper-atmosphere temperature is exercised primarily through the heating effect of extreme ultra-violet radiation from the Sun (wave-lengths probably about 200–1000 Å), which is absorbed mainly at heights between 100 and 200 km[15,18]. The quiet solar wind, the stream of charged particles continually flowing out from the Sun at speeds of order 500 km/sec, also probably causes heating as a result of its interaction with the outer boundary of the Earth's magnetosphere; and this heating effect due to charged particles is greatly enhanced when the Sun is very active and the particles ejected from it are more numerous and energetic.

Other variations in upper-atmosphere density. Although the main variations in upper-atmosphere density and temperature are the day-to-night effect and the fluctuations dependent on solar activity, there is a smaller but

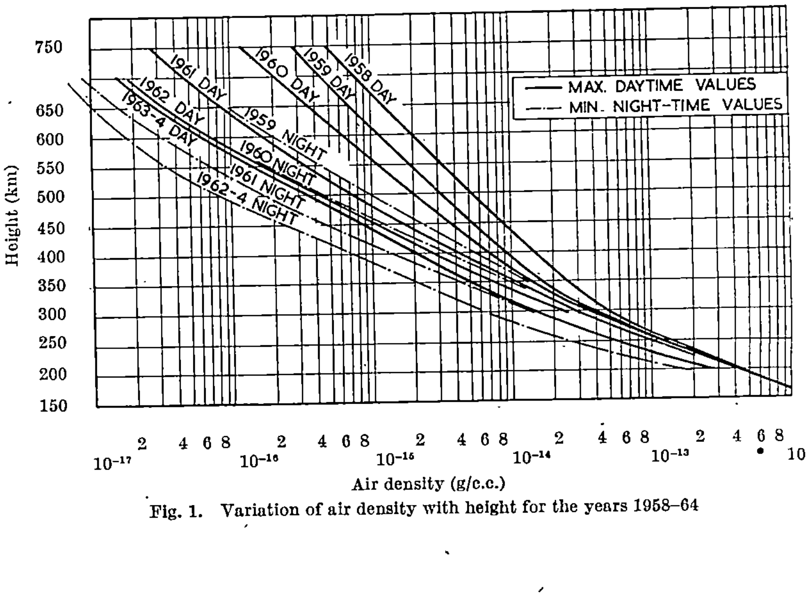

Fig. 1. Variation of air density with height for the years 1958–64

still appreciable effect associated with the time of year. Upper-atmosphere density and temperature undergo a semi-annual variation with minima in January and July, and maxima in April and October. Generally, however, the July minimum is lower than that in January, and the October maximum is higher than that in April[19,20]. These variations amount to about ± 100° K in temperature, and ± 20 per cent in the density at a height of 350 km[8,20]. The effects are probably linked with the variation in the Earth's distance from the Sun during the course of a year, and variations in the interaction of the solar wind with the Earth's outermost atmosphere.

At heights outside the range 200–800 km the results obtained from satellite orbits are less satisfactory. For heights of 150–200 km, the lifetimes of the satellites are uncomfortably short and the drag coefficients are somewhat uncertain. However, it is established that the day-to-night effect reverses at heights of 150–200 km, the density being slightly greater by night than by day[21–24], and it seems that the density is much less dependent on solar activity than it is at greater heights. At heights above 800 km, data from satellites are as yet rather sparse, since only balloon-satellites exhibit orbital changes large enough to be accurately measured. The main variations at heights up to 2,000 km, however, still appear to be due to solar activity and the day-to-night effect[5,16,25,26].

D. G. King-Hele

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ASTRONOMY

Lunar Brightness Distribution at 1·5 mm Wave-length

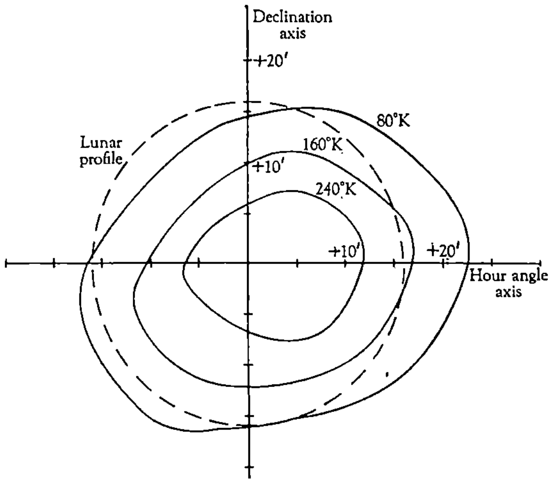

Fig. 1. Lunar micro-wave isotherms at 1·5 mm. Phase 136° after new moon. Detector, Golay cell. Entrance aperture of collecting cone 15 mm, giving angular response function of approximately gaussian form, with standard deviation 8·4 min of arc

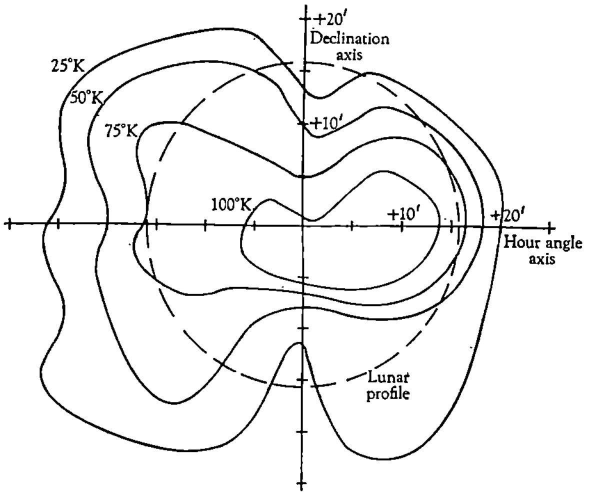

Fig. 2. Lunar micro-wave isotherms at 1·5 mm. Phase, 45° after new moon. Detector, indium antimonide photo-conductor at 11·7° K. Entrance aperture of 8 mm giving angular response function of approximately gaussian form with standard deviation 5·0 min of arc

WE have recently measured the microwave brightness temperature over the lunar disk using a new 1·5 m aperture Cassegrain telescope with both cooled indium antimonide and Golay cell detectors. The measurements cover a fairly broad band centred at 1·5 mm and with a mean wave-length deviation of about 0·2 mm[1]. The results of solar scans show that the angular response function of the telescope when used with either detector is of approximately Gaussian form. The results of two series of lunar drift curve measurements are shown in isothermal form in Figs. 1 and 2. The results have been corrected for the time constant of the detector circuit but not for the angular response function of the telescope.

The measurements show a much greater poleward darkening than that found from infra-red measurements[2], and in this respect our results agree with previous microwave measurements at higher wave-lengths[3,4].

Measured microwave temperatures are a function of the electrical and thermal properties of lunar material (since the surface is partially transparent to radiation at these wave-lengths). In the case of a plane homogeneous or stratified model, however, theory[5] predicts that the mean microwave temperature $\overline{T}_\lambda$ over a whole lunar cycle is related to the mean surface temperature $\overline{T}_0$ by the relation:

$$\overline{T}_\lambda = (1 - R_\xi)\overline{T}_0 \qquad (1)$$

where ξ is the zenith angle of the point of observation at the lunar element being considered and R_ξ is the reflexion coefficient corresponding to this angle. For two small regions, one located on the lunar equator and the other on the lunar meridian passing through the centre of the lunar disk both having the same angle ξ, equation (1) shows that:

$$\frac{\overline{T}_{\lambda 0}}{\overline{T}_{\lambda\psi}} = \frac{\overline{T}_{00}}{\overline{T}_{0\psi}} \qquad (2)$$

In this equation the second suffix refers to the lunar latitude ψ in each case, so that $\overline{T}_{\lambda\psi}$ and $\overline{T}_{\lambda 0}$ are the microwave temperatures of the two regions and $\overline{T}_{0\psi}$ and $\overline{T}_{00}$ are the corresponding surface temperatures which can be calculated from the infra-red measurements[2]. Each of the microwave measurements shows a systematic disagreement with equation (2) and in each case the ratio $\overline{T}_{\lambda 0}/\overline{T}_{\lambda\psi}$ is significantly greater than the value of $\overline{T}_{00}/\overline{T}_{0\psi}$ determined from infra-red measurements.

We think that this disagreement between the microwave results and the theory of plane stratified model is due to surface roughness. Near either lunar pole only a small fraction of the facets of a rough surface will receive solar radiation and because of the T^4 radiation law the mean temperature of the lunar material (from which the microwave radiation comes) will be lower than that from a macroscopically smooth surface. The same considerations do not apply to nearly the same extent for a point on the lunar equator since a much larger fraction of facets is illuminated during a lunation. The size of the roughness elements is somewhat less than that suggested recently from the results of other measurements[6], thus implying a size distribution of linear dimensions of the roughness elements. The properties of the lunar surface thus indicated are not dissimilar from those of the hierarchical-void model proposed by Troitskii[7], but they do not seem to be consistent with those theories which suggest a uniform flat layer of fine dust.

We thank Dr. E. H. Putley and his colleagues of the Royal Radar Establishment, Malvern, and Dr. D. H. Martin of this Department for their help in the installation and operation of the cooled indium antimonide detector.

Note added in proof. It is hoped that the recent American close-range lunar photographs, obtained since the communication of this letter, can be used, by extrapolation, to give an estimate of the density of microcraters of size below 1 cm, which might give rise to the roughness described here.

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Rotation of the Globular Cluster ω Centauri

A long series of observations and measurements of photographic plates taken on the globular cluster ω Centauri has now been completed by members of the staff of the Royal Greenwich Observatory at Herstmonceux. The observational material was obtained at the Radcliffe Observatory in Pretoria and at the Royal Observatory at the Cape of Good Hope, with the co-operation of the Radcliffe Observer, Dr. A. D. Thackeray, and of H.M. Astronomer at the Cape, Dr. R. H. Stoy, respectively. Details will be published in the *R.G.O. Annals* and *Bulletins* shortly, but special attention is directed here to the fact that the cluster has been observed to be rotating, and that the rate of rotation agrees with a theoretical value deduced from the ellipticity of the cluster. The view is therefore supported that the structure of the cluster is given by an appropriate modification of the isothermal gas sphere, and, moreover, that the elliptical shape of the cluster is a consequence of a rotation considerably greater than galactic rotation. This latter result disposes of the view that the elliptical shape is a consequence of galactic tidal forces.

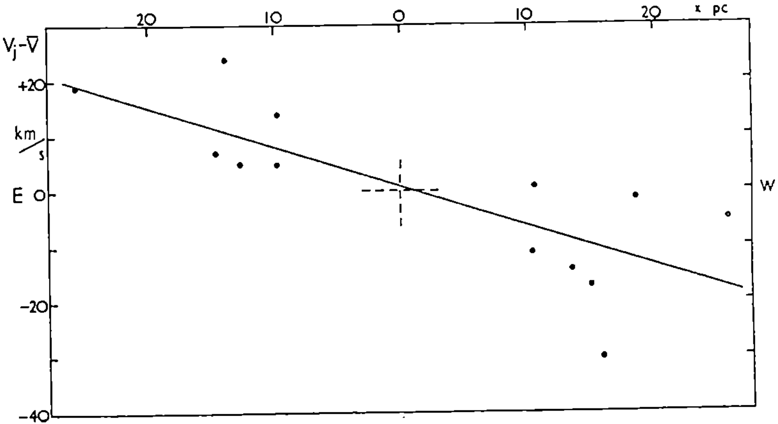

Fig. 1. Radial velocity plot. Excess radial velocity against distance from axis of rotation

As the brightest stars of the cluster are of the thirteenth magnitude, it is no easy matter to determine their radial velocities individually, but the work was done by Mr. G. A. Harding, using fast cameras on the Cassegrain spectrograph of the 74-in. telescope at the Radcliffe Observatory. As the result of two seasons' observing, he secured three spectra of each of thirteen stars known to be cluster members from other studies at Herstmonceux. The results were analysed to determine the velocity dispersion due to instrumental error, the true dispersion of the velocities of the star, and also the projection of the rate of rotation of the cluster normal to the line of sight. The latter two quantities were found to be:

$$\sigma = 10 \pm 4 \text{ km/sec}$$

$$\text{and} \quad V - \bar{V} = (0{\cdot}72 \pm 0{\cdot}18)x + (0{\cdot}9 \pm 2{\cdot}8) \text{ km/sec} \qquad (1)$$

where x is the distance from the axis of rotation in parsecs. The observations on which this result is based are illustrated in Fig. 1.

All the stars brighter than the 16th visual magnitude have been catalogued (except for the centre of the cluster where the count terminated at the 14th magnitude on account of the crowding of images) and the counts were corrected for the presence of non-cluster members on the plate by an appeal to an extensive series of proper motion measurements. From an analysis of the count it was found that the lines of equal star density are roughly circular at the centre and in the extreme outer parts, but that the eccentricity rises to a maximum at about 8 parsecs from the centre of the cluster where the ratio of the axes is 0·81 as seen in projection. This corresponds to 0·86 if the axis of the cluster is normal to the line of sight. The ratio of the axes is plotted against distance from the centre in Fig. 2. The detailed reductions have been carried out for the most part by Mr. R. J. Dickens.

If the cluster behaves like a (truncated) isothermal gas sphere, it is characterized by a scale-length l and a characteristic velocity j^{-1}. These are observed to be $l = 1$ parsec and $j^{-1} = 8$ km/sec from the observed velocity dispersion. With these constants established we can make use of a theoretical argument by Woolley and Dickens[1] to determine the rate of rotation of the cluster from the observed eccentricity. The result of this calculation is $\omega = 0{\cdot}55 \pm 0{\cdot}25$ km/sec/parsec, in very reasonable

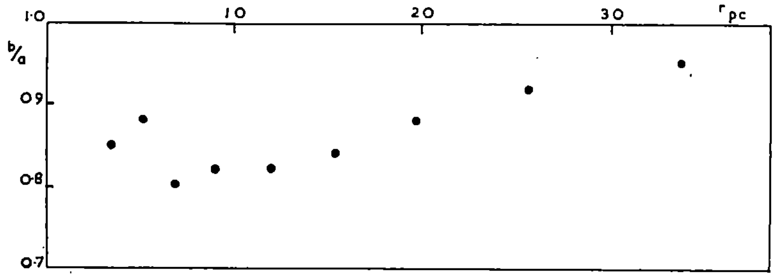

Fig. 2. Ratio of axes as a function of radius

agreement with the observed value of 0·72 ± 0·18 km/sec/parsec. In both cases it is assumed that the axis of rotation is normal to the line of sight. In comparison, the rate of rotation of the galaxy is only about 0·025 km/sec/parsec.

Since the outer parts of the cluster are more or less spherical, it must be supposed that they have not yet been brought into equilibrium with the inner parts by interstellar collisions, and indeed one might attempt to estimate the age of the cluster by calculating the time of relaxation at that point where the eccentricity begins to fall away. Only approximate figures can be given, but the time of relaxation 8 parsecs from the centre is about 4×10^9 years.

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RADIOPHYSICS

Observations of Solar X-ray Emission in the Band 44–60 Å from U.S. Naval Research Laboratory Satellite *1963-21-C*

The U.S. Naval Research Laboratory has instrumented a series of satellites for the investigation of solar X-ray and ultra-violet emissions[1,2]. It has generously allowed the Radio Research Station, Slough, to participate in these experiments and has provided the necessary information for reducing telemetry data. This communication presents some results for X-rays in the band 44–60 Å derived from telemetry recorded from satellite *1963-21-C* at the D.S.I.R. Radio Research Minitrack Station at Winkfield during June–July 1963. The principle of the experiment has been used on previous satellites and has been described by Acton *et al.*[3].

The satellite was launched into an elliptical orbit with perigee of approximately 190 km, but, in spite of the relatively low satellite heights, measurements made at small solar zenith angles showed little evidence of atmospheric absorption. In Fig. 1 are shown the X-ray fluxes measured at ½-min intervals during a satellite pass on July 23, 1963. These X-ray fluxes have been derived assuming a spectrum corresponding to a source temperature of $0{\cdot}5 \times 10^6$ °K (ref. 3). The accuracy of these measurements is estimated to be better than 5 per cent[4]. Although the satellite height changed by almost 20 km over the pass, no significant change in flux was noticed.

In order to estimate the possible attenuation of the fluxes by atmospheric absorption, calculations of the optical depth, τ, at each position of the satellite have been made using the relation:

$$\tau = \sum_s (n_s H_s \sigma_s) \sec \chi \qquad (1)$$

where n_s and H_s represent the number density and scale height of the absorbing gas at the satellite and σ_s the absorption cross-section; the summation includes the effects of O_2, N_2 and O constituents in the atmosphere. In this calculation use has been made of the atmospheric model appropriate to local noon at sunspot minimum conditions, as proposed by Harris and Priester[5], and the following values of mean absorption cross-sections have been adopted:

$$\sigma_{O_2} = 4 \times 10^{-19}\ \text{cm}^2$$
$$\sigma_{N_2} = 2 \times 10^{-19}\ \text{cm}^2$$
$$\sigma_{O} = 4 \times 10^{-20}\ \text{cm}^2$$

These values have been taken from a summary of theoretical and experimental results given by Van Zandt[6] with account being taken of the variation in response of the detector over the wave-length range 44–60 Å.

This calculation has shown that the attenuation of X-ray fluxes presented in Fig. 1 is expected to be less than 0·3 per cent. These and other observations near noon have indicated that the solar flux remains fairly constant during the 5 to 7 minutes of the satellite pass.

Measurements near sunrise and sunset have shown marked changes in flux during a single pass. Fig. 2 summarizes the measurements made at intervals of 0·5 min during a pass beginning at 0252 G.M.T. on June 25, 1963; the first observation shown at 0253·20 G.M.T. was above the limit of the X-ray detector. Also shown is the variation during the satellite pass of the grazing height of solar rays, that is, the height of nearest approach to the Earth's surface; a reduction in this height implies an increase of atmospheric thickness traversed by X-rays received at the satellite. In order to examine whether the variation of X-ray flux during the pass can be explained quantitatively by atmospheric absorption, estimates of the optical depth at the satellite have been made using the Nicolet approximation[7]:

$$\tau = \sum_s n_s' \sigma_s \sqrt{\frac{\pi(R + h')}{2H_s'}} \left[1 + \operatorname{erf}\left\{ \tan\left(\chi - \frac{\pi}{2}\right) \sqrt{\frac{R + h'}{2H_s'}} \right\}\right] \qquad (2)$$

where R represents the radius of the Earth, h' the grazing height, and n_s' and H_s' the number density and scale

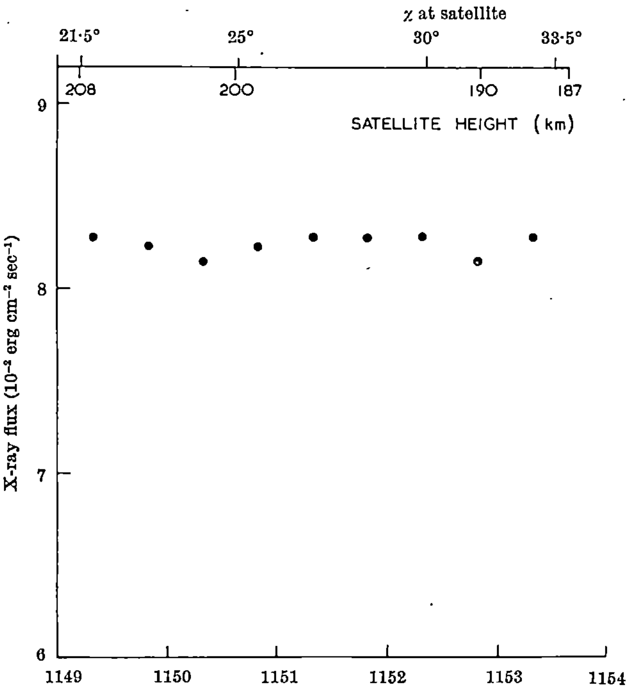

Fig. 1. Measurements of solar X-ray flux in the band 44–60 Å during a pass near noon on July 23, 1963

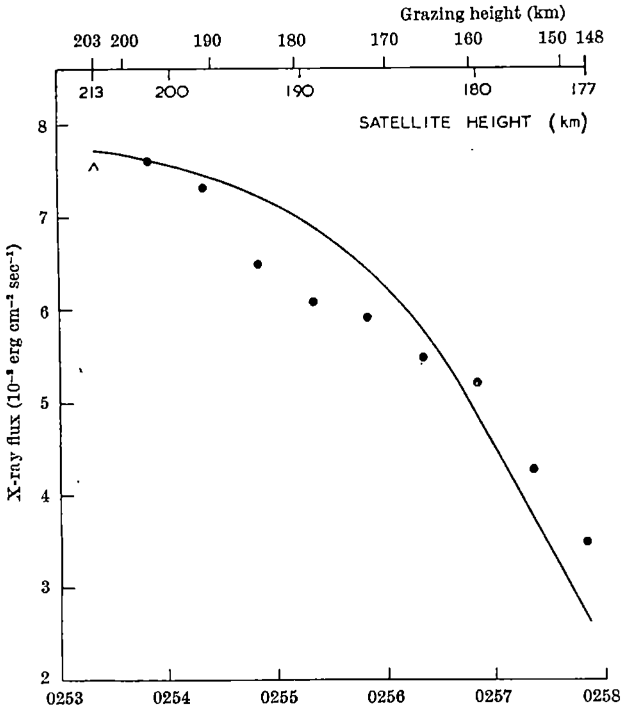

Fig. 2. Measurements of solar X-ray flux in the band 44–60 Å during a pass near sunrise on June 25, 1963, compared with the variation computed for a theoretical model of atmospheric absorption

height of the absorbing gas at the grazing height. Again the summation includes the effects of O_2, N_2 and O constituents. In these calculations the atmospheric model of Harris and Priester[5] applicable to early-morning hours at sunspot minimum has been adopted. The smooth curve of Fig. 2, which is normalized to the measurement at 0253·50 G.M.T., represents the variation of X-ray flux expected during the pass because of atmospheric absorption, assuming that the flux incident at the top of the atmosphere remains constant as observations near noon have indicated. It is evident that the slope of the theoretical curve depends on the accuracy of τ, given by expression (2), and hence on the values of absorption cross-section adopted and on the atmospheric model used. However, it seems reasonable to conclude that the variations of flux observed during passes near sunrise and sunset are almost entirely due to atmospheric absorption.

In conclusion, the observations indicate that the flux of solar X-rays in the band 44–60 Å during the period of low solar activity of June–July 1963 was near 8×10^{-2} erg cm^{-2} sec^{-1}. This value lies within the range of values found from rocket observations at sunspot minimum and sunspot maximum conditions[3]. In general, measurements made near 190 km show little evidence of atmospheric absorption except near sunrise and sunset.

The work described here was carried out as part of the programme of the Radio Research Board, and this communication is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research.

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GEOPHYSICS

Fluctuations in Ionospheric Absorption Events at Conjugate Stations

BROWN[1] has recently presented observations of those ionospheric absorption events observed at Kiruna which fluctuate over periods of several minutes, and from it has concluded that the fluctuations are consistent with modulation of the electron precipitation by hydromagnetic waves. However, no detailed mechanism for this interaction has yet been proposed. The purpose of this communication is to present a complementary feature which any theory must take into account: namely, the phase relation between these fluctuations in magnetically conjugate regions.

A sample of the records from the conjugate stations Great Whale River, Canada, and Byrd, Antarctica, has been inspected for these fluctuations. From 48 active days (that is, with activity lasting at least 6 h) during the period March–October 1963, seven cases of fluctuation have been found. They occurred between 0500 and 1300 local time, with a mode at about 1100 local time. They are therefore somewhat later in the day than are those seen at Kiruna. Nevertheless, they have the same property of association with the 'slowly varying' type of absorption. The different time of occurrence is probably due simply to the changing diurnal pattern of absorption with magnetic latitude[2], this being 66·6° at Great Whale River and Byrd, and 65·3° at Kiruna. It seems very probable, therefore, that these fluctuations represent the same phenomenon as that reported by Brown.

Of the seven cases, one has a regular sinusoidal form and the others show periodic features with varying degrees of irregularity. The periodicities are in the range 3–16 min. In all cases, the fluctuations are in phase rather than in antiphase at the conjugate stations. A typical example is shown in Fig. 1, which gives the absorption in decibels, slowly varying components having been removed. The general similarity between the two records, in both amplitude and phase, is obvious. There are also some small phase differences, which seem to grow and then decay progressively over several cycles.

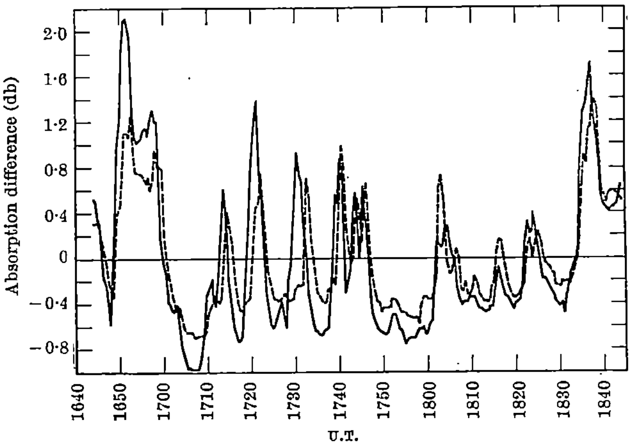

Fig. 1. Detail of absorption at Great Whale River and Byrd on October 14, 1963. The mean trend of the event has been removed. ——, Byrd; - - -, Great Whale River

Our observation presented here appears to be consistent with Brown's suggestion about the cause of the fluctuations. The magnetic observations of Sugiura[3] and Nagata *et al.*[4] indicate that the hydromagnetic disturbance originates near the equatorial plane, whence it propagates along the field-lines to arrive simultaneously in the two conjugate regions. If this propagation of hydromagnetic energy towards the Earth can modulate the flux of precipitated particles, then, provided the phase paths are equal, that modulation should be in phase at conjugate points. The observed periodicities also accord reasonably with Dungey's[5] treatment of hydromagnetic oscillations in the exosphere. From this we should expect the periods of 2·5–8·5 min observed at Kiruna to be scaled up by a factor of about 2·6 at Great Whale River and Byrd. The observed periodicities, 3–16 min, are indeed longer than those at Kiruna, though perhaps not quite as long as expected. On the other hand, our observation does not in itself prove that hydromagnetic waves are the cause of the fluctuations. It only limits the choice to mechanisms acting symmetrically with respect to the equatorial plane.

It is interesting to note that these absorption fluctuations, which are in phase at conjugate points, occur in just that part of the day when the slow fluctuations, which have periods up to 2 h and are in antiphase at conjugate points[6], are least common. This might mean that different parts of the exosphere tend towards different modes of oscillation. However, further phenomena will no doubt come to light before the picture is complete.

The riometers were operated by W. M. McMurtry (Great Whale River) and L. D. Lewis (Byrd). The field operation was made possible through the co-operation of the Canadian Government and the U.S. Navy. The work was financially supported by the National Science Foundation through grant 23788.

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PHYSICS

Metastability of Amorphous Structures

IN earlier papers[1–4] we developed a free-volume model for the amorphous phase according to which the liquid and glassy states of a given substance together comprise a single, thermodynamically well-defined phase. In this model the transition to the glass state results ideally from the freezing out of the free volume, and hence the configurational entropy of the liquid[3]. This transition should occur in all classical liquids, including monatomic ones, provided crystallization is by-passed[2]. Implicit in these ideas is the hypothesis that the glassy state is truly metastable and not unstable; consequently, by the Nernst theorem, it would have vanishing entropy at 0° K. For the hypothesis to be true, each microscopic structural unit of the glass must lie at a position of static equilibrium, the totality of which is randomly distributed. If one such structure exists there must be a large number of similar random structures of equal energy. Nevertheless, the entropy of each is zero, because all these structures are mutually inaccessible. One particular structure would be picked out in a given cooling process, and the system would remain in that structure. The increase of entropy on heating back into the liquid state would then occur because the equivalent random structures become mutually accessible[5].

This hypothesis of metastability is supported by some recent observations on model systems and by recent computations of Rahman.

It had been found[6] that a random arrangement of hard spheres will compress to a rigid and apparently amorphous structure (dense random structure) with a unique density (0·86 times that of ideal close packing). The detailed observations of nearest neighbour contacts in this structure carried out by Bernal *et al.*[7] indicate that each sphere is rigidly locked into position within a truly amorphous structure. It follows that the Bernal dense random structure satisfies the condition of mutual inaccessibility of equivalent structures and hence is metastable; it therefore constitutes the prototype of the monatomic glass predicted in our earlier papers. Stillinger, DiMarzio and Kornegay[8] have shown that a random two-dimensional assembly of uniform hard disks also collapses to a rigid dense random structure which they consider to be a model for a two-dimensional glass.

Recently, Rahman[9] carried out machine calculations of the trajectories of a collection of 864 spherical atoms interacting via a 6–12 potential appropriate to argon and held at a density equivalent to that of crystalline argon at 40° K and one atmosphere pressure. Under the conditions of his calculations, he finds that the liquid state of this system can be 'quenched' in a period of 10^{-12} sec to a temperature of 10° K without crystallization. In effect, these calculations of Rahman may be regarded as equivalent to a laboratory experiment in which minute droplets of liquid argon are quenched rapidly. They further confirm the high resistance of amorphous systems, even when monatomic, to the initiation of crystallization.

Inasmuch as the hypothesis of metastability is intuitively most difficult to accept for monatomic systems, we regard these two instances of its correctness as convincing evidence for its general validity.

We thank Dr. A. Rahman for permitting us to refer to his work prior to its publication and for his advice.

This work was supported in part by the Office of Naval Research contracts at the University of Chicago and at Harvard University.

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Electrical Conduction in Amorphous Films

WHILE carrying out general exploratory work on the electrical properties of vacuum-deposited thin films, an interesting phenomenon was observed in boron layers. Similar effects have since been observed in other materials; however, most of the work so far has been with boron films. The purpose of this communication is to describe the observed phenomenon, leaving the explanation to a later date when more facts have been collected.

The boron layers were deposited by direct electron bombardment in a vacuum of about 10^{-5} mm mercury. An electron beam was focused on an extremely pure boron rod which rested on a carbon pedestal. Every precaution was taken to bombard only the boron and not the pedestal. The carbon pedestal was of spectroscopic grade purity. A beam current of approximately 120 m.amp at a voltage of about 10 kV was found sufficient to obtain a deposition rate of 100–200 Å/min.

The complete sample preparation consisted of first depositing metal electrodes, usually aluminium, in the form of strips (a few mm wide) on an in.^{2} glass substrate. The boron was then deposited on the substrate (at ambient temperature), leaving only the ends of the electrodes free. Finally, the top electrodes were deposited using the same mask as the first electrodes, but rotated 90°. This formed a cross-grid structure with boron separating each cross-over. The electrodes were deposited in a different chamber from the boron in order to prevent contamination. This procedure allowed oxidation of the bottom electrodes when the substrates were exposed to air. However, since gold electrodes yielded similar results and no asymmetry was observed in the samples, it is not believed that oxidation plays a part in the effect. During each step of sample preparation appropriate vacuum outgassing was conducted.

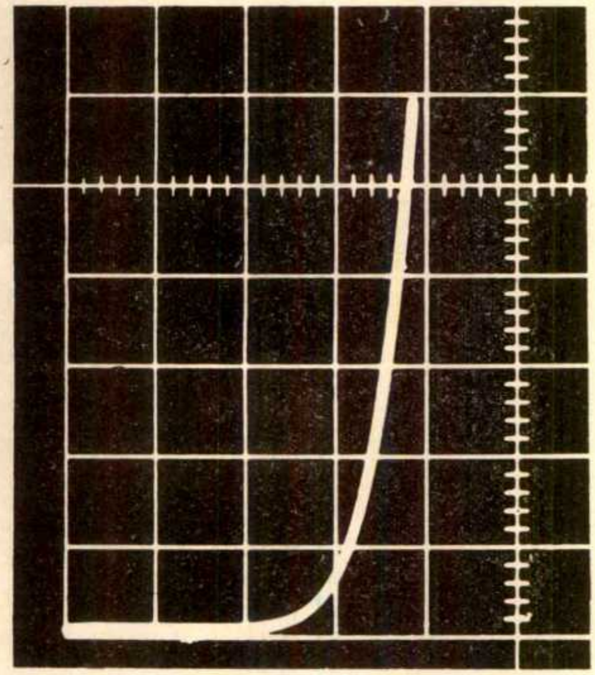

Fig. 1. Oscilloscope trace showing current-voltage relationship in a thin boron film (Sample No. 74). Scales: vertical, 0·5 m.amp/div.; horizontal, 10 V/div.

A typical current-voltage oscilloscope trace of a boron sample is shown in Fig. 1. Reversal of the voltage produces the same trace in the opposite direction, indicating that the curves are completely symmetrical about the origin. The current density through the sample can be described by the following relation:

$$i = KV^n \qquad (1)$$

The constants K and n vary considerably between samples. At very low voltages, depending on the sample, the currents tended to ohmic behaviour. Some typical values of K and n are shown in Table 1 along with appropriate physical parameters. K is calculated to yield the current density in amp/cm^2. As can be seen from Table 1, n is larger than that usually found in symmetrical varistors[1]. Sample No. 76, listed in Table 1, which used gold alloy electrodes showed similar results to the aluminium electroded samples.

Table 1. PARAMETERS OF BORON FILM SAMPLES

Boron film sample No.	n	K	Thickness (Å)	Electrodes	Electrode area
28	6·2	$2·2 \times 10^{-10}$	2100	Aluminium	$7·7 \times 10^{-3}$ cm^2
34	7·2	$1·0 \times 10^{-11}$	1300	Aluminium	7·7
53	6·6	$8·1 \times 10^{-12}$	4500	Nichrome	7·7
64	8·2	$2·6 \times 10^{-13}$	2400	Nichrome	7·7
74	8·3	$9·5 \times 10^{-14}$	2700	Aluminium	2·0
76	6·1	$4·3 \times 10^{-9}$	2700	50% Au–50% Al (alloy)	0·4

A planar electrode configuration was examined in which the boron was placed between two deposited nichrome tabs separated by about 13μ. The same general behaviour was observed. This geometry enabled the examination of the photoconductive properties of the layers. The boron films showed some photoconductivity when exposed to a tungsten light source, and the magnitude of the photoconductivity was directly dependent on the voltage applied across the sample.

Optical transmission data revealed a band edge at approximately 1·6 eV. The sample resistance increased with decreasing temperature; however, the relationship between temperature and the constants n and K has not yet been determined. An exact dependency on film thickness has likewise not been found. This is probably due to the strong dependence on purity and deposition parameters which masks other effects.

The sample purity and structure seemed to play a major part in determining the constants K and n of equation (1). The samples which gave the highest n were made from the purest boron obtainable (L. Light and Co., Ltd., Colnbrook, England). Samples made from impure source material yielded very poor results and, if the material was less than 99·99 per cent pure, the effect was not observed at all. X-ray diffraction did not reveal any crystal structure and it must be assumed that the films are completely amorphous. Films deposited at higher temperature in which some crystal structure was discernible did not show the observed non-linear current-voltage relationship.

A similar, but less-pronounced, effect has been observed in amorphous germanium and silicon films formed in the same manner. The effect likewise disappears when the films are heated and become crystalline in structure. Based on the foregoing observations it is apparent that the phenomenon is related to the amorphous nature of the layers. These amorphous semiconductor films have high resistivities and low charge carrier mobilities, enabling a high field strength to be maintained across them. This high field probably causes the observed effect either through an increase in the mobility, and/or the number of charge carriers in the sample. It is hoped that continued work will reveal the exact nature of the behaviour and will also lead to a better understanding of the properties of amorphous semiconducting films.

This work was supported in part by the U.S. Department of the Navy, Bureau of Naval Weapons.

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Load Discontinuity Method for Pressure Calibration : the Tetrahedral Anvil Apparatus below 25 Kilobars

PRESSURES in ultra-high pressure apparatus of the belt or anvil[1] types are commonly inferred from a subsidiary load/pressure calibration using the well-known resistance discontinuities in bismuth at 25·4 kilobars[2], in thallium at 36·7 kb[2] and in barium at 59 kb[3]. Possible uncertainties of this method are associated with: (*a*) variations in the anvil[4] and test-cell materials[5]; (*b*) the influence of sample geometry[6]; (c) the influence of temperature, particularly when generated within the test-cell[1]; (*d*) the rather wide region between 0 and 25·4 kb for which no very suitable resistance transition is yet available. In this communication a load discontinuity method is described which extends load/pressure calibration for ultra-high pressure apparatus. It is applied here to a ¾-in. edge tetrahedral unit of the N.B.S. type[1].

For a normal pressure calibration the wires of bismuth, thallium or barium are surrounded by a sleeve of low shear strength material, typically silver chloride. This minimizes the effects of stress gradients, non-hydrostatic stresses and deformation within the pyrophyllite, which is generally used as the solid pressure transmitting material in high-pressure test-cells. Curve *A* of Fig. 1 shows a typical load/resistance relation for a bismuth wire in a silver chloride sleeve: the sample was mounted between opposite faces of the tetrahedron as shown, and electrical contact was made directly to the anvil faces via soft copper end plugs. The anvils, of cemented tungsten carbide with 6 per cent cobalt binder, were painted with jeweller's rouge. Fig. 1 is taken from an *XY* recorder trace; the load signal was derived from a strain gauge load cell, and the resistance from the potential difference along the wire at constant current. Load was increased steadily at about 1 ton/min in the experiment, using a continuously pumped hydraulic press. Bismuth shows a rapid increase of resistance with pressure up to the onset of the transition at 25·4 kb. Curve *B* shows the corresponding load/pressure relation, when the silver chloride is replaced by a pressure-equalizing sleeve made from cuprous iodide: this has a rapid reversible transition at 14·1 kb at 20° C (ref. 7). In this case the monotonic resistance increase of the bismuth has been interrupted by a horizontal *t*, and, at the same time, the 25·4 kb transition has been displaced to higher loads, by an amount approximately equal to *t*. Both effects are due to the volume change accompanying the transition in the cuprous iodide sleeve (in this case ~ 0·006 cm^3/g)[7], and *t* represents the load necessary to 'take up' this change. It will be noted that this effect will be most obvious in high-pressure apparatus of the belt or anvil types, where there is a definite relation (through the compressibility of the test-cell) between the test-cell volume, the applied load and the pressure generated. Silver chloride itself has a transition at ~ 13 kb[7]. This transition, however, is sluggish and has only a small volume change (0·0001 cm^3/g); it has not been detected in these experiments.

A load/pressure relation for the tetrahedral anvil apparatus was obtained by using the following sleeves with suitable reversible volume transitions in the 0–25 kb range: potassium nitrite at 6·2 kb[8], silver perchlorate at 7·5 kb[7], cuprous iodide (as aforementioned) at 14·1 kb, and potassium bromide at 18 kb[9]. Except for the last-mentioned, which was machined directly from the solid, samples were precompacted from the powder, using a simple die with a removable core to provide the hole for the wire specimen. Bismuth was used as the sensing resistance in each case; there is no reason in principle why other materials with an appreciable pressure coefficient of resistance (or indeed of another suitable property) might not be used. Fig. 2 shows the load/pressure relationship obtained for the various sleeves: the beginning of the horizontal was taken in each case to indicate attainment of the appropriate transition pressure. Each point in Fig. 2 is a mean of three points except for cuprous iodide with four. Successive runs were repeatable and the estimated load error is ± 1·0 ton. With the exception of one run in silver perchlorate there was little evidence of negative slope in the interval *t*, which would indicate metastability of the low-pressure phase. Furthermore, the slopes of the resistance/load curves prior to the transitions (Fig. 1) were very similar to that for silver chloride, so that differences in the compressibilities of the sleeve materials do not appear to be important in this pressure range.

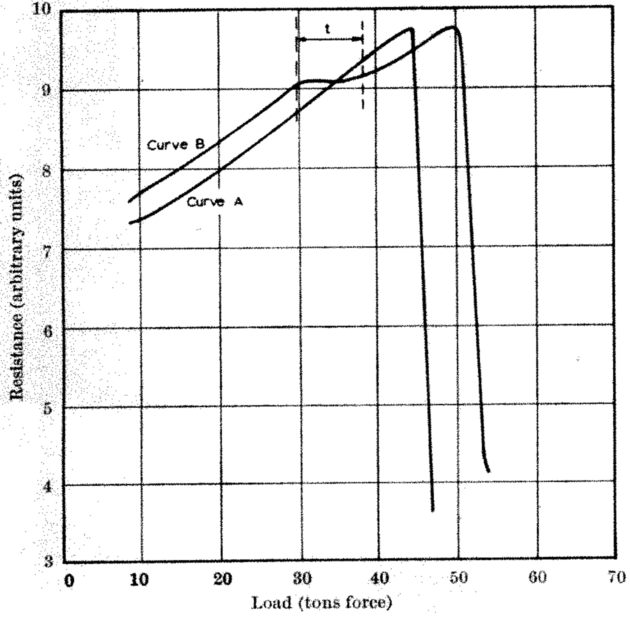

Fig. 1. Pressure variation of resistance of bismuth wire in sleeves of: *A*, silver chloride; *B*, cuprous iodide with a transition at 14·1 kb

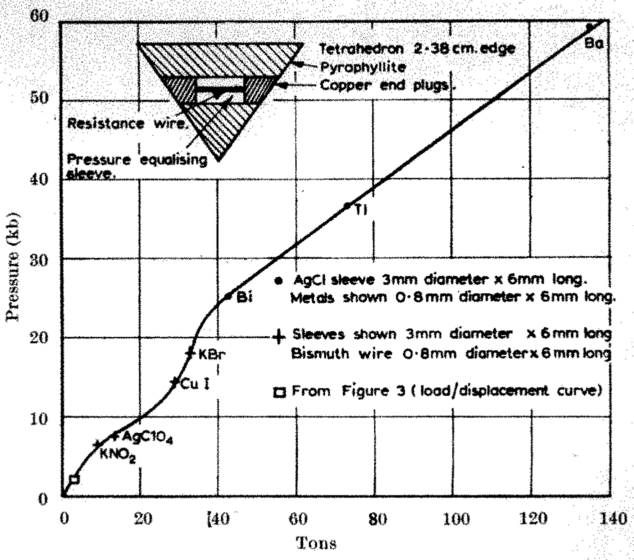

Fig. 2. Load/pressure curve for 3/4 in. edge N.B.S. type tetrahedral anvil apparatus with (insert) tetrahedron assembly

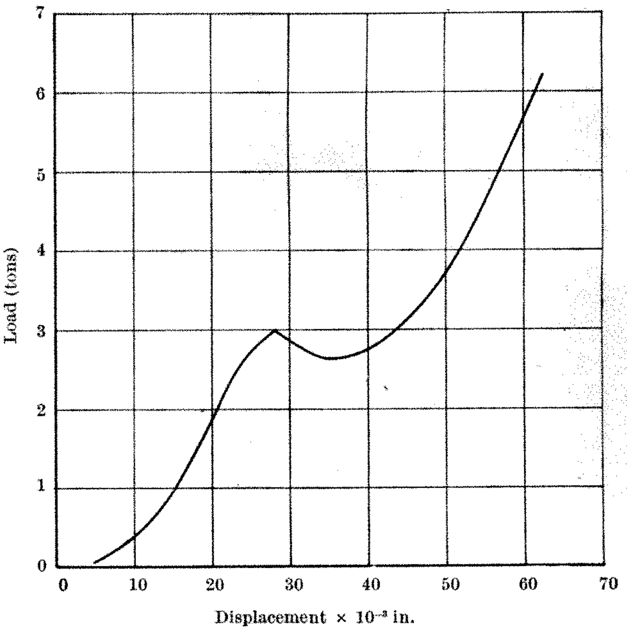

Fig. 3. Load versus displacement; pyrophyllite tetrahedron in N.B.S. tetrahedral anvil apparatus

Further information on the pressure generation in the tetrahedral apparatus can be derived from Fig. 3, which shows the load/displacement characteristic during the initial stages of compression of a plain pyrophyllite tetrahedron, displacement being measured by means of an inductive transducer mounted between the top anvil and the base plate of the apparatus. This type of curve, with load rising to a maximum, a fall to lower value and a subsequent rise, is typical, as might be expected, of extrusion in the presence of friction[10]. It is reasonable to suppose that the observed maximum in the load at 3·0 tons corresponds to the beginning of the extrusion process which forms the compressible gaskets: at this load the mean pressure on the anvil faces is 1·8 kb. This point is plotted in Fig. 2.

The conventionally obtained bismuth, thallium and barium points are also plotted, and it may be seen that the combined curve obtained by these methods has a fairly complicated shape. It may be divided into three regions: (*a*) the initial pressure rise, with a slow falling off in slope up to ~ 12 kb; (*b*) a rapid increase in slope between this pressure and 23–24 kb; (*c*) a fall off thereafter to a fairly linear relation up to the barium point. While it is possible that metastability of the low-pressure phases could have affected the transition loads, we believe that the straightforward and consistent experimental results obtained make this unlikely. Fig. 2 then indicates that the load/pressure characteristic for the N.B.S. type tetrahedral anvil apparatus is very similar to that obtained by Litster and Benedek[11] for a 'belt' type apparatus, using the pressure variation of the ^{57}Fe nuclear resonance frequency.

The complicated shape of both curves is undoubtedly related to the precise mechanism of pressure generation.

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CRYSTALLOGRAPHY

Two New Binary Chromium Oxides

WELL-DEVELOPED black crystals of two chromium oxides (*A* and *B*) with composition between CrO_3 and CrO_2 have been obtained by heating CrO_3 in a high-pressure autoclave at 200°–300° C. The oxygen pressure was 1·5–3 kilobars.

Single crystal photographs of substance *A* indicate the Laue symmetry *mmm*, and the conditions limiting possible reflexions suggest the space group *Pbcn* (No. 60). The unit cell dimensions of *A* derived from single crystal and powder photographs are:

$$a = 12{\cdot}04\ \text{Å};\ b = 8{\cdot}21\ \text{Å};\ c = 8{\cdot}18\ \text{Å};\ V = 808\ \text{Å}^3$$

The observed density, 3·68 g cm^{-3}, corresponds to a formula weight of 1,790 per unit cell, suggesting a cell content of 4 Cr_5O_{12} (1808).

The Laue symmetry of the substance *B* was also found to be *mmm*, and the missing spectra were those characteristic of the space groups *Cmcm* (No. 63), *C2cm* (No. 40) and $Cmc2_1$ (No. 36).

The unit cell dimensions of *B* as obtained from single crystal and powder photographs are:

$$a = 8{\cdot}47\ \text{Å};\ b = 12{\cdot}88\ \text{Å};\ c = 10{\cdot}09\ \text{Å};\ V = 1101\ \text{Å}^3$$

The observed density, 3·34 g cm^{-3}, corresponds to a formula weight of 2,210 per unit cell, suggesting a possible cell content of 12 Cr_2O_5 (2208) or 8 Cr_3O_8 (2278).

The powder patterns of *A* and *B* are distinctly different from those reported earlier[1] for specimens with the gross compositions ~ Cr_3O_8 and ~ Cr_2O_5.

Crystal structure determinations of these two new chromium oxides as well as further preparative investigations in this field are in progress.

This investigation forms part of a research programme on the crystal chemistry of chromium compounds, which is supported by the Swedish Natural Science Research Council.

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METALLURGY

Role of Silica in the Oxidation Resistance of Molybdenum Disilicide

MOLYBDENUM disilicide is of particular importance in the field of high-temperature materials, both as a structural material in its own right and as an oxidation resistant coating for molybdenum alloys. Apart from having a high melting point of 2,000° C (ref. 1) and good oxidation resistance up to 1,700° C, $MoSi_2$ possesses high-temperature mechanical properties which make it adequate for many applications under static high-temperature oxidizing conditions[2].

However, a relatively high elastic modulus coupled with low thermal conductivity and high thermal expansion render it prone to crack on thermal cycling, so that a molybdenum test-piece silicided by pack cementation at

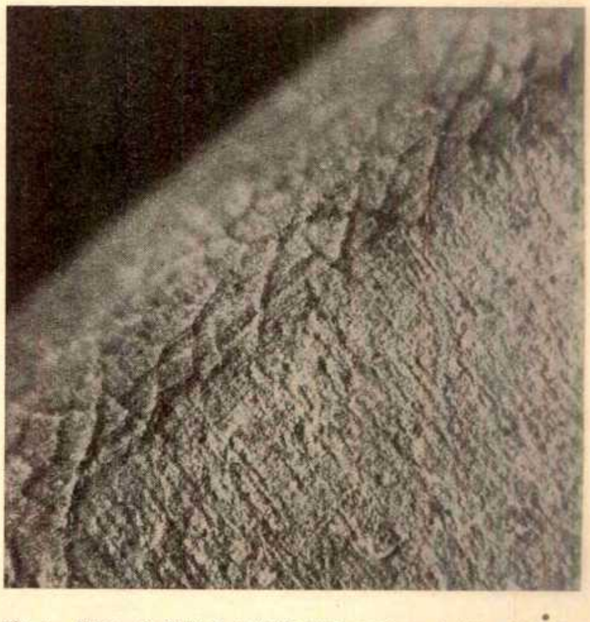

Fig. 1. Edge of silicided molybdenum specimen (×30) which has craze-cracked as a result of thermal cycling from 1,100° C to room temperature

1,100° C (ref. 3) will rapidly 'craze' if repeatedly thermally cycled to this temperature (Fig. 1).

Although such cracks are obviously detrimental to oxidation protection, an interesting and puzzling feature is that the cracking of a $MoSi_2$ coating does not necessarily result in failure of the part. We believe that this must be largely due to the secondary protection afforded by lower silicides of molybdenum which exist as an intermediate system between disilicide and substrate in most as-prepared coatings or are later developed by thermal diffusion (Fig. 2). This intermediate system, which may be identified by X-ray analysis as compounded of Mo_5Si_3 and Mo_3Si, is not oxidation resistant and decomposes on heating to silica. On theoretical grounds this should occur with a large volume increase and, in fact, if a sectioned specimen is heated in air at 650° C and observed microscopically, the decomposition product is seen to protrude from the surface as a siliceous ridge (Fig. 3). At this temperature the silica produced occurs as a low-density frit of loosely bound particles, and this may well exaggerate the volume increase, but it is considered that a similar mechanism operating at higher temperatures will root-fill any crack that develops in the outer $MoSi_2$ coating and hence arrest further ingress of oxygen (Fig. 4).

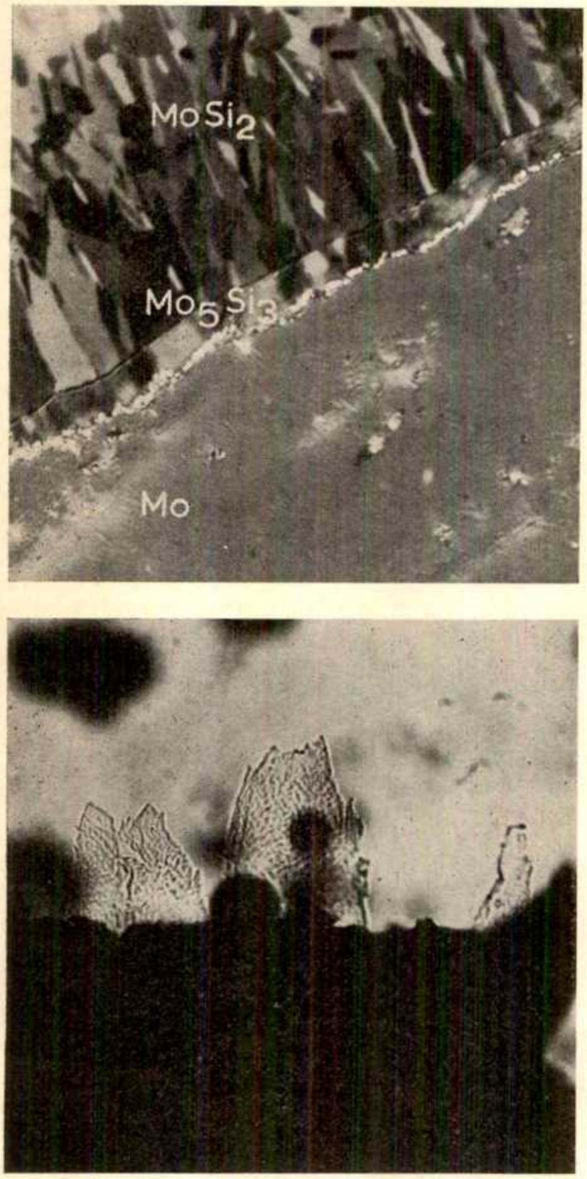

Fig. 2. Above, cross-section of silicided molybdenum specimen (× 1,500) showing the columnar outer $MoSi_2$ coating superimposed on a narrower intermediate layer of Mo_5Si_3. Below, the specimen has been lightly etched with nitric acid and the very narrow bright region between the Mo_5Si_3 and molybdenum substrate probably represents a thin oxidized Mo_3Si zone. Prolonged oxidation in acid causes the whole intermediate system to decompose to silica gel and to project from the surface (× 90)

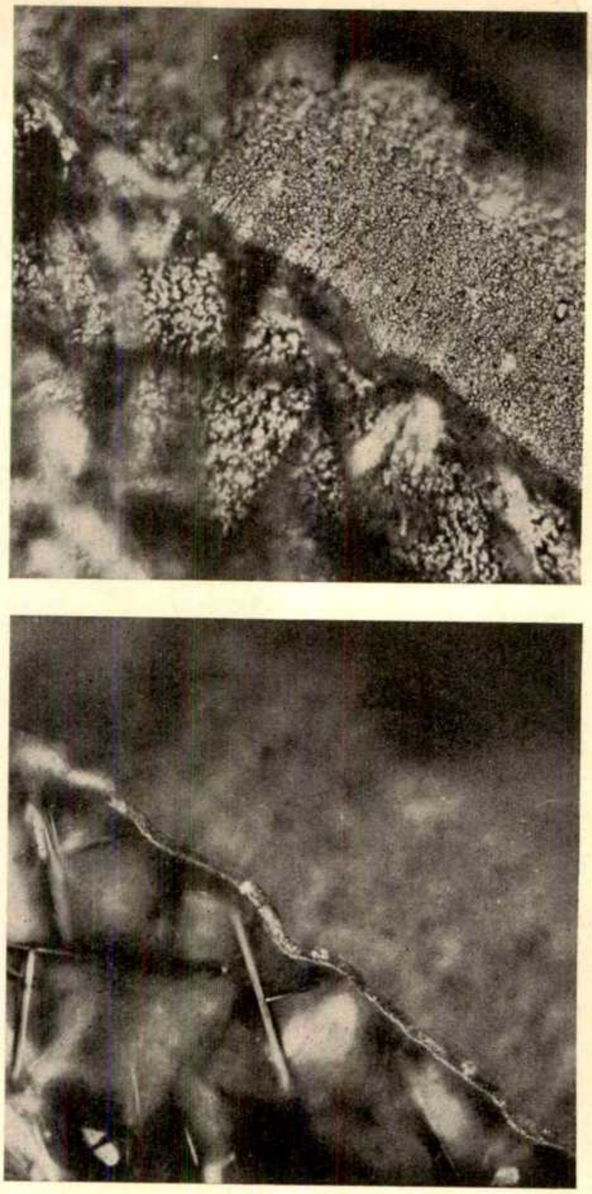

Fig. 3. Cross-section of a silicided molybdenum specimen (× 750) oxidized in air at 650° C. Above, the microscope has been focused on the plane of the specimen; below, the photograph shows detail 5μ above the plane and demonstrates the expansion of the intermediate layer of Mo_5Si_3 on oxidation. Crystals of MoO_3 are also evident

It is possible that at higher temperatures a low-melting mixture of MoO_3 and SiO_2 forms initially and that this flows before losing MoO_3 by volatilization and finally solidifying. This would substantially aid the crack-filling process, and it is noteworthy that a particularly effective coating results if molybdenum boride is laid down between the silicide and metal substrate. This composite coating, which will survive many thousand thermal cycles without failure, soon becomes covered with a system of cracks from which siliceous material erupts and later devitrifies to cristobalite (Fig. 5). It is probable in this case that the exaggerated crack-filling process results from the fluxing action of boron oxides derived from molybdenum boride.

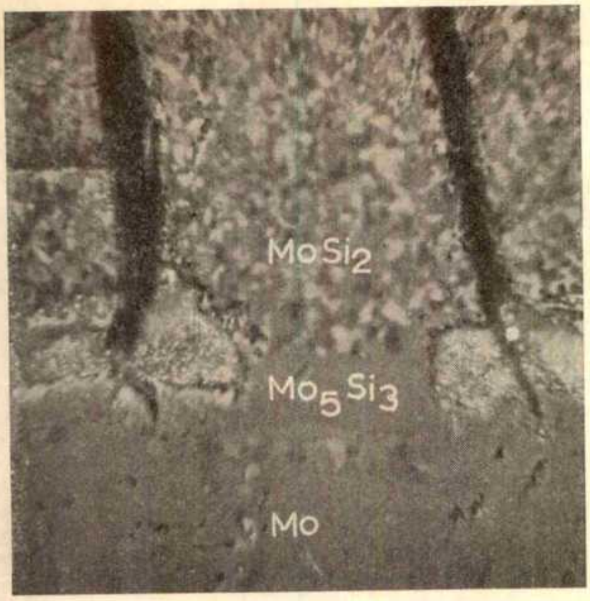

Fig. 4. Cross-section of a silicide multilayer on molybdenum (× 720). The outer MoSi$_2$ and Mo$_5$Si$_3$ layers have cracked and air has found its way to the intermediate system to decompose the Mo$_5$Si$_3$ layer to silica

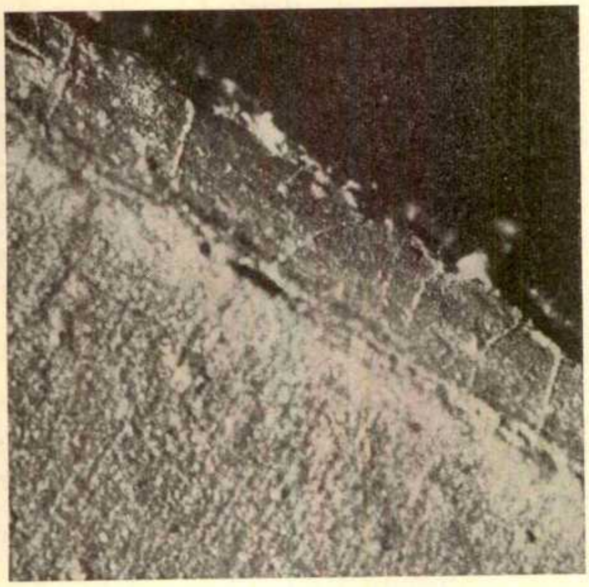

Fig. 5. Edge of silicided molybdenum specimen (× 30) which has had molybdenum boride located at the interface between the MoSi$_2$ and the molybdenum substrate. The fluxing action of oxides of boron has caused silica from the decomposition of the Mo$_5$Si$_3$ intermediate layer to erupt to the surface through the cracks in the MoSi$_2$

A mechanism involving oxidation of Mo$_5$Si$_3$ may also explain the unique oxidation resistance of molybdenum disilicide among the silicides at temperature extremes of up to 1,700° C. Molybdenum disilicide develops an appreciable vapour pressure as this temperature is approached[4] and in vacuum has been shown to lose silicon to acquire a surface layer of Mo$_5$Si$_3$ (ref. 1). In air this product will decompose and expand to silica as soon as it is formed and the disilicide will rapidly become covered with a protective siliceous crust.

The unique resistance of molybdenum disilicide to oxidation is presumably due to the fact that the metal oxide MoO$_3$ is volatile and rapidly evolves to leave a solid residue of silica melting only above 1,700° C. This is apparently substantiated by the fact that the introduction of metal oxides to the coating has a disadvantageous effect on oxidation resistance, probably as a result of persistent fluxing action[5].

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GEOCHEMISTRY

Palæoclimatic Significance of δ-Oxygen-18-time Trends observed by Oxygen Isotopic Analysis of Freshwater Limestones

AFTER McCrea[1] and Epstein *et al.*[2] established the carbonate–water palæotemperature technique whereby the temperature of calcium carbonate precipitation can be determined by measuring the oxygen-18 content of the calcite, this tool has been widely utilized by a large number of workers for marine carbonates ranging in age from early Mesozoic to the present. Since the fractionation factor, that is, the isotopic composition of calcite divided by that of sea-water, is temperature dependent, deposition temperatures can be determined by: (1) measuring the $^{18}O/^{16}O$ ratio of ancient carbonate; (2) assuming a value for the isotopic composition of sea-water at that time.

So far, this technique has not been applied to fresh-water carbonates, presumably because of large present-day variations in the isotopic composition of fresh-water, shown, for example, by the data of Dansgaard[3], and Epstein and Mayeda[4]. If the $^{18}O/^{16}O$ ratio of fresh-water varied to this extent in large ancient lakes, temperatures determined by this method would be meaningless.

This need not be the case, however, at times when ice caps were non-existent, presumably over much of geological time. The isotopic composition of meteoritic water formed by the evaporation of sea-water is approximately −7 per mil. relative to the mean ocean water standard, and Epstein and Mayeda claim that isotopic fractionation which changes the oxygen-18 content of meteoritic water from about −7 per mil. to about −20 per mil. occurs in areas close to and possibly in the permanently glaciated regions. These authors further suggest that precipitation over the larger part of the Earth has an oxygen-18 content similar to that resulting from the initial evaporation of sea-water from the surface of the ocean, that is, −7 per mil.

Thus, in times of non-glaciation, the isotopic composition of large bodies of fresh water, especially in latitudes where calcium carbonate precipitation is possible, should not be a large variable. If enough samples of fresh-water limestones are used to suppress random errors or variations, it should be possible to determine palæotemperatures for these carbonates. Moreover, since these bodies of water are much smaller than the oceans, the response of water temperature to changes in climate are more rapid. Hence, fresh-water carbonate sequences may provide very detailed climatological trends and serve as sensitive palæoclimatic indicators.

In this light, a select collection of 157 samples of fresh-water limestones assembled by Keith and Weber[5] might be examined. Almost all these specimens are from North American localities, and range in age from Devonian to the present. Each sample was identified on the basis of fresh-water fossils and was analysed for $^{18}O/^{16}O$ ratio in

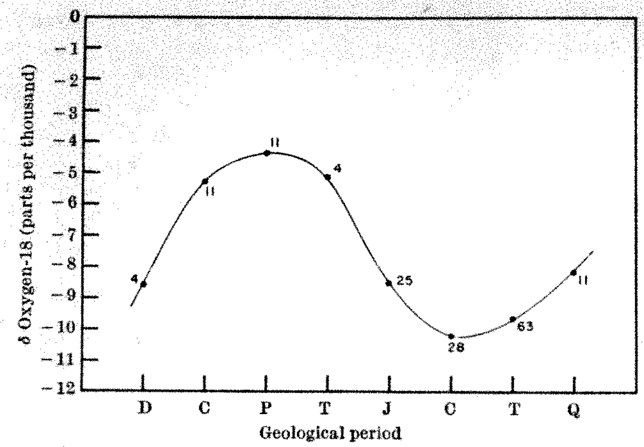

Fig. 1

duplicate using a 6 in., 60° sector mass spectrometer with isotope ratio recording apparatus. Grouped according to age, mean δ-oxygen-18 values relative to the Chicago P.D.B. standard, with the standard deviation given in brackets, are: Devonian −8·57 (1·3), Pennsylvanian −5·25 (1·6), Permian −4·36 (3·5), Triassic −5·12 (0·91), Jurassic −8·52 (3·0), Cretaceous −10·22 (3·5), Tertiary −9·65 (4·5), and Quaternary −8·15 (2·1). The results illustrated in Fig. 1 appear to generate a sinusoidal curve with $^{18}O/^{16}O$ maxima in the periods Permo-Carboniferous and Quaternary, and a minimum in the Cretaceous. The striking fit of the mean δ-oxygen-18 values to the curve shown in Fig. 1, the magnitude of the difference (about 6 per mil.) between the maximum and the minimum, and the occurrence of relatively high oxygen-18 contents in the carbonates precipitated during geological periods (Permo-Carboniferous and Quaternary) when glaciation was known to have occurred, strongly suggest that the data based on 314 analyses of 157 samples reflect temperature differences in large lakes located in central North America.

There are a number of variables on which the isotopic composition of fresh-water carbonate depends; these are discussed by Keith and Weber[5]. If the effects of some of these variables can be eliminated, for example, by proper sampling, random variation should cancel if enough samples are taken.

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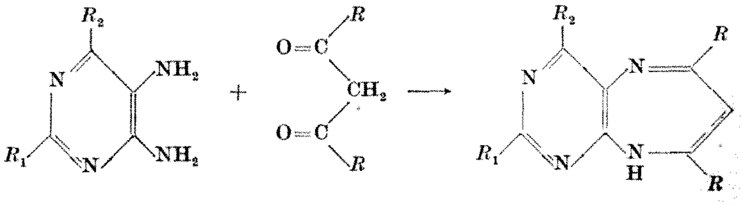
CHEMISTRY

Structural Investigation of 4-[(4-Amino-5-pyrimidinyl)imino]-2-penten-2-ol

As part of a continuing programme of synthesis of condensed pyrimidine ring systems as potential antimetabolites and antitumour agents, the preparation of the hitherto unreported 1,5-pyrimido[4,5-*b*]-diazepine ring system (I) was undertaken in these laboratories[1]. This ring system is analogous to the 1,5-benzodiazepines extensively examined by a number of investigators[2-8]. The preparation of the pyrimidodiazepines (I) was attempted by condensation of 4,5-diaminopyrimidine derivatives with β-diketones, as in the synthesis of the corresponding 1,5-benzodiazepines



I

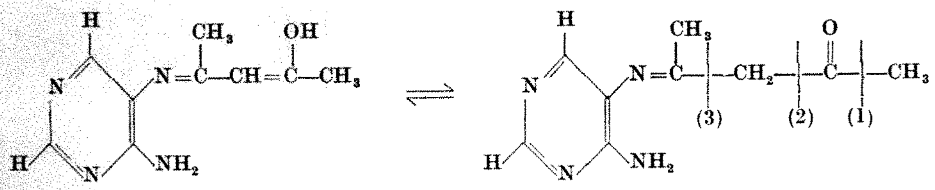
Reaction of a number of substituted 4,5-diaminopyrimidines with β-diketones under neutral or weakly acidic conditions afforded good yields of yellowish reaction products that are soluble in non-polar solvents and crystallize from benzene or methanol. These compounds possess definite melting-points and display two ultraviolet absorption maxima at *p*H 10, the longer wavelength peak being in the range 305–315 mμ. The infra-red spectra show sharp bands in the NH and OH regions and exhibit no carbonyl absorption.

The elemental analyses of the condensation products consistently show the presence of the elements of water, which could not be shown to be water of crystallization by the Karl–Fischer test. Therefore, an investigation of structure was undertaken in order to determine whether the compounds are hydrates of structure I or isomeric forms with covalent carbon–oxygen bonds, such as II–IV. For this purpose the simplest derivative was examined, namely, the condensation product of 4,5-diaminopyrimidine and 2,4-pentanedione.

4,5-Diaminopyrimidine (0·05 mole) was condensed with 2,4-pentanedione (0·055 mole) in 50 ml. of refluxing absolute methanol for 9 h. After removal of solvent, the residual reaction mixture was crystallized from methanol with the aid of activated carbon and a 61 per cent yield of off-white crystalline material (compound *A*) was obtained. Sublimation at 120°–140° (0·005 mm) afforded analytically pure, off-white monoclinic needles, m.p. 180°–182° dec., which are photosensitive and easily oxidizable. (Calc. for $C_9H_{12}N_4O$: C, 56·2; H, 6·3; N, 29·1; found: C, 56·3; H, 6·4 N, 28·9.)

The ultra-violet spectrum is consistent with extended conjugation involving the pyrimidine ring: λ_{max} in mμ (ε): *p*H 1–280 (10,700); *p*H 10–231 (9,600), 314 (19,100); EtOH – 232 (10,300), 314 (16,400). In the infra-red the principal features are λ_{max}^{KBr} 3·03μ (OH, NH); 3·20μ (CH); 6·05, 6·19, 6·35, and 6·70μ (C=N, C=C, pyrimidine ring). No carbonyl bands were seen either in KBr or in chloroform.

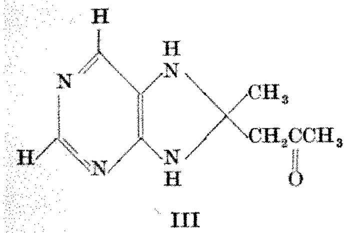
Inasmuch as the foregoing chemical and physical properties do not permit a definite assignment of structure, the compound was investigated by nuclear magnetic resonance spectroscopy, mass spectrometry, and X-ray structure analysis. The nuclear magnetic resonance spectrum fails to support structure I and is consistent with the open-chain structure II. (The isomer of II with a free amino group at pyrimidine position 5 is unlikely to have formed. Since the 5-amino group of 4,5-diaminopyrimidine has a greater degree of nucleophilic character than the 4-amino group, involvement of the 5-amino group in Schiff's base formation is the preferred reaction[9].) The nuclear magnetic resonance spectrum in deuterochloroform, taken with a Varian *A*-60 spectrometer with tetramethylsilane as the internal reference, shows two dissimilar methyl groups at 8·2τ (3 hydrogens) and 8·0τ (3 hydrogens), and no methylene group in the 6·0–6·5τ region. One enolic hydrogen at −1·8τ, one vinyl hydrogen at 4·9τ, and a broad peak at 4·3τ (2 hydrogens), corresponding to a primary amino group, are observed. The two pyrimidine ring hydrogens appear at 2·2 and 1·9τ. Both peaks at −1·8 and 4·3τ exchanged rapidly with deuterium when the compound was treated with deuterated acetic acid (d_4), and the vinyl hydrogen peak at 4·9τ exchanged slowly. Except for the absence of the

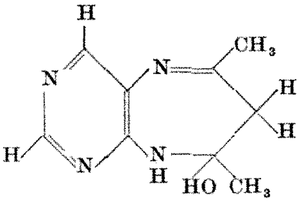

II ⇌ II*a*

methylene band, the nuclear magnetic resonance spectrum of the non-nitrogenous side-chain of the molecule is generally similar to that of 2,4-pentanedione[10].

The mass spectrum (determined with a G.E.C. 21–103*C* mass spectrometer, equipped with a heated inlet system, operated at 170°; ionization potential 70 eV; ionizing current 50 μamp) of this compound indicates the molecular weight to be 192, rather than 174 which would correspond to the anhydrous ring system I. Furthermore, the successive elimination of mass units 15, 43 and 57, along with the appearance of a strong peak at m/e 43 (CH_3CO^+), is consistent with fragmentation of the side-chain of the uncyclized structure II*a*, tautomeric with the enolic form II. At the low pressure prevailing in the mass spectrometer, the less associated keto form II*a* is evidently favoured and the side-chain undergoes the indicated cleavages at (1), (2), and (3).

Major mass ions (m/e)	Fragments lost	Observed peaks
M	None	192
M-15	(1) $CH_3\cdot$	177
M-43	(2) $CH_3CO\cdot$	149
M-57	(3) $CH_3COCH_2\cdot$	135


III


IV

It is possible, then, to reject structures I, III and IV for compound *A* on the basis of the combined ultra-violet, infra-red, nuclear magnetic resonance, and mass spectral data. The X-ray analysis recently performed by Yannoni and Silverman[11] provides final confirmation of structure II (Fig. 1) and proves that the 5-amino group of 4,5-diaminopyrimidine did indeed participate in the reaction. The X-ray analysis establishes the positions of the heavy atoms in the molecule. The molecule lies in two planes and the crystal structure is characterized by an extensive network of hydrogen bonds. Cyclization to diazepine structure I or IV is rendered unlikely because, in the solid state, the primary amino group of the pyrimidine ring not only lies in a separate plane but is also far removed from the oxygen function. The X-ray analysis does not clearly indicate the enol (II): keto (II*a*) tautomeric ratio in the crystal state.

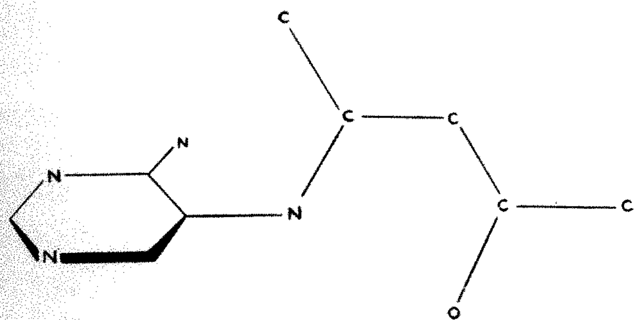


Fig. 1. Molecular structure of 4-[(4-amino-5-pyrimidinyl)imino]-2-penten-2-ol (ref. 11)

In summary, compound *A* has been assigned the structure 4-[(4-amino-5-pyrimidinyl)imino]-2-penten-2-ol (II). All the chemical, physical and spectral data presented are consistent with this assignment, except for the existence of the keto tautomer II*a* in the vapour phase during mass spectrometry and in the crystal state.

We thank Dr. K. Biemann, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass., for making available the mass spectrometric facilities in his laboratory; Dr. B. C. Das, of the same Department, for carrying out and interpreting the mass spectrometric analysis; and Drs. N. F. Yannoni and J. Silverman, of the Space Physics Laboratory, U.S. Air Force Cambridge Research Laboratories, Bedford, Massachusetts, for collaborating with us and keeping us informed on the status of the X-ray structure analysis.

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Pentasulphur Triimides: Completion of a Class of Cyclic Sulphur Imides

AMONG the compounds theoretically obtainable by replacing sulphur atoms in the octasulphur ring by imino groups, heptasulphur imide and tetrasulphur tetraimide have long been known. Last year the 1–3, 1–4 and 1–5 isomers of hexasulphur diimide were reported independently by Tavs, Schultze-Steinen and Colchester[1], and by one of us[2], and we have since confirmed the structures proposed in the latter reference by measurement of dipole moments[3]. Excluding compounds with adjacent imino groups, none of which is known, there remained to be made only two sulphur imides derived from octasulphur in its normal 'crown' conformation, namely, the 1–3–5 and 1–4–6 isomers of pentasulphur triimide. We now report the preparation of two compounds which we think are these.

The crude reaction product from 420 ml. of disulphur dichloride and ammonia in formdimethylamide[4] was extracted with diethyl ether[1], which took up the sulphur imides. The diimides and triimides were concentrated and separated from the bulk of the heptasulphur imide by fractional crystallization. They were then isolated by chromatography on silica gel, eluting at first with carbon disulphide.

1·35 g of the compound thought to be 1–4–6 pentasulphur triimide came off at $R_F \sim 0·16$, immediately after the last (1–3) diimide. It formed long colourless needles, m.p. 131° (decomp.) (found S, 78·3 per cent; N, 21·1 per cent; H, 1·45 per cent; m.wt. (cryoscopic in benzene) 180; calc. for $S_5N_3H_3$, S, 78·0 per cent; N, 20·5 per cent; H, 1·46 per cent; mol. wt. 205). The solubility in carbon disulphide at 18° was 0·049 molal. The saturated solution in a 0·2 mm cell gave strong infra-red bands at 3·01μ (*OD* 0·21), at 11·99μ (*OD* 0·26) and at 12·28μ (*OD* 0·41). The dipole moment in carbon disulphide was difficult to measure accurately owing to association, but approached a value of 1·0 ± 0·3 Debye units with diminishing concentration; the value calculated by vector addition, assuming that each imino group contributes 1·28 Debye units (our measurement of the moment of heptasulphur imide) is 1·4 Debye units.

The other isomer had an R_F of only about 0·09 in carbon disulphide; it was more quickly eluted by changing the solvent to 5 : 2 carbon disulphide–benzene. 0·33 g of colourless rhombs, m.p. 124° (decomp.), were obtained (found S, 78·4 per cent; N, 19·9 per cent; H, 1·57 per cent; m.wt. 210 (cryoscopic in tertiary butanol)). The solubility in carbon disulphide was only 0·008 molal at 18°. The saturated solution in a 0·2 mm cell gave infra-red bands at 3·00μ (*OD* 0·03), at 11·82μ (*OD* 0·04) and at 12·12μ (*OD* 0·07). The dipole moment, in carbon disulphide solution, was also difficult to measure because of the small solubility, but the value found, 2·8 ± 0·6 Debye units, corresponded with the calculated value of 3·0 Debye units for 1–3–5 pentasulphur triimide.

Note added in proof. We have recently heard that Dr. H. Tavs also made the presumed 1-4-6-triimide in unpublished work.

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BIOCHEMISTRY

Magneto-optical Rotation Spectra of Cytochrome c

MANY spectroscopic investigations have been reported on cytochrome *c* in defining its role in biochemical electron transport systems[1,2]. These include the ultra-violet and visible absorption measurements of Keilin and Hartree[3] and the optical rotatory dispersion (ORD) data of Eichorn and Cairns[4]. In this laboratory, we have been investigating the application of the new technique of magneto-optical rotation spectroscopy[5] to the examination of different oxidation states of the cytochrome *c* molecule. In this type of measurement, the optical rotations induced by an external magnetic field (Faraday effect) are obtained as a function of wave-length to provide a magneto-optical rotation (MOR) spectrum. The results for cytochrome *c* (Figs. 1–4) are quite pronounced and show large changes with the oxidation state of the molecule.

The MOR spectra were measured for aqueous solutions (essentially the same results were obtained in 0·01 M *p*H 7 phosphate buffer) in a 1-cm cell at a 10,000-gauss field and corrected for the background natural optical activity of the molecule, measured in the absence of a magnetic field. The reduced cytochrome *c* samples were commercial grade preparations (Nutritional Biochemicals, Inc., Cleveland, Ohio) from beef heart muscle. The oxidized samples were prepared by oxidation of the reduced form with potassium ferricyanide and purified by dialysis for four days at 4° C to remove excess salts and by-products. The results in Figs. 1–4 show the plot of specific magnetic rotation, as defined by equation (1), as a function of the wave-length in mμ:

$$[\alpha]^M_{sp} = \frac{\theta}{lc}\left(\frac{10,000}{H}\right) \qquad (1)$$

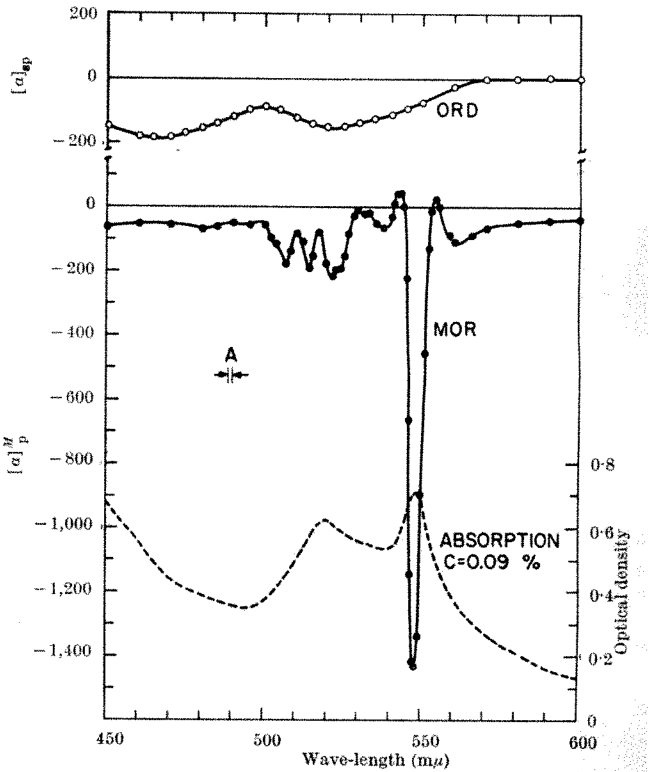

Fig. 1. MOR and ORD spectra of reduced cytochrome *c* at 23° C in water $[\alpha]^M_{sp}$, specific magnetic rotation; *A*, monochromator band pass

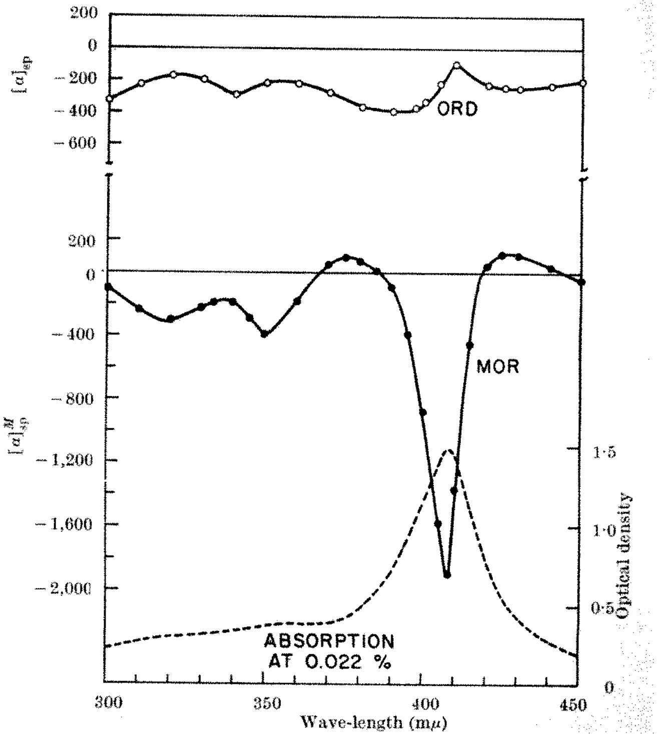

Fig. 2. MOR and ORD spectra of reduced cytochrome *c* at the Soret band region in water at 23° C

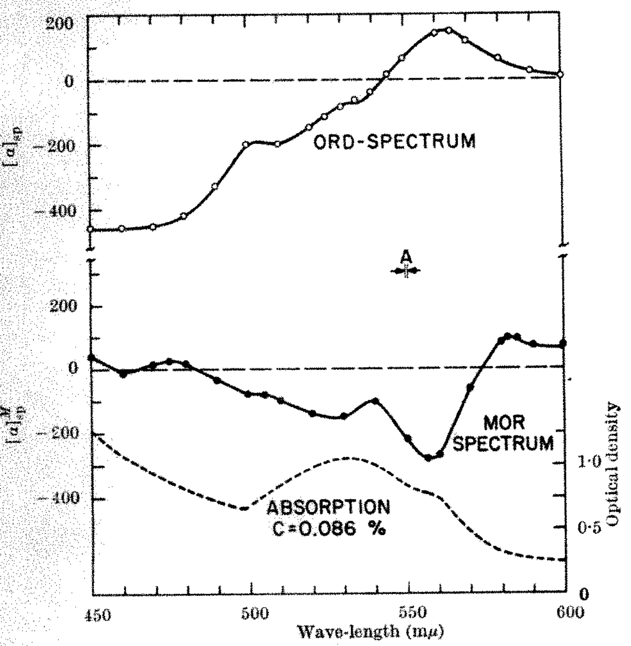

Fig. 3. MOR and ORD spectra of oxidized cytochrome *c* at 23° C in water. $[\alpha]_{sp}^{M}$, specific rotation; *A*, monochromator band pass

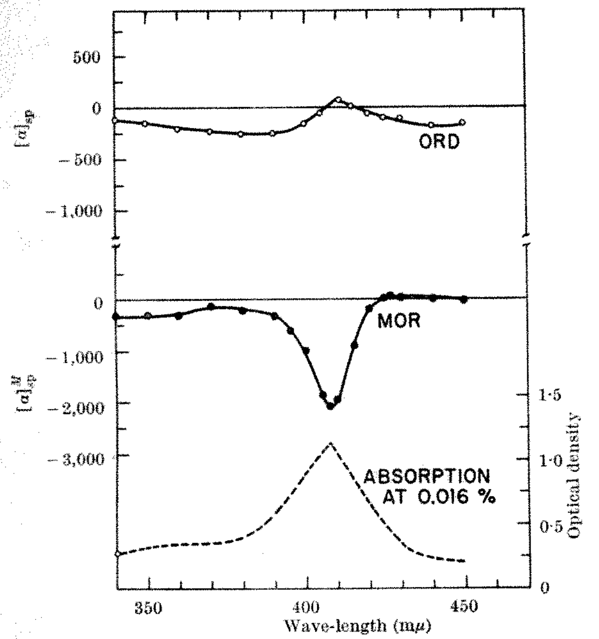

Fig. 4. MOR and ORD spectra of oxidized cytochrome *c* at the Soret band region at 23° C

where: θ is the angle of rotation in degrees; *l* is the path length in decimetres; *c* is the concentration in grams per ml.; and *H* is the magnetic field (gauss).

The apparatus and the techniques of measurement of the MOR spectra were the same as previously described[7].

Figs. 1 and 2 summarize the results for reduced cytochrome *c* at the absorption band regions corresponding to the α, β and γ (Soret) bands of the molecule. A comparison of the MOR spectrum with the optical rotatory dispersion (ORD) and the absorption spectrum at the α and β bands (549 and 520 mμ) clearly illustrates the greater fine structure of the MOR spectrum. The presence of six negative magnetic rotation peaks indicates that the two absorption bands of the molecule must be composite in structure and consist of at least six separate absorption peaks. The remarkable feature of the MOR spectrum is the large negative rotation peak at 549 mμ, with a specific magnetic rotation of − 1,450, at least 10 times as large as any other MOR peak of the spectrum. As shown in Fig. 3, this rotation is reduced to − 160 for the oxidized molecule, indicating the possibility of using the magnetic rotation to examine the kinetics of the reaction. Moreover, the fine structure of the MOR spectrum completely disappears when the molecule is oxidized to the ferric state.

Figs. 2 and 4 compare the measurements at the Soret band region of the molecule. Here a large negative rotation occurs in the MOR spectrum at the 410 mμ band with no distinctive changes on oxidation of the molecule. The natural optical activity measurements are also nonspecific in this respect. These results suggest that the more significant information about the electron transport function of cytochrome *c* is to be obtained from investigations at the long wave-length region of the spectrum, that is, at the α and β bands rather than γ (Soret) band. These observations indicate that the MOR spectral data may be quite useful for the investigation of the electron transport properties of cytochrome *c*.

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Non-enzymatic Interactions of Reduced Coenzyme I with Inorganic Phosphate and Certain Other Anions

We previously reported[1] changes of the absorption spectrum of nicotinamide adenine dinucleotide (coenzyme I, abbreviated NAD^+) in solutions containing orthophosphate. In an extension of those investigations[2], it has been observed that dihydronicotinamide-adenine dinucleotide (reduced coenzyme I, abbreviated NADH) also reacts with orthophosphate, but in an entirely different manner. Similar changes were observed with dihydronicotinamide adenine dinucleotide phosphate (reduced coenzyme II, abbreviated NADPH) and certain other NADH-analogues. This is a preliminary report of our findings[3].

In spectrophotometric investigations, maximal changes were observed at 340 mμ and at 280 mμ. This is shown in the difference spectra of Fig. 1. It is apparent that the changes progress with time. Kinetic analysis[3,4] revealed that the decrease in absorbancy at 340 mμ fitted apparently first-order kinetics. However, the apparent first-order velocity constant was dependent on the concentration of the $H_2PO_4^-$ ion. In the *p*H range 6–8, a velocity constant independent of the phosphate concentration and the *p*H could be obtained by use of the second-order rate equation for the special case where one reactant is in great excess of the other, that is:

$$\frac{2\cdot 3}{b_0}\log\frac{a_0}{a} = kt \qquad (1)$$

In this equation, b_0 is the $H_2PO_4^-$ ion concentration (as obtained from the Hasselbach equation), and a_0 and a the absorbancies of NADH (equivalent to its concentration) at 340 mμ, at zero time and time *t*, respectively. At 34·4° C, the rate constant, *k*, was equal to $2\cdot 8 \times 10^{-4} \times M^{-1}$ sec^{-1}. Other polybasic anions with *pK′* values in the region between *p*H 6·0 and 8·0 were also reactive. This included $H_2AsO_4^-$ ($k = 4\cdot 9 \times 10^{-4}$); HSO_3^- ($k = 6\cdot 4 \times 10^{-4}$); and $H_2PO_3^-$ ($k = 3\cdot 0 \times 10^{-4}$). Monobasic (for example, Cl^-) or dibasic (for example, $SO_4^{=}$) anions fully dissociated at this *p*H range were almost non-reactive[3].

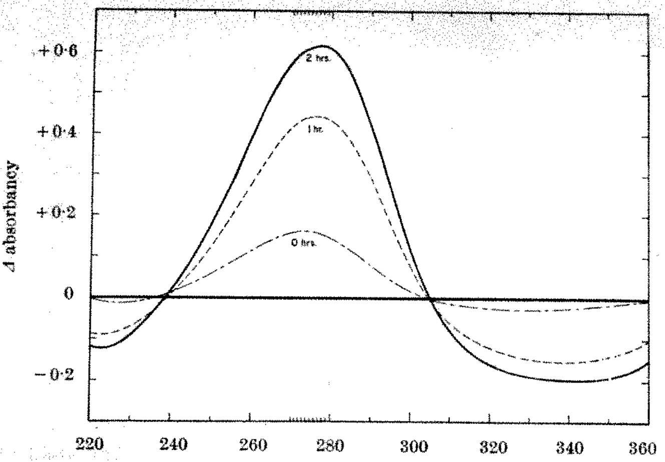

Fig. 1. Difference spectra of NADPH ($6{\cdot}17 \times 10^{-4}$ M) in 1·5 M potassium orthophosphate at *p*H 6·62 and 34·4° C, versus a reference solution containing NADPH in 0·05 M potassium orthophosphate. Solvent absorption was corrected for by the tandem-cell technique (ref. 12) and the sample in high concentration of phosphate was positioned in the sample beam of a Beckman *DK*-1 spectrophotometer. The light-path was 1 mm

Kinetic treatment of the changes at 280 mμ (Fig. 1) was also possible at a fixed *p*H and potassium orthophosphate concentration. After an initial and apparently instantaneous change in absorbancy (Fig. 1) which can be tentatively attributed to electrostatic interactions similar to those reported for NAD⁺ in high orthophosphate concentration[1,3], the subsequent time-dependent absorbancy changes could be described most simply by a reaction pathway involving two pseudo-first-order steps. Thus, the overall process can be written as follows:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad (2)$$

In this proposed mechanism, A is considered to be the species of NADH (still capable of oxidation by acetaldehyde-alcohol dehydrogenase), as it occurs at essentially zero time in 1·5 M potassium orthophosphate ($\varepsilon_{280} = 3{,}960$; $\varepsilon_{340} = 5{,}550$). The transformation from A to B was assumed to proceed at the same velocity that characterized the decrease in absorbancy at 340 mμ, that is, $k_1 = k$ of equation (1). The (phosphorylated ?) intermediate B ($\varepsilon_{280} = 18{,}250$) was further transformed (one or more steps?) to a final product C ($\varepsilon_{280} = 6{,}340$) at much slower speed. These interrelations are shown in Fig. 2.

From such considerations[4] it was demonstrated[3] that for the reaction of NADH in 1·5 M potassium phosphate at *p*H 6·62 and 34·4° C, $k_2/k_1 = 0{\cdot}07$. It, therefore, follows that a lag period (about 60 min., Fig. 2) should precede the appearance of a product C in measurable quantities. In other words, the intermediate B is reasonably stable under the conditions specified. It is hoped that determination of the second velocity constant in the presence of different anions and under various conditions of *p*H and anion concentration will contribute to the clarification of the nature of the decomposition of B.

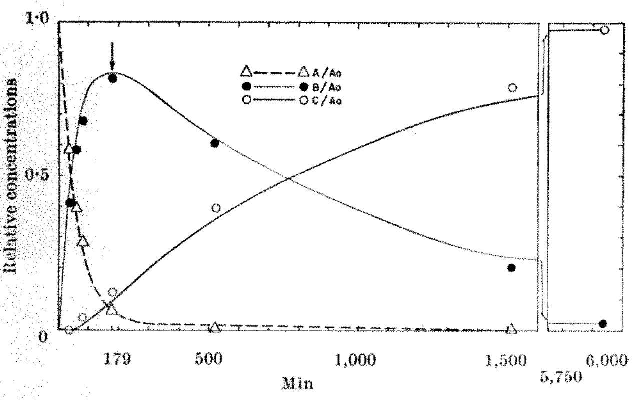

Fig. 2. Concentration-time curves for A, B, and C. Concentrations were computed from the molar extinction coefficient for each substance (see text) and from the relation $A + B + C = A_0$. (A_0 was the original quantity of NADH present in the medium.) The points assigned to its curve were calculated from equations for first-order reactions in series (ref. 4) and from the time of maximum accumulation of B (ref. 3) indicated in the figure by the arrow

A comparison of the rate constants for the reaction of various enzymatically reduced 3-substituted, 1,4-dihydropyridine derivatives in 1·5 M orthophosphate at *p*H 6·62 revealed that k_1 decreased over 300-fold in the order given here in the series: NADH; thiodihydronicotinamide–adenine dinucleotide; 3-acetyldihydro-pyridine–adenine dinucleotide and dihydropyridinealdehyde–adenine dinucleotide. Evidently the rate of reaction from A to B becomes slower with increasing effectiveness of the side-chain at position three of the dihydropyridine to act as an electron sink. The variation in k_1 with structure is thus in agreement with the changes of an overall velocity constant for the reaction of certain model compounds (for example, 1-benzyl-3-acetyl-1,4-dihydropyridine) in acid[5], and favours a reaction mechanism similar to that proposed by Anderson and Berkelhammer[5] involving β-protonation of 1,4-dihydropyridines, followed by reaction of the intermediate carbonium ion (at position 6) with a nucleophilic species (see also ref. 6). However, in contrast to the experimental conditions of Anderson and Berkelhammer, the hydrogen ion concentration in our experiments was extremely low (about $2{\cdot}4 \times 10^{-7}$ M) and the reaction rate apparently depended only on the anion concentration[3]. Furthermore, only polybasic anions with a second dissociation constant in the *p*H range between 6 and 8 were reactive. These considerations and a comparison of our findings with those of Wallenfels *et al.*[7] with model compounds in organic solvents have led us to the conclusion that the mechanism of the transformation from A to B involves a concerted action of the anion supplying a proton at the 5 position simultaneously with an attack of the resulting nucleophilic species at the 6 position of the 1,4-dihydropyridine (Fig. 3).

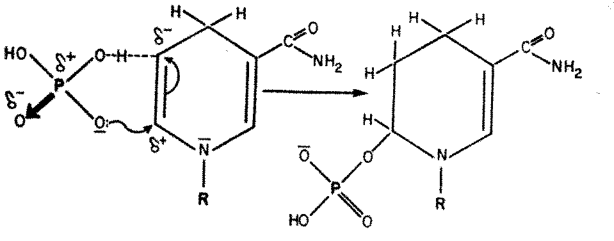

Fig. 3. Proposed mechanism of the spontaneous NADH–orthophosphate interaction at *p*H 6·62 (steps *A–B*)

It has also been demonstrated that 1,4-dihydronicotinamide ribonucleotide reacts more than twice as fast as NADH. This might be related to the folded state of NADH in solution[8]. The rate of reaction of the hypoxanthine analogue of NADH was the same as with NADH[3]. This would indicate that at *p*H 6·62 folding is independent of the presence of either a hydroxyl group or an amino group in the 6 position of the purine moiety.

It is probable that 'DPNH-*X*' of Chaykin, Meinhart and Krebs[9] may be identical or closely related to the product(s) described here. In their investigations, the chemical change of NADH in the presence of polybasic anions was catalysed by triose phosphate dehydrogenase, while in our experiments a reaction occurred spontaneously at relatively high anion concentrations. It is more difficult to evaluate the relation of the interactions and products described above to the phosphorylated NADH 'intermediate' of Griffiths[10]. The manner of decomposition of the latter[10] is reminiscent of the change between *B* and *C* as already described here, but more work is required to correlate these findings. In this connexion, it is interesting that in certain circumstances[11] the intramitochondrial concentration of orthophosphates might rise through an 'active transport' mechanism.

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Infra-red Spectrophotometric Measurement of the Kinetics of the Action of β-Glucosidase

IN recent reports[1-3] it was shown that infra-red spectrophotometry could be applied to the measurement of the kinetics of enzymatic reactions. The purpose of this communication is to give data concerning the use of infra-red spectrophotometry to determine kinetics of a β-glucosidase reaction.

β-Glucosidase (emulsin) was purchased from Mann Research Laboratories, New York. The substrate, β-phenyl-D-glucoside, was prepared by the method of Montgomery *et al.*[4]. The system consisted of 0·5 ml. of enzyme solution added to 2·0 ml. of substrate solution, producing concentrations of 3·2 per cent β-phenyl-D-glucoside (w/v) and 0·2 per cent β-glucosidase (w/v) in *p*H 5·2 acetate buffer ($\mu = 0{\cdot}1$) at the beginning of the reaction, which was stirred magnetically.

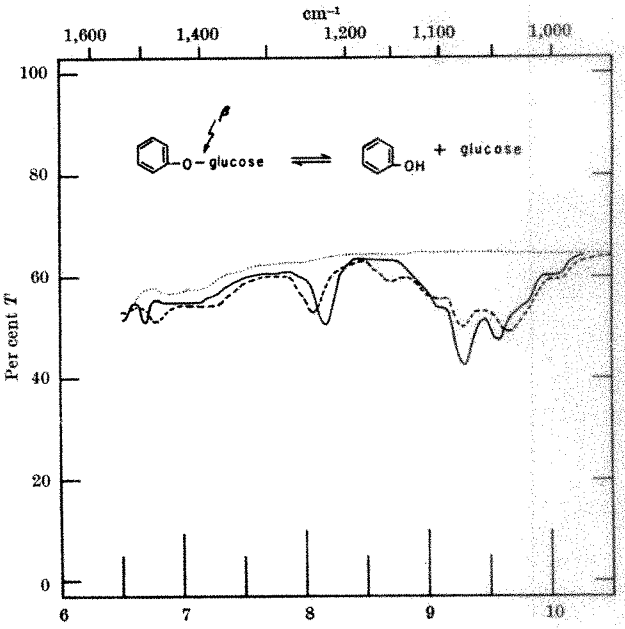

Fig. 1. The action of β-glucosidase on 3·2 per cent (w/v) β-phenyl-D-glucoside. · · · ·, water in an 'Irtran-2' cell, thickness 0·025 mm, versus water in the reference beam; ——, reaction mixture near beginning of the reaction (see text); – – – –, reaction mixture at end of enzymatic action (170 min)

Fig. 1 shows the course of action. The dotted line is the spectrum of water in an 'Irtran-2' cell of thickness 0·025 mm, measured against water in a reference 'Irtran-2' cell. The solid line is the spectrum of the enzymatic system near the beginning of the reaction, recorded against acetate buffer in the reference beam at a rate of 1μ per min. This curve was begun 2·5 min after the mixing of enzyme and substrate. The enzyme itself does not display a spectrum at 0·2 per cent concentration. As the reaction proceeds, several changes in the spectrum occur. The new band at 6·78μ is characteristic of free phenol in aqueous solution[5]. The band at 8·15μ (solid line), which is characteristic of the β-phenyl group linked to glucose through an oxygen atom, gradually disappears and becomes a band at 8·06μ (dashed line), characteristic of phenol[5] which is no longer bonded to glucose. As the reaction proceeds, a new band appears at 8·70μ, which does not exist in the spectrum of the original β-phenyl-D-glucoside. This band has been shown earlier[6] to be characteristic of the glucose molecule, and its intensity increases gradually as more free glucose appears. The spectrum of the final solution is identical with a solution made up of stoichiometric amounts of phenol and glucose, assuming complete hydrolysis.

The 'Irtran-2' (ZnS) does not interfere with the assay of the enzymatic reaction, since it was demonstrated that spectral changes were the same whether they were determined on aliquots removed from the reaction mixture at various time intervals, or were determined directly on the reaction mixture contained in the cell. First-order kinetics were calculated from absorbance data of the band at 8·70μ from the equation:

$$k = \frac{1}{t} \log \frac{A_0 - A_\infty}{A_t - A_\infty}$$

In a typical run A_∞, the absorbance of the band at the end of the reaction, was 0·2864; A_0, the initial absorbance of the band (extrapolated to time 0), was 0·2528; and A_t was the absorbance of the band at time t, varying from 0·2557 to 0·2857. Some typical examples of k were 0·0106, 0·0096, 0·0134 and 0·0112, with an average k of 0·0112 min⁻¹.

The methods reported here should be useful in the application of infra-red spectrophotometry in other enzymatic reactions and other chemical reactions occurring in water.

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Inhibitory Levels of Fluoride on Mammalian Cells

As a preliminary to more detailed investigations of the cellular effects of fluorides, growth inhibitory levels have been determined on mammalian cells. Because of the widespread concern about possible toxic effects of fluoride when used for water fluoridation, these levels are of more than theoretical interest.

Murine leukæmic lymphoblasts, *L5178Y* strain, were cultured following the method of Fischer[1], at 37° C, in 16 mm × 125 mm closed culture tubes, containing serial dilutions of sodium fluoride. Below a concentration of 5×10^5 cells/ml. these cells reproduce logarithmically approximately every 12 h. Each tube contained 5 ml. of culture medium with approximately 2,000 cells/ml. At the end of four days cell population was determined using a Coulter counter. This was readily accomplished since the cells grow freely in the medium without attaching to glass. The number of cell generations that have occurred was plotted as a function of the logarithm of the concentration of fluoride in the medium. By extrapolation the molarity of fluoride was determined which would result in a 20 per cent or a 50 per cent reduction in cell generation production (*Gd* 20 and *Gd* 50).

Significant inhibition has not been found at a concentration of 3×10^{-4} M F^- (5–6 p.p.m. F^-), and might possibly have been detected at $4{\cdot}4 \times 10^{-4}$ M F^- (8–9 p.p.m. F^-). Growth depression was rapid from $5{\cdot}9 \times 10^{-4}$ M F^-, and was linear above a concentration of 10^{-3} M F^- (19 p.p.m.) (Fig. 1). The amount of fluoride presumably required to inhibit the rate of cell reproduction by 20 per cent (*Gd* 20) was $1{\cdot}1 \times 10^{-3}$ M F^- (20 p.p.m.) (range 1·04–1·13) and by 50 per cent (*Gd* 50) was $1{\cdot}5 \times 10^{-3}$ M F^- (30 p.p.m.) (range 1·38–2·04). It is apparent that much higher concentrations of fluoride were required to demonstrate growth inhibition in these mammalian cells than the $5{\cdot}5 \times 10^{-5}$ MF^- (1–1·2 p.p.m. F^-) usually advocated for water fluoridation.

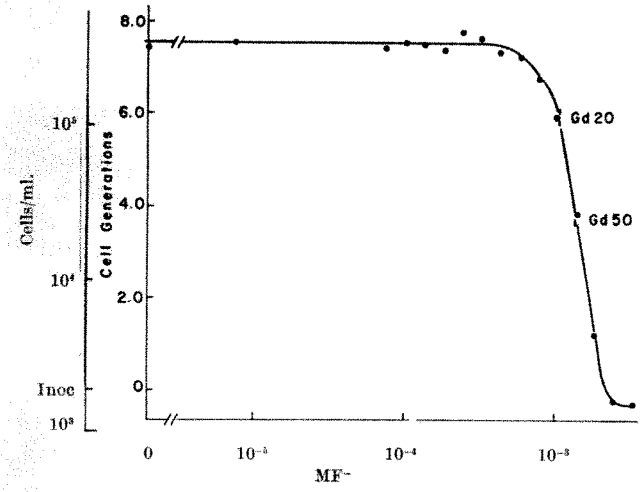

Fig. 1

These results differ from those reported by Berry[2], who interpreted his data to indicate an effect on mammalian cells (strain *L* mouse fibroblasts and HeLa S–3_{oxf} cells) with a fluoride level of 0·1 p.p.m. ($5{\cdot}3 \times 10^{-6}$ M F^-).

Although the anti-caries effect resulting from the recommended 1–1·2 p.p.m. F^- ($5{\cdot}3$–$6{\cdot}3 \times 10^{-5}$ M F^-) in the water supply may very well be due to a local effect on the teeth[3], the changes which occur in chronic fluorosis, such as mottling of the teeth and skeletal effects, are probably secondary to cellular dysfunction caused by toxic levels of fluoride in the immediate environment of the affected cell. This interference of cell function may be related to inhibitory effects of fluoride on specific enzyme systems, some of which are known to be affected by levels of fluoride which were found to inhibit the cells in the work recorded here[4,5].

Water levels of fluoride differ from blood or tissue levels. Blood levels of fluoride tend to be considerably lower than water levels and bone levels tend to be considerably higher[6,7]. Most of the fluoride in bone, however, appears to be bound with calcium as fluoroapatite and may not be physiologically active. Even so, it seems reasonable to assume that the effect on bones and teeth is related to the high concentration of fluoride in these structures and that pathological abnormalities occur when the level of ionized fluoride (which differs from total fluoride) rises above a critical level (affecting cell function). The kidney, which excretes fluoride, and therefore contains higher levels than other soft tissues, is the organ affected when the water-level is increased (approximately 125 p.p.m. in the water supply is required to cause pathological changes). This would indicate that, above a critical level of fluoride, cell function may be affected in the kidney as well as in bone[8].

Other soft tissues do not demonstrate pathological changes except with an intake of excessive amounts of fluoride, and levels of fluoride in these tissues are below those required to affect the reproduction of the cells used in this work[9].

An effect on any tissue, therefore, may depend on a sufficient level of fluoride in that tissue to affect cell function, although the critical level of fluoride necessary to produce an adverse effect may or may not differ in different tissues. This toxicity study is in agreement with those findings which indicate that 1–1·2 p.p.m. fluoride in the drinking water will produce no adverse effects.

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Transfer of Melanin Granules from Melanocytes to the Cortical Cells of Human Hair

THE manner by which the cortical cells of developing hair fibres acquire melanin pigment has been the subject of much speculation. Some authors have postulated that the pigment granules are actively inoculated into the prekeratinized cells by way of the dendritic processes of the melanocyte (cytocrine activity)[1,2], whereas others have suggested that pigment granule transfer involves the active ingestion (phagocytosis) of the granules by the cortical cells[3,4]. Birbeck and Mercer[3,4], who have found some evidence for this latter process, observed small 'pseudopods' enveloping the pigmented processes of black hair melanocytes at a point adjacent to the developing cortical cells of hair.

If the phagocytosis of pigment granules does indeed occur, then remnants of cell membranes ought to be apparent in the vicinity of the pigment granules in the cortex of mature hairs. That there is a close association

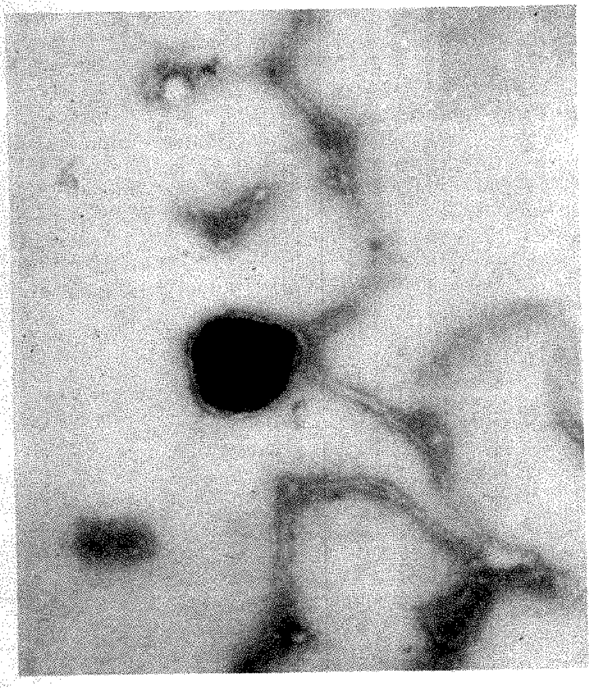

Fig. 1. Electron micrograph of a transverse section of human hair

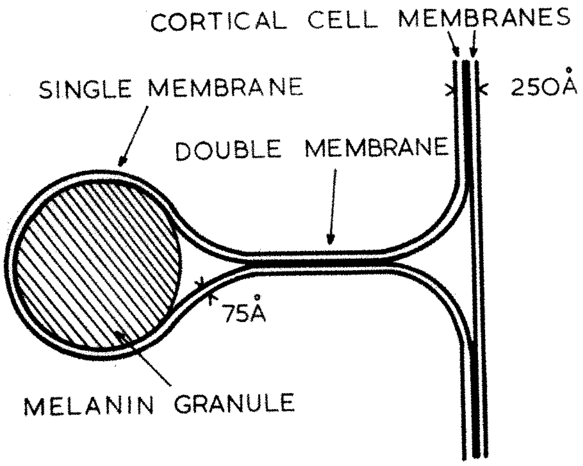

Fig. 2. Schematic diagram illustrating the association between melanin granules and the cortical cell membranes of hair

between pigment granules and the cortical cell membranes of hair has already been demonstrated in electron micrographs of transverse sections of osmium-stained hair[5]. This latter work, however, failed to reveal cell membranes around the pigment granules themselves. The reason for this is now thought to be that, because osmium tetroxide reacts so vigorously with melanin, the periphery of the pigment granules is obscured from view in the electron microscope. To overcome this shortcoming in technique, a method for staining thin sections of hair, which reveals the cell membranes in the cortex of hair without obliterating any of the detail adjacent to the pigment granules, was used in the present work.

Untreated human hair was dehydrated in ethanol and embedded in a prepolymerized mixture of methyl and *n*-butyl methacrylate (1 : 1 v/v) in the normal manner. Thin transverse sections of the embedded fibres were obtained with a Porter Blum ultramicrotome and collected on collodion-covered gold electron microscope grids. The specimen grids were then immersed in a centrifuged 10 per cent solution of dodeca tungstophosphoric acid in 50 per cent ethanol for 1 h and then washed in fresh 50 per cent ethanol for a further hour.

Using the staining technique already described here, the cell membranes and nuclear material in electron micrographs of the hair cortex were very well defined. Single granules and groups of two or three granules were seen to be intimately associated with adjacent cortical cell membranes. In a few cases the pigment granules were connected by a long finger of double membrane to the cortical cell membrane, and frequently single granules or groups of granules were enclosed by a single unit membrane (Fig. 1) similar in structure to that of the cell membrane. The reason why not all the pigment granules were apparently connected to a nearby cortical cell membrane is thought to be that some of the connecting membranes were either above or below the plane in which the sections were cut. A schematic diagram illustrating the observed association between pigment granules and cortical cell membranes is given in Fig. 2.

It is considered that this close association between the pigment granules and the cell membranes in mature hairs is a consequence of phagocytosis. It is envisaged that melanin granules exude from the ends of melanocyte dendrites into the space between pre-keratinized cortical cells of the hair follicle, where they are ingested singly or in groups by the cortical cells by phagocytosis. The ingestion of the pigment-laden tips of melanocytic dendrites in this manner would also explain the regular end-to-end arrangement of pigment granules, which is frequently encountered in electron micrographs of longitudinal sections of hair.

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Synthesis of Cystathionine and its Control in *Salmonella typhimurium*

CYSTATHIONINE formation in *Escherichia coli* involves the functioning of two enzymes[1-3]. The first enzyme is present in certain cystathionine-requiring auxotrophs such as *E. coli* strain 7/9 but absent from others (such as strain 2/2) and catalyses the formation of *O*-succinyl homoserine from homoserine and succinyl coenzyme *A*. The nature of the product was originally deduced from the chemical and biological properties of the compound[3] and this was confirmed by comparison with chemically synthesized *O*-succinyl homoserine[4]. The second enzyme which is absent from strain 7/9 but present in strain 2/2 forms cystathionine from *O*-succinyl homoserine plus cysteine[2]. Further evidence for the functioning of *O*-succinyl homoserine as a methionine precursor in *E. coli* was afforded by the observation[5] that strain 7/9 accumulated *O*-succinyl homoserine when grown with limiting amounts of homocysteine although the addition of methionine abolished the accumulation. Nutritional and genetic investigations with methionine-requiring strains of *Salmonella typhimurium*[6] suggested that the biosynthetic pathway might be the same as in *E. coli*. Two other observations support this view. First, organisms of *S. typhimurium* strain *B*23, a cystathionine-requiring mutant, accumulate *O*-succinyl homoserine in the culture fluid[5], while a genetically dissimilar cystathionine-requiring strain (strain *A*15) shows no accumulation. Secondly, extracts from *S. typhimurium* strain *A* form cystathionine from *O*-succinyl homoserine plus cysteine[7]. These data supported the view that cystathionine and *O*-succinyl homoserine are methionine precursors in *S. typhimurium* and, as the accumulation of *O*-succinyl homoserine by strain *B*23 (like that with *E. coli* strain 7/9) was prevented by methionine[5], it appeared that control of the biosynthetic pathway might be similar to that in *E. coli*[2]. The present work shows that *S. typhimurium* contains both enzymes of cystathionine formation and that enzymatic repression and feed-back inhibition occur.

Ultrasonic extracts of *S. typhimurium* strain *B*23 grown with limiting amounts of homocysteine catalysed the synthesis of *O*-succinyl homoserine from homoserine and succinate; the activity was assayed by the uptake of ^{14}C-succinate into *O*-succinyl homoserine as described by Rowbury and Woods[4]. The amount of enzyme present in strain *B*23 was much less than that in *E. coli* strain 7/9, but, as strain 7/9 accumulates 20 times as much *O*-succinyl homoserine as strain *B*23[5], this was not unexpected. Homoserine *O*-trans-succinylase of strain *B*23 was subject to enzymatic repression as growth with methionine (1·5 mM) reduced the enzyme level to 20 per cent. Methionine also inhibited the activity of the enzyme in extracts from organisms grown without methionine. Activity was reduced to 50 per cent by 1 mM DL-methionine and to 20 per cent by 5 mM methionine (Table 1). The formation of *O*-succinyl homoserine by intact organisms of strain *B*23 is also subject to feed-back inhibition by methionine, and the enzyme *in vivo* is much more sensitive to methionine. Thus accumulation was reduced to 6 per cent by 0·2 mM DL-methionine (Table 1). Homoserine *O*-trans-succinylase of whole organisms is also much more susceptible to inhibition by methionine than the cell-free enzyme in *E. coli* strain 7/9 (ref. 8).

Table 1. FEED-BACK INHIBITION OF HOMOSERINE *O*-TRANS-SUCCINYLASE BY METHIONINE IN *Salmonella typhimurium* STRAIN *B*23

DL-Methionine (mM) added to the reaction mixture	Homoserine *O*-trans-succinylase of intact organisms	Homoserine *O*-trans-succinylase of ultrasonic extracts
0	100	100
0·2	5·8	—
0·5	—	60
1	3·0	49
5	1·0	20

Values are given as percentages of the control (without methionine).

Extracts from *S. typhimurium* strain *A*15 formed cystathionine from *O*-succinyl homoserine plus cysteine, and in this case the level of enzyme activity was similar to that in *E. coli* strain 2/2 (Table 2). Activity was absent from *S. typhimurium* strain *B*23, and in strain *A*15 was greatly reduced by the omission of cysteine. This enzyme was also subject to repression by methionine; growth with 1·5 mM DL-methionine reduced activity to 22 per cent in extracts from strain *A*15 (Table 2). From these data it appears that *S. typhimurium* strain *B*23 resembles *E. coli* strain 7/9 while *S. typhimurium* strain *A*15 is like *E. coli* strain 2/2.

Table 2. ENZYMATIC REPRESSION OF CYSTATHIONINE SYNTHETASE OF *Salmonella typhimurium* STRAIN *A*15

	Cystathionine formed from *O*-succinyl homoserine (μmmole/mg protein/h)			
	Strain *A*15		Strain 2/2	
	Cysteine present	Cysteine absent	Cysteine present	Cysteine absent
Organisms grown without methionine	165	12·5	160	15
Organisms grown with methionine (1·5 mM)	36	12·0	30	12

S. typhimurium strains *A*15 and *B*23 also show homocysteine methylase activity, and in each strain formation of the enzyme was repressed by growth with methionine. Growth with 1·5 mM DL-methionine reduced enzyme synthesis to 15 per cent in strain *B*23 and to 25 per cent in strain *A*15. *S. typhimurium* has cystathionase activity also, but this activity in strain *B*23 is not appreciably reduced by growth with methionine.

Thus methionine synthesis in *S. typhimurium* appears to be like that in *E. coli* and to involve the intermediate formation of *O*-succinyl homoserine, cystathionine and homocysteine. The two cystathionine-forming enzymes and the homocysteine methylase complex are subject to enzymatic repression by methionine in *S. typhimurium*, and the first enzyme of the pathway (homoserine *O*-trans-succinylase) is also regulated by feed-back inhibition of its activity by methionine.

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PHYSIOLOGY

Effect of Thiocyanate on Secretion of Acid and Uptake of Oxygen by Gastric Mucosa of the Frog

THE ratio of acid secretion by gastric mucosa to the oxygen uptake involved in the secretory process does not seem to have a value significantly greater than four[3,4] as predicted by the redox theory of acid secretion[2,6]. Forte and Davies[5] have recently reported a series of experiments on electrically short-circuited gastric mucosæ of the bullfrog, in which thiocyanate produced a decrease in oxygen uptake and acid secretion. The ratio $\Delta q\text{H}^+/\Delta q\text{O}_2$ ($q\text{H}^+$ = rate of acid secretion, $q\text{O}_2$ = rate of oxygen uptake in the same units) was calculated from the mean rates of acid secretion and oxygen uptake before and after thiocyanate in each experiment. This ratio had high mean values which varied inversely with the concentration of thiocyanate from 5·1 to 12·0.

This finding was not confirmed in the experiments described here, which show that thiocyanate lowers the value of the stoichiometry between acid secretion and oxygen uptake. An everted sac technique was used[1]. Everted sacs of gastric mucosa obtained from starved frogs were prepared and filled with a medium of the following composition: 112·2 mM sodium chloride, 4·5 mM potassium chloride, 1·1 mM calcium chloride, 0·6 mM magnesium sulphate, 0·7 mM sodium phosphate buffer (*p*H 7·4), 11 mM glucose. The sacs were incubated initially in the same medium. Uptake of oxygen was measured manometrically at 25° C (direct method of Warburg; gas phase air), and acid secretion was measured by renewal of the incubating medium at intervals of 45 min and electrometric titration of the medium back to its initial *p*H. Twelve experiments were performed. Each sac was first incubated 5–7 times in the glucose–saline, and afterwards 3–4 times in the same saline containing 6 or 12 mM sodium thiocyanate. Acid secretion occurred spontaneously in all experiments.

The observations made were pooled and acid secretion before and after the addition of thiocyanate to the incubating medium was plotted as a function of oxygen uptake. The regression of acid secretion on oxygen uptake appeared to be linear both before and after thiocyanate. The following linear equations were obtained by the method of least squares:

before thiocyanate

$$q\text{H}^+ = 2{\cdot}59\ (q\text{O}_2 - 0{\cdot}029)\ \mu\text{M/mg dry wt./h} \qquad (1)$$
$$(n = 67,\ r = 0{\cdot}7)$$

after thiocyanate

$$qH^+ = 0{\cdot}89\,(qO_2 - 0{\cdot}029)\ \mu\text{M/mg dry wt./h} \qquad (2)$$
$$(n = 45,\ r = 0{\cdot}6)$$

The coefficients of correlation are not high but both are significant ($P < 0{\cdot}001$). There is also a significant difference between the regression coefficients ($P < 0{\cdot}001$). The variance of the coefficient of regression before thiocyanate indicates a range (mean ± 3 *S.D.*) of 1·69–3·49 for this parameter. These figures compare with the 99 per cent confidence limits obtained by Davenport[3], namely, 0·95 and 3·71.

The coefficient of regression of acid secretion on oxygen uptake gives the ratio of acid secretion to the associated oxygen uptake, as may be seen from the regression equations given here. It would appear that this ratio does not have values exceeding four with a high degree of probability, and that thiocyanate reduces its value.

The following mean rates of acid secretion and oxygen uptake were observed before and after thiocyanate in the present experiments.

	$\overline{qH^+}$ (μM/mg dry wt./h)	$\overline{qO_2}$ (μM/mg dry wt./h)
(1) Before thiocyanate	0·102	0·069
(2) After thiocyanate	0·022	0·053

Thiocyanate produced a 78 per cent decrease in the mean rate of acid secretion, but only a 23 per cent decrease in the mean rate of oxygen uptake. The oxygen uptake associated with the acid secretion abolished by thiocyanate would seem to remain largely unaffected by this agent. For this reason Forte and Davies[5] could have obtained high values for the ratio $\Delta qH^+/\Delta qO_2$.

I thank Mr. J. R. P. O'Brien for his advice.

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Radiation Inactivation of the Immunological and Biological Activities of Human Chorionic Gonadotropin

RADIATION of protein hormones or enzymes results in loss of activity. The rate of inactivation can be used to estimate the volume and weight of the active portion of the molecule if this rate is exponentially related to the dose of ionizing radiation and low-density ionizing radiation is used[1]. Previous investigations[2] have demonstrated that the rate of loss of hormonal activity of human chorionic gonadotropin (HCG) exposed to a 2·0-MeV electron beam was exponential and was identical when measured by four bioassays: in intact immature rats by (1) uterine weight, and (2) ovarian hyperæmia responses; in hypophysectomized rats by (3) testes, and (4) prostate weight responses.

Since hormonal and immunological activities of HCG may vary independently in certain circumstances[3], we have undertaken to compare the rate of radiation inactivation of HCG measured by immunoassay as compared to bioassay.

Detailed methods of preparation and radiation of HCG and of radiation dosimetry will be reported in a separate publication[2]. In brief, 2,000–5,000 I.U. of HCG were placed on stainless-steel planchets, lyophilized and exposed to a 2·0-MeV electron beam for varying lengths of time. The radiated hormone was bioassayed by means of the prostate and testes weight responses in immature hypophysectomized male rats[4,5] and loss of activity calculated as reported[2]. 250–500 animals were used to assay each radiation batch of HCG. Antisera to a Freund's adjuvant emulsion of a partially purified (7,000 I.U./mg) preparation of HCG were made in New Zealand white rabbits, such that 1 ml. of antiserum was capable of neutralizing the biological activity of 1,000–4,000 I.U. of HCG as measured by the prostate weight response of the hypophysectomized rat. A single line was observed when the antisera were analysed by double diffusion in agar against HCG. Immunological assays of HCG were performed by two methods: (1) by hæmagglutination inhibition as modified by Wide[5] from the technique of Boyden[6] utilizing HCG-sensitized, tanned, formalinized sheep red blood cells; (2) by a modification of the radioimmunoassay described by Feinberg[7] and by Skom and Talmage[8] utilizing an HCG preparation of 12,000 I.U./mg for radioiodination. In the latter assay, the presence of hormone is detected by its competition with radioiodinated hormone for reaction with rabbit antibody. The immune complex is afterwards precipitated with sheep anti-rabbit γ-globulin. Supernatant radioactivity is an index of amount of hormone. In order to eliminate the possibility that non-hormonal urinary contaminants were being measured by the immunoassays, standard amounts of HCG were prepared in an extract of the urine of a hypophysectomized human and in phosphate buffered saline. Results of simultaneous assays of both preparations in both immunoassay systems were essentially identical.

Multiple assays were performed at each radiation dosage and biological results were verified on eight separately radiated batches of hormone. In ten assays on two separately radiated batches of hormone, loss of immunological and hormonal activity was measured on the same preparations.

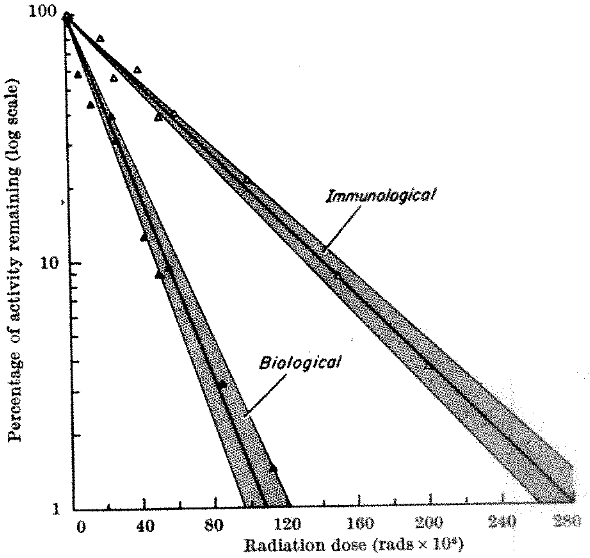

Fig. 1. The rates of inactivation are represented by regression lines; shaded areas represent 95 per cent confidence limits of the regression line. ▲, ventral prostate assay; △, hæmagglutination inhibition assay

Results are summarized in Table 1 and Fig. 1. Fig. 1 demonstrates a linear relationship between the logarithm of percentage of activity remaining and the radiation dosage obtained for both immunological and biological data. This indicates that in both instances a population of uniform-sized active units is being measured; significant heterogeneity in the size of active units would result in departure from linearity[1].

The rate of inactivation was consistently greater when assayed biologically than when measured immunologically.

Table 1. RADIATION INACTIVATION OF IMMUNOLOGICAL AND BIOLOGICAL ACTIVITIES OF HCG

Assay	D_{37} × 10^{-6} rads (95% limits)	Target volume-mμ³ (95% limits)	Mol. wt. × 10^{-3} (95% limits)
Biological			
Prostate weight	25·67 (23·10—28·91)	32·46 (28·57—36·36)	26·9 (23·7—30·1)
Testes weight	25·63 (23·12—28·76)	32·54 (28·74—36·35)	27·0 (23·8—30·1)
Immunological			
Hæmagglutination inhibition	63·01 (58·90—67·73)	12·90 (11·95—13·87)	10·7 (9·9—11·5)
Radioimmunoassay	58·31 (50·63—68·74)	14·01 (11·76—16·28)	11·6 (9·8—13·5)

The radiation dose required to reduce activity to 36·8 per cent (D_{37}) as measured biologically by prostate weight response was 25·67 × 10^6 rads ($\pm$ 1·29 × 10^6 (*S.E.M.*)) and as measured by testes weight response was 25·63 × 10^6 rads ($\pm$ 1·26 × 10^6). The D_{37} measured by hæmagglutination inhibition was 63·01 × 10^6 rads ($\pm$ 2·09 × 10^6) and by radioimmunoassay was 58·31 × 10^6 rads ($\pm$ 4·27 × 10^6). The target volume calculated[1] from biological data averaged 32·5 mμ³. The target volume calculated from immunological data averaged 13·5 mμ³. Assuming a density of 1·376 g/c.c. the molecular weight calculated from biological data was 27·0 × 10^3, which agrees well with previous physical measurements[9]. The molecular weight calculated from immunological data was 11·2 × 10^3.

These findings are interpreted as demonstrating that the structural unit required for immunological activity of HCG is considerably smaller than the unit required for biological activity.

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Origin of Neuraminic Acid in Human Seminal Fluid

THE occurrence and possible function of the neuraminic acids in mammalian tissues and fluids is a matter of great interest. Large amounts of *N*-acetylneuraminic acid have been demonstrated in the seminal fluid of man[1,2], bull[3] and boar[4]. Analysis of the secretions of the accessory genital organs of the boar showed that the mucin from the bulbourethral glands contained 4–4·5 per cent (fresh weight) of neuraminic acid. The concentration was less in the seminal vesicles and negligible in the other glandular secretions[5]. In man the average concentration of neuraminic acid was in the seminal vesicle fluids 231·9 mg/100 ml., in the prostatic secretions 60·6 mg/100 ml., and in the seminal fluid 124·2 mg/100 ml. From these data, Warren[2] calculated that approximately two-thirds of the seminal fluid came from the prostate gland. It is, however, difficult to calculate the relative contribution of the various glandular systems to the ejaculate by using data obtained from extracts of autopsy material.

It is known that during ejaculation the three main glandular systems contributing to the ejaculate are discharged successively. The first portion comes from the prostate gland, the second from the testes and epididymis, and the third from the seminal vesicles. During ejaculation the secretions from these three glandular systems become more or less mixed. By estimating the concentration of acid phosphates (from the prostate gland), spermatozoa (from the testes, epididymis and vas deferens) and fructose (from the seminal vesicles) in each portion of a 'split-ejaculate' it is possible to calculate the amount secreted by each of these glandular systems[6].

In the present investigation the origin of neuraminic acid was examined by analysing each portion of split-ejaculates for its content of acid phosphatase, fructose and neuraminic acid. The procedure and analytical methods have been described earlier[6,7]. Neuraminic acid was determined by the thiobarbituric acid method[3]. The various seminal fractions were not examined for their content of spermatozoa since the secretions of the testes, epididymis and ductus deferens are relatively small and probably add little to the neuraminic acid content of the semen[2,6]. Analysis of the sperm cells by the Ehrlich or resorcinol test showed the presence of only very small amounts of neuraminic acid[2]. Seminal fluid was received from five healthy male volunteers aged 25–30.

The analytical data from a typical experiment is presented in Fig. 1. The results indicate that neuraminic acid is secreted in nearly equal amounts from both the prostate gland and the seminal vesicles. This secretory pattern is not usual since most substances investigated are secreted mainly or entirely from a single gland.

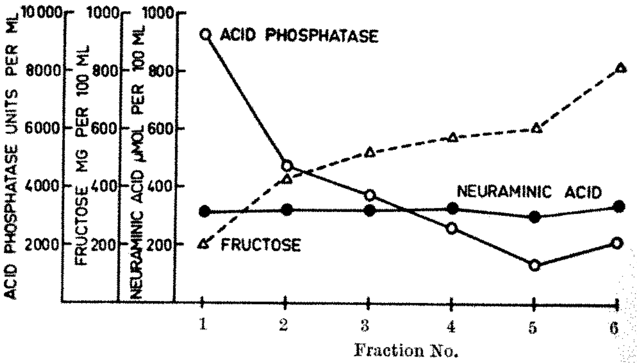

Fig. 1. Concentration of acid phosphatase, fructose and neuraminic acid in different portions of human seminal fluid collected with the 'split-ejaculation method'

The highest concentration found in the seminal vesicle secretions (last fractions) was 430 μmoles/100 ml. This is lower than the mean value (750 μmoles/100 ml.) given by Warren[2] for the seminal vesicle fluid obtained at autopsy. Moreover, the mean value given by Warren[2] for the prostatic fluid (196 μmoles/100 ml.) is below the concentrations found in the first fractions of the ejaculates (250–350 μmoles/100 ml.).

The concentration of neuraminic acid in the total ejaculates varied between 200 and 350 μmoles/100 ml. This is in the same order of magnitude as found by Svennerholm[1] and Warren[2].

The significance of neuraminic acid for human fertility is not known, but work on this problem is in progress.

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PHARMACOLOGY

Neural and Photic Regulation of 5-Hydroxytryptophan Decarboxylase in the Rat Pineal Gland

THE mammalian pineal gland has high concentrations of serotonin[1-3] and of 5-hydroxytryptophan decarboxylase (5-HTPD) (ref. 4), the enzyme which synthesizes serotonin. Innervation of the rat pineal gland is derived largely or solely from sympathetic nerve fibres the cell bodies of which are located in the superior cervical ganglia[5]. Fluorescent microscopic findings indicate that a large part of pineal serotonin content is localized in sympathetic nerve endings[2]. Following superior cervical ganglionectomy, the specific indole fluorescence in these nerves disappears[2] and there is a 50 per cent decrease in pineal serotonin[2,3].

When rats are placed in constant light, there is a considerable reduction in both pineal weight[6] and in the activity of the enzyme in the pineal gland which makes melatonin[7]. These effects of constant light can be eliminated by bilateral orbital enucleation or by bilateral superior cervical ganglionectomy[8]. It has been shown that the serotonin content of pineal glands of rats in constant light for six weeks is one-half that of rats under diurnal lighting conditions[1]. On the other hand, an increase in pineal serotonin content was observed during the light phase of diurnal illumination[9].

We have recently developed a sensitive assay for the estimation of 5-HTPD activity which enabled us to measure the 5-HTPD activity of individual rat pineal glands[4]. Investigations were carried out to determine if the serotonin-synthesizing capacity of the rat pineal gland, as measured by its 5-HTPD activity, was subject to photic regulation. Our experiments indicate that exposure of rats to constant light results in a marked increase in pineal 5-HTPD activity as compared to rats kept in constant darkness. In addition, the effects of constant light on 5-HTPD activity can be eliminated by blinding, by interrupting the sympathetic innervation of the pineal gland, or by treatment with bretylium, a drug which blocks sympathetic nerve impulses[10].

Groups of 10–20 Sprague–Dawley female rats weighing 160–180 g were placed in constant light or in constant darkness (except for a 25-W red light bulb which was used while the cages were cleaned) in rooms equipped with double door light baffles and air conditioning. Animals were killed while still in the light or dark rooms. Control animals in constant light or darkness were killed at the same time as drug treated or operated animals, between 9 and 10 a.m. Pineal glands were quickly removed, weighed, and homogenized in 1 ml. of 0·05 M phosphate buffer, *p*H 7·4. 5-HTPD (ref. 4) and monoamine oxidase[11] activities were assayed by procedures previously described. None of the experimental treatments altered body-weight.

Rats kept in constant light for six days had almost twice as much pineal 5-HTPD activity as litter-mates placed in constant darkness for the same time-period (Table 1). The difference in enzyme activity was significant whether computed per pineal or per unit weight. Pineals of rats in constant light weighed less than those of animals in constant darkness, but the difference was not statistically significant. Since, in other experiments from this laboratory using large numbers of rats[7], light exposure for 6 days produced statistically significant decreases in pineal weight, the failure to obtain significant alterations in pineal weight in this experiment may have been due to the small number of animals used.

It has been shown that the regulation by light of pineal melatonin-synthesizing capacity is mediated by the retina and the superior cervical ganglia[8]. In order to ascertain whether light influences pineal 5-HTPD via the same pathway, animals were subjected to bilateral orbital enucleation or bilateral superior cervical ganglionectomy. One day after the operations, the rats were placed in continuous light or darkness for 30 days along with control animals. The eyelids of the ganglionectomized rats were removed to correct for possible effects of ptosis.

Table 1. EFFECT OF CONSTANT LIGHT OR DARKNESS FOR SIX DAYS ON RAT PINEAL 5-HTPD

Group	No.	Pineal weight (mg)	mμM ^{14}C-serotonin formed/h/pineal	mμM ^{14}C-serotonin formed/h/mg
Dark	7	1·14 ± 0·31	12·5 ± 0·9	11·7 ± 1·5
Light	6	0·99 ± 0·10	17·7 ± 2·1*	18·1 ± 2·3*

* Differs from dark value $P < 0·05$.

5-HTPD activity was determined as described (ref. 4) by incubation of pineal tissue with 5-hydroxytryptophan-^{14}C and measuring the ^{14}C-serotonin formed.

Control animals placed in constant light for 30 days had significantly smaller pineal glands ($P < 0·01$) with almost twice the 5-HTPD activity of their litter-mates in constant darkness (Fig. 1). The increase in 5-HTPD was statistically significant whether expressed per unit weight ($P < 0·001$) or per whole pineal gland ($P < 0·01$). In confirmation of previous findings[7], light produced no difference in the activity of monoamine oxidase, an enzyme presumably involved in the metabolism of serotonin. Blinding or removal of the superior cervical ganglia eliminated the difference in pineal weight between light and dark exposed groups, confirming earlier findings[8], and blocked the enhancement by light of pineal 5-HTPD activity. These observations indicate that the path whereby light affects pineal 5-HTPD activity involves the retina and the sympathetic nervous system.

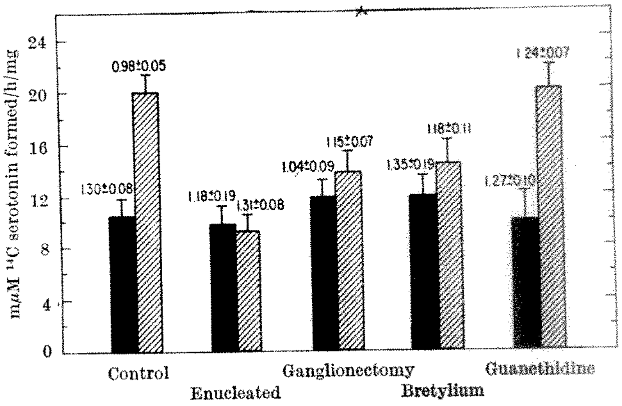

Fig. 1. The effect of bilateral orbital enucleation, superior cervical ganglionectomy, or treatment with bretylium or guanethidine on the response of rat pineal weight and 5-HTPD activity to constant light or darkness. The vertical bars represent the standard error of the mean (*S.E.M.*). The numbers over the bars show pineal weight (mg ± *S.E.M.*). Black, constant dark; hatched, constant light

Other groups of rats were placed in continuous light or dark environments two days after beginning the daily intraperitoneal injections of bretylium (20 mg/kg) or guanethidine (20 mg/kg). Daily drug treatment was continued for 30 days in the light and darkness. Bretylium and guanethidine are known to block sympathetic nerve impulses[10,12]. Guanethidine, in addition, depletes a number of tissues of norepinephrine[13,14] and serotonin[15,16]. If the effect of light on pineal 5-HTPD activity were mediated by sympathetic nerve impulses, these drugs might be expected to counteract them. Both bretylium and guanethidine abolished the effect of light on pineal weight (Fig. 1). Bretylium also eliminated the light-induced increase of pineal 5-HTPD activity, while guanethidine was without effect. It would appear from these results that these drugs, which interfere with nerve impulses, also interfere with some of the effects of light on the pineal gland, for example, weight. However, bretylium, but not guanethidine, abolished the effects of light on pineal 5-HTPD activity. Whether the failure of guanethidine to block the effects of light on pineal 5-HTPD activity is due to accessory effects of this drug, for example, amine depletion, cannot be ascertained from this work.

Light has now been shown to have a marked effect on two enzymatic processes in the rat pineal gland. It stimulates the activity of 5-HTPD, an enzyme which makes serotonin, and inhibits the activity of hydroxyindole-*O*-methyl transferase, an enzyme localized only in the pineal gland[17] which makes melatonin. It is interesting that the enzymatic synthesis of serotonin, a precursor of melatonin, is out of photic phase with the enzymatic formation of melatonin. Whether the effects of light on these two enzymatic processes are directly related remains to be established. The experiments described here and elsewhere[7,8] demonstrate that light information reaches the pineal gland and affects both enzymatic activities by the same anatomic pathway: the sympathetic nervous system. Since light also affects pineal weight via the sympathetic nervous system, it is possible that many other biochemical events in the pineal are regulated by light through this anatomic route.

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Mechanism of the Antihypertensive Effect of α-Methyldopa

It has recently been reported that a decarboxylase inhibitor, α-methyl-3,4-dihydroxyphenylalanine (α-methyldopa), lowers the tissue norepinephrine level of various species of animals[1,2]. Moreover, this decarboxylase inhibitor has been demonstrated to be antihypertensive in man[3].

It seemed that these actions of α-methyldopa could be explained by the inhibition of decarboxylation of 3,4-dihydroxyphenylalanine (dopa). However, norepinephrine depletion produced by α-methyldopa continued long after the activity of the decarboxylase had been restored[1,2]. Thus the persistent effect on tissue norepinephrine appears to be due to another mechanism. Furthermore, as Clark stated previously in his review[4], substances which strongly inhibit the enzymatic decarboxylation of dopa *in vitro* do not always play parts as blood-pressure reducing agents *in vivo*. Therefore, not only the inhibitory effect of α-methyldopa on dopa decarboxylase as a direct result of tissue norepinephrine depletion should be considered.

Recently, we have become interested in the uptake and storage of catecholamines in various tissues. In the investigation recorded here, we have carried out model experiments on the uptake of norepinephrine by rabbit blood platelets and examined the effects of α-methyldopa and its decarboxylated metabolite, α-methyldopamine, on these processes. It was demonstrated that α-methyldopa was itself decarboxylated *in vivo*, in animals and in man[5,6].

As shown in Table 1, radioactive norepinephrine is taken up and accumulated by the blood platelets by an active transport mechanism. This uptake of norepinephrine was slightly inhibited by α-methyldopa and strongly inhibited by α-methyldopamine. It is of interest that we found α-methyldopamine to be much more potent than its corresponding amino-acid, α-methyldopa, in the inhibition of uptake of norepinephrine by blood platelets. Thus, it appears that the action of α-methyldopa is in large part, if not entirely, due to the amine derived from it metabolically. Reserpine, known as a catecholamine releaser, also strongly inhibited the uptake of norepinephrine by blood platelets, even at as low a concentration as 10^{-7}–10^{-9} M. It may be suggested that reserpine lowers the content of catecholamine in tissues primarily by blocking the uptake of amines into storage site. Further investigations have shown that the inhibitory effect of reserpine on the norepinephrine uptake has still remained even after reserpine had been removed from the medium. On the contrary, α-methyldopamine has an inhibitory effect when present in sufficient concentration in the medium. It seems that the action of α-methyldopamine might be of a 'reversible' type, rather than 'irreversible' as exemplified by reserpine. However, it is not yet known whether α-methyldopamine inhibits the uptake of norepinephrine into blood platelets by the same mechanism as reserpine does.

Table 1. Effect of α-Methyldopa, α-Methyldopamine and Reserpine on ^{14}C-Norepinephrine Uptake by Blood Platelets

	Norepinephrine uptake (c.p.m./mg of dry weight)	Inhibition %
Control (°C)	80	—
Control (37° C)	3,160	—
α-Methyldopa (10^{-3} M)	2,820	10
,, (10^{-4} M)	3,050	3
α-Methyldopamine (10^{-3} M)	630	80
,, (10^{-4} M)	1,170	63
,, (10^{-5} M)	2,040	36
,, (10^{-6} M)	2,750	13
Reserpine (10^{-6} M)	790	75
,, (10^{-7} M)	1,170	63
,, (10^{-8} M)	1,640	48
,, (10^{-9} M)	2,340	26

Rabbit blood platelets were suspended in 3 ml. of phosphate saline containing 12 μg of ^{14}C-norepinephrine (0·2 μc. of radioactivity) and different concentrations of each substance. The mixture was incubated at 37° C for 60 min. Values given represent the mean of six experiments.

Our results suggest that the depletion of tissue catecholamines produced by α-methyldopa is attributable to two factors. First, to the inhibition of an intermediate step in the biosynthesis of catecholamines. Secondly, catecholamines, synthesized from dopa, are prevented from being taken up by its storage sites by α-methyldopamine which is the decarboxylated metabolite of α-methyldopa. Thus there is an increase in the destruction of unbound catecholamines.

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RADIOBIOLOGY

Variations in Radiosensitivity of a Mammalian Cell Line with Phase of the Growth Cycle

In recent years dose-survival curves have been published for a number of mammalian cell lines in culture following X-irradiation, and it is often assumed that the extrapolation number (n) and the 37 per cent survival dose (D_0) that characterize these curves are constants for a particular cell line. However, Lockart *et al.*[1] noted small differences in n and D_0 for different samples of HeLa *S*3 cells, and Bases[2] found that of two strains of HeLa cells only one gave consistent values of n and D_0. This communication describes variations in radiosensitivity with the phase of the growth cycle in rat sarcoma cells.

This cell strain was originally established in culture by Alexander and Mikulska from a benzpyrene-induced sarcoma in a *CBRI* rat; six months ago it was cloned in this laboratory. One of the clones (*S*6/1) was used for this work. The culture medium consists of calf serum (15 per cent) plus a 1 : 1 mixture of Fischer[3] and 199 media. Cultures were incubated in a water-saturated atmosphere of 95 per cent air and 5 per cent carbon dioxide at 37° C.

To prepare a series of bottles with the same initial inoculum of cells, approximately 5×10^5 cells in 100 ml. culture medium were divided equally between twelve bottles and left overnight. The cells were removed from one bottle with trypsin, counted in a hæmocytometer, re-suspended in culture medium and diluted to give concentrations of five and one thousand cells per ml. 1 ml. aliquots of the cell suspension were X-irradiated (140 kV, 5 m.amp, no extra filtration, 280 r./min) to total doses of 0, 300, 400, 500 r. (1,000 cells per ml.), 600, 700 and 800 r. (5,000 cells per ml.). The irradiated cells were plated into 35-mm plastic Petri dishes (Messrs. Falcon Plastics, Ltd.); 5 dishes per dose point, 10 dishes for controls. This procedure was repeated over the next 7 days at the intervals shown in Fig. 2. The Petri dishes were incubated for 7 days, fixed, stained and colonies counted with the aid of a low-power microscope.

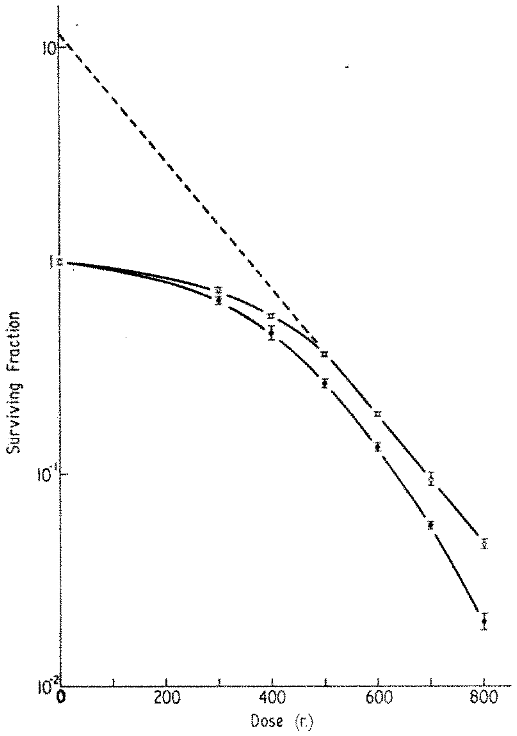

Fig. 1. Dose-survival curves for the *S*6/1 cells after X-irradiation. Upper curve (open circles) is for cells irradiated on the morning of the fourth day (lag phase): $D_0 = 144 \pm 3{\cdot}6$ r.; $n = 12 \pm 1{\cdot}3$; $7{\cdot}5 \times 10^5$ cells in the bottle. Lower curve (closed circles) for cells irradiated on the morning of the seventh day (stationary phase): D_0 estimated from the 700 and 800 r. points as 95 r. and n as of the order of 100; $2{\cdot}6 \times 10^6$ cells in the bottle. Vertical bars indicate *S.E.M.*

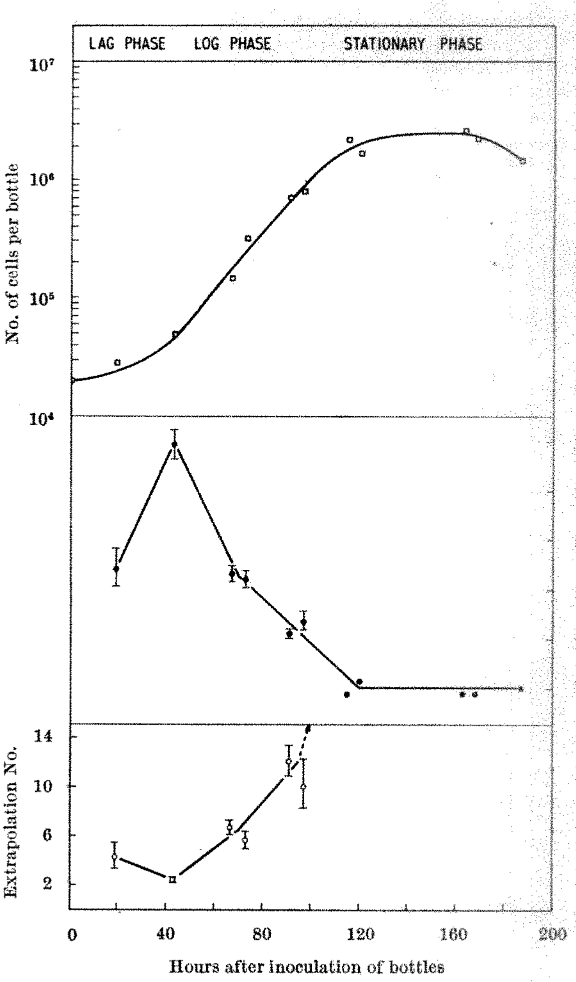

Fig. 2. Variations of D_0 and extrapolation number (n) of cells taken from different phases of the growth cycle. Abscissa, common to all three curves, is the time of trypsinization expressed as hours after inoculation of the bottles. The upper curve (open squares) is the growth curve—number of cells per bottle against time. The centre curve (closed circles) is a plot of D_0 against time. Vertical bars denote the standard errors; no errors are shown for values of D_0 corresponding to cells in the stationary phase, as these values were estimated from only two points (see text). Lower curve (open circles) is a plot of n against time; for cells in the stationary phase n can only be estimated but is of the order of 100. This steep rise in n is denoted by the dotted line and arrow

The dose-survival curves for cells irradiated on the first 4 days were of the typical form, that is, an initial shoulder followed by an exponential decrease in colony number with dose, whereas the curves for cells irradiated on the last three days showed a continuous curvature. Examples of these two types are shown in Fig. 1. Fig. 2 shows the values of n and D_0 plotted against the time of trypsinization (hours after inoculation of bottles). For comparative purposes the values of 'n' and 'D_0' for the last 3 days were estimated from the survival after 700 and 800 r. Fig. 2 includes a growth curve for these cells, that is, a plot of the number of cells in the bottle at the time of trypsinization against time; there was some loss of cells on the last day when they became overgrown and some detached from the glass.

It can be seen that the radiosensitivity fluctuates through the phases of the growth cycle. The value of D_0 is similar for cells in the middle of the lag and log phases. There is a pronounced maximum in D_0 as the cells pass through the lag to the log phase. A similar pattern was observed in a preliminary experiment. Cells in the stationary phase are the most radiosensitive and have the different type of survival curve described here. The extrapolation number follows a course that is the inverse of that of D_0.

Ludovici *et al.*[4] reported a difference in the radiosensitivity for HeLa cells irradiated in the lag and log phase of growth. Their criterion of radiosensitivity—change in cell count following irradiation—was shown by Glinos and North[5] to be unsatisfactory unless the growth of the cells after irradiation is followed for a time sufficient to eliminate the influence of 'doomed' cells. When this was done, these latter authors found no difference between this criterion and that of colony formation for mouse L cells. They also compared the radiosensitivities of these cells taken from the log and stationary phases and found no difference. However, the cells were irradiated some time after they had been plated when in both cases the cells may have been in the lag phase. Elkind and Sutton[6] reported a small decrease in D_0 for hamster cells in the log phase compared with cells in the lag phase which is compatible with the results shown in Fig. 2.

The fluctuation in radiosensitivity through the phase of the growth cycle is presumably related to changes in the metabolic state of the cells. The maximum in D_0 as cells pass from the lag to the log phase may be the result of an increased potential for repair. Increased rates of DNA, RNA and protein synthesis were found by Salzman[7] for HeLa cells at this point in the growth cycle.

If other cell lines show similar variations in radiosensitivity, care must be taken when comparing the radiosensitivities of different cell lines or of one cell line irradiated on separate occasions.

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Quantitative Relations between Doses of Chemical Protective Agents and Doses of X-irradiation

IN a previous paper[1] we quoted a number of results appearing in the literature which demonstrate quantitative relationships between the dose of protective agent and radiation dose. This applies especially to sulphydryl substances. In assuming that the sulphydryl protective agents act as radical scavengers this can be easily understood. Higher radiation doses may produce higher concentrations of radicals in the tissue and for this reason the concentration of radical scavengers must also be higher. Contrary to this, high radical scavenger doses may have relatively less protective effect in lower radiation doses. The increase rate of the protective effect of such agents is larger for higher radiation doses than for lower ones.

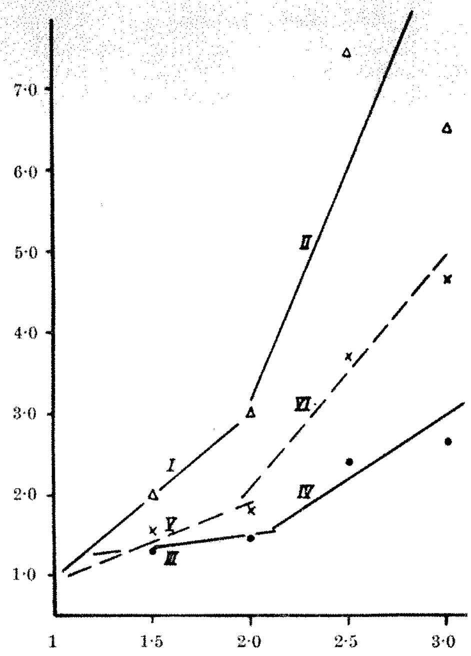

Fig. 1. Curves of the increase rates after whole-body X-irra 810, 750 and 690 r. Abscissa: doses of the intraperitone cysteamine in mg/mouse. Ordinate: Dx/D_1 = percenta animals at the cysteamine dose x divided by the percent animals after application of the lowest cysteamine dose, mouse. The regression lines were computed by the formul $y - y_1 = \frac{y_2 - y_1}{x_2 - x_1}(x - x_1)$ and the mean lines drawn

△, 810 r.; ×, 750 r.; ●, 690 r.

In contrast to these ideas the possible behaviour of pharmacodynamically acting protective substances is to be discussed. Here the radical-quenching effect mechanism is not present, but, for example, changes in the blood circulation in radiosensitive organs are observed[3]. It is assumed that in the usual conditions there are no dose relationships between the protective agent and the radiation dose. If our assumption were correct, considerable differences would exist in the increase rate tective effect of radical-quenching substance which act on the basis of their pharmacodynan

As typical substances for both possibiliti chosen cysteamine as a radical scavenger[2] an as a pharmacodynamically active substance[3]. ments were carried out with male mice (19–21 g inbred strain. In the experiments with cyst mice received 690, 750 and 810 r., respectiv body irradiation. The protective effects of th doses of cysteamine were tested for each radi 1·0, 1·5, 2·0, 2·5 and 3·0 mg/mouse 5 min bef tion intraperitoneally. Each group of these and those with histamine was carried out with of 50 and a maximum of 200 animals. In th investigations the radiation doses applied 950 and 1,000 r. respectively. The correspond histamine were: 0·5, 1·0, 2·0 and 5·0 mg hista 5 min before irradiation intraperitoneally.

In order to find the increase rate of the tw agents the percentage of living animals after each radiation- and agent-dose was divided b sponding lowest dose of the protective agent. the relation D_x/D_1 was found; D_x stands for higher doses, and D_1 for the lowest one, of the

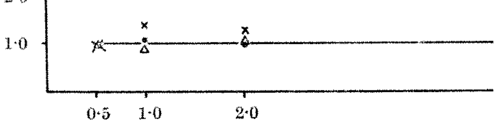

Fig. 2. Curve of the increase rate after whole-body irra 1,000, 950 and 810 r. Abscissa: doses of the intraperiton histamine in mg/mouse. Ordinate: Dx/D_1 corresponding lowest dose of histamine (D_1) is 0·5 mg/mouse. The re has been computed and drawn out corresponding t

●, 810 r.; ×, 950 r.; △, 1,000 r.

tective agent. The relationship between D_x/D_1 (ordinate) and the different doses of the protective agents (abscissa) is shown in Figs. 1 and 2.

Fig. 1 shows the position after application of cysteamine. Between the doses of 1 and 2 mg the increase rate grows only a little in the three cases of 810, 750 and 690 r. The turning point is found at 2 mg. From here it is clearly demonstrated that the increase rate becomes much bigger in the case of 810 r. than in that of 690 r. The differences are statistically significant. For this reason, an increase of protective agent produces an increase in the protective effect in the case of cysteamine. In the lower dose range this effect is only very small; the increase rate directly depends on the radical concentration in the tissue. In other words, the dose of the protective agent and the radiation dose are closely related, thus confirming our assumption.

In Fig. 2 the findings of the histamine experiments are illustrated in the same manner. It is possible to demonstrate for all radiation doses and for all doses of histamine that there is no growing increase rate. The increase rate (D_x/D_1) is one or nearly one in all cases. The explanation for this is that the pharmacodynamic action of 0·5–5·0 mg histamine is sufficient to produce the optimal protective action for all radiation doses.

The experiments demonstrate the differences in the mode of action of various chemical protective agents, and would seem to make clear the mode of action of chemical protective agents, at least certain ones.

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Efficient Labelling of Tissue-soluble Proteins with Iodine-131 using Chloramine T

Following the reports of Hunter and Greenwood[1] and Reith and Tampion[2], the chloramine *T* radioiodination procedure has been applied[3,4] for labelling, with iodine-131, serum proteins from several animal species. High labelling efficiency (about 65 per cent) could be obtained provided oxidation was allowed to proceed for at least 30 min. The advantage of using a protein: chloramine *T* ratio of about 250 : 1 has been stressed[3,4] in order to avoid even small damage to the proteins, undetectable by electrophoretic and immunological analyses but evident from metabolic behaviour *in vivo*.

Because of its utility in disposing of tissue-soluble ^{131}I-proteins an attempt to adapt the procedure for labelling with radioiodine tissue-soluble proteins has been made and results are reported here.

Selected organs were removed from saline-perfused animals and homogenized with an equal volume of 0·15 M phosphate buffer, *p*H 7·6. The homogenates, after having been frozen at −30° C and thawed six times, were centrifuged at 34,000*g* for 60 min, and the supernatants were filtered through paper. Half of each tissue-soluble protein solution was dialysed in 'Visking' tubing 8/32 in. for 2–3 days. The whole procedure was carried out at +2°. After dialysis both protein solutions were again centrifuged (3,000*g*, 90 min) and adjusted with phosphate buffer at the same protein concentration. Proteins were estimated either by the method of Gornall *et al.*[5], or by micro-Kjeldahl procedure.

The final procedure adopted was as follows: to a solution (1·0 ml.) of tissue-soluble proteins (13–31 mg) was added 0·15 M phosphate buffer (*p*H 7·6) containing the required amount (0·025–0·5 mc.) of carrier free iodine-131 (*IBS*. 3, the Radiochemical Centre, Amersham) and then chloramine *T* solution (B.D.H.; 10·0 mg/ml. in *p*H 7·6 phosphate buffer). The test tube was stoppered with 'Parafilm', gently shaken and, in order to increase labelling efficiency[3,4], oxidation was allowed to proceed for 60 min. At this stage the proteins were diluted 3·5 times. Afterwards sodium metabisulphite solution (6·25 mg/ml., w/v solution in *p*H 7·6 phosphate buffer) and carrier sodium iodide (0·2 ml. of a 10 per cent solution) were added separately with mixing. 0·50–0·62 mg of freshly prepared sodium metabisulphite was sufficient to prevent any iodination due to 1·0 mg of chloramine *T*. The reducing agent was in slight excess in order to avoid over-iodination on adding carrier iodide.

T.ble 1. Tissue Soluble and Sera ^{131}I-Protein Recoveries* using the Chloramine *T* Radioiodination Procedure

	Proteins (mg)	Chloramine *T* (mg)	Percentage ^{131}I-recovery	^{131}I-TPA soluble radioactivity before dialysis	after dialysis
Rabbit					
Sera	74·6	0·6	74·3	1·6	1·1
Sera	74·6	—	0·4	97·5	—
Brain	20·8	2·0	34·6	6·9	3·3
Lung	13·7	2·0	36·5	4·2	2·5
Heart	14·3	2·0	38·6	2·0	1·7
Spleen	13·2	2·0	30·7	5·1	2·8
Kidney	22·8	3·0	35·9	8·9	4·1
Liver	30·9	3·0	52·9	4·3	2·9
Rat					
Sera	80·6	0·6	71·3	2·2	1·2
Sera	80·6	—	0·2	94·3	—
Brain	21·1	2·0	40·0	7·1	3·6
Spleen	22·0	3·0	36·6	7·9	3·4
Kidney	20·1	3·0	22·9	18·7	9·8
Liver	29·4	3·0	51·3	6·6	3·2

The reaction mixture was made up with proteins in phosphate buffer containing 0·5 mc. iodine-131 per each sample. Percentages of ^{131}I-TPA soluble radioactivity were estimated on the eluates before and after dialysis.

* Each value, except when chloramine was not added, is an average of at least two determinations.

The reaction mixture, after having been counted to determine the total radioactivity, was passed through a

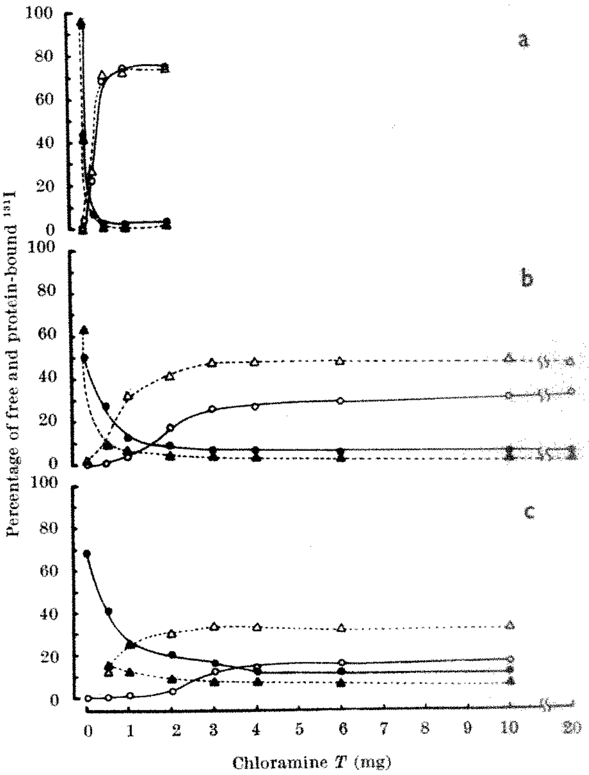

Fig. 1. Rabbit serum (*a*), liver (*b*) and kidney (*c*) ^{131}I-protein recoveries using the chloramine *T* procedure. Percentages of total iodine-131 recovery of undialysed (○) and dialysed (△) protein samples. Percentages of ^{131}I-TPA soluble radioactivity of undialysed (●) and dialysed (▲) samples. The reaction mixture was made up with proteins in phosphate buffer containing 25 μc. iodine-131 per each sample

column (8·0 × 1·0 cm) of the anion-exchange resin 'De Acidite FF' in the chloride form. The eluates, including two 0·15 M sodium chloride washings, were counted to ascertain the percentage recovery of radioactivity. To attain higher recoveries the exchange resin columns were presaturated with 5–10 mg of tissue soluble proteins. If necessary, protein solutions were concentrated by dialysis[6].

^{131}I-tungsto-phosphoric acid (TPA, final concentration: 1·7 per cent in 0·68 N hydrochloric acid) soluble radioactivity as percentage of the original radioactivity was measured in a well-type scintillation counter in the presence of carriers NaI and bovine serum proteins (35 mg).

Labelling efficiencies were lower for rabbit liver and kidney soluble proteins compared with those for serum proteins, particularly if protein solutions were not dialysed, due perhaps to presence of reducing agents (Fig. 1). However, dialysis of serum proteins was not beneficial. No binding of iodine-131 to proteins was observed if chloramine *T* was omitted. An inverse relationship is apparent between labelling efficiency and ^{131}I-TPA soluble radioactivity.

It may be observed that by increasing the amount of chloramine *T* (over 3–4 mg) the labelling efficiency is reduced. The increase in oxidizing agent determined, after addition of sodium metabisulphite, the appearance of an increasingly intense opalescence and of visible protein aggregates in the protein solution. The radioactive larger aggregates trapped by the exchange resin, or discarded after centrifugation, lowered the recovery. Either chloramine *T* or sodium metabisulphite or carrier iodide used singly were ineffective.

Labelling efficiency is diminished by using the same amount of oxidizing agent but higher protein concentration.

31–53 per cent of ^{131}I-protein-bound was obtained by labelling dialysed samples with 0·5 mc. of iodine-131. Only soluble proteins from rat kidney were scarcely labelled (Table 1). After removing most of the iodide-131 by ion-exchange resin, dialysis can further reduce the unbound radioactivity.

Rabbit and rat protein samples (of Table 1) were tested by starch-gel electrophoresis[7] and a contact autoradiogram of the bottom slice was taken with X-ray film ('Kodirex'). Radioactivity appeared bound to tissue proteins, prealbumin, albumin and globulin-like components. Immunological examination of rat and rabbit protein samples showed little or no contribution of serum proteins to tissue soluble proteins.

By using the chloramine *T* procedure the distribution and fate of soluble ^{131}I-proteins from rabbit and rat liver have been studied[8].

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BIOLOGY

Mating Preferences in Wild Birds

Hayman[1], describing mating preference in a merino ram, states that mating preference has long been postulated as a mechanism concerned in the development of new species, but that such preference does not appear to have been observed in wild-type populations.

Mating preference has, in fact, been demonstrated among at least three species of polymorphic birds. Populations of the snow goose, *Chen caerulescens*, are polymorphic for plumage colour: individuals may be blue or white. Strongly assortative mating (mating of like phenotypes) has been demonstrated in wild populations of this species[2]. The arctic skua, *Stercorarius parasiticus*, has a pale- and a dark-plumaged form, and assortative mating among these forms has been demonstrated in at least some wild populations[3]. In the white-throated sparrow, *Zonotrichia albicollis*, there are two colour forms, one with a brownish and the other with a white crown stripe. In this species, strongly disassortative mating has been demonstrated[3].

In general, assortative mating increases and disassortative mating decreases the genetic variance of a population. But whether mating preference is important in sympatric speciation remains to be shown. Theoretically, complete or nearly complete assortative mating could give rise to new species within a single population.

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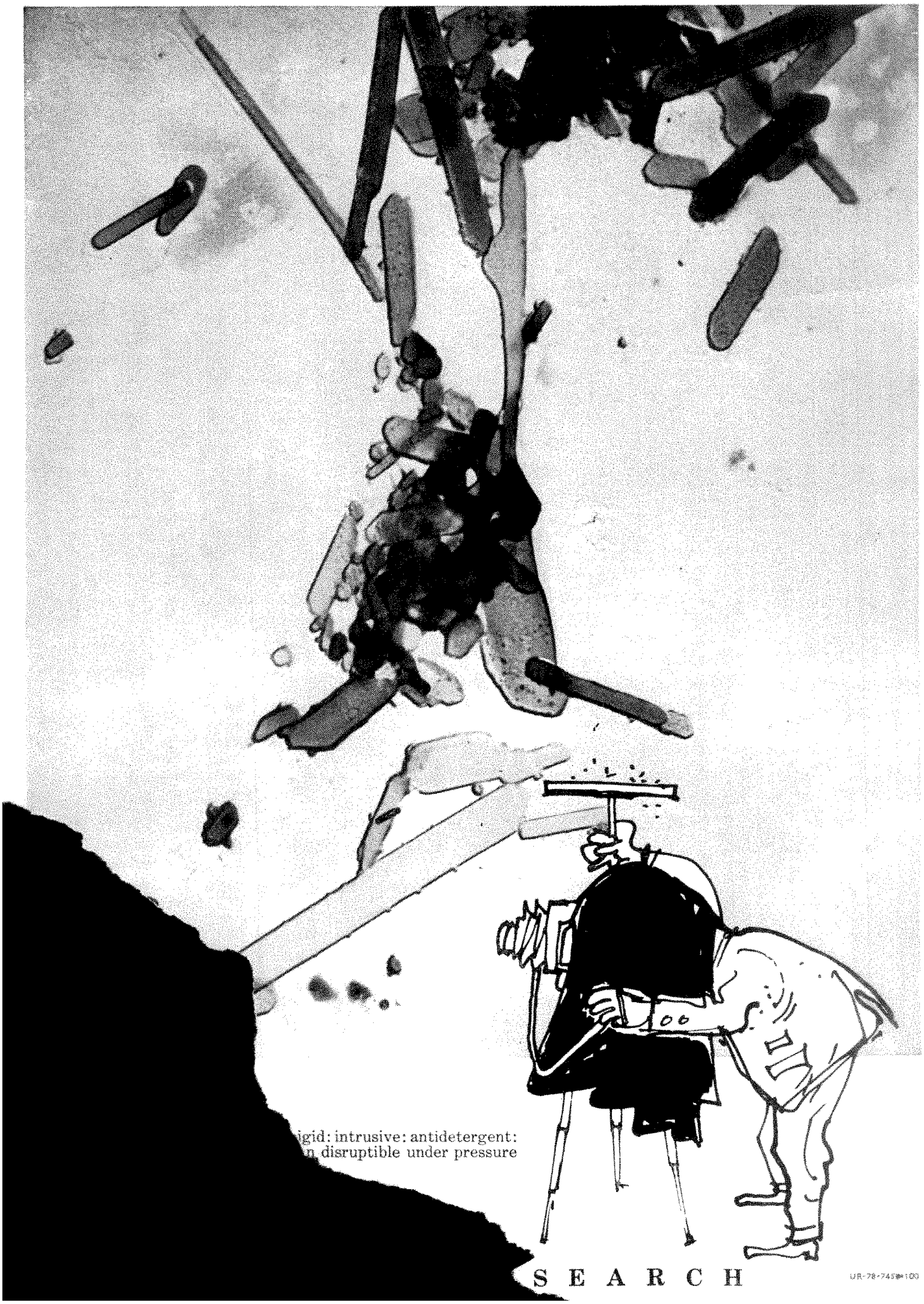
Evidence for a Dynamic Equilibrium in the Incidence of Cestoda and Acanthocephala in the Intestines of Freshwater Fish

As Hopkins observed[1], following an examination on the seasonal variations in the incidence and development of the cestode *Proteocephalus filicollis* (Rudolphi, 1810) in the stickleback *Gasterosteus aculeatus* L., the significant point in the host–parasite relationship, usually overlooked, is that the incidence of the parasite in the host throughout the year is a dynamic equilibrium. Hopkins clearly showed that less than 1 per cent of the worms which became established in the intestine of the stickleback survived to become gravid. Thus, from the evidence, Hopkins deduced that the parasite population in the intestine of the stickleback was in dynamic balance, that is, the resultant point of equilibrium was the product of gain and loss of worms from the host.

A similar dynamic situation was postulated by Chubb[2] for the life of the cestode *Triaenophorus nodulosus* (Pallas, 1781) in the intestine of the pike *Esox lucius* L. The results in this instance were complicated by difficulties in obtaining sufficient pike of each length group, and by changes of diet of the pike in relation to length. Nevertheless, it was clear that the parasite population was in dynamic balance. Data on the occurrence of the acanthocephalan *Echinorhynchus clavula* Dujardin, 1845, nec. Hamann, 1892, in the fishes of Llyn Tegid[3] also indicated the presence of a dynamic equilibrium between gain and loss of worms.

Recently, additional evidence for a dynamic equilibrium in the incidence of Acanthocephala in the fish host has become available.

During a study on the parasites of the brown trout *Salmo trutta* L. in the Afon Terrig, North Wales[4], particular reference was made to the biology of the acanthocephalan *Echinorhynchus truttae* (Schrank, 1788). Experimental infections of *E. truttae* were established in the brown trout and the rainbow trout *Salmo gairdneri* Richardson, and movements of the worms were found to occur in the intestines. It was found that the acanthocephalans were first attached in the pyloric region of the intestine, and occupied a more posterior region of the intestine as they matured; gravid females were found in the rectal region[4]. A similar observation has recently been recorded for *Acanthocephalus jacksoni* Bullock, 1961 (ref. 5). In addi-



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ion to a loss of spent males from the trout, female *E. ruttae* at all stages of maturation and immature males were passed out in the fæces at irregular intervals. In this connexion it was interesting to note that in trout autopsied immediately after killing with a blow on the head, a number of *E. truttae* of both sexes were found free in, or attached to, the contents of the intestinal lumen. It thus appears that movement of position of the acanthocephalans in the intestine of the host may be a regular phenomenon, and may be an important factor in the loss of worms from the host.

As worms were passed from fish experimentally infected and kept in an artificial environment, it might be thought that the conditions in which these fish were maintained were contributory to the loss of the worms. While this point cannot be ignored, it is probable it is not of major significance. In this connexion, it was of interest to note the finding of specimens of *Acanthocephalus lucii* (O. F. Müller, 1776) in a general limnological sample collected in the Grand Canal, County Dublin, Eire, on September 18, 1963. Two worms, a male and an ovarian ball stage female, were taken, which indicates a loss of Acanthocephala from their hosts even in natural conditions.

While the loss of gravid and spent worms is understandable, the factors evoking the passing out of immature forms, of both sexes, are not known. It is clear that the evidence indicates that the phenomenon of a dynamic equilibrium between gain of worms from the intermediate host, and loss of worms from the intestine of the definitive host, is found in some species of Acanthocephala and Cestoda of freshwater fishes. It seems probable this phenomenon is of wide occurrence, although it has rarely been commented on by previous workers.

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Transmission of Pig Lungworms (*Metastrongylus* sp.)

THE life-cycle of the pig lungworm (*Metastrongylus* sp.) has been known since Hobmaier and Hobmaier[1] incriminated earthworms as the obligatory intermediate host. Lungworm infestation in pigs is therefore likely to be a problem only where animals have access to pasture and hence earthworms.

A recent survey (1961–63) at a North Wales abattoir showed that the incidence of lungworms in pigs varies from 8 to 65 per cent of pigs examined, the figure depending on the season. The majority are *M. apri* (syn. *M. elongatus*), *M. pudendotectus* being very rare.

During an investigation of the effects of lungworms on pigs a litter of seven minimal disease Welsh pigs (obtained by hysterectomy) were penned in groups of three (Nos. 1, 2 and 3) and four (Nos. 4, 5, 6 and 7) in accommodation which excluded earthworms. Pigs 2, 3 and 4 were then separately isolated and fed an initial infective dose of 4,000 infective larvæ in a gelatine capsule. The larvæ had been removed from earthworms by dissection. After infection the pigs were given food and water in an attempt to ensure that the infective dose had been cleared from the mouth, then returned to their pens and the company of their litter-mates.

The three infected animals started to cough 10 days after the date of infection, and the first lungworm ova were detected in the fæces by the flotation technique of Dunn and White[2] 32 days after infection. Fæcal samples of uninfected animals were negative and the animals did not cough.

Clinical symptoms of coughing were more severe in the minimal disease pigs we have kept than in similarly housed non-minimal disease animals and a bout of coughing always preceded the effort to come to the trough at feeding time. Fifty days after the initial infection, besides retaining their cough, the infected animals developed the habit of vomiting shortly after they had ingested the first few mouthfuls of food at feeding times, the convulsions continuing until all the ingested material was regurgitated. The vomited material was always eagerly devoured by all other pigs in the pen. This has not been observed in other infected animals but the pigs described here retained this habit until slaughter.

Sixty-eight days after infection, pigs 2 and 3 were re-infected with 2,500 larvæ. Twenty-nine days after re-infection, pig 3 showed an increase in fæcal egg counts, in contrast to a previous experiment where much larger re-infection doses were used and very few lungworm eggs were recovered, presumably because the animals had become immune. Pig 2 showed no increase in fæcal egg counts although eosinophilia indicated that at least a few infective larvæ had invaded the host as a result of the re-infection. At this time, however, the supposedly uninfected animals yielded fæcal samples that were positive for lungworm ova (2–5 eggs/g fæces), these being passed until slaughter. These animals developed a slight cough and eosinophilia and skin sensitization tests were positive.

Pigs 2 and 3 were re-infected again with 2,500 larvæ 43 days after the first re-infection. These worms did not have time to mature and produce eggs, since the pigs were killed 2 weeks later, but a few did reach the lungs. Table 1 details the numbers of lungworms fed and those recovered from all animals at slaughter.

Table 1

	Pen 1			Pen 2			
Pig No.	1	2	3	4	5	6	7
No. larvæ dosed							
Initial dose	0	4,000	4,000	4,000	0	0	0
First reinfection	0	2,500	2,500	0	0	0	0
Second reinfection	0	2,500	2,500	0	0	0	0
No. of worms recovered from the lungs at slaughter	46	74	233	42	10	51	5

In previous experiments where pigs were individually penned the control animals did not become infected, but Table 1 shows that lungworms may be passed from one pig to another when pigs are housed together. Just how this comes about is a matter of speculation at the moment but three possibilities may exist: (*a*) that infective larvæ are retained in the stomach of the experimentally infected animals for some time after their introduction through the mouth and later regurgitated and made available to other animals; (*b*) that infective lungworm larvæ are passed through the gut of the infected pigs and become available to other animals from the floors of the pens or in contaminated food and water; (*c*) that infective larvæ having migrated to the lung of the host may then be passed to a second pig in saliva or sputum.

The habit of regurgitation developed in infected animals meant that when animals 2 and 3 were re-infected the chances of viable infective larvæ being vomited were high and possibly this accounts for the infection in pig 1. This could not account for the infection of three extra animals in the second pen from pig 4 because this was not re-infected, and indeed the size of the lungworms recovered at post-mortem from these control pigs and pig 1 suggests that infection had occurred much earlier than the date of re-infection.

One cannot discount the second possibility, for the ultimate burden of worms is very much lower than the initial dose and might suggest losses with the fæces. This phenomenon has been frequently observed by other workers in the past in that the number of worms successfully invading and completing development in a host compared with the dose fed is often very low.

The frequency of this type of transmission of lungworm from one animal to another is a matter of some conjecture, but it may explain how the disease is transmitted to animals which have not had access to the earthworm intermediate host.

It is conceivable that the foregoing methods of transmission may apply to other parasites and hosts, and this is not difficult to visualize among ruminant animals when they lick each other mouth to mouth. Indeed, this is the known method of transmission of rumen protozoa from adult animals to young stock.

One of us (S. E. J.) acknowledges the financial support of the Pig Industry Development Authority while engaged in this work. Any views expressed here are not necessarily those of the Authority.

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Isolation and Bioassay of a Fungal Morphogen

THE staling of fungal cultures is a topic which has not received much attention recently. Pratt[1] described two types of staling agent, organic acids and potassium bicarbonate. She suggested that staling due to the formation of bicarbonate by the carbon dioxide of respiration is of primary importance and occurs whenever the medium is such that a basic radical is set free. She determined that the organic acid radicals were not effective as staling agents in the alkaline-staled medium. Pratt investigated the staling of a species of *Fusarium* in Richards's solution and her criterion of staleness was based on the average germ-tube length of conidia of *Botrytis cinerea* sown in drops of the filtered medium in which the *Fusarium* had grown. More recently Park[2] has investigated staling in *F. oxysporum* using agar cultures of *Aspergillus niger* as the test organism. Drops of culture medium were pipetted on to the margin of colonies of *A. niger* and the criterion of staleness was the cessation of extension-growth of the marginal hyphæ with accompanying vacuolation. Vacuolation has since been adopted as the best indication of ageing, since, whereas the stale culture fluid would always induce vacuolation, extension-growth was not permanently inhibited under all conditions of test. It is also important to note that a wide range of substances will cause hyphæ to branch or to cease extension-growth, but no substance among several tried has yet been found to reproduce the vacuolation effect caused by the stale medium from which the bulk of the mycelium has been removed by filtration through nylon gauze. It is of additional interest that both extracts of soil and of pond-water can also induce vacuolation of our test organism. The substance or substances responsible may therefore be associated with microbial habitats of different sorts.

Vacuolation is normally considered an indication of senescence in fungal hyphæ and so the factor causing vacuolation may be of importance in the consideration of morphogenesis, since a few minutes after its application it induces this symptom of ageing and other morphological effects of maturation in the juvenile hyphæ of the test organism. The present report gives a brief description of the method of assay used for the detection of the factor and methods so far utilized for its purification.

The standard producer fungus for the vacuolation factor in these investigations has been *F. oxysporum*, but under the same conditions of culture the effect has been found to be given by isolates of *F. moniliforme*, *F. culmorum* and *F. solani*, and also by *Trichoderma viride* and *Penicillium expansum*. The test organism used is *A. niger* grown for 3–4 days at 25° C on the agar medium described by Park[2]. Drops of the solution to be tested are pipetted on to the colony margin so as to wet the hyphal tips which are then examined under the microscope for cessation of extension-growth and for the appearance of vacuolation beginning near the tips and extending back.

The time to appearance of vacuolation after application of the culture filtrate gives some measure of the activity of the factor. The temperature of the test culture affects this time. The time to vacuolation is at a minimum at 25°–30° C and increases with lower temperature. The times recorded from one such experiment were from 3·5 min at 25° to 14 min at 4° C. Dilution of the culture filtrate increases the time to vacuolation. A solution giving vacuolation in 2·5 min gave vacuolation in 16 min when diluted to 1/24. Further dilution allowed extension-growth to continue and there was no vacuolation. The time to appearance of vacuoles in treated hyphal tips is influenced also by other materials present in solution. Glucose, for example, at a concentration of 0·001 per cent (w/v) gives a smaller time to vacuolation than that in the absence of glucose. Increasing the glucose concentration over the range 0·001–1·0 per cent gives a progressive decrease in the time to vacuolation, that is, increases the rate of action of the factor.

The *p*H of the culture filtrate has a marked effect on the response of the treated hyphæ. Normally the *p*H of the culture filtrate is 3·2, and at this *p*H cessation of extension-growth occurs within about 1 min. The first stages of vacuolation together with the cessation of particulate movement in the cytoplasm are observed at 2–3 min. The extent of vacuolation increases for a further 3 min and the condition then remains static for some time. Further growth arises by the origin of lateral branches behind the affected portion. The extent of vacuolation is smaller at a higher *p*H and the lateral branches arise sooner and closer to the tip. At *p*H 8 no vacuolation occurs, but there is cessation of extension-growth at the original growing point and a slight swelling of the tip. Usually two, but sometimes three, small protuberances become visible after about 10 min just behind the original growing point. These rapidly grow out as new sub-apical branches giving a sympodially branched structure.

An effect of the factor on spore germination has been demonstrated. Uninoculated culture solution is inappropriate as a control for these experiments because its nutritional level is higher than that of a culture filtrate. A suitable control solution is prepared by adsorbing the vacuolation factor from the culture filtrate with activated charcoal and then filtering through paper. Culture filtrate treated in this way should not be nutritionally more favourable for germination than a similar solution not treated. Germination of spores incubated in the active culture filtrate is not totally inhibited, but a lower germination rate than that in the control has been demonstrated in sporangiospores of *Absidia glauca*, *Mucor hiemalis*, *M. plumbeus*, *Rhizopus stolonifer* and *Zygorhynchus vuilleminii*, in conidia of *Alternaria tenuis*, *F. caeruleum*, *F. solani* and *Stemphylium lanuginosum*, and in icospores of *Chaetomium globosum*. Sporangiospores of *unninghamella elegans*, and conidia of *Botrytis cinerea*, *. culmorum* and *T. viride*, showed no detectable reduction germination in active culture filtrate. Sporangiospores *R. stolonifer* were germinated in a control solution for h to 4 per cent and for 5 h to 8 per cent germination-vels, and then transferred to active culture filtrate. ermination-levels of the transferred spores remained onstant and there was no increase in germ-tube length ver a period of 22 h. Thus, whereas spores incubated hroughout in active culture filtrate were able to germin-te, although more slowly than in control solution, artially germinated spores were completely inhibited fter transference to active culture filtrate.

In order to produce large quantities of the vacuolation actor it is convenient to culture *F. oxysporum* in 20–40 l. atches in the light at 20°–22° C with constant aeration. 'he medium used has the following composition; glucose, ·0 g; $MgSO_4.7H_2O$, 0·5 g; KH_2PO_4, 0·2 g; NH_4NO_3, ·1 g, distilled water to 1 l. Under these conditions the acuolation factor can be demonstrated after five days of ulture. It is convenient to filter the culture medium hrough a fine nylon gauze to remove most of the mycel-um. The filtered medium can be concentrated to 1/20th ts original volume by vacuum distillation, and the *p*H fter concentration is 2·8. The *p*H of the medium is djusted to 10·0 with 0·1 N sodium hydroxide solution nd the medium extracted with diethyl ether. The *p*H s then adjusted to 3·0 with 0·1 N hydrochloric acid and he medium again extracted with ether. Bioassay of hese fractions indicates that the active factor has acidic roperties since no activity is detected in the basic- and ieutral-ether fraction, but the amount of activity present n the acidic-ether fraction is equivalent to that in the riginal culture filtrate.

It was important to know whether vacuolation was lue to one or more closely related substances or several nrelated substances. It was considered that counter-urrent fractionation would give an indication as to whether more than one substance was involved. An cidic-ether extract from 2 l. of filtered medium was listributed between diethyl ether and 0·02 M phosphate ouffer (*p*H 6·1) for 50 transfers in a counter-current pparatus. Both the aqueous and ethereal phases from each tube were bioassayed for vacuolation activity. Marked activity was present in both the aqueous and ethereal phases of tubes 7–11 (inclusive) with peak activity in tube 9. Very minor activity occurred in the aqueous phase of tube 1 and in the ethereal phases of tubes 44 and 45. The main vacuolation effect can thus be ascribed to one or more related substances which are acidic in nature.

Perhaps the main difference between staling under our conditions and those of Pratt is that, whereas the culture filtrate she used was fairly alkaline, that used at Belfast is acidic. Consequently, bicarbonates are certainly not a contributing factor to cultural staling in *F. oxysporum* under our conditions. It seems likely that our factor is composed of one or more acidic substances. Moreover, the factor has been shown to be less effective at alkaline *p*H in our assay. It is significant that the factor described in this communication is produced by several unrelated fungi and also exists in some natural microbial habitats. A number of fungi also respond to the factor in a similar way to *A. niger*, and the factor isolated from the culture filtrate of *F. oxysporum* will cause vacuolation and cessation of extension-growth when applied to young cultures of *F. oxysporum*.

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Formation and Metabolism of Lactic Acid during Germination of Pea Seedlings

EARLIER work[1-4] has indicated that the production and metabolism of ethanol during the germination of pea seeds are accompanied by changes in the organic acid contents of the cotyledons. Paper chromatography of the organic acid fraction[3] showed that, in addition to large changes in the malate content, the amounts of lactic acid fluctuated as ethanol was formed and metabolized.

In the work recorded here, changes in the lactic acid content of germinating peas have been examined in more detail. In addition, slices of pea cotyledons have been supplied with micromolar amounts of lactate-1-^{14}C to determine whether the changes observed in lactate content during germination were due to the metabolism of this compound. Such experiments have indicated that temporary increases in the lactate content occur during the earliest stages of germination. With emergence of the radicles, the amounts of lactic acid are decreased and the cotyledons are able to metabolize supplied lactate-1-^{14}C. After short periods of incubation with lactate-1-^{14}C, the cotyledons incorporated the carbon-14 into carbon dioxide, amino-acids and organic acids. Extracts of the cotyledons suggest that lactate metabolism is due to activity of a lactic dehydrogenase system located in the mitochondria.

Pea seeds, variety 'Homesteader', were germinated in vermiculite[4]. Samples of the seeds were removed daily over a five-day period and killed in ice-cold 10 per cent trichloroacetic acid. After adjustment to *p*H 8, the lactic acid contents were determined by the colorimetric procedure of Barker and Summerson[5]. Following the initial 18-h soaking period, the seeds contained 105 μmoles of lactate/100 g fresh wt. This increased to a maximum of 120 μmoles/100 g fresh wt. after 10 h germination and fell abruptly to 40 μmoles/100 g fresh wt. when the radicles had emerged.

In the experiment shown in Table 1, samples of peas, in which the lactate content was decreasing, were supplied with lactate-1-^{14}C solutions. The cotyledons were sliced[3] and placed in buffered lactate-1-^{14}C solutions as indicated. At the end of the experimental period, the tissues were killed in boiling 80 per cent ethanol and, following homogenization, were extracted using dilute ethanol and water. The soluble material was then separated into four fractions using ion exchange columns[4]. The organic acids were further separated by the method of Palmer[6]. The radioactivity of the fractions separated was assayed using a 'Mylar' window continuous gas flow Geiger–Müller tube.

It is evident from Table 1 that the small amounts of supplied lactate-1-^{14}C were rapidly metabolized. After 5 min of lactate-1-^{14}C metabolism, glutamic and aspartic acids contained five times as much radioactivity as the carbon dioxide released. Furthermore, small amounts of carbon-14 were detected in the acids of the tricarboxylic acid cycle, especially malate. These results might indicate a rapid fixation of carbon dioxide derived from the 1 position of the lactate-1-^{14}C supplied. Similar small amounts of $^{14}CO_2$ have been observed when ethanol-1-^{14}C

Table 1. METABOLISM OF LACTATE-1-^{14}C BY PEA COTYLEDON SLICES*

Fraction	Incubation time (min) 5 ^{14}C (c.p.m.)	10 ^{14}C (c.p.m.)	20 ^{14}C (c.p.m.)	30 ^{14}C (c.p.m.)
Organic acids				
Glycollate fraction	225	230	260	270
Succinate	590	860	1,100	1,200
Malate	990	1,050	1,100	1,150
Pyruvate	1,600	3,200	5,400	7,800
Sugars	Not active	2,000	4,400	8,000
Amino-acids				
Glutamic and aspartic	1,500	8,000	14,000	19,000
Neutral and basic	10,000	26,000	31,000	40,000
Carbon dioxide	300	1,440	7,000	14,000
Total ^{14}C incorporated	15,200	42,780	64,260	91,420

* 0·5-g samples of 3-day-old pea cotyledons sliced and incubated at 30° C in darkness with 0·82 μmoles lactate-1-^{14}C (2·5 μc. of ^{14}C) in 50 μmoles phosphate buffer at *p*H 5·5.

was fed to these tissues[4]. Furthermore, feeding $^{14}CO_2$ to these tissues has indicated that organic acids and amino-acids are readily synthesized by a process of dark carbon dioxide fixation[7]. Paper chromatography of the neutral and basic amino-acid fraction[4] revealed the presence of alanine-^{14}C. Degradation[8] of samples of alanine formed during lactate-1-^{14}C feeding indicates that approximately 92 per cent of the radioactivity is located in the 1 position. These results are therefore consistent with an oxidation of lactate-1-^{14}C to pyruvate-1-^{14}C which would then yield alanine-1-^{14}C by a transamination reaction. Pyruvate-^{14}C is present in the organic acid fractions in large amounts, and mitochondria, isolated in sucrose-phosphate buffer[9], readily form pyruvate-^{14}C and $^{14}CO_2$ when supplied with lactate-1-^{14}C.

More detailed experiments on lactate metabolism in these and other plant tissues are now in progress. This work was supported by National Research Council grant A-1747.

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CYTOLOGY

Chromosome Abnormality in Three Cases of Lymphatic Leukæmia in Cattle

In three cases of overt lymphatic leukæmia in cattle a very specific change in the chromosome complement has been found.

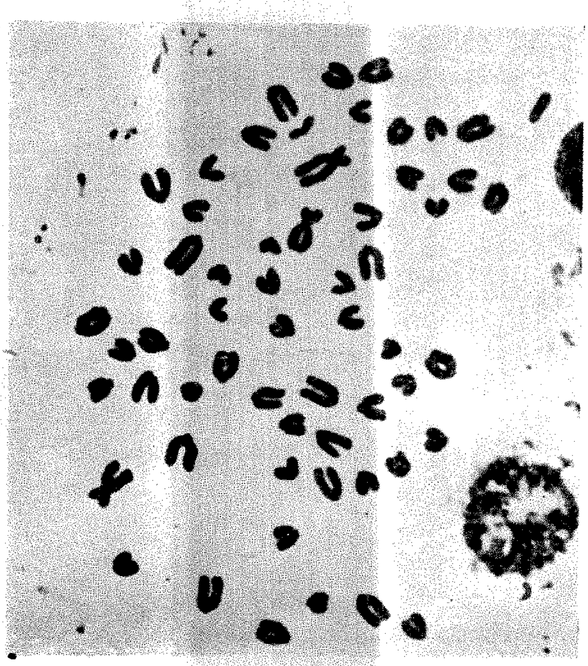

Fig. 1. The mitotic chromosomes of a leucocyte in one of the cases. (× c. 1,600)

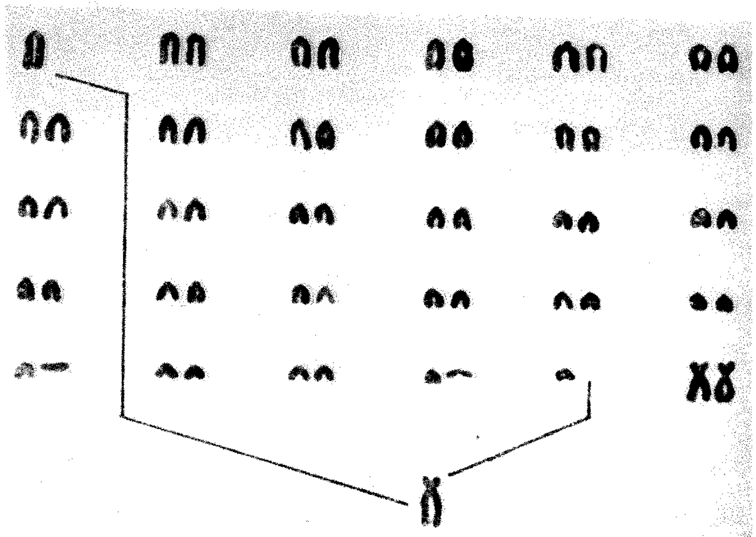

Fig. 2. The karyogram with the autosomes arranged as nearly as possible according to decreasing size. The lines show the origin of the abnormal chromosome

Sedimentation of the erythrocytes in the blood sample was obtained after centrifuging at 1,000 r.p.m. for abou 15 min. Leucocytes and serum were removed and sus pended in tissue culture medium Parker 199. Phyto hæmagglutinin was added just before incubation. Eve on the second day, many leucocytes were in mitosis The cells were treated with colchicine and hypotonic flui and were fixed in 9 parts 60 per cent acetic acid plus part 0·1 N hydrochloric acid. After staining with aceto orcein, manual squash preparations were made in th usual way.

More than 100 mitoses from every animal were analysed and all were found to contain 59 chromosomes, with on diverging morphologically from the normal complemen (Fig. 1). This chromosome had a subterminally situate centromere and presumably originated from a chromosom fusion or translocation, the real nature of which cannot a yet be established. The chromosome pairs affected ar numbers 1 and 29, the largest and the smallest pair, fact that has been confirmed by chromosome measure ments (Fig. 2).

The same chromosome abnormality was found in fœtus from one of the cows. The fœtus was about si months of age and looked normal. Mitoses obtaine *in vivo* from the bone marrow and chromosomes of kidne cells after eight days incubation were examined.

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Electron Microscope Observations on Mitotic Chromosomes in Erythroblasts of the Newt, *Triturus cristatus cristatus*

The structure of DNA is now known and the externa form of mitotic chromosomes of higher organisms i fairly well characterized in the light microscope. However the way in which chromosomes are constructed from DNA histones and other proteins is not yet clearly understood although there are many observations and several theories of chromosome structure[1-9]. In chromosomes of new erythroblasts we have found structures, intermediate in size between DNA molecule and chromosome, which may provide clues to the way these chromosomes are organized

Pieces of newt spleen were fixed in buffered glutaraldehyde and OsO_4 (ref. 10) and embedded in 'Araldite'. Sections were stained in uranyl acetate and lead citrate and examined in an Elmiskop 1 and Zeiss *EM*9.

Fig. 1 is an electron micrograph of a section through an anaphase chromosome selected to show a special feature, namely, a long projection of chromosome material, 1–2μ in length, limited on either side by a lighter zone. Each zone consists of two membranes separated by a space and is essentially similar in structure to the nuclear envelope in interphase erythroblasts. The unusually light appearance of the space between the two membranes, and their low visibility, is mainly due to the high density of hæmoglobin. Although many sections through mitotic figures do not show such projections, they are not uncommon and have been seen in as many as five chromosomes per section; there were, at the most, two projections per chromosome. Membrane-limited projections have been encountered in cells at prometaphase, metaphase, anaphase, telophase and interphase. Examination of serial sections showed that such projections are in fact sections through sheets of membrane-limited chromosomal material. In one series a chromosome projection, 2μ long, persisted through seven silver sections, constituting a sheet 2μ by, at least, 0·5μ. Although the dimensions in the plane of the sheet are variable, the width of the chromatin sheets, at all stages, is approximately constant with an average value 464 ± 35 Å (mean of 74 measurements). The fine structure and staining properties of the sheets are similar to those of the main chromosome body, namely, fibrils and granules about 20–40 Å diameter.

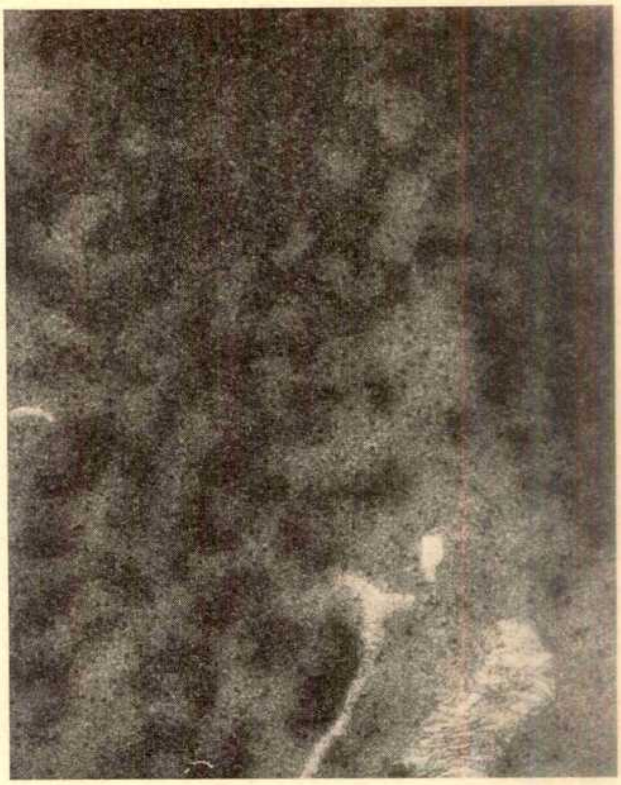

Fig. 2. Electron micrograph of part of an anaphase chromosome, one cut tangential to surface (× 30,000)

A second feature of these chromosomes is the irregularity of their surface, which is especially apparent when the section is cut tangential to the surface (Fig. 2). Although the surface structure has not yet been examined extensively, serial sections suggest that many of the irregularities correspond to relatively short (2000–6000 Å) projections, approximately cylindrical in shape, with a diameter of about 1000 Å. These projections are apparent from prometaphase onwards, until the stage of telophase clumping when they cannot be distinguished. Surface irregularities occur in mitotic chromosomes from both plant and animal species[11–14].

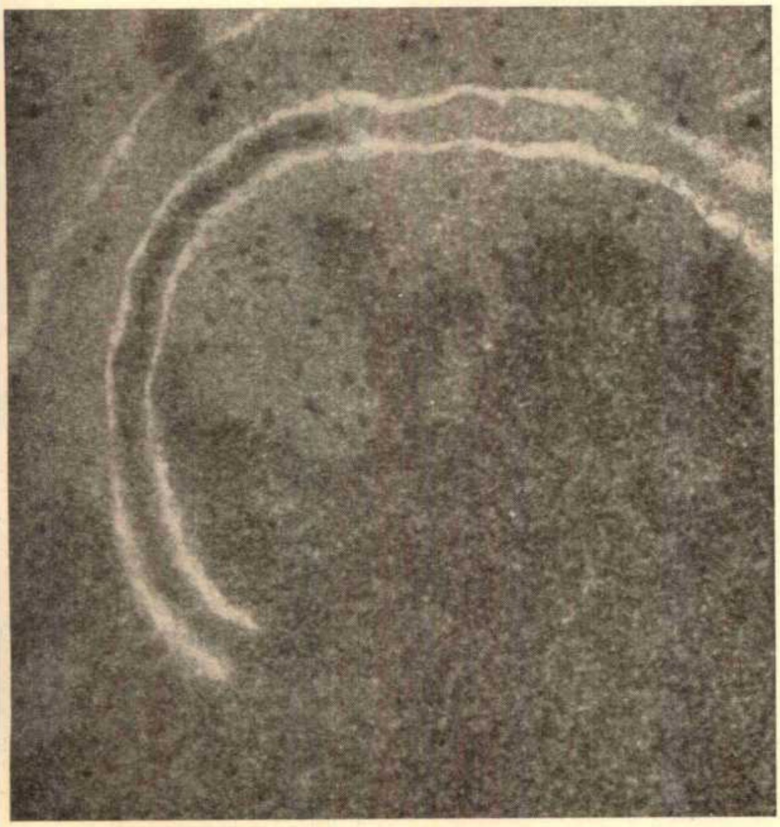

Fig. 1. Electron micrograph of part of an anaphase chromosome (× 80,000)

The most interesting aspect of our observations is the constant thickness of the membrane-limited sheets of chromosome material. Almost certainly the membranes are part of the nuclear envelope. Although the mode of origin and significance of the sheets are not yet established, the following suggestions are made. Although the constant thickness of the chromosome sheet may merely be the result of some peculiar transformation, and depend solely on the properties of the nuclear envelope, it is more likely that the width of the sheet depends on the properties of chromatin itself and is some aspect of a constructional unit, intermediate in size between DNA molecule and chromosome. We may suppose a sheet or sheets of chromatin, each 450 Å thick, to be actually present in the interphase nucleus. During the formation of the chromosome in prophase the sheet of chromatin, apposed to the envelope, remains stable under its influence and is not assembled into the main cylindrical body of the chromosome. During the motion of the chromosomes, a piece of free nuclear envelope becomes attached to the other side of the chromosomes sheet, resulting in a stable sheet complex. The occurrence of sheets in these chromosomes may be due to the high concentration of cytoplasmic hæmoglobin affecting the interaction of nuclear envelope with chromatin.

The surface topography of chromosomes appears to provide clues about their organization. For example, our data are consistent with a model in which the chromosome is formed by successive coiling of a thread 450 Å in diameter. In the interior of the chromosome these threads are packed so close together that their presence is not disclosed. However, at the surface, due to irregularities in coiling, the hypothetical 450 Å thread forms a loop, the elements of which twist round one another to form a projection about 1000 Å in diameter. Furthermore, a thread laid down on a surface may fold to form a sheet. Sheets may rise at the so-called clumping stage[15,16] of telophase when the chromosome is supposedly partially uncoiled into the 450 Å threads. New nuclear envelope associates with the chromosomes at about this stage and we assume that sheets so formed persist during interphase as heterochromatic regions attached to the nuclear envelope, only to appear in the subsequent division as the sheet structures we have figured. However, a model[8] formed out of coiled sheets cannot be excluded.

We thank Dr. J. H. Frenster for directing our attention to electron micrographs[17] of interphase nuclei in certain human neutrophils, which contain projections probably corresponding to sections through sheets of chromatin about 500 Å wide. We thank Miss Pamela Rush for her assistance.

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VIROLOGY

Isolation of Louping Ill Virus from Small Mammals in Ayrshire, Scotland

THE natural history of louping ill, particularly the role of small mammals, has been little examined although the disease is still a major veterinary problem in many hill sheep-farms in Scotland. Findlay and Elton[1] showed that the short-tailed vole, *Microtus agrestis*, is susceptible to intracerebral inoculation with the virus. The occurrence of natural infections in wild animals such as red deer, *Cervus elaphus*[2]; red grouse, *Lagopus scoticus*[3]; and hares, *Lepus* sp.[4], suggested that the virus had a wider natural host range than sheep and cattle.

The rough pasture of coarse grass and rushes on many hill farms supports a large population of small mammals, particularly voles, shrews and wood mice. This is also the typical habitat of the tick, *Ixodes ricinus*, the arthropod host of louping ill. Most species of small mammals in such biotopes are infested with immature stages of *I. ricinus* and, in areas where louping ill occurs, they are exposed to the bite of infected ticks.

In March–April of 1962 and 1963, small mammals were collected from three farms around Dalmellington, Ayrshire, where louping ill is known to be enzootic, and wher investigations of the ecology of the disease in sheep are als being made[5]. The shrews, voles and wood mice wer caught in Longworth traps and the moles in Duffus traps The common shrew (*Sorex araneus*) made up 44 per cen of the collections, the short-tailed vole (*Microtus agresti* 27 per cent, and the wood mouse (*Apodemus sylvaticu* 13 per cent. The mole (*Talpa europea*), pygmy shre (*Sorex minutus*), bank vole (*Clethrionomys glareolu* and water shrew ((*Neomys fodiens*)—in that order— accounted for the rest of the collections (Table 1).

Table 1. ISOLATION OF LOUPING ILL VIRUS FROM BRAINS AND SPLEEN OF SMALL MAMMALS COLLECTED IN AYRSHIRE, SCOTLAND, IN 1962 AND 196

Species*	Total collected	Virus isolations from brains and spleens No. processed	No. positive	Infectio rate %
Mole (*Talpa europea*)	13	13	0	0
Pygmy shrew (*Sorex minutus*)	10	9	0	0
Common shrew (*Sorex araneus*)	100	68	1	1·5
Water shrew (*Neomys fodiens*)	6	3	0	0
Short-tailed vole (*Microtus agrestis*)	61	32	0	0
Bank vole (*Clethrionomys glareolus*)	8	6	0	0
Wood mouse (*Apodemus sylvaticus*)	30	26	2	7·7
Total	228	157	3	1·9

* Common names are those given in Southern (ref. 6)

After collection, the animals were lightly anæsthetize and examined for ectoparasites. Blood for antibody investigations was collected under anæsthesia from the cavernous sinus through the optic foramen. The animals were killed, and the brains and spleens removed, then sent on dry ice to London, where they were stored at −60° C until processed. In the 1962 investigation, the brain and spleen from each animal were ground up separately, each in 1·0 ml. of 0·75 per cent bovine albumin in borate saline buffered at pH 9. The suspensions were pooled, centrifuged and the supernatant inoculated intracerebrally into mice 2–3 days old, each mouse receiving 0·01 ml. The rest of the suspension was stored at −60° C in sealed ampoules. In the 1963 investigation, the brain and spleen from each animal were processed separately.

In 1962, virus was isolated twice: from the pooled brain and spleen of a wood mouse (*A* 596) collected on April 12 and from the pooled brain and spleen of a common shrew (*A* 603) collected on April 13. In 1963, virus was isolated only once: from the spleen of a wood mouse (*A* 743) collected on March 30. All three isolates were shown to be identical with louping ill virus, and they were successfully re-isolated from the original suspensions.

Table 1 shows the number of brains and spleens processed and the number of isolations. The overall infection rate in the small mammals was 1·9 per cent; the infection rate in the wood mouse was 7·7 per cent and in the common shrew 1·5 per cent.

There seems to be no doubt that some of the small mammals in louping ill areas become infected with the virus. This is supported by the finding of antibodies to the virus in their blood and the fact that they are infested with larvæ and nymphs of *I. ricinus*. However, to show whether these small mammals can maintain foci of infection in Nature in the absence of sheep, or whether they influence the frequency of infection in sheep, depends on further investigation of a number of factors: their population densities, their degree of tick infestation and the levels of viræmia which occur in them.

Larval infestation rates of small mammals appear to be lower in Britain than in Europe[7], where small mammals have been found naturally infected with Central European tick-borne encephalitis virus which is also transmitted by *I. ricinus*[8]. Moreover, the small mammals examined in Ayrshire are not maintenance hosts of adult *I. ricinus* and therefore could not themselves maintain a population of infected ticks.

Little is known about louping ill viræmia in small mammals. In one experiment, a wood mouse inoculated intraperitoneally with louping ill virus had viræmia on the first, second, third and fifth day after inoculation and survived to develop antibodies. The highest titre, 1·1 log mouse LD_{50} per 0·03 ml., was obtained on the third day. This may be below the threshold required to infect feeding larvæ. Small mammals such as shrews, voles and wood mice may be of particular importance in their peak population years when they probably act as amplifiers of the infection. They may do this in two ways: (1) The larger number of larval hosts present in these years probably substantially increases the proportion of larvæ which succeed in feeding and thus in surviving. (2) At the same time a proportion of the larvæ probably become infected. Together these will increase the risk of infection in sheep in subsequent years. A similar phenomenon has been shown to happen in Czechoslovakia, where outbreaks of tick-borne encephalitis in human beings have followed 'mouse years'[9].

We thank the late Mr. James Murdoch, and Mr. John Murdoch, for their unfailing hospitality and for permission to trap on the farms; Mr. J. M. Ferguson for advice on trapping, Messrs. J. Smart, R. Kinloch, J. C. Cunningham, R. J. C. Page, S. C. Srivastava and Miss Iva Albert for assistance with the trapping; Mr. A. L. Wilson of the West of Scotland Agricultural College for providing laboratory facilities at Auchincruive, Ayrshire; Dr. Chan Yow Cheong and Miss Margery Dorr for examining and dealing with the trapped animals, and the Agricultural Research Council for a grant towards the field expenses of this work.

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PSYCHOLOGY

Sensitivity of Hands to Visible Light

Recently, reports have appeared in the press of experiments demonstrating the existence of a dermal sensitivity to intensities and wave-lengths of visible light. Many of the experiments do not appear to have precluded thermal effects. and B. Konstantinov has suggested[1] that the results may be explained by the perception of reflected infra-red radiation emitted by the hand. In the experiment recorded here these effects have been reduced, and college-age subjects have been required to discriminate between black and white.

In the experiment the subject placed one hand in a dark box, above two (2 in. × 4 in.), 1 in. deep, black chambers, one of which contained a white lint-covered plate and the other a black. The interchangeable plates were illuminated by an external fluorescent lamp, and were separated from the subject's hand by a 3-mm thick window of heat-absorbent glass (Chance *ON*20 which transmits less than 5 per cent of the incident infra-red). The intensity, above the window, of the light reflected from the white plate was 0·00012 lumens/cm², this being 3·5 times the intensity above the black.

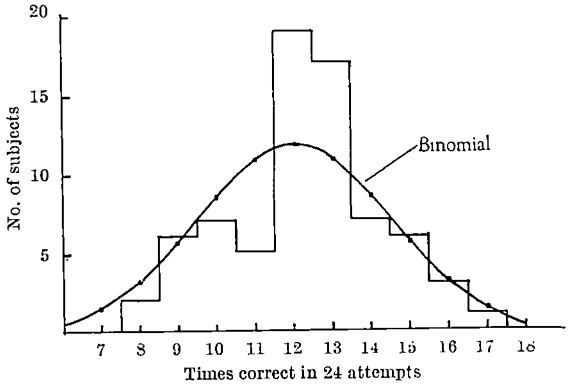

Fig. 1

The subject was given a few minutes in which to learn the difference between black and white before being tested 24 times. Before each test the plates were placed in the chambers on a pre-determined random basis by an experimenter invisible behind a screen. The subject was given no knowledge of her performance until the end.

The results for a total of 73 subjects (66 women) are shown in Fig. 1. No individual showed significance at the 5 per cent level. The results were combined and a significance level of 8 per cent was obtained ($\chi^2 = 14·4$, 8 *d.f.*)—reflecting a slight tendency for the subject to be right rather than wrong. A test merely for discrimination (curve folded) gave a significance level of 11 per cent ($\chi^2 = 7·6$, 4 *d.f.*).

At this relatively low light intensity the experiment does not appear to support the proposition that hands are sensitive to visible light.

We thank Mr. J. D. Valentine and Dr. M. J. Pickersgill for useful discussions.

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[1] *Soviet News*, Feb. 10, 1964.

Central Adaptation in Mechanisms of Form Vision

Perception of any visual pattern is subject to three well-known forms of adaptation. In the first place a 'negative after-image' is formed which tends to reduce the contrast between the brightnesses of neighbouring areas. Secondly, after more prolonged fixation, peripheral parts of the visual field tend to fade from view in large patches. With a stabilized retinal image, it is well known that a third type of adaptation can occur, in which the whole field fades and reappears intermittently.

With a randomly structured field such as an enlarged photograph of sandpaper (Fig. 1*a*) I have recently observed a fourth type of adaptation which seems to be distinct from all of those mentioned. After ten or twenty seconds' fixation, monocular or binocular, the clarity of one's visual image is unimpaired but the texture of the field assumes a curiously uniform character. The coarser features disappear and leave a mottled pattern reminiscent of a tufted carpet. Some observers report that the mottling has a roughly periodic character, the unit cell subtending an angle of the order of 1 degree. There is evidence also that the character of the uniform field seen is partly determined by the form of the region of the random field fixated. During monocular fixation a kind of rivalry is observed between the mottled image and a dark field, subjectively associated with the occluded eye, criss-crossed by a highly active population of randomly distributed dots and lines in motion.

The original pattern at once reappears, with enhanced vividness, if fixation is displaced by a few degrees. If the

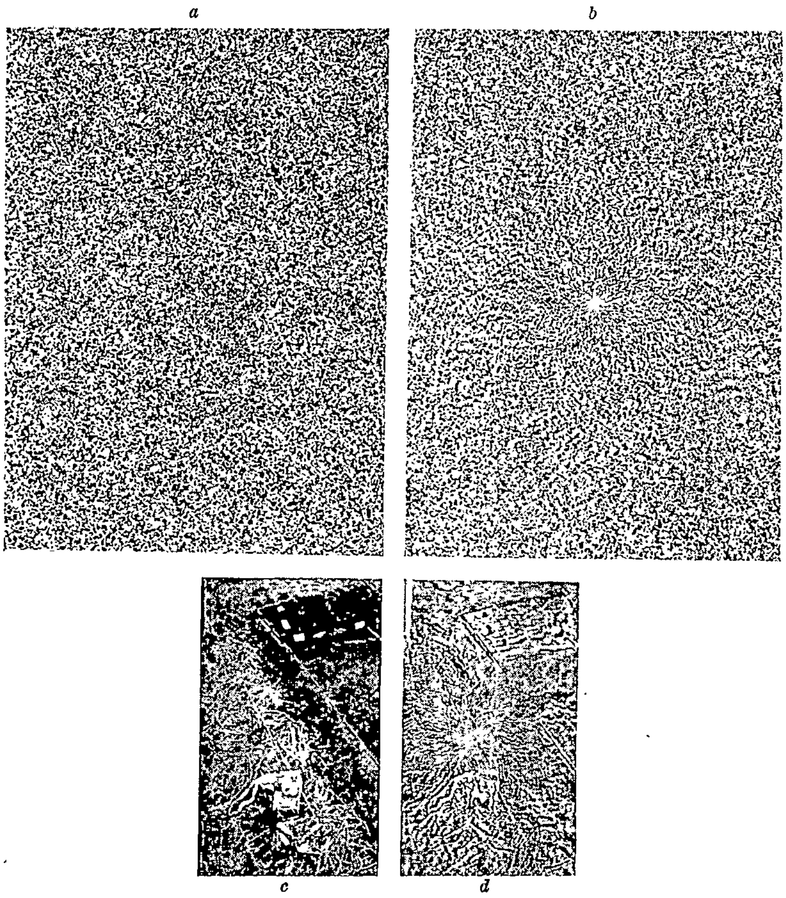

Fig. 1

originally fixated position is resumed within 1–2 sec, the uniformly mottled appearance returns, proving that the adaptations concerned are local to each area of the visual field stimulated. Though this might at first seem in some way to implicate local retinal adaptation to brightness, two further observations indicate that the phenomenon is different in origin. In the first place, the use of intermittent illumination, which partially overcomes brightness adaptation, does not restore the original form. Secondly, the same tendency towards homogeneity is found with the 'all-or-none' pattern of Fig. 2, in which simple brightness adaptation could affect only the contrast.

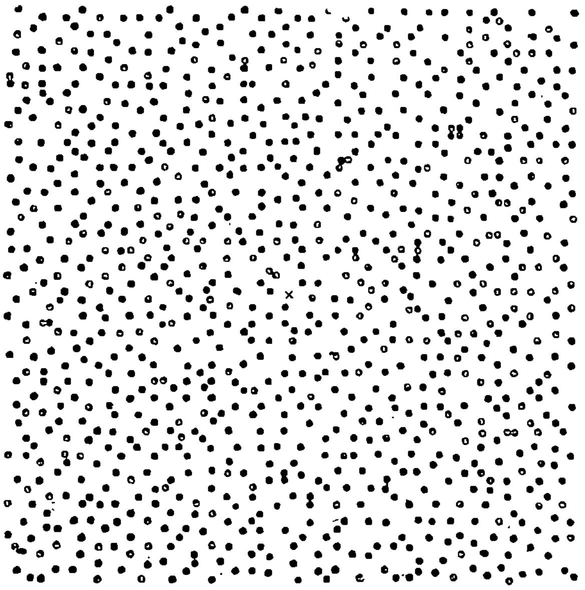

Fig. 2

It is tempting to interpret this phenomenon in terms of the contour-detecting and other form-sensitive neural mechanisms adumbrated by earlier observations[1] and reported recently in lower animals[2–4]. It would not be surprising if the long-range interactions between receptive fields required for the computation of form were more vulnerable to adaptation than those concerned with the signalling of brightness contrast, particularly when the redundancy of the pattern is low. The latter qualification is reinforced by the observation that with pages of newsprint, and other massive stimuli in which regular contours are present, such 'figural' adaptation is not observed to anything like the same extent.

The foregoing effects should be distinguished from another visual phenomenon observable on prolonged fixation of such random fields, namely, the transient appearance of spiral patterns. These seem to have a simpler explanation. As the reader can easily verify, they can be produced at will by suddenly rotating the fixation field by a degree or two. It seems reasonable, therefore, to attribute them to small torsional eye-movements.

It is not perhaps obvious at first sight why rotation in this case should generate spirals rather than circles. The reason becomes clear, I think, if one superimposes positive and negative photographic images of identical fields, such as Figs. 1*a* and *c*, with a slight relative rotation. The results are shown in Figs. 1*b* and *d*. Close to the centre of rotation, only edges which happen to lie radially are sufficiently displaced to be enhanced. At greater distances from the centre of rotation the optimal displacement for contour enhancement is exceeded for edges lying radially, but is achieved for those lying at progressively larger angles to the radius. The loci suggested by these enhanced contours thus tend to be spiral. Figs. 1*c* and *d* have been included to indicate that the effect is strengthened when the primary image is rich in long black or white edges at all angles. In the production of spirals observed when viewing a field such as Fig. 1*a*, the part of 'negative' is presumably played by the adapted retina.

Notwithstanding its irrelevance to the first phenomenon described, this mechanism of spiral-production may possibly be of interest in connexion with another type of central adaptation—the generation of spiral 'complementary images' when a field of random visual noise is viewed through or against a pattern of radial lines[1,5]. Since it is known that in this case eye movements are not responsible[6], the appearance of spirals raises the question whether lateral inhibitory action along the radial contours produces in effect a displaced 'negative' of the figure of neural excitation, which combines with the primary figure in analogous fashion to Figs. 1*b* and *d*.

This work was supported by the Department of Scientific and Industrial Research.

D. M. MacKay

Department of Communication,
University of Keele.

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FORTHCOMING EVENTS

Sunday, August 30—Wednesday, September 2

British Association for the Advancement of Science (at Southampton)—Continuation of 126th Annual Meeting.

Sunday, August 30

At 10.30 a.m.—Official Service in St. Mary's Church. Preacher: The Very Reverend the Dean of York, Canon Alan Richardson, M.A., D.D.

Monday, August 31

At 10 a.m.—Dr. W. Marshall: "The Exploration of Solids Using Thermal Neutrons" (Kelvin Lecture).

At 10 a.m.—Mr. W. H. Wilcockson: "Some Aspects of East African Vulcanology" (Presidential Address, Section C).

At 10 a.m.—Dr. C. A. Ralegh Radford: "History and Archæology" (Presidential Address, Section H).

At 11.30 a.m.—Prof. Lucy Mair: "How Small-scale Societies Change" (Presidential Address, Section N).

At 2.30 p.m.—Dr. H. A. Thomas: "Automation" (Young People's Lecture).

At 8 p.m.—Prof. A. S. Parkes: "Biological Aspects of the Population Explosion" (Evening Discourse).

Tuesday, September 1

At 2.30 p.m.—Dr. E. J. Ambrose: "Cells at Work" (Young People's Lecture).

Wednesday, September 2

At 10 a.m.—Dr. G. R. Coope: "Insect Populations in the Ice Age" (Darwin Lecture).

At 10 a.m.—Dr. V. B. Proudfoot: "Bringing Archæology to Life" (Lister Lecture).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer or Lecturer (preferably with experience in radiation or radioactive isotope measurements) in the Department of Biophysics, for activation analysis and trace element work—The Secretary, St. Thomas's Hospital Medical School, London, S.E.1 (September 5).

Research Engineer (with an honours degree in mechanical engineering and experience in the design or testing of engines) in the Department of Mechanical Engineering, to work on a project concerning the assessment of lightweight I.C. engines—The Registrar, The University, Sheffield (September 5).

Assistant Lecturer (with a degree in mathematics or the physical sciences and preferably some postgraduate experience) in Computing—The Registrar, University College of North Wales, Bangor, North Wales (September 7).

Lecturer in Pathology (Morbid Anatomy)—The Registrar, The University, Bristol, 8 (September 7).

Assistant Statisticians (2) (with a degree or diploma in technology in mathematics, or in pure or applied biology with "A" level in mathematics), for work on the planning and analysis of experiments—The Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex (September 8).

Demonstrator (graduate with qualifications in dairying and dairy microbiology) in Dairy Science—The Registrar, University College of Wales, Aberystwyth (September 12).

Horticultural Economist (graduate, preferably with research, teaching or advisory experience in horticultural economics) in the Department of Agricultural Economics—The Registrar, The University, Nottingham (September 12).

Laboratory Superintendent (with a degree or equivalent qualification, and substantial experience with the equipment and supervision of mechanical engineering laboratories) in the Department of Mechanical Engineering, for duties which will include the supervision of technical staff; supervision, maintenance and development of laboratories and apparatus; ordering and accounting of equipment and stores; and implementing safety regulations—The Registrar, The University, Sheffield (September 12).

Lecturer and an Assistant Lecturer (with special interests in pure or applied mathematics or numerical computation or mathematical statistics) in Mathematics at Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (September 12).

Senior Lecturer or Lecturer in the Department of Materia Medica and Therapeutics—The Secretary, The University, Aberdeen (September 12).

Graduate Research Assistant (with a good honours degree in a biological subject, and preferably experience in genetics or cytogenetics) in the Cytogenetics Group of the Pædiatric Research Unit, to work on a population survey on human autosomal abnormalities—The Secretary, Pædiatric Research Unit, Guy's Hospital Medical School, London Bridge, S.E.1 (September 14).

Lecturer (either botanist specializing in biochemistry or biochemist preferably with research interests in plant pathology; or botanist specializing in plant virology) in Botany—The Registrar, The University, Manchester, 13 (September 17).

Senior Lecturer in Psychology and a Senior Lecturer or Lecturer in Statistics at the University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, September 18).

Assistant Lecturer or Demonstrator (with medical qualifications registrable in the United Kingdom) in Pathology—The Registrar, The University, Manchester, 13 (September 19).

Senior Lecturer in Medical Statistics in the Department of Public Health and Social Medicine—The Registrar, The University, Edinburgh (September 19).

Senior Lecturer or Lecturer (with medical qualifications registrable in the United Kingdom) in Pathology—The Registrar, The University, Manchester, 13 (September 19).

Lecturer (preferably with research interests in lipoproteins or cytochemistry) in Biochemistry at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 25).

Lecturer in Animal Production—The Secretary, Wye College (University of London), near Ashford, Kent (September 30).

Professor of Geography at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 30).

Professor in the Department of Agricultural Biology, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 30).

Professor of Mathematics at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 30).

Research Assistant (preferably with an honours degree in zoology or medical sciences and a knowledge of German; persons graduating this year will be considered) to Prof. J. Z. Young in the Department of Anatomy, for work mainly on the central nervous system—The Secretary, University College, Gower Street, London, W.C.1 (September 30).

Research Fellow and a Senior Research Fellow in Social Anthropology and Sociology at the Institute of Advanced Studies, Australian National University—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

Scientific Officer (with a good honours degree in a biological subject and preferably with research experience in microbiology) to study the soil-surface microflora, particularly nitrogen-fixing algae—The Secretary, Rothamsted Experimental Station, Harpenden, Hertfordshire (September 30).

Senior Lecturer in Psychiatry at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

Senior Lecturer (with experience in any field of botanical science) in the Department of Botany, Monash University—The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia; or the Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 30).

Chair of Forestry at the Australian National University, Canberra—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 1).

Chair of Veterinary Clinical Studies at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 2).

Lecturer/Senior Lecturer in the School of Anatomy, The University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (October 5).

Assistant Lecturer or Lecturer in Textiles and Clothing in the Department of Domestic Science, University College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 8).

Lecturer/Senior Lecturer in Animal Production in the Faculty of Agricultural Science, University of Tasmania—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 9).

Senior Lecturers (Clinical) (2) in Pathology, University of Queensland, Brisbane, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 16).

Assistant Director of Research (with a degree in agriculture or agricultural science or suitable postgraduate qualifications in these subjects, and substantial experience of the agriculture of developing countries) in Comparative Agriculture—The Secretary, School of Agriculture, the University, Downing Street, Cambridge (October 31).

Lecturer (with a university degree in chemical engineering, plus five years' approved industrial experience) in Chemical Engineering—The Director, The Swinburne Technical College, John Street, Hawthorn, E.2, Victoria, Australia (October 31).

Senior Fellow or Fellow (with several years' research experience in some branch of astronomy) on the Academic Staff of the Mount Stromlo Observatory—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Director, Mount Stromlo Observatory, Australian National University, Canberra, A.C.T., Australia (Australia and London, November 2).

Assistant Lecturer (graduate in science or engineering with a special interest in physical or physico-chemical properties of polymers) in Polymer Science in the Department of Materials—The Registrar, The College of Aeronautics, Cranfield, Bedford.

Experimental Officer (with basic scientific knowledge to at least G.C.E. "A" level or H.N.C., and preferably experience in electronic circuitry and high-vacuum methods with engineering design and drawing capability) for participation in researches in plasma spectroscopy, supported by U.K.A.E.A. (Culham)—Dr. W. R. S. Garton, Physics Department, Imperial College, London, S.W.7.

Lecturer in the Department of Organic Chemistry—The Registrar, The University, Liverpool, quoting Ref. CV/266.

Livestock Officer (with a degree in agriculture with animal husbandry or animal health as a major subject) in the Bahamas, to advise small farmers on methods of improving pastures and the quality of local sheep, goats and cattle, and to supervise co-operative livestock associations and pasture development schemes—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. No. RC 213/14/01.

Physicist (between 25 and 35, with a good science degree and preferably experience in research and experience of textiles), to take charge of investigations of fundamental importance to the hosiery industry—The Secretary, Hosiery and Allied Trades Research Association, Thorneywood, 7 Gregory Boulevard, Nottingham.

Post-Doctoral Research Fellow (with experience in adsorption studies or in mass spectrometry) for the spectroscopic investigation of molecules absorbed on surfaces—Prof. N. Sheppard, School of Chemical Sciences, University of East Anglia, Wilberforce Road, Norwich, NOR 77H.

Post-Doctoral Research Fellows (2) (physicists or engineers) for work on magnetic and optical properties of metals and non-metals—Dr. R. R. Birss, Physics Laboratory, University of Sussex, Brighton, Sussex.

Scientific Officer (with an honours degree or equivalent in engineering or physics) for investigation of behaviour of tractors and implements on sloping land, particularly effects of forces arising from slopes on motion of machines—The Secretary, National Institute of Agricultural Engineering, Scottish Station, Bush Estate, Penicuik, Midlothian, Scotland.

SENIOR RESEARCH ASSOCIATE IN THE SCHOOL OF CHEMISTRY for research into reaction mechanisms in boron chemistry—The Registrar, The University, Newcastle upon Tyne, 2.

TECHNICAL ASSISTANT (female, 20 or over, with advanced level G.C.E. in physics and mathematics, or the equivalent) IN THE PHYSICS DEPARTMENT, for duties concerned with the medical application of radioactive isotopes, and training will be given in the specialized techniques involved—The Secretary, The Royal Infirmary, Leicester.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Electrical Research Association. Report No. 5059: Dielectric Losses in Polymer Films Formed by a Discharge. By M. Stuart. Pp. 5+5 figures. (Leatherhead: Electrical Research Association, 1964.) 7*s*. 6*d*. [147

Department of Scientific and Industrial Research. Report of the Research Council for the year 1963. Pp. iv+66. (Cmnd. 2394.) (London: H.M. Stationery Office, 1964.) 4*s*. 6*d*. net. [147

Fourth Annual Report of the Council of the Institute of Physics and the Physical Society, 1963. Pp. 26. (London: Institute of Physics and the Physical Society, 1964.) [147

General Register Office. The Registrar General's Quarterly Return for England and Wales: Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, Quarter ended 31st March, 1964. (No. 461, 1st Quarter, 1964.) Pp. 28. (London: H.M. Stationery Office, 1964.) 2*s*. 6*d*. net. [147

Henry Wiggin and Co., Ltd. Hot-Working Tools in Nimonic and Nimcast Alloys. Pp. 8. (London: Henry Wiggin and Co., Ltd., 1964.) [147

The Grassland Research Institute. Experiments in Progress No. 16: Annual Report for 1962–63. Pp. 100+4 plates. (Hurley, near Maidenhead: The Grassland Research Institute, 1964.) 10*s*. [147

Department of Scientific and Industrial Research. Building Research 1963: The Report of the Building Research Board with the Report of the Director of Building Research. Pp. iv+90+16 plates. (London: H.M. Stationery Office, 1964.) 8*s*. 6*d*. net. [147

British Chemicals and their Manufacturers—1964 Supplement. Pp. 32. (London: Association of British Chemical Manufacturers, 1964.) [147

Committee of Privy Council for Medical Research. Report of the Medical Research Council for the year 1962–1963. Pp. viii+280+3 plates. (Cmnd. 2382.) (London: H.M. Stationery Office, 1964.) 16*s*. 6*d*. net. [147

British Radio Valve Manufacturers' Association. British Valve and Semiconductor Industry. Second edition. Pp. 8. (London: B.V.A., 156 Oxford Street, 1964.) [147

The South Yorkshire Glass Industry. By Geoffrey D. Lewis. Pp. 4+2 plates. (Sheffield: The City Museum, 1964.) 6*d*. [147

An Introduction to the Work of the British Steel Castings Research Association. Pp. 24. (Sheffield: The British Steel Castings Research Association, 1964.) [147

Department of Scientific and Industrial Research: National Physical Laboratory. Notes on Applied Science, No. 12: Calibration of Temperature Measuring Instruments. By J. A. Hall and C. R. Barber. Third edition. Pp. iv+55. (London: H.M. Stationery Office, 1964.) 4*s*. 6*d*. net. [147

Furniture Development Council. Fifteenth Annual Report for the year ended 31st December, 1963. Pp. 12. (Stevenage: Furniture Development Council, 1964.) [147

The Scientific Proceedings of the Royal Dublin Society. Series A. Vol. 1, No. 15 (May 5, 1964): Irish Salmon—a Review of Investigations up to 1963. By Arthur E. J. Went. Pp. 365–412. 12*s*. Vol. 2, No. 1 (April 30, 1964): Compartment Deformation near Buttevant, County Cork, Ireland, and Its Relation to the Variscan Thrust Front. By M. E. Philcox. Pp. 1–12+1 plate. 4*s*. 6*d*. Vol. 2, No. 2 (April 30, 1964): The Geological Structure of North County Cork, Ireland. By R. A. Monkhouse. Pp. 13–20+plate 2. 4*s*. Series B. Vol. 1, No. 11 (April 24, 1964): Evaluation of Tick Beans (*Vicia faba* L.) as a Protein Supplement in Pig Feeding. By F. X. Aherne and Prof. D. M. McAleese. Pp. 113–122. 3*s*. (Dublin: The Royal Dublin Society, 1964.) [147

Department of Scientific and Industrial Research. Torry Research Station Annual Report on the Handling and Preservation of Fish and Fish Products: Report of the Torry Research Station Steering Committee and Report of the Director of the Torry Research Station. Pp. iv+51+4 plates. (London: H.M. Stationery Office, 1964.) 4*s*. 6*d*. net. [147

Review of Coal Tar Technology, July–December 1964, Vol. 15, Part 2. Pp. ii+79–149. (Gomersal, Leeds: The Coal Tar Research Association, 1964.) 30*s*. per volume. [147

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Other Countries

Coconut Research Institute, Ceylon. Bulletin No. 21: Biological Control of Coconut Leaf Caterpillar (*Nephantis serinopa* Meyrick) in Ceylon. By Edwin Dharmaraju. Pp. 46. Cumulative Index to *Ceylon Coconut Quarterly*, Author and Subject, Volumes 1–10, 1954–1959. Compiled by M. J. C. Perera. Pp. 13. (Lunuwila: Coconut Research Institute, 1963.) [256

Mutation Research, Vol. 1, No. 1 (May 1964). Pp. 1–112. Published bi-monthly. Annual subscription 130*s*.; 65 D.fl.; 18 dollars. (Amsterdam: Elsevier Publishing Company, 1964.) [256

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South African Council for Scientific and Industrial Research: National Building Research Institute. Proceedings of the Building Research Symposium, 16–17 May 1963, Red Cross Hall, Windhoek; (Theme: Building Research and Its Significance to South West Africa). Pp. v+129. Urban Bantu Townships. By C. Tod Welch. Pp. 17. (Supplement to *S.A. Architectural Record*, December, 1963). C.S.I.R. Research Report No. 193: Komprehensiewe Hoërskool vir 1,200 Leerlinge: Aanbevelings van die Skoolgebouekomitee. (1,200-Pupil Comprehensive High School: Recommendations of the School Buildings Committee.) Pp. 48. C.S.I.R. Research Report No. 209: School Lighting. Pp. iv+47. (Pretoria: South African Council for Scientific and Industrial Research, 1963 and 1964.) [256

Museum of Comparative Zoology, Harvard University, Breviora. No. 200, (April 10, 1964): An Anguid Lizard from the Leeward Islands. By Garth Underwood. Pp. 10. No. 201, (April 10, 1964): Food Habits and You Stages of North Atlantic *Alepisaurus* (Pisces, Iniomi). By Richard Haedrich. Pp. 15, No. 202, (April 10, 1964): The Blind Snakes (*Typhlops*) Haiti with Descriptions of Three New Species. By Neil D. Richmond. P 12. No. 203, (April 10, 1964): A New Capromyid Rodent from the Qu ternary of Hispaniola. By Clayton E. Ray. Pp. 4. No. 204, (May 15, 196 The Status of *Pseudogekko shebae* and Observations on the Geckos of t Solomon Islands. By Walter C. Brown. Pp. 8. (Cambridge, Mas Museum of Comparative Zoology, Harvard University, 1964.) [2

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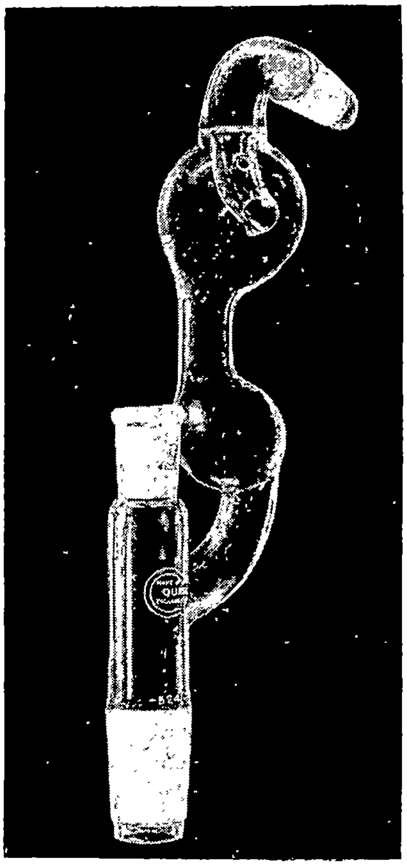

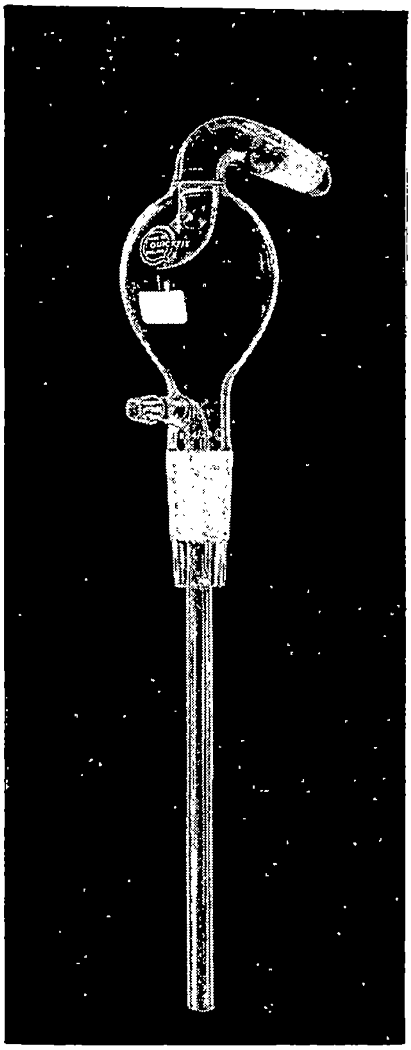

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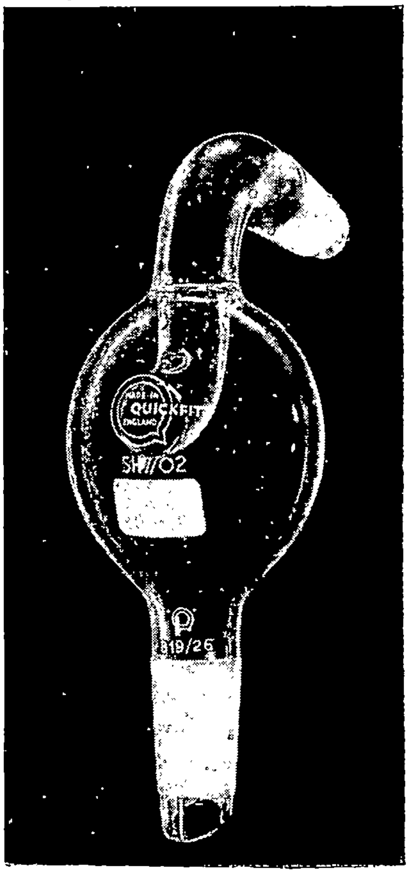

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SCIENTIFIC AND INDUSTRIAL RESEARCH UNDER REVIEW

THE annual report of the Council for Scientific and Industrial Research for 1962 was discussed in these pages in the context of the report of the National Economic Development Council, *Conditions Favourable to Faster Growth* (*Nature*, 199, 207; 1963). That report, as well as the further report, *Growth of the United Kingdom Economy to 1966*, is now noted in the report of the Council for Scientific and Industrial Research for 1963*, as are also the reports both of the Trend Committee and of the Robbins Committee and further reports which have been issued by the Federation of British Industries on *Civil Research Policy* (*Nature*, 199, 725; 1963), and by Political and Economic Planning on the *Government's Role in Applying Science to Industry* (*Nature*, 200, 503; 1963). The Research Council does not comment on these reports, but its latest annual report reflects nothing of the near hysteria with which the Trend Report, for example, was received in some quarters.

Conscious that the future of the Department of Scientific and Industrial Research is under review, the Research Council has chosen instead to make a dignified and considered statement setting forth what it considers as a set of guiding principles which it has evolved over the past seven years. These it regards as sufficiently flexible to meet any demands which may be made on the Council, and the statement records also the progress of the Council's thinking so far, in the light not only of the experience of the Executive Council but also of almost half a century of achievements by the Department. Moreover, the account of activities is better balanced than that given in some of the Council's annual reports and appropriately attests the soundness of the Council's claim that, throughout its seven years' existence, it has been at pains to emphasize the responsibility of the Department across the whole spectrum of research and development from basic science to industrial application and the dangers inherent in any arbitrary division of this responsibility.

The tendency detected in one or two of the Council's reports to arrogate responsibility that properly belongs to the Advisory Council on Scientific Policy is absent from this report, nor is it dogmatic as to the relations with the National Research Development Corporation or the National Economic Development Council. It is admitted that flexible means should be found, as experience develops, of harnessing public and private means for research and development to support established national requirements and priorities. There is no further evidence as to the effectiveness of the research associations or any inherent weaknesses in the conception, but here also there is a reasonable and a flexible outlook. The whole statement is not only dignified but also reflects a confidence arising from conscious strength and proved achievement that cannot be disregarded; it is a distinctive contribution to impartial decision as to the future of the Department and its relations with other bodies.

The statement suggests that a main task of the Government is to secure that the right proportion of resources is put into the conduct of research and its application by British industry, and that these resources are distributed as wisely as possible between the various branches of science and technology, and between the main types of activity—basic research, applied research, development and information and advisory services. The Trend Committee was, of course, charged with the responsibility of advising how far existing arrangements were appropriate for that purpose, and its recommendations are intended to provide a more efficient and effective structure, capable of meeting an increasing competition for scarce resources. Since the Research Council was first established as an executive body, expenditure on scientific and technological research and development in Britain has doubled from about £350 million in 1956–57 to about £700 million in 1962–63. However, the gross expenditure of the Department of Scientific and Industrial Research has increased from just more than £8·25 million to nearly £19·5 million. Although in earlier days the separate activities of the Department in encouraging academic and industrial research, and conducting research in the national interest, were separately planned, and control was kept to the minimum to ensure public funds were not wasted, closer attention to the selection and control of research programmes is now essential.

Recently, the Research Council has come to the conclusion that there are some shortcomings in the traditional pattern of control which could only be remedied if the Government took greater initiative. While these shortcomings are not precisely identified in the report, the Council states that it found that this task of considering the implications of such wider responsibility in a democratic society and finding effective tools for its discharge involved, first, identifying areas where the scale or pace of work is inadequate; secondly, discovering the reasons and suggesting possible ways of dealing with them; and thirdly, ensuring appropriate action. Within the orbit of the Council's own responsibilities, important parts of the first two of these tasks have been entrusted to its standing Economics Committee, which reviews industries to discover where research and development are lacking, and its Research Grants Committee, which is directing special attention to important research fields where increased effort and better co-ordination of existing effort may be desirable. The Council's statement is mainly concerned with the outcome so far of these enquiries.

First, while techniques for surveying requirements, industry by industry, are still being worked out, the Council is satisfied that surveys by economists and technologists afford a promising method: the findings can make a noticeable impact on key people in the industries concerned, and a careful follow-up of the recommendations can secure a larger and more balanced effort in research and development in which teaching and research institutions and industrial firms can all play their part. Clearly this activity is related to the activities of the National Economic Development Council, and the Research Council is now considering what help the Department could give to the economic development committees being set up by the National Economic Development Council for particular industries. This aspect, which is particularly concerned with faster economic growth and economic performance, may well assume greater importance in view of the evidence that, over the first five months of 1964, industrial output has ceased to grow.

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* Department of Scientific and Industrial Research. Report of the Research Council for the year 1963. Pp. iv+66. (Cmnd. 2394.) (London: H.M.S.O., 1964.) 4*s*. 6*d*. net. See also p. 1019 of this issue of *Nature*.

The Economics Committee is also attempting to establish sound economic criteria for national priorities in research and development. The Research Council reports that it is too early to say how successful this attempt may be, but in the absence of any details it would seem that such investigations are more appropriately made directly for the Advisory Council on Scientific Policy. This Committee has also been considering the national need for research and postgraduate investigations in applied economics. The Research Council's own experience indicates that available knowledge of the workings of the industrial economy is insufficient to provide a sure guide to policy. Difficulties have also been encountered in recruiting appropriately trained and experienced economists for work under the Committee, and the Council has transmitted its conclusions on the urgent need for more support for research and training in economics to Lord Heyworth's Committee on Social Studies.

As regards research in universities and colleges, the Research Council records a disappointing response to the indication that it would welcome applications for research grants within those areas indicated by a survey made in 1960 for the Advisory Council on Scientific Policy, which identified or confirmed important gaps in biology, technology and some subjects on the fringe of two or more disciplines. It is clear that some expansion of research is needed in some branches of technology and biology, and the Council has modified its procedures where necessary to assist this purpose. Here it is assisted by a series of sub-committees, which review applications and make recommendations to the Research Grants Committee, and three of these sub-committees—technology, metallurgy and metals, and biology—are conducting reviews, sometimes with the help of special panels, to stimulate more research in areas where it is especially timely and promising. The technology sub-committee has now been divided into two panels, and a new sub-committee has been set up for computer science.

Maintenance of the balance in research effort implies reconsideration of the level of support for work in established areas, particularly work requiring large teams or expensive equipment. Here the Research Council is particularly concerned with nuclear physics, which still absorbs about a quarter of the money available for its research grants. This it does quite apart from expenditure by universities and colleges out of general funds and the large sums going to the National Institute for Research in Nuclear Science and the European Organization for Nuclear Research. Since important advances can only be made with large and very expensive equipment and a single project may need a large part of available resources, the establishment of priorities in nuclear research has become important. The Research Council states that it will not feel justified in assigning further large sums to nuclear physics unless it is first assured that its support for other branches of science and technology is adequate.

It might be observed that the Secretary of State for Education and Science, or the Advisory Council on Scientific Policy, seem better placed and more appropriate for such decisions. However, clearly the problem is one that the Council for Scientific and Industrial Research cannot escape, any more than it can escape involvement in some problems of policy to which it next directs attention. With expensive apparatus or large research teams, ensuring that the work is done in the most suitable place and in the most effective way may involve a choice between new or existing locations and cannot always be divorced from the personality of the professor directing it. Should such a professor move elsewhere, the Research Council must clearly satisfy itself that his successor is able and willing to develop the work successfully. Here and in other ways it may be difficult for the Research Council to avoid trespassing on academic autonomy, but the Research Council is manifestly anxious to face and solve such problems in close collaboration with the universities themselves.

In determining changes necessary in the Department's duties to achieve a balance of effort in its work for Government and industry, the Council has directed special attention to its research stations, the research associations and civil research and development contracts with industry. In the whole field the Department's present main responsibilities are: to undertake scientific research to advise Government departments, local authorities and other public bodies in the exercise of their responsibilities; to provide industry with basic scientific and technological data and standards of measurement; to be the vehicle for Governmental financial support for industrial research and development in the Department's own stations and in industry; and to further the dissemination of the results of researches and their practical application. As regards the Department's own stations, the Council expresses satisfaction with the results of the experiment with small compact steering committees in place of the large advisory boards, and considers that there is much to be said for small closely-knit groups when trying to keep research programmes flexible in the face of changing needs. This is contrary to the view of the Zuckerman Committee, which did not think that such steering committees should be a normal feature in running a research establishment. Nevertheless, the Research Council is convinced that independent review committees can be useful in taking a wider look at the scientific and technological needs against the background of university and industrial resources and the developing needs of the community. Such a committee is looking at the functions of the Water Pollution Research Laboratory.

On the research associations, the statement records that the scheme has again been examined to ensure that the policy of support is rational and that the research associations themselves—to the expenditure of which (more than £9 million a year) the Government contributes some £2 million—are as vigorous as possible. The Council regards the traditional pattern of grant for associations which have passed their first stage of growth as inadequate. While there is now no suggestion that these associations are being forced to grow faster than their industries wish, the new grant policy still involves progressive stiffening of terms but in relation to the industrial circumstances of each association. Apart from seeing the associations develop their service to industry to the full and encouraging them to undertake sponsored work, the Council has now offered a new form of support in earmarked grants enabling them to undertake work for which they are best fitted, which is important in the national or long-term industrial interest, but which is unlikely to receive the necessary support or priority. The first of such grants was to the British Welding Research Association in upport of fatigue investigations of welded construcion.

On research and development contracts the Council omments that its policy has been to confine support to rojects representing a major technical advance and or which another department is not already responsible. t recognizes that inevitably only a small proportion f those submitted would be accepted, but it is disappointed that so few contracts have yet been placed. Accordingly, it is seeking to bring together the industrial users, the manufacturers of machinery and equipment, and scientists working in related fields, for example machine tools and printers' machinery, to specify projects which are likely to be technically feasible and commercially desirable. Again, a thorough survey has been made of the field of superconducting materials and as a result proposals are now being considered for research both in universities and in industrial laboratories in the three main directions in which intensified effort is needed: fundamental research into the properties of thin films and the mechanism of operation of superconductors; the production of materials; and the construction and operation of superconducting magnets.

Little is said in this section of the report about relations with the National Research Development Corporation. In spite of the evidence of the competence of the present Council for Scientific and Industrial Research, there are wider considerations not touched on in, or within, the scope of this report to which regard should be had before a final decision is taken on the relations between the Council and the Corporation, or whether the latter should be placed within the Board of Trade, or be responsible to the Secretary of State for Education and Science. This present report should ensure that the achievements and the limitations of the Council for Scientific and Industrial Research are fairly assessed when decisions are taken as to the future organization of civil research; however, the report by no means disposes of the arguments of the Trend Report.

One other matter calls for comment. This year the Council gives a little more space to its activities and responsibilities in the field of technical information, where it considers a transformation is needed in the traditional means of encouraging the adoption of new ideas by industry. Nevertheless, there is only the briefest account of the National Lending Library for Science and Technology, now making loans at the rate of 5,500 a week, and making a survey of the use made of its resources and an examination of the resources of the 2,000 organizations (more than half of them industrial concerns) to which it lends. The Council admits that the problem of communicating to small firms that have no technical expertise is still largely unsolved, and that an efficient network of information centres requires far more resources than can be obtained from local industrial contributions. It has now assisted the Scottish Education Department to foster a scheme whereby the four central technical institutions in Scotland and the Royal College of Science and Technology, Glasgow (now the University of Strathclyde), appoint out of their general funds officers of senior lecturer rank whose main responsibility is industrial liaison in the surrounding area.

So much interest has been aroused by this innovation that the Council has made arrangements with the Ministry of Education under which existing regional technical information centres in England will be brought into the scheme during the next three years. The Council does not regard this step as offering a final solution to the problem, but it is of interest in indicating that the Council now realizes the importance of measures on a national scale if the library and information needs of Britain as a whole are to be served effectively. The same outlook can be seen in the new arrangements for the grant to the Association of Special Libraries and Information Bureaux (Aslib), under which the Council will be responsible for virtually the full cost, up to an annual limit of £20,000, of a programme of research into documentation problems of national interest, beginning with investigations on information storage and retrieval. This research grant is intended to enable Aslib to concentrate more of its resources on longer-range investigations and to build up a strong research team, and is independent of a second grant to the Association for other activities. The Council also concurs in the view of the Advisory Council on Scientific Policy as to the serious neglect of documentation research in Britain. It would be reassuring as well as welcome if both Councils showed a keener awareness of the way in which the various aspects of library development and information services are interlocked in Britain. Moreover, it would be pleasing to find that they were fully alive to the significance of the work of the Committee on Libraries appointed by the University Grants Committee and the Ministry of Education, of the early development of the new National Reference Library for Science and Invention, and of the development of the public libraries under the Public Libraries and Museums Act which received Royal Assent on July 31.

FINANCING MEDICAL RESEARCH

THE Office of Health Economics was founded in 1962 by the Association of the British Pharmaceutical Industry to undertake research to evaluate the economic aspects of medical care, investigate from time to time other health and social problems, collect data on experience in other countries, and publish relevant results, data and conclusions. The Office has now issued a paper* which puts the expenditure on medical research in the United Kingdom in 1961–62 at about £24 million, with a further £3 million for providing new buildings for research. More than half this came from Government funds, one-third represents the research expenditure of the pharmaceutical industry in Britain—about £7·8 million, compared with £2·8 million in 1953—and the balance is made up by medical research charities and trusts, such as the British Empire Cancer Campaign or the Wellcome Trust. Medical research accounts for about 4 per cent of the total expenditure on research and development in the United Kingdom; about 3·5 per cent of both Government and industrial research expenditure is devoted to medical ends.

There was a rapid increase in expenditure on medical research in Britain in the 'fifties, that of the Medical

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* *The Finance of Medical Research.* Pp. 32. (London: Office of Health Economics, 1964.) 2*s*.

Research Council and of the pharmaceutical industry increasing more than three-fold, and expenditure by trusts and foundations probably even more rapidly. Expenditure by the Medical Research Council expanded faster than the expenditure on the National Health Service, and that of the pharmaceutical industry rose much more rapidly than the industry's total sales or sales to the health services. The rate of expansion in research expenditure by the Government and by industry appears to be slackening since the early 1960's. Half the medical research charities in Britain were founded or reconstituted during the 1950's, and the trend appears to be continuing. Eight charities were responsible for £1·8 million of the total expenditure on medical research from private sources of just more than £2·3 million in 1962 (there was a further £1·6 million for buildings)—a figure which does not include direct donations from the public to universities or other medical research units—and, of this, £1·2 million came from the British Empire Cancer Campaign. Both the Imperial Cancer Research Fund and the National Fund for Research into Poliomyelitis contributed £400,000, and although the expenditure on research by the remaining charities is small, it increased from £128,000 in 1961 to £208,000 in 1962. The Mental Health Research Fund also increased its expenditure on research, in spite of a fall in income.

The distinguishing feature of these charities is that they approach medical research in terms of diseases rather than along disciplinarian lines such as endocrinology or pathology as in the universities, or by the pharmacological investigation of chemical substances as in the pharmaceutical industry. This varied approach gives them an important place in medical research and, although the magnitude of their contribution is not great in specific fields, its marginal effect can be significant. The paper instances research into multiple sclerosis, where, although voluntary societies have supported only 1 per cent of the effort, this 1 per cent has permitted a few research workers to work intensively and draw together the research effort in this field.

Of the major trusts concerning themselves with the medical field, namely, the Wellcome Trust, the Nuffield Foundation, the Nuffield Provincial Hospitals Trust, the Leverhulme Trust and the Wolfson Foundation, the latter two tend to concentrate on providing buildings for general medical research purposes, rather than financing the conduct of research. However, it is worth noting that almost half the £2 million allocated by the Wellcome Trust in 1960–62 was for new buildings for established departments with research reputations, and £450,000 of the £647,000 allocated by the Nuffield Foundation in 1961–62 was for buildings. Both the trusts and the charities have a crucial advantage over Government agencies in the flexibility of their support, both in form and in direction, and the paper suggests that the volume of private benefaction in the United States may have been an important factor in the lead established there in medical research.

Medical research expenditure in the United States for 1961 is estimated at about £370 million, and at the beginning of the 1960's it amounted to 3·6 per cent of the expenditure on health, compared with an expenditure in Britain on medical research of 2·7 per cent of the costs of the National Health Service. A total expenditure on medical research in 1961 of £35 million, instead of the £27 million, would have been needed to reach the ratio of medical research expenditure to all health expenditure prevailing in the United States, and, to match the pace of expansion in that country, this would need to rise to £5 million by 1963. Furthermore, the expenditure on medica research in the United States in 1963 was almost half the total cost of the National Health Service, but the paper points out that, whereas the latter places the main emphasis on satisfying present-day needs, in the United States the Government's responsibility for the health of the community is discharged primarily through research aimed at long-term improvement.

Precise comparisons of national expenditure on research are difficult and can be misleading, and probably the value of this survey lies rather in its analysis of the structure of the finance of medical research and its assessment of the several factors that determine not simply the scale but the objectives of research. The mere fact that the pharmaceutical industry in the United States spends some £81 million on research, or ten times the amount spent by British industry, has little meaning apart from such further analysis. The share of support between Government and industry is very similar in both countries: the most important difference in the sources of support is the larger proportion coming from charities and trusts in the United States—13·8 per cent compared with 9·7 per cent in Britain. Furthermore, since the beginning of the 1960's, the Federal Government has been increasing expenditure on medical research at almost twice the rate of increase of Government expenditure for this purpose in Britain.

This is particularly relevant in considering the expenditure on medical research of the pharmaceutical industry itself, and in noting the tendency for such expenditure by British industry to slow down its rate of expansion in the 1960's. It is also noted that this has been attributed to the greater economic risks which the industry faces as a result of the erosion of patents and the general discouragement of the prescribing of branded products, from which the pharmaceutical firms undertaking medical research draw much of their revenue. The section which discusses the economic problems of industrial research is one of the most useful in the paper, for it brings out the issues involved in the general question of research and economic growth and the weakness of the patent protection which the products of medical research really enjoy. Scientific knowledge, research and innovation have yet to find a place in the framework of economic analysis, and the economic model of a firm based on marginal costs and returns has little relevance in understanding the problems and growth of science-based industry.

The present pharmaceutical industry is principally a creation of research, but research is not a direct cost of production: it is an overhead cost, and marginal analysis tends to ignore the implications of overhead expenditure. The paper explains clearly the difficulties that arise in considering the impact and place of research, and particularly the nature of research overheads, which have been consistently ignored in recent debates in the House of Commons when the supply of drugs to the National Health Service has been under consideration. Little appreciation was then shown of the greater risks of losing the advantages that the patent system was intended to afford in support of research and which the holder of a patent originating from pharmaceutical research faces through his greater liability to compulsory licence.

Here again the paper brings to the forefront the real problem. It is true that the magnitude of the financial support for medical and allied research is a key factor in determining the speed of medical progress, but the financial backing is by no means the only factor. Moreover, while inadequate support may limit the rate of advance, an increase in funds does not necessarily lead to an acceleration. The intractable character of some problems may mean that advance depends on progress in other fields, not obviously related, and even so, new understanding makes no impact on health until it is translated into new medical procedures.

The paper does well to point out that under the National Health Service there is now no danger that the public will be deprived for economic reasons of the benefits of medical research, a danger which the safeguard of compulsory licensing in the patent system was originally intended to avert. There is, on the contrary, the question how far the system now affords sufficiently effective protection to ensure a flourishing and progressive pharmaceutical industry in Britain, but behind this lie the more fundamental questions asked in this paper: how much should be spent by the Health Service on medical goods, and where does the balance of public advantage lie? Does it lie in stimulating medical research or in obtaining short-term economies in National Health Service expenditure?

These questions dig at the very roots of the issues concerned with the relations between the research-based industries supplying medical goods and the Health Service. Public accountability for expenditure has tended to emphasize the Government's present responsibility at the expense of the long-term commitments with which the Federal Government is primarily concerned in the United States. Moreover, appraising the economics of these industries in traditional terms, with excessive emphasis on costs of production, and failing to take into account the relation between research and production, only aggravates the problem. Nor does it help to ignore the implications of the use of trade-marks or brand names by industrial firms to protect their discoveries. To the inventor these also are part of the means of financing further research, and some 95 per cent of branded medicines prescribed under the National Health Service are manufactured by companies undertaking major research programmes. While some of these products are not the results of present-day research, all carry a share of its cost.

There is no simple way to judge whether Britain's present expenditure of some £27 million on medical research is either too low or too high or approximately right. There can be no objective standard for the right level for expenditure on medical research. This arises from the nature of research. What should be remembered is that the progress of medical science is a dominant factor affecting expenditure on the National Health Service, but the motive force of this progress is not economic. Where research is financed through revenue from the Health Services, the search for economies can impede research. In recent years finance for medical research which does not come from sales to the National Health Service has risen faster than that which does.

The conflict between the claims of present-day responsibility for health expenditure and long-term responsibility for the health of the community was implicit in the discussions in Parliament over the costs of drugs. Here it is brought out explicitly, with the warning, too, that depression of one sector of medical research may lead to false economies. When the question of the costs of medical care predominates, there may be a strong argument for encouraging lines of research which combine lower costs with more effective care, and the paper instances, as a promising field, lines of investigation making feasible domiciliary rather than hospital treatment.

The final assessment of the level and direction of medical research must depend on the broad issues of health and welfare of the community, not on narrow economic considerations. The level, moreover, may be adequate, but the allocation of resources may be poor, and this is at present tied closely to the system of financial support. We should be asking whether the right opportunities are being taken, whether funds at present spent on cancer or poliomyelitis research might return greater benefit if directed to the field of mental health, and again, whether if so much research is pursued along the lines of medical disciplines there may not be excessive concentration on the therapeutic rather than the preventive aspects of medicine. The paper does not pretend to give the answers to the searching questions which it asks, but it puts the whole problem of medical research into a perspective which should facilitate realistic and reasonable discussion.

FIBRE STRUCTURE

Fibre Structure

Edited by Dr. J. W. S. Hearle and Prof. R. H. Peters. Pp. xxv + 667. (Manchester: The Textile Institute: London: Butterworth and Co. (Publishers), Ltd., 1963.) 126*s*.

FOR the fifteen years since it was first published, Preston's *Fibre Science* has been a standard text in university courses in textiles. In recent years, however, its usefulness has been diminished by progress in the development of new fibres and in the understanding of old ones. The time is ripe, therefore, for a replacement, and this book on *Fibre Structure* is designed to that end. The general scheme of the new volume is similar to that of the old (it comprises a number of separate contributions which are, in fact, based on lectures delivered in a course at Manchester in 1959), but in one important respect there is a change. *Fibre Science* was aimed at the ordinary undergraduate or non-specialist; to a large degree it retained the philosophy of Astbury's *Fundamentals of Fibre Structure* that the basic sciences, as well as their special application to fibres, were unfamiliar to the reader. This volume is, in general, more sophisticated, and should appeal rather to honours students or postgraduates.

The title phrase is taken to cover not only molecular and fine structure but also grosser features of morphology and external characteristics, and the book is thus divided into three sections dealing with texture on three different levels. The first section opens with a discussion by Sharples of chain configuration in cellulose solutions and the possible chemical inhomogeneity of cellulose. In connexion with this latter topic I noted one of the few instances of discrepancies between different contributors, for Sharples can accept no more than about 1 per cent of non-glucose sugar in cellulose hydrolysates, whereas Preston, in a subsequent chapter, cites one source of what he defines as cellulose which contains as much xylose as glucose. The other chapters in this section deal with synthetic polypeptides and proteins (Bamford and Elliott), with nothing new but giving a good review, particularly of the chemistry and physics of the polypeptides; and with synthetic fibre-forming polymers (Goodman) and tactic polymers (Gordon), two chapters which I thought exceptionally good. My only adverse criticism of this section on molecular structure, and I hope that this is not tendentious, is that it would have been nicely rounded off by a chapter on wool chemistry. Certainly those (there are still a few of us) who regard the study of wool as something of a cult will agree that in this one respect *Fibre Science* surpasses its successor.

The second section of the book is, perhaps, the most important, since it deals with topics which are more controversial. It starts with an introductory chapter (Hearle) of a historical character, which surveys the development of micellar/fibrillar hypotheses and describes the author's own fringed fibril concept. A feature of this chapter is the attention directed to the ideas of Kargin and his school; but, in trying to be fair, Hearle has been too objective in his treatment, quoting extensive extracts from the Russian papers without criticism. In particular, to offer to students without comment the notorious fragment "Electron microscope investigation of cellulose and its ethers reveals that they are amorphous and that the entire electron diffraction pattern is determined by the ordered arrangement of atoms in the molecule of cellulose and this gives evidence of the absence of strict order in the mutual distribution of their molecules" is distinctly unwise. In my opinion, the apparent unorthodoxy of Kargin's views arises largely out of a lack of agreement as to the proper definition of such terms as "crystalline", "amorphous", and "oriented".

The chapters on fine structure in plant fibres (Preston) and animal and man-made fibres (Sikorski) emphasize the part which electron microscopy plays in structural investigations nowadays; since both emanate from Leeds it would be improper for me to do more than mention their scope. Preston's contribution is largely based on his own work on algae and wood tracheids, and Sikorski's does something to redress the comparative neglect of keratin in previous chapters. Treloar (the non-crystalline state) and Keller (crystallinity) have complementary topics. Treloar deals somewhat briefly with mechanical properties, with a good introduction to transition phenomena. Keller gives a very readable account of a fascinating field of study only marred by a teasing tendency to stop a discussion with the apology that space does not permit more. He is, of course, chiefly concerned with crystallization from the melt or from solution, where the evidence for folded chains is now conclusive; the phenomenon of fibrillation in drawn fibres he regards as one which illustrates the inadequacy of our knowledge of fibres at present, an opinion which is echoed by Thompson in a later chapter when he notes that ". . . the final fibre structures are very similar whether crystallization occurs first at spinning or is delayed until drawing".

The remainder of the book deals with grosser structural characteristics, surface and fibre morphology, skin and core effects, and other properties which are important in end-product use or in the technology of fibre manufacture. It also includes, as a welcome luxury, chapters on glass (Jellyman) and asbestos (Whittaker) fibres.

There is no doubt that *Fibre Structure* will exert a very considerable influence on the teaching of fibre science at the higher levels for a long time, and the standards of topic and presentation are such that this influence will be mostly for the good. It is a pity, therefore, that the editorial introductory chapter should fall below the general level of excellence; it is here that I noted the few examples of inadequate proof reading (dicarboxylic acids $HO(CH_2)_n$ COOH on p. 5, wrong formulæ III (p. 6) and VI (p. 8); and if II (p. 6) is not wrong it seems particularly inapt for student consumption). There are also one or two examples of factual error, particularly in references to keratin; but perhaps more disturbing, since we often grumble about the standard of student English, is the number of inelegances in the editorial English, of which one example will suffice: "The disposition of the cells varies with the fibre and may be peripheral, as in mohair, or composed only of one kind". Having just finished marking degree scripts, I am in no mood to stomach this sort of thing in a text-book! H. J. WOODS

BRYOPHYTE BIOLOGY

The Structure and Life of Bryophytes

By E. V. Watson. Pp. 192. (London: Hutchinson and Co. (Publishers), Ltd., 1964.) 15*s*. net.

THE writing of a small book on a very large subject is not an enviable task; it is a task, however, which Dr. E. V. Watson has fulfilled and fulfilled admirably. In spite of the fact that this is one of the "University Library" series, it may be recommended whole-heartedly to other than university students—a tribute in itself in days when 'student' and 'university student' are often thought of as synonymous, and books so tailor-made for syllabuses as to deter the 'amateur' who learns for the pleasure of learning, and whose existence is frequently ignored. Dr. Watson obviously enjoys his subject, and thus writes enjoyably. Abundant references for those who wish to take any point further are skilfully interpolated without losing the thread of a discussion or the flow of reading matter.

The title of the book gives a true idea of its contents, and every aspect of bryophyte biology is dealt with. Taxonomy, physiology, ecology . . . all disciplines are given their rightful, equal place, and Dr. Watson takes a thoroughly balanced view. As with most modern biological investigations, the phylogenetic aspect is stressed, though some might consider the opening sentence of the concluding chapter—"The gross morphology of a bryophyte is chiefly of interest in an evolutionary context"—to be going a bit far. Few will quarrel with the statement that the clue to phylogeny is to be found in the aberrant (taxonomic entities); rather might this be extended to the downright teratological. It is good to see (p. 87) natural relationships stressed against technical characters even of the sporophyte. Too often natural genera have been split on such characters (*Tortula*, *Polytrichum*). On the other hand, through lack of them, even the family of a well-known species, for example the plant known as *Trichostomum sinuosum*, may remain somewhat in doubt.

It may be noted (apropos of the differences between *Calobryum* and *Haplomitrium*, p. 45) that Berrie (*Proceedings of the Linnean Society of New South Wales*, 87) has already aired Schuster's conclusion that the distinctions between these two genera are invalid. *Orthotrichum* does not always have the peristome teeth reflexed (p. 86). Of the mosses with 'amphigastria', the Rhacopilaceae simulate hepatics rather more than the Hypopterygiaceae. I myself feel that the number of bryophytes found in the tropics and temperate zones of both hemispheres will be higher than that of flowering plants as the monographic revisions so much desired by Dr. Watson and all bryologists are accomplished (p. 156). Unfortunately, since "bryophytes are unlikely to achieve great economic importance" (p. 175), these revisions are likely to remain as pathetically small as the number of full-time bryo-taxonomists. . . . Practically cosmopolitan species are not confined to the 'weedy species'. *Campylopus introflexus*, for example, has a tremendous area. The occurrence of the otherwise Australasian genus *Echinodium* in Macaronesia (p. 158) looks even more remarkable when we credit the area with *Gollania* (mainly far eastern) and *Tetrastichium* (South American affinities). Among the adaptations to xerophytic conditions, mention might have been made of strongly contorted leaves in the dry state, exposing chiefly the multistratose nerve to the influence of the atmosphere (for example, *Tortella nitida*, *Timmiella* spp.). The selection of material for the ecological section must have caused as much heart-searching as for that on geography, but it is a pity that mention could not have been made of Tutin's remarkable photographs of zonation of epiphyllous liverworts in the article by Richards in the *Manual of Bryology*.

These, however, are some of a legion of thoughts arising from Dr. Watson's book rather than criticisms. No doubt all its deservedly many readers will be similarly stimulated, and many postgraduate research workers will be grateful for the fruitful leads which the author supplies.

C. C. TOWNSEND

WHAT IS COMPARATIVE ENDOCRINOLOGY?

Comparative Endocrinology

Vol. 2. Edited by U. S. von Euler and H. Heller. Part 1: Invertebrate Hormones. Part 2: Tissue Hormones. Pp. xiii + 282. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 86s.

IN reviewing Volume 1 of *Comparative Endocrinology* (*Nature*, 201, 120; 1964) I emphasized the exciting phase into which the subject has now entered, but pointed out that it has not, in Britain, yet gained that recognition which is its favoured lot throughout the world. However, this comment does not apply to insect endocrinology which, largely due to Sir Vincent Wigglesworth, has been a recognized and dynamic field for three decades or more. It was the more surprising and disappointing to find that Part 1 of Volume 2, "Invertebrate Hormones", occupies only some 62 pages.

In the first section, L. I. Gilbert considers "Hormones Controlling Reproduction and Molting in Invertebrates". He deals mainly with arthropods and particularly with the insects. Crustacea are cursorily dismissed in five pages. In the part on insect metamorphosis, the Holometabolous insects are emphasized to the almost complete exclusion of the Hemimetabola. The text arouses the curiosity, but by lack of information fails to satisfy it. For example, ". . . thorough study of the subspiracular glands in the Cecropia silkworm during all stages of development and obtained evidence of secretion at several critical stages". The nature of the studies is not described, nor are the critical stages given. The addendum is very scrappy and incomplete and should not, in any event, occur in this type of treatise. As a whole, instead of being a critical synthesis of the present state of knowledge, this part is a collection of selected findings from which the reader will have difficulty in discerning any emergent pattern. The second part, which includes insects and is a description of gross structure of neurosecretory systems by F. G. W. Knowles, is too short and superficial to be of any real value.

Part 2 comprises a miscellaneous collection of articles, including those on kinins, heparin, lipid anions, 5-hydroxytryptamine, cholinergic and adrenergic neurohormones and histamine. Whether all these varied compounds should be classed as endocrine is in doubt and certainly not enough is known to justify their presence in "Comparative Endocrinology". It is the strength of the comparative approach that a variety of animals, especially those below the sub-class Eutheria, can be considered. By so doing, light is thrown on fundamental processes of wide applicability, in animals wherein there is greater ease of access and fewer complicating factors.

A rag-bag of so-called 'tissue hormones', knowledge of which stems almost completely from a few mammalian laboratory animals, certainly makes no contribution to 'comparative endocrinology'. The articles might be considered useful if they urge comparative physiologists to look further. If this were the rationale of the editors it would have been justified if they had been included in Volume 1. As it is, Volume 2 serves comparative endocrinology ill in being neither particularly comparative nor unequivocably about hormones.

I. CHESTER JONES

NUCLEAR PHYSICS

Progress in Nuclear Physics

Vol. 9. Edited by Prof. O. R. Frisch. Pp. v + 310. (London and New York: Pergamon Press, 1964.) 90s. net.

THIS ninth volume deals with a variety of detection methods and with theoretical and experimental investigations, mostly, but not wholly, in the field of high-energy physics. Though research, in most of the matters considered, is still moving fast, a great deal of data exist, and the present book provides valuable information on the subjects considered.

The first two articles are devoted to electronic methods of particle detection, namely, "Spark Chambers" by J. G. Rutherglen, and "Semiconductor Counters" by G. Dearnaley. In the former the mechanism of spark production is fully discussed. A short description of some of the essentials of spark chamber operational techniques follows, and a number of valuable references are given for those wishing to set up complete systems. Of particular interest is the account given of the effects of clearance field on spark alignment, and of the assessment of track accuracy. In the second article the essential characteristics of the depletion layers of semi-conducting counters are outlined. The relative merits of this type of counter are well exemplified, in particular the energy measurement of short-range, slowly moving and heavily ionizing particles. Some of the disadvantages are also referred to, such as the very high noise background, experienced in fast rise time work, and the methods of combating this by fast–slow coincidence methods.

Then there is an article on "Theoretical Techniques of High Energy Beam Design" by N. M. King. It is often difficult to have at one's disposal a physical account of the principles of beam design, since beam design manuals often list only the computational parameters. This article provides such a physical description admirably. Details are given of the trajectories and phase space parameters of the particle beams for quadrupole and main bending magnets, and of fringe field effects. The properties and difficulties of electrostatic separators are outlined.

The "Introduction to the Structure Analysis of Collision Amplitudes" by R. J. Eden deals with the development of dispersion theory and the relation of its singularities to perturbation theory. Starting with derivations from causality, it leads to a discussion of the analyticity properties which hold in collision problems. Some of these sections may not be found easy, as the author himself seems to fear, but there are many references available. Two important interaction problems are helpfully mapped out and provide some of the underlying steps of a full analysis. The role of Regge poles in asymptotic behaviour is briefly indicated.

This is followed by a detailed 100-page article on "The Interaction of Strange Particles with Nuclei" by E. H. S. Burhop, D. H. Davis and J. Zakrzewski. A valuable introductory account is given on the interactions of kaons and hyperons with nucleons, and on mesic capture. The generalizations made to nuclei provide data on neutron reactions and, of particular interest, on those yielding Y particles among the final states. But in the presence of the other nucleons, other processes such as internal absorption can intervene, and these are detailed. Hyperfragments are discussed separately and a useful short analysis is given of Λ-nucleon binding forces.

Finally, there is the article on "Electromagnetic Properties of the Muon" by F. J. M. Farley. It is a very comprehensive and exciting story of recent electromagnetic measurements leading to definite specifications of the spin, mass and, particularly, the magnetic properties of this particle, and raising throughout the intriguing question of muon-electron differences.

Throughout the book there are abundant references, though these end mainly in 1962. The print is of good size, and the book is a pleasure to read.

G. M. LEWIS

PROGRESS IN NUCLEIC ACID RESEARCH

Progress in Nucleic Acid Research
Edited by J. N. Davidson and Waldo E. Cohn. Vol. 1. Pp. xiv + 424. 93*s*. Vol. 2. Pp. xiv + 346. 88*s*. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.)

THE rapid advance of biochemistry has been accompanied by an increase in both the number and size of journals; if one had to read every paper written in the field, there would be no time left for research work. This is especially true of the central field of nucleic acids; the scientific literature in this field has been growing exponentially since 1947 with a doubling time of about two-and-a-half years. As a result of this rapid growth, specialization has increased greatly, and there is a real danger that the fragmentation of the field will hamper scientific advance. There is, therefore, a great need for publications in which specialists can summarize and critically assess new developments, and this is precisely the main aim of the editors of *Progress in Nucleic Acid Research*. Volume 1 contains eleven articles which cover a large range of subjects. F. J. Bollum discusses the role of primer in DNA polymerase reactions. He draws a careful distinction between two kinds of 'primer'. The first, shown by oligodeoxyribonucleotides for calf thymus polymerase, leads to chain extension; in this case the primer is incorporated into the product and acts as an initiator in a linear condensation polymerization. The second type of priming is the better-known one; here the DNA primer is used as a template and guides the structure of the replica. It is, however, still not clear whether the polymerases extracted from cells are indeed the enzymes which act in DNA replication in the cell. In the case of the *E. coli* polymerase of Kornberg, the polymerization proceeds in only one chemical direction, whereas we know that in the cell both chains of DNA are copied together in one direction. It is conceivable that the polymerases may be enzymes used to repair DNA in the cell and the real replication enzymes remain to be discovered. The fact that many polymerases have an absolute requirement for denatured DNA as a primer may reflect their repair function, and Bollum discusses the difficulties involved in constructing models of replication based on the enzyme experiments.

Two articles deal with RNA synthesis. R. M. S. Smellie gives a useful general review of the subject, and directs attention to a group of enzymes with very curious and as yet unexplained functions. For example, there is an enzyme which is responsible for the incorporation of UMP residues alone, and there are other enzymes which can form homopolymers of adenylic acid. The second article by Hurwitz and August discusses the biochemistry of the DNA primed-RNA polymerase, the enzyme which is responsible for the transcription of DNA into RNA. This article contains an excellent summary of the careful experiments done by Hurwitz and his collaborators. M. Grunberg-Manago writes on polynucleotide phosphorylase. This is a most valuable article, since it collects in one place all the essential findings about this enzyme.

The paper on messenger RNA by F. Lipmann reviews the background of this subject and also discusses the interaction of messenger RNA with ribosomes, and the possible mechanisms of protein synthesis. The details of this process are still not clearly understood.

The review of the coding problem by F. H. C. Crick is a clear analysis of the status of work in this field, and discusses both the genetic studies and the experiments using synthetic polynucleotides. It becomes clear that the genetic code may be cracked, but it is not yet broken, and much further rigorous work will have to be done to provide the definitive codon dictionary.

Bendich and Rosenkranz present "Some Thoughts on the Double-Stranded Model of Deoxyribonucleic Acid"; their main theme is that all is not yet solved. They suggest that DNA structures other than the classical Watson–Crick structure may occur in cells and they direct attention to the possibility that DNA molecules may be linked by amino-acids or small peptides. These ideas run against the main trends in this field, and the evidence on which they are based will have to be very carefully evaluated. They claim that in some viruses and spermatozoa the volume occupied by the DNA, if it were exclusively in the doubled-stranded form, is larger than the volume available. The table illustrating this contention on p. 226 contains much misleading information particularly in the cases of $T2$ and $S13$.

There are three articles on the structure and physical chemistry of nucleic acids. Marmur, Rownd and Schildkraut have written an excellent review of "Denaturation and Renaturation of DNA"; Spirin reviews the structure of RNA and Luzzati writes on the structure of DNA as determined by small angle X-ray scattering. Finally, A. Wacker reviews the work on molecular mechanisms of radiation effects.

The editors have set a high standard in this first volume of the series. They are to be commended for the international character of the book. Above all, they must be congratulated on their powers of persuasion: everybody knows how difficult it is to elicit review articles from busy investigators.

Volume 2 maintains the standards set by the editors in the first volume. It contains a very important article on chemical mutagenesis by D. R. Krieg. We now know that there are major difficulties in trying to identify the nucleotide changes at induced mutational sites. The degeneracy of the genetic code and the possibility that many amino-acid changes will not produce defective proteins make the genetic analysis almost intractable. In addition, we just do not know all the chemical changes produced by mutagens in DNA, and in many cases there is no justification for assuming that the major chemical reaction has anything to do with mutation induction. Kreig's article is very clear and balanced and includes an analysis of all the mutants of the $T4$ r_{II} genes.

The article by Cavalieri and Rosenberg challenges the importance of Watson–Crick base pairing in guiding nucleic acid copying. They propose an alternative scheme in which specificity in copying is given by stacking interactions between bases rather than by hydrogen bonding. The article is speculative.

H. Harris's article centres around the question of the stability of messenger RNA. Must unstable RNA always be called messenger RNA and must messenger RNA always be unstable? Harris emphasizes that there is a nuclear RNA in animal cells which appears to be unstable but does not appear to be transferred into the cytoplasm.

R. Markham writes on plant virus nucleic acids, and he critically reviews the role of the RNA as a messenger, and the reports claiming synthesis of infectious tobacco mosaic virus RNA.

The other articles in the volume deal with chromatography of oligo- and poly-nucleotides (Stahelin), the mechanism of action of aza-pyrimidines (Skoda), *s*RNA (Brown) and an analysis of the mechanism of RNase (Witsel).

S. BRENNER

Encyclopedia of Chemical Technology

By Kirk-Othmer. Vol. 3: B to Calcium. Second, completely revised edition. Pp. xvi + 927. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1964.) 338*s*.

FOR an encyclopædia of chemical technology to be useful to the chemist and chemical engineer in industry, university or in a research organization, it must be as near perfection as possible. To achieve this perfection can be no easy task, since over the past decade, chemical technology has expanded to unbelievable levels. The editors of "Kirk-Othmer", supported by a large team of expert authors and reviewers, are to be congratulated on providing a compendium of information of chemical technology that is as near to perfection as possible, up to the time of printing. Every article, expertly written, contains a mass of information of industrial methods, materials, processes, equipment and, where possible, information on storage, transport, health and safety aspects. In certain cases details are given of new industrial research processes that are both in operation and at the experimental stage.

The third volume of the revised *Encyclopedia of Chemical Technology* covers 43 articles ranging from "Bacterial Infections" to "Calcium Alloys". The subjects reviewed follow closely those chosen in the first edition. In general, new articles cover materials which over the past decade have become of increasing industrial importance. For example, there is an article on "Bentonite", a clay that displays strong colloidal properties, and which is put to a multiplicity of uses in the pesticide, paint, abrasive, detergent and food industries. Also included are "Optical Brighteners", an article that summarizes the various types of fluorescent organic chemicals that are of utmost importance to the manufacturers of detergents, plastics and textiles. The omission of a selection of trade names in this article is to be regretted. In the analytical field, there is a good review on "Bioassays", where special reference is given to underlying theories and calculations.

It is disappointing to note the omission of the collective article on "Building Materials", a subject that is of world-wide importance. Although no cross-references are given, one must assume that this omission will be partially remedied by the inclusion in later volumes of separate articles on individual materials. K. FIELD

Local Rings

By Masayoshi Nagata. (Interscience Tracts in Pure and Applied Mathematics, No. 13.) Pp. xiii + 234. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1962.) 83*s*.

THIS book presents an up-to-date account of a flourishing branch of abstract algebra, to which the author has made notable contributions. It is No. 13 in the series of *Interscience Tracts in Pure and Applied Mathematics*. After an introductory chapter on "General Commutative Rings" there are chapters on "Completions and on Multiplicities", followed by a chapter on the "Theory of Syzygies". The latter is a new theory, taking the place of homological methods. As a result, homological algebra is not used in the book and the author claims that his theory is simpler than that based on homology theory. Later chapters cover the theory of complete local rings, geometric local rings and Henselian rings and Weierstrass rings. In an appendix, there is a valuable historical account of many of the important results in the text; there is also a comprehensive bibliography.

The exposition is somewhat condensed, although it is systematic, and it is assumed that the reader is already familiar with a good deal of the necessary background, in abstract algebra. The book is, in fact, for the specialist, but since it is virtually the only account of the subject it will be indispensable to every mathematician doing research on the theory of local rings. It is perhaps a little disappointing that the book does not reveal more of the geometric motivation behind the subject, and the algebraist who wishes to link up with this will have to study independently the subject of algebraic geometry. L. S. GODDARD

Aspens

Phoenix Trees of the Great Lakes Region. By Prof. Samuel A. Graham, Robert P. Harrison and Casey E. Westell, jun. Pp. xiii + 272. (Ann Arbor: University of Michigan Press; London: The Cresset Press, Ltd., 1963.) 7.50 dollars; 50*s*. net.

THIS book is devoted entirely to the two aspens that occur in the Great Lake Region of North America, that is, the trembling aspen (*Populus tremuloides*) and the bigtooth aspen (*Populus grandidentata*), so named because of its deeply indented leaves. The trembling aspen is a very common and widely distributed tree in North America, where it reaches 100 ft. It is transcontinental in its range and occurs from northern Mexico or California to the frozen tundra of Alaska. It is the only Californian tree to do so. The bigtooth aspen is less widely distributed, occurring from Maine and southern Canada to Tennessee and North Carolina. These two trees are of great importance in the forest ecology of some parts of North America and it is pleasing to note that they have received such close attention and study. Would that other trees in other parts of the world that are important constituents of the native forests could receive similar attention.

Both these species have been cultivated in other countries, including the British Isles, where they have not shown great promise or grown as well as other North American species of *Populus*.

In this work, the two trees have been studied from all angles, with the main emphasis on their ecology. Pests, diseases, sylviculture and utilization—all figure prominently. In recent years the trees have become of more and more importance as sources of paper pulp. Some of the work of postgraduate students, who have worked on specific problems concerned with aspens, is incorporated in the book and is acknowledged, as is financial assistance gratefully received from the forest industry of Lower Michigan. F. N. HOWES

Vegetative Anatomy of Plants

By H. G. Burström and Camilla Odhnoff. Pp. 148. (Stockholm: Svenska Bokförlaget, 1964.) n.p.

IT is no mean achievement to write a good introduction to plant anatomy in a book of only 148 pages. It is true that the authors' task has been made easier by the omission of any reference to floral anatomy, but, even so, they have succeeded in presenting the essentials of the subject very effectively. The language is simple, technical terms are not needlessly numerous and the meaning of each is clearly defined wherever it is first presented. The book is clearly and adequately illustrated. The text is based on a course of lectures given as part of the curriculum for a Swedish M.Sc. degree. The authors' special hope is that it will give an adequate introduction to plant structure to those botanists whose interests are mainly in plant physiology. The subject is covered in four main sections dealing respectively with the plant cell, the forms of cells and their mode of origin, the histological differentiation (ontogeny) of the plant body and ecological anatomy. There is a selected bibliography of 240 titles and an index.

Although the brevity of the book inevitably imposes limitations on its content, it is an admirable text for the type of readers for whom it is intended. It is to be hoped that the spelling will be more carefully checked in future editions. C. R. METCALFE

LIFETIMES OF GEOMAGNETICALLY TRAPPED ELECTRONS OF SEVERAL MeV ENERGY

By JAMES A. VAN ALLEN

Department of Physics and Astronomy, State University of Iowa, Iowa City

THE loss of charged particles from trapped orbits in the magnetosphere is a complex physical process, which is not characterized by any simple law. Hence it is not possible, in the strict sense, to define a single parameter which characterizes their lifetime. Nevertheless, there is now a large body of observational knowledge of the monotonic decrease with time of the intensities of trapped particles following a natural or artificial injection of such particles. The most definitive knowledge on the loss of energetic electrons comes from investigations of the time history of nine artificial radiation belts which have been produced by U.S. and U.S.S.R. high-altitude nuclear explosions in 1958 and 1962. In Fig. 1 is shown a sample set of curves of intensity versus time for the fission-decay electrons from the *Starfish* nuclear explosion of July 9, 1962, as observed with satellites *Injun* I and III. The counting rate is that of a small, heavily shielded Geiger tube (designated *SpB*) which detects the trapped electrons via their intermediate bremsstrahlung in the shield. The response of the *SpB* detector[1] is due primarily to electrons the energies of which lie in the range 1·5–5 MeV. The curves of Fig. 1 are for a particular value, 1·25, of the McIlwain magnetic shell parameter L and for a succession of values of the scalar magnetic field B within that shell. It is clear that there is a relatively rapid decrease of intensity within the first $\sim 1{,}000$ h after injection and that this transient period is followed by a much longer one characterized by an approximately constant slope on a semi-logarithmic plot. It is noteworthy that the value of this slope is independent of B.

For $L \lesssim 1·25$, an essentially complete theoretical understanding of the time-decay history of the *Starfish* radiation belt has been achieved by Walt[2] in terms of scattering and energy loss in the atmosphere.

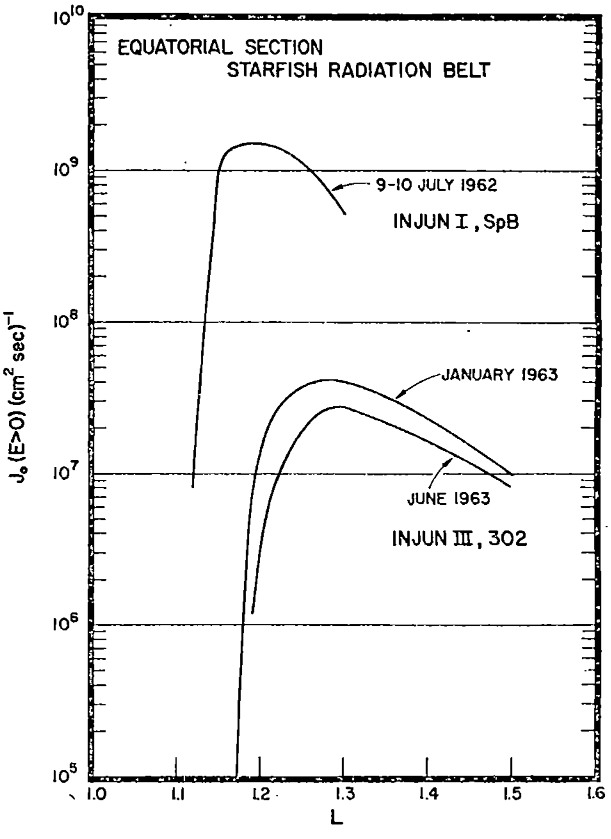

Fig. 2. An abridged summary of the time-history of the *Starfish* radiation belt. The ordinates are in absolute units

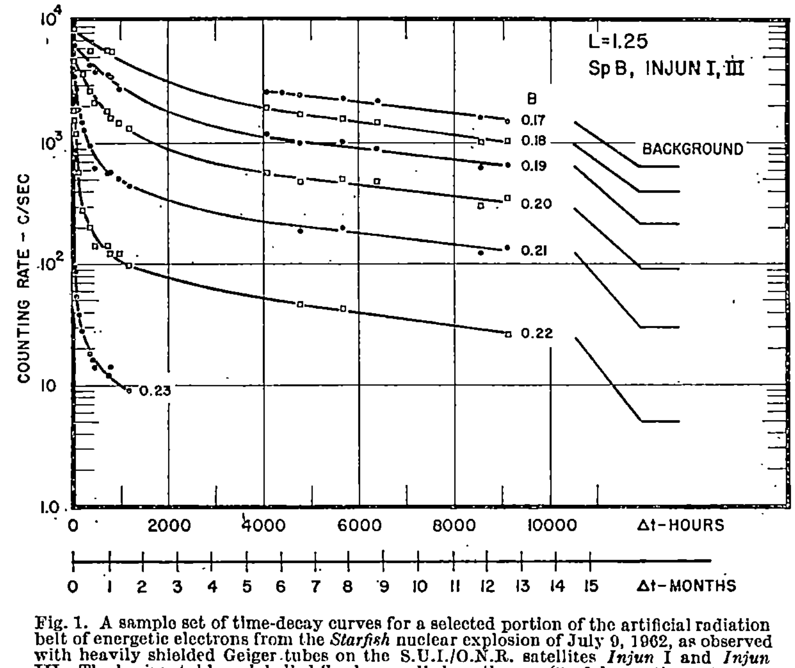

Fig. 1. A sample set of time-decay curves for a selected portion of the artificial radiation belt of energetic electrons from the *Starfish* nuclear explosion of July 9, 1962, as observed with heavily shielded Geiger-tubes on the S.U.I./O.N.R. satellites *Injun* I and *Injun* III. The horizontal bars labelled 'background' show the pre-*Starfish* counting rates corresponding to the several members of the family of curves. Δt is the elapsed time after the detonation

For the purposes of this article the L-dependent quantity τ in $\exp(-\Delta t/\tau)$ is termed the 'apparent mean lifetime' of ~ 2-MeV electrons during the main decay-phase, that is, for $\Delta t > 1{,}000$ h (Fig. 1).

The overall decay of the *Starfish* radiation belt is shown in Fig. 2.

An example of the decay of the intensity of energetic electrons in the composite radiation belt produced by two Soviet nuclear detonations on October 22 and 28, 1962, is shown in the lower panel of Fig. 3, taken from *Explorer* XIV observations by Frank, Van Allen and Hills[3].

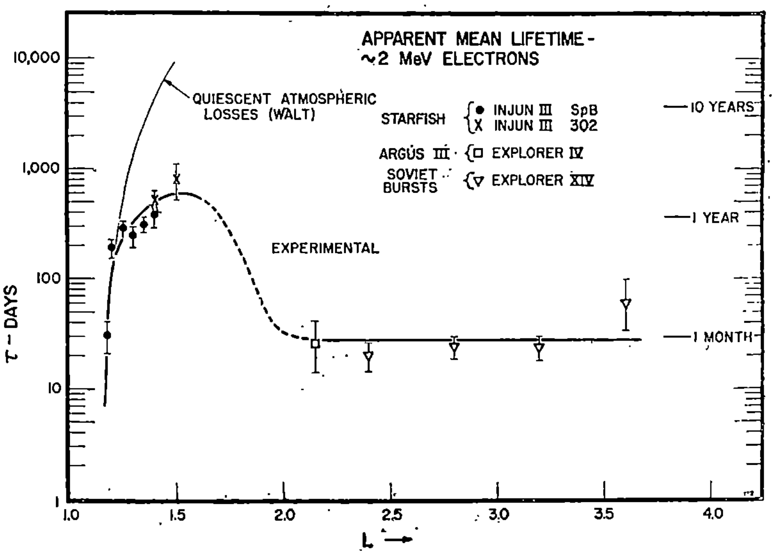

Fig. 4. The L-dependence of observed values of the 'apparent mean lifetime' of ~ 2-MeV electrons from two U.S. and two U.S.S.R. nuclear detonations at high altitudes. Note that the theory of quiescent atmospheric losses is adequate for $L < 1·25$ but departs heroically from the experimental curve for $L > 1·25$ (see text)

Fig. 4 gives a summary of the L-dependence of experimental values of the apparent mean lifetime of ~2-MeV electrons. The general nature of this dependence has been known for some years[4] from investigations of the time variations of the intensity of energetic electrons in the natural radiation belts. For $L \lesssim 1·25$, the loss of electrons is dominated by 'quiescent' scattering and energy-loss processes in the outer atmosphere. For greater L values such processes become of relatively trivial importance and the loss of particles is dominated, presumably, by electromagnetic and magnetic processes in the geomagnetic field as it is subjected to perturbations by terrestrial sources (for example, whistlers, and other very-low-frequency phenomena) and by erratic buffeting by the solar wind at its outer boundary. The observed lifetime of ~2-MeV electrons has a maximum value of about 2 years at $L = 1·5$, then declines rapidly to values of the order of one month for L from 2·0 to 3·5. Examination of time variations in the population of energetic electrons of similar energy in the natural radiation belts[3,5] shows that, even during periods of relatively low solar and geomagnetic activity, the effective lifetime declines at greater L to values of the order of a few days at $L \sim 8$. Beyond $L \sim$ 8–10, durable trapping is not possible. Fragmentary investigations in the region of the 'slot' between the inner and outer radiation belts ($L \sim 1·8$) have shown that there are large, sporadic time variations there. This fact suggests that the effective value of τ may fall considerably below the dashed portion of the experimental curve of Fig. 4 in the range $1·6 < L < 2·1$.

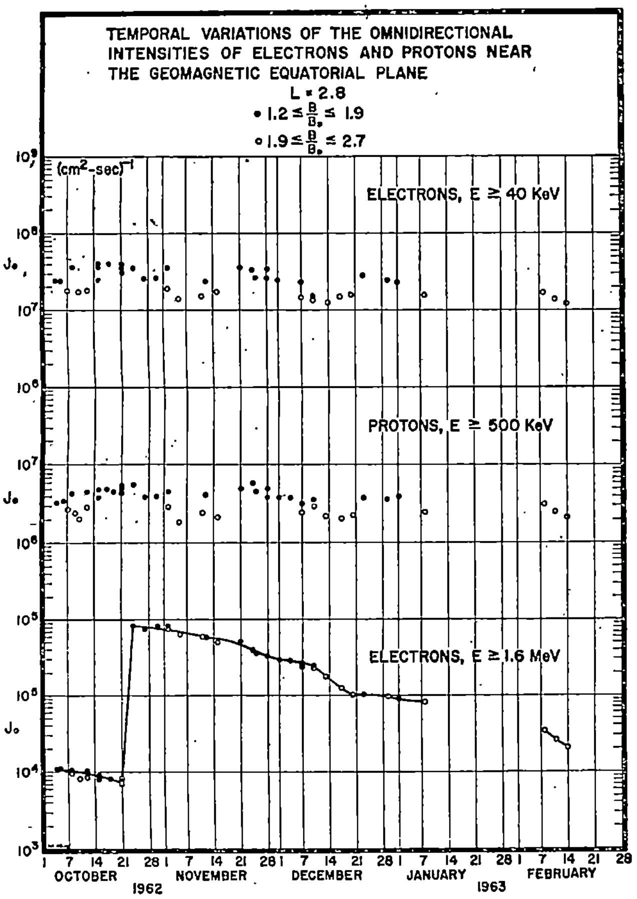

Fig. 3. In the lower panel is shown the time-decay of the intensity of energetic electrons from the U.S.S.R. nuclear detonations of October 22 and 28, 1962. Note, in the upper two panels, the negligible effect on the intensities of electrons of energy greater than 40 keV and of protons of energy greater than 500 keV (ref. 3)

Finally, it is noted that there is no established theory of the loss of trapped particles for $L \gtrsim 1·25$. The observational evidence suggests that the time-history of the intensity of electrons at the greater L values consists of a sporadic sequence of discontinuous perturbations, some of which result in the loss of particles and some of which result in replenishment[4,5]. The overall tendency is for specifically injected electrons to lose their identity and to be assimilated into the general mêlée. Hence, effective values of τ for $L \gtrsim 1·25$ are almost certainly dependent on the general level of solar activity and on the consequent level of geomagnetic activity, τ being expected to be less during periods of high solar activity. An incidental aspect of this line of thought is the tentative 'explanation' of the outer boundary of the inner-zone-distribution of energetic protons ($E \gtrsim 20$ MeV) which has been suggested previously[1,4].

I thank the U.S. Office of Naval Research (contract *Nonr* 1509(06)) for its support and Drs. W. C. Lin and L. A. Frank for their assistance.

[1] Van Allen, J. A., Frank, L. A., and O'Brien, B. J., *J. Geophys. Res.*, 68, 619 (1963).

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[4] Van Allen, J. A., *Trans. Intern. Astro. Union*, 11, B, 99 (1962).

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INFLUENCE OF THE SATELLITE *Io* ON JUPITER'S DECAMETRIC EMISSION

By E. K. BIGG*

C.S.I.R.O. Radiophysics Laboratory, Sydney, Australia

A CONSIDERABLE amount of experimental and theoretical work has been performed on the bursts of radio emission emanating from Jupiter at frequencies of about 20 Mc/s since this radiation was discovered by Burke and Franklin[1]. It was realized by Shain[2] that the signals tended to recur at fixed intervals which could be associated with Jupiter's rotation. Subsequent work has fixed this period with considerable precision, and a co-ordinate system, known as System III longitudes, based on it, is now in use. The emission is thought to be associated with the motion of electrons in Jupiter's vicinity, the enhanced probability of radiation when particular parts of the planet face the Earth being due to the orientation of the planet's magnetic field.

Jupiter has four large satellites, of which the closest, I*o*, has a mass and distance from Jupiter almost identical with the mass of the Moon and its distance from the Earth. Since the Moon exerts at least some control on the Earth's magnetic field through the electrons in or beyond the ionosphere, it is reasonable to ask if I*o* influences the signals coming from Jupiter.

I thank Dr. James W. Warwick, who has made available to me his four-year catalogue of Jupiter events before its publication. In this catalogue is recorded the U.T. start and finish of each event, its frequency range, intensity on a scale of 1, 2 or 3, character and frequency drifts. In 1960 there were only 52 events recorded, but in 1961, 1962 and 1963 there were 240, 334 and 380, respectively. The last three years contain sufficient material for analysis by separate years and have therefore been exclusively considered.

To find whether Io has any effect on the emission, the following procedure was adopted. Time zero was taken as a moment, known as superior geocentric conjunction, when Io crossed the Earth–Jupiter line on the far side of Jupiter, and is given in the *American Ephemeris and Nautical Almanac* of the appropriate year. Each Jupiter event was then recorded in terms of elapsed time from the last zero. A table of 2,550 columns was then constructed (one for each minute of Io's period as seen from the Earth) and, for each minute during which a signal was observed, an entry 1, 2 or 3 corresponding to signal intensity was made in the appropriate column. The columns were then summed in consecutive groups of 10, the last group being adjusted for the fact that Io's mean period is only 2,548·5 min. The procedure gives more weight to the more intense events, but similar results are obtained if one considers only the frequency of occurrence.

The results for the individual years are shown in Fig. 1, and for three years combined in Fig. 2. While there are some differences between the years, there is a remarkably close correlation, particularly with the positions of the two largest peaks.

I*o* therefore appears to control the decametric emission which reaches the Earth to a surprisingly large degree. It is of theoretical importance to know whether the position of the satellite with respect to the Earth–Jupiter line or the Sun–Jupiter line is the more relevant. Seen from Jupiter the Earth's orbit subtends an angle of more than ± 11°, so that if the Sun–Jupiter line were used to

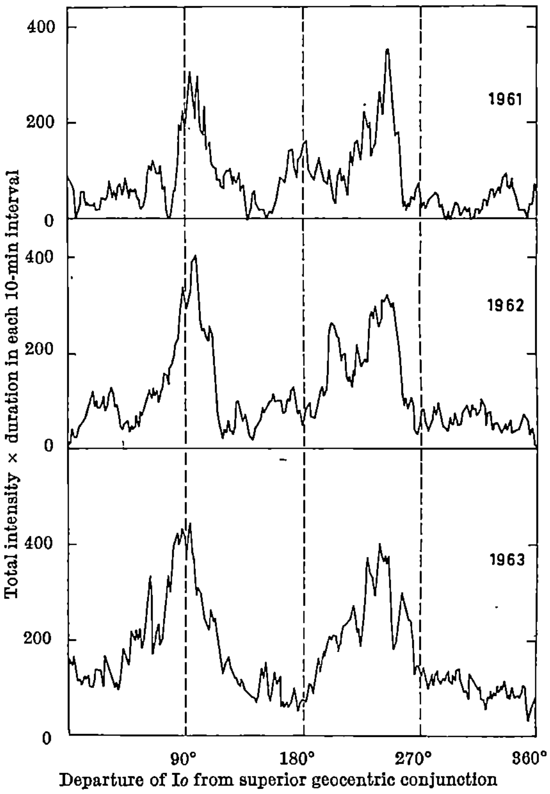

Fig. 1

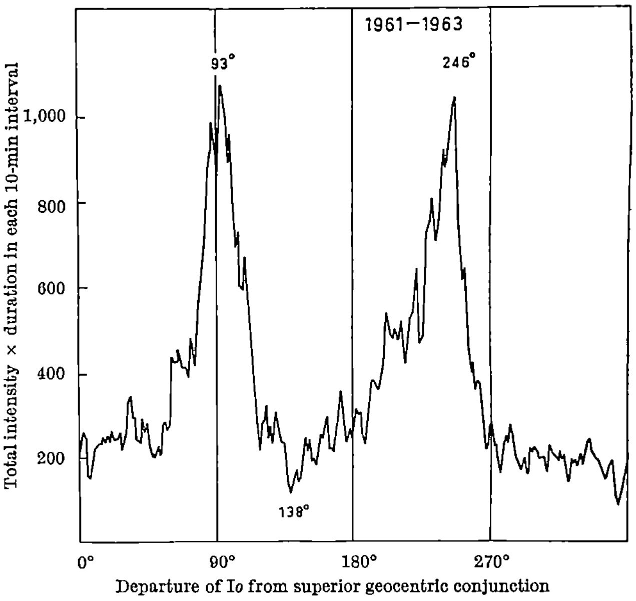

Fig. 2

Figs. 1 and 2. Dependence of Jupiter's decametric radio; emission on the position of the satellite I*o*

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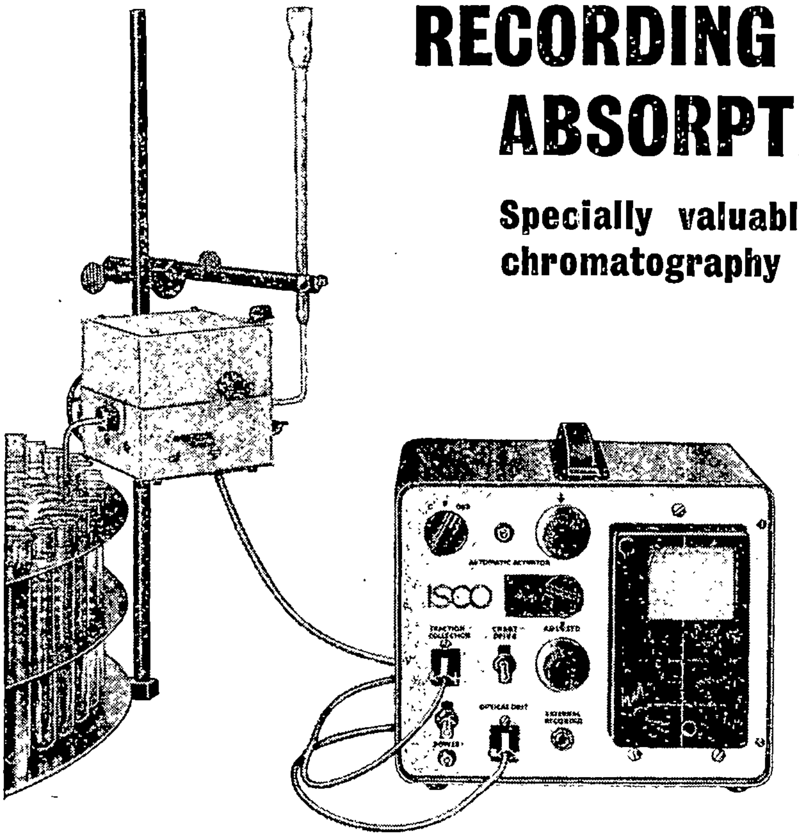

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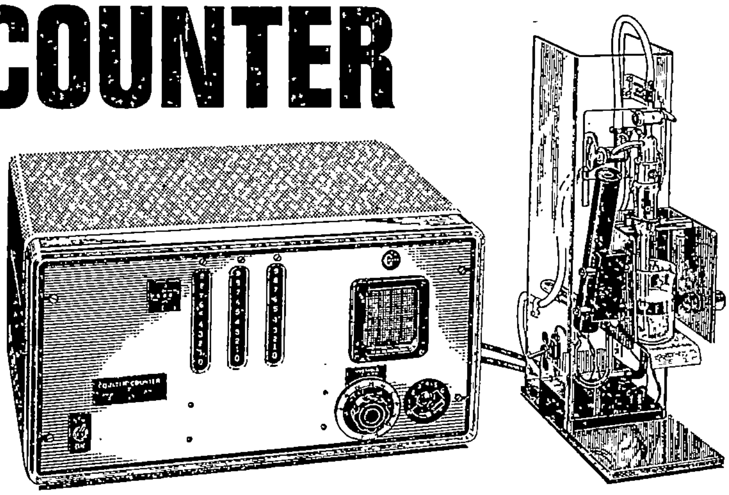

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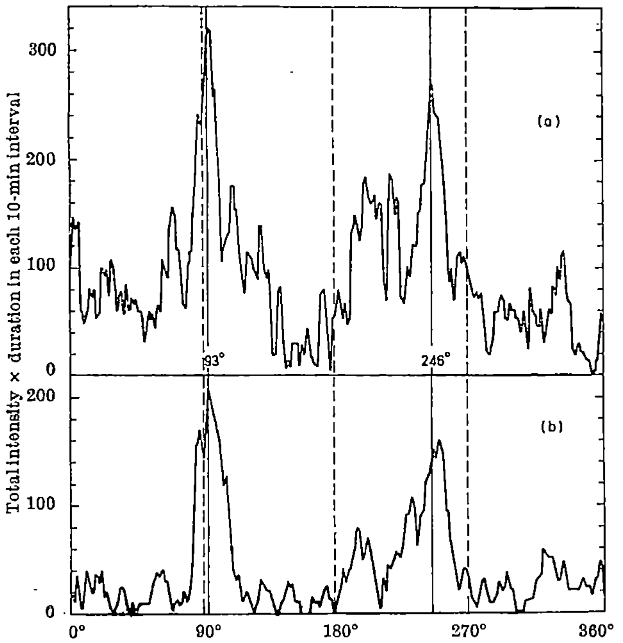

Fig. 3. The dependence of Jupiter's emission on the position of *Io* when (*a*) the Earth was ⩾9° to the east of the Sun as seen from Jupiter and (*b*) the Earth was ⩾9° to the west of the Sun as seen from Jupiter

define *Io*'s position, the peaks of Fig. 2 could be shifted by as much as ± 11°.

To test this, all cases when the Earth was more than 9° to the east of the Sun (Fig. 3*a*) were compared with all cases when the Earth was more than 9° to the west of the Sun (Fig. 3*b*). Clearly, the two highest peaks do not change their positions appreciably. Hence, the effect as detected on Earth depends on the position of *Io* relative to the Earth–Jupiter line.

Table 1
Co-ordinates of:

Io	Jupiter	Relative intensity
85°	115°	940
85°	145°	860
235°	225°	860
235°	335°	660

The apparent control of the emission by *Io* is far more complete if only events showing emission at frequencies ⩾ 30 Mc/s are considered. This is illustrated by Fig. 4, which is a re-analysis of the data in Fig. 2, selecting only events at the higher frequencies.

The relation of this analysis to earlier work on Jupiter's rotation is obviously important. A grid was constructed in which the vertical lines represented Jupiter's System III longitudes and the horizontal lines represented *Io*'s angular departure from superior geocentric conjunction. (*Io*'s orbit is circular and its velocity uniform except for small perturbations, so that times and angles are interchangeable.)

Each disturbance was plotted as a line on this grid and the total line-length in each 10° × 10° square calculated. Intensity '2' lines were counted twice, and intensity '3' lines three times. The results are shown in Fig. 5, the main features being confined to two quite limited portions of the map. The co-ordinates of the squares having highest intensities and their relative intensities are given in Table 1.

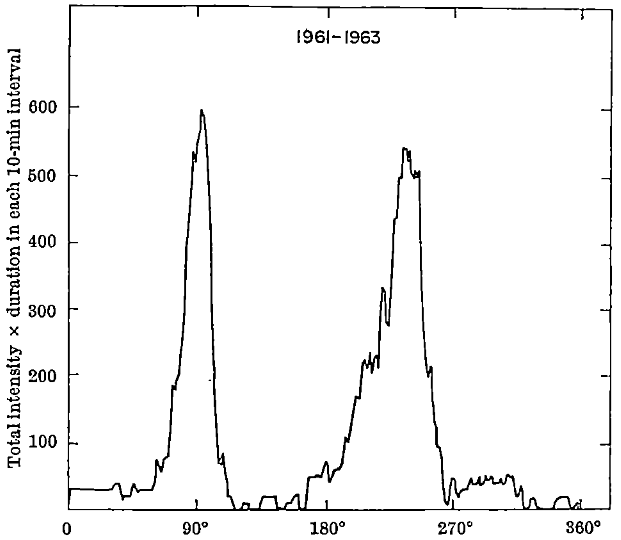

Fig. 4. Dependence of Jupiter's emission on the position of *Io* when only cases having top frequencies ⩾30 Mc/s are considered

Fig. 1 shows that, for the 600 cycles of *Io* represented, the highest peaks shift in position by less than 20°. The 2,548·5-min period chosen is therefore accurate to better than one part in 10,000, making it unlikely that the result is fortuitous. It cannot be the result of a direct coincidence with Jupiter's period of rotation, the ratio being 4·28 : 1. Warwick, in private discussions, has suggested that, because observation periods are spaced by about 24 h, the third harmonic of 24 h may beat with Jupiter's rotation period to give the observed effects. This would require a mean spacing of observation periods of 24 h 7·5 m, about 1 per cent longer than the mean spacing of 23 h 56 m. While this and other possible beats require close examination, it will obviously be difficult to match the sharply defined peaks which are observed.

There is a possibility that Satellites II, III, IV (Europa, Ganymede and Callistro) contribute to this effect; but in view of the approximate 1 : 2 : 4 : 8 ratio of the periods of the four satellites, it would require a more sophisticated treatment than that given here to reveal the extent to which they are involved. It is at least certain that *Io*'s

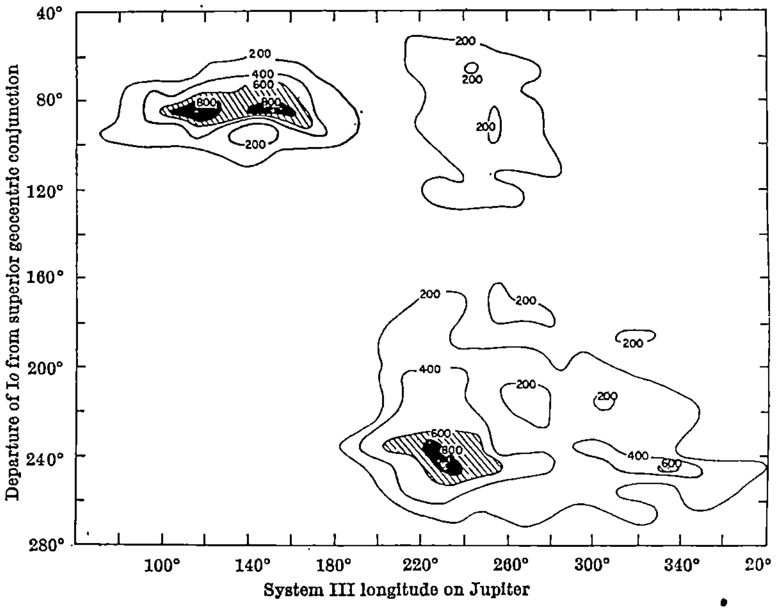

Fig. 5. The relationship between the position of *Io* and the orientation of Jupiter for the reception of decametric emission at the Earth

period has much the strongest influence. The very small Satellite V, which is the closest satellite to Jupiter's surface, has a period within 0·1 per cent of half the mean period between observations and consequently all parts of its cycle are not equally represented in one year. Since the observations are not equally distributed throughout each year, or between years, it is impossible to deduce whether Satellite V has a real effect or not. Once again, however, it is safe to conclude it is not responsible for the influences attributed to Io.

It is perhaps premature to consider why Io should influence Jupiter's radiation until other causes of a 2,548·5-min periodicity are eliminated. However, assuming that Io is responsible, then it seems possible that only one main 'source region' on Jupiter exists, and that the orientation of the line joining source and satellite determines whether the radiation will reach the Earth. This concept requires a considerable revision of ideas on the origin of the radiation, and probably of the orientation, of Jupiter's magnetic field.

I thank Dr. James W. Warwick, the High Altitude Observatory and Colorado University for permission to use their Jupiter results; Dr. Walter Orr Roberts for inviting me to the National Center for Atmospheric Research, Boulder, Colorado, where this work was carried out; and Dr. E. G. Bowen for his advice.

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SCIENCE AND MANKIND*

By S. P. CHAMBERS, C.B., C.I.E.
Chairman, Imperial Chemical Industries, Ltd.

IF you look up the *Oxford English Dictionary* or the *Shorter Oxford English Dictionary*, you can get quite lost looking for any definition of science; almost everything seems to be covered, including any craft, trade or occupation or any branch of learning or skill. The best we can do is take from the *Oxford Dictionary* a definition of science in a more restricted sense which reads as follows: "A branch of study which is concerned either with a connected body of demonstrated truths or with observed facts systematically classified and more or less colligated by being brought under general laws, and which includes trustworthy methods for the discovery of new truths within its own domain". Even that is very general and seems to bear little relationship to all those branches of science, for example, mathematics, physics, chemistry, which we think of when we talk about science. Nevertheless it is important to recognize that when we are considering the impact of scientific thought and scientific discovery on mankind we must avoid putting the various branches of science into water-tight compartments. The body of scientific knowledge to-day is so vast that individuals must specialize in one sector or another; but the impact of discovery in one field on another can be seen in almost every walk of life.

It is for this reason that, while concentration on one aspect of science in a narrow field can be rewarding in that it may lead to fresh discoveries in that field, nevertheless it is the relationship of discoveries in some fields to the whole body of scientific knowledge which really matters.

Those of you who have been interested in the history of science must have been struck by the unevenness of the progress made in different fields over the centuries. In mathematics great progress was made centuries ago, and the same is true of discovery in the fields of astronomy and elementary mechanics. But in other fields, of the most vital importance to humanity, centuries passed before anything of great value was discovered.

In general, the need for protection against enemies, particularly human enemies, seems to have been the incentive for the application of science to the development of both offensive and defensive weapons; specially tempered metals for armour, gunpowder, cannons and cannon balls are obvious examples. The modern equivalent is the application of science to warfare on a world-wide scale at enormous cost, including the cost of developing nuclear weapons.

In the eighteenth and nineteenth centuries, with the Industrial Revolution, there was a great acceleration in the rate of progress in the application of science to more peaceful pursuits and a recognition of the immense benefits to be gained by applying scientific methods to almost every activity which could put up standards of living. By the use of mechanical power, the productivity of many industrial operations was raised sharply. The power was, for the most part, derived from coal, the energy of which was harnessed mostly by the use of steam engines. Standards of living throughout the World have indeed been improved tremendously by the application of science to industrial production, using the word 'production' in its widest sense to include transport and distribution.

One of the great problems, and it is still with us in one form or another, is that the advantages of more scientific production are not evenly spread throughout the population, and some sections may at first suffer through the consequential unemployment of those whose jobs have disappeared because of mechanization. The famous 'Luddite' revolts in which new textile machinery was smashed afford an illustration of the hardships and bitterness which industrial progress can cause. In the long run, anything which increases man's output of the things which man wants must put up the standards of living; but for those who are adversely affected it is the short run that counts. As Lord Keynes, the economist, once said: "in the long run we are all dead". The solution of this problem lies in the humane treatment of those who suffer from changes of this kind, and assistance which enables them to fit themselves with new skills and new jobs for which there is a demand in the new conditions. The solution can never be the holding back of economic progress based on scientific discovery. If we jump now straight from the remarkable surge forward in the eighteenth and nineteenth centuries to conditions as we see them to-day, it is clear that, so far from the Industrial Revolution having come to an end, it is continuing and, if anything, at an accelerated pace.

Perhaps the simplest illustrations are in the field of transport, whether on land or in the air. The steam engine has given way to the Diesel engine and the fast electric train; in the air we have seen successively the progress from the simple, slow bi-planes of fifty years ago to heavier, faster, multi-engine pistoned planes, to turbo-jets and pure jets. Returning from Australia two weeks ago the captain of the aircraft in which I was travelling announced as we left Rome that owing to a strike of air control officials in Paris we would have to make a detour of an extra 100 miles over Germany; that, he said, will take us another ten minutes. Now there is the prospect of further change from sub-sonic to super-sonic air travel.

In the manufacturing industry there is similar progress, particularly in those sections of industrial production which lend themselves to large-scale operation.

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* Substance of an address to the fifth Annual Conference for Sixth Forms, Eltham Green School, on July 14.

Here again, there has been a steady transition from manual operation to mechanical operation and from the simple power-driven machinery of the nineteenth century to far more complicated and more efficient machinery, driven by electric power, whether generated from coal, oil or atomic energy. To-day we are now seeing the introduction of computers into almost every aspect of industrial operations, including research, production, distribution and the control of marketing operations. Some industrial plants to-day are being controlled by computers which not only determine which are the best operating conditions but set the various plant controls so that these conditions are achieved.

Perhaps I can give here an illustration of the inter-relation of different scientific techniques in practical applications in modern industry. Most of the simple starting materials for the synthesis of organic chemicals are now obtained by pyrolysis (or cracking) of low-boiling petroleum fractions usually referred to as naphthas. These various components have to be separated and purified to make them suitable for further chemical operations, and this is done mainly by fractional distillation at very low temperature and high pressure, an operation which would not have been possible without the tremendous progress which has been made in fields of refrigeration and the physics of mass transfer.

At present the main product from the Imperial Chemical Industries crackers is ethylene, the raw material for polythene; but other gases, including methane, propylene, butylene and so on, are produced—about ten in all—together with petrol and some heavy material. Some of the gases produced are more valuable than others, and it is frequently necessary to vary the proportions of the components in the primary cracker gas: this can be done by modifying the reaction conditions. To do this effectively, however, rapid analysis of the primary gas is essential, and it is only relatively recently that this has been made possible by the use of the techniques of infra-red, ultra-violet and mass spectroscopy which can give an answer in minutes whereas older chemical methods required hours. Thus we have here a case in which important advances in the chemistry of naphtha cracking could not have been put to full industrial use without advantage being taken of equally important discoveries in the fields of physics and physical chemistry.

Another example is nylon, which was originally discovered by Carothers in the United States, who was engaged in highly fundamental research on what are called 'long-chain polymers'. This was a most important discovery, but by itself it could never have led to the universal use of nylon which we see to-day. A whole series of new chemical processes had to be uncovered in order to be able to produce nylon cheaply enough from readily available raw materials. New dyes had to be found which would provide the requisite range of colours for this new fibre. Entirely novel machinery had to be developed for spinning the fibre and many basic investigations in the field of physics had to be made to elucidate the relationship between molecular alignment and mechanical strength, the effect of cross-sectional geometry on the handle of the final fabric and so on. Control of the spinning, drawing and twisting of fibre processes would have been impossible on a large scale but for the development of many new physical techniques.

The work of original discovery is one step only; the scientific work of developing a product for full-scale production goes on for years rather than months.

All this work in industry requires a very high level of scientific knowledge and inventiveness. For able men the work is exciting and rewarding.

It is natural that I should be thinking more of industrial production than of other aspects of man's activities; but there has, of course, been great progress in many other fields. Medical science, for example, is to-day moving forward rapidly and, with a far better understanding of all the factors that determine health in both man and other animals, some of the worst diseases which plagued the world for centuries have little more than historical interest to-day. Others, such as malaria, are beginning to disappear even from those countries, such as, for example, India, where they have probably been the greatest source of human misery. Much remains to be done; there are still diseases prevalent in temperate or tropical climates for which there are no adequate remedies.

Agriculture is also becoming far more scientific than it was, and the changes from hand-work to mechanical work have their counterparts in a growing understanding of botanical conditions and of the means of getting rid of plant diseases and putting up productivity in terms of food crops. But the haphazard use of chemicals for the destruction of weeds or insect pests can do as much harm as good. Here again, the right attitude is not to condemn all herbicides and pesticides but to produce and use these substances in a scientific way. Only after patient scientific analysis, experiment and trials can we be sure of their long-term effects. Properly used, the resulting benefit can be dramatic. For example, a few years ago Ghana was faced with a cocoa blight which threatened almost her whole crop. That crop represented half of Ghana's export trade and was therefore vital to her economy. The timely erection of a small insecticide plant and the application of this insecticide at the right time saved the whole crop. Some of the greatest crop disasters in history which involved starvation on a large scale would have been avoided if our present knowledge of plant diseases and their remedies had been available then.

The problems of water erosion and of wind erosion to the soils of vast areas in the Americas, Asia, Africa and, perhaps to a lesser extent, Europe are also other cases in point. To-day new methods are being devised whereby weeds and the remains of a previous year's crops can be destroyed by chemicals which leave no harmful residues in the soil. Following this destruction of the existing vegetation, it has now been found possible to sow fresh seed directly into the ground without ploughing, and this means that the fibrous materials and roots from the older plants remain in the ground and act both as food for the new plants and as a protection against erosion, whether by wind or water. The possibility of increasing food production substantially on large areas of land which were, and are still becoming, steadily impoverished by erosion is of the greatest importance. By the adoption of more scientific practices in agriculture, including the application of appropriate fertilizers, the fears expressed by Malthus and others that food production would be unable to sustain the growing population of the world can, I believe, be dispelled.

There is, of course, the other side of this particular problem, namely, the growth in population which some pessimists still think will outstrip any possible food production. Here again, I think the pessimists will be proved to be wrong. It has already been demonstrated that as the standards of living of people in a particular area rise above a certain level, there is a tendency for the rate of growth of population to fall, and I am sure that other methods will be found to keep the population of the World within reasonable limits.

I have ranged over many activities in a very sketchy manner to indicate that there are openings and opportunities for scientists in many walks of life. When, however, a boy or girl entering a university to read for a science degree is thinking about his or her career after graduating, other questions are bound to arise.

Sometimes fairly sharp distinctions are drawn between pure science and applied science. There are, of course, certain aspects of scientific work which can be regarded as pure in the sense that they are of general or, indeed, universal application. This is certainly true of some scientific work in the field of, say, mathematics, physics or astronomy; but the borders between the pure and the applied are not clearly defined, nor should they be.

Moreover, I think it is desirable to dispel any idea that the 'purer' side of science is in some way superior or requires a higher intellectual ability than the more applied side. There may be in some minds a vague idea that the pursuit of scientific research solely for the sake of adding to the sum of human knowledge is more intellectually satisfying than a career which is concerned with scientific research leading more directly towards something of material value in our daily life. Personally, I would regard such a notion as false.

In many countries the importance of scientific work in the great task of raising living standards is clearly recognized, and there is, among scientific workers engaged on work which may lead to the reduction of disease and the improvement of nutrition, a sense of purpose which is at least equal to that of the pure scientist seeking knowledge for its own sake. This is particularly true of such scientific workers in poorer countries such as India or Pakistan.

It does sometimes happen that a scientist engaged in purely academic work discovers something which is of outstanding practical importance. On the other hand, the need to solve some practical problem, whether in the sphere of industrial production, transport, agriculture, medicine, or in some other aspect of our daily life, will often be the spur to work of a more fundamental character. Indeed, so often it is the practical problem which leads us to realize that our basic knowledge is imperfect and which can give a sense of purpose and direction to this kind of research.

In the chemical industry the competition between one large chemical company and another which reveals either relative weaknesses in existing products or the possibility of new products very often throws up work to be done in our laboratories which is of a highly scientific character, the results of which can sometimes have an application far beyond the solution of the original problems. The same happens in the drive to improve the processes for the manufacture of existing products. Once embarked on a revolutionary method of producing something which has been made by traditional methods for years, we find the problems to be solved can lead back to basic questions related to the structure of molecules or to the mechanism of reactions which occur often under abnormal conditions of temperature or pressure.

I mention this because I think it is most desirable to get away from any idea that the most brilliant scientists should remain in research establishments in universities and should not go into industry. There is need for such scientists both in the universities and in industry. From the over-simplified statements sometimes made, one might imagine that discoveries of new products are made only or mainly in universities, leaving industry to take the new product, to develop it and then to produce it on full-scale plants. In practice, most discoveries of new products are made in the laboratories of industrial firms where the scientists are struggling to solve particular problems arising from the production and sale of their existing products. This is true, for example, of polythene, nylon, 'Terylene', 'Paludrine' (a drug which is of outstanding importance in combating malaria), and of a whole range of chemical products. In the Imperial Chemical Industries alone we are spending £18 million a year on scientific research and development work. There is room, therefore, in industry for growing numbers of scientists, and the better the scientists the better it is for industry.

Science in one form or another permeates the whole of industry and transport and has indeed entered the sphere of office work. We have in the Imperial Chemical Industries more than half a million stockholders, and there are thousands of transfers of stock every year which have to be recorded in the Company's stock registers. Twice a year dividends have to be paid to the stockholders on the registers. This used to require a large amount of clerical work in preparing twice a year the cheques, or 'dividend warrants' as they are called, and many people were employed on this rather laborious work. To-day the whole of the stock register has been put on an electronic computer which itself produces the dividend warrants complete with the names and addresses of the stockholders. The number of people needed is much smaller; but there is a growing need for electricians who can build and maintain such computers, and for operators who can devise what are known as the programmes for the computers. In other words, the trend is for a reduction in routine work and an increase in work which requires higher educational standards, and particularly higher standards on the scientific side.

This transformation from hand-work to mechanical work and computer-controlled work is going on steadily throughout large-scale industry. There will, of course, always be smaller businesses where it is more economical to use less highly developed and complicated processes; but in many sections of industry and commerce the movement towards scientific methods of control and accounting is accelerating rapidly. This movement extends to banking and insurance, where much of the routine work is being taken over by machines and electronic computers.

In the manufacturing industry, scientific progress invariably means that fewer operatives are needed for the same or a larger output of products. Sometimes large plants are involved, but not always. In one case in Imperial Chemical Industries a revolutionary method of producing the gases from which ammonia is made has meant a great reduction in the amount of plant needed as well as a reduction in the number of process workers. This ammonia is needed for agricultural fertilizers and has many other uses. Until a few years ago, a plant of 20,000 tons would have been regarded as large and would have required a whole battery of coke ovens as a first step towards producing these gases. To-day we are scrapping plants put up only a few years ago and putting in two, each of 300,000 tons a year, and plants with this tremendous capacity look small by comparison with those which a few years ago produced a mere fraction of that quantity. Externally a modern plant of this kind appears to consist of a lot of pipe-work carrying gases, some large cylindrical reactors, and other plant which is completely enclosed, and therefore appears to contain no machinery in the accepted sense or anything other than gases and a catalyst to assist the reaction. The whole plant is controlled from a control room with a large number of dials showing flow rates, temperatures and pressures at different stages in the process. There are no manual workers in the ordinary sense, just one or two people in the control room watching the dials, and the men required for the maintenance of the plant. This is becoming a common pattern in sections of the chemical industry, and indeed a similar pattern is emerging in other industries. Routine work of a repetitive character and laborious manual labour are both on the decline; but the need for intelligent men who can understand and control highly automated operations is growing.

Other questions arise in this connexion which one may feel are not of a scientific character; but they are, in fact, of importance in considering the place of scientists in industry. With the growth of the size of industrial organizations to cope with the growing scale of production, the scientist may find himself one of a number of employees in a large concern such as Imperial Chemical Industries. One may wonder whether life in such a concern is likely to be less interesting than in a smaller organization, and perhaps feel that scientists may get lost in a vast bureaucratic machine. This, in fact, is not true. It is now recognized that there is a growing need to apply scientific methods to the organizational problems of a large business. The object is to give every man full scope to do his work to the best of his ability and with the minimum of detailed instructions. An intelligent man works better if he is given personal responsibility for his section of the work. Some instructions, some control and a good deal of co-ordination there must be, but this need not be inconsistent with the freedom of the individual to get on with his own job largely on his own responsibility. Personal relationships between different people in a large organization are of outstanding importance, and the object is not only to get the highest productivity in the direct or physical sense but also to get workers of all kinds, and particularly the higher grade workers, including scientists, to do their work with a sense of purpose and enthusiasm. No organization is in this respect perfect, and the task of getting many people to work happily and harmoniously is never finished. Indeed, this task of organizing and co-ordinating the work in the many sections of a large organization requires men of the highest intelligence and character.

So much of the work has a scientific foundation that it is desirable that many of the higher posts, probably the majority, should be filled by men with a scientific background. It is quite wrong to assume that the place of the scientist in industry is only in the laboratory, or that the scientist need not bother much about matters outside his particular scientific field. All the operations of a company which is making products of importance to world trade have to be closely co-ordinated, and there is need for an understanding of the relationships between the marketing of products, their manufacture and the research and development work that goes on all the time to improve the products, to reduce their cost and to find new products. In Imperial Chemical Industries we employ some thousands of men and women with scientific degrees. A proportion of these work in the laboratories; but many of them are engaged on other operations, controlling large sections of work which may be on the production side or on the marketing side, or their tasks may straddle all the operations, from manufacture to sale, of a group of products.

A good scientist who can comprehend not only the problems in a laboratory but also the problems involved in determining the scale of production, the erection of a new plant which may cost millions of pounds, or in the control of men either at home or overseas, is the kind of man or woman which is needed in the higher posts in industry. The careers open to scientists are varied and interesting, particularly for those who reach a high academic standard in their particular field of science and also have the capacity to understand the relationship of this work to all the other work in a large industrial organization. The chemical industry has been uppermost in my mind, but much of what I have been saying is equally relevant to other industries, such as aircraft manufacture, the metal industries, radio, television, transport, and so on: the requirements of high academic standards plus the right character are similar throughout modern industry.

I have said that the specialist scientist is a better man if he has also a good understanding of the relationship of his work to the world of science as a whole, but even this is not enough. An understanding of the way in which the country's economy works and, if exports are involved, of social and economic conditions throughout the World is invaluable. I am not suggesting that every chemist, physicist or engineer must also be an economist; but I do know that those chemists, physicists or engineers who have a broad background knowledge of economic principles can do their work with a better understanding of its significance and are likely to be better candidates for higher executive posts where they have to control and co-ordinate work over a wide field than the specialists who lack any such background. The future for scientists in industry is bright; the best prospects are for those good scientists who have just something more than their scientific qualifications.

There is another general point which is inescapable. With the growing productivity of human effort as scientific methods are applied throughout industry and commerce, we shall be able to produce the material things of life with ever-shortening working hours, even if this shortening is a gradual process, extending over many years. The shortening of hours, whether we are considering either the shortening of the number of hours worked per day, or the number of days worked per week, or the number of weeks worked per year, will mean, I believe, an increase in leisure time, and with rising standards of living an increase in the money that can be spent in leisure time. This itself will raise certain problems. In particular, how will these leisure hours be utilized? This is a matter which has to be answered by each individual for himself; but I am convinced that higher educational standards can make for a greater enjoyment of life as a whole and not only that part of life which is concerned with work. In cultural matters, in the appreciation of art, music, literature, the better and broader the education, the greater is the capacity for appreciation and enjoyment of all the things that can be done in leisure hours. As our material standards of living rise, the need to preserve a balance in education between science and the humanities and arts becomes more apparent.

OBITUARY

Dr. M. J. Cross

MICHAEL CROSS died in an air crash in Tennessee on July 9 on his way to a scientific meeting.

Cross possessed a rare combination of fine qualities of character and intellect, and his death at the age of thirty-seven is a serious loss for medical research. He received his early education at Cranley School, where he won an open exhibition and the St. Nicholas Scholarship. During 1944–47 he was in the Royal Naval Volunteer Reserve which he left as sub-lieutenant. In 1947 he went up to New College, Oxford, with an open exhibition and read for an honours degree in chemistry. After graduating in 1951, Cross took up research in Oxford as Guinness Research Fellow in microbiology, working under Prof. D. D. Woods, and in 1954 he obtained the D.Phil. degree for a thesis on the metabolism of serine by micro-organisms. At first his work was concerned with the interrelationship of carbon dioxide and citrovorum factor in the requirement for serine by *Leuconostoc*; he showed that carbon dioxide prevented the breakdown of citrovorum factor. Later he worked on the synthesis of methionine by *Escherichia coli*, and he was largely responsible for the development of a cell-free system which has been the basis for many subsequent advances. The results of this work were published between 1951 and 1960 in a series of papers in the *Journal of General Microbiology* and in the *Biochemical Journal*.

Although Cross was then well on the way towards a fine career in biochemistry he took the unusual step of becoming a student again to study medicine in the Oxford Medical School. During that time he kept himself up to date in his earlier subject by tutoring his fellow medical students at New College in organic chemistry. After graduating in December 1959 Cross was successively house physician to Prof. L. J. Witts at the Radcliffe

Infirmary and house surgeon to Mr. Frank Ellis at the Churchill Hospital. Cross's work in clinical medicine was highly thought of and he was happy doing it. However, it became clear to him that his deepest interests lay, after all, in research. His successive training in chemistry and medicine was ideal for starting him in medical research with every hope that he would be able to make important and useful contributions to knowledge. From what he had already achieved when he died, this hope was fully justified.

Cross often said that he would like any research on which he was engaged to have a good chance of being clinically useful. He was therefore happy when he was asked to join an investigation into biochemical aspects of blood coagulation and thrombosis. For this he came to the Department of Pharmacology at the Royal College of Surgeons of England in London. The College made him Research Fellow, and he was supported by the Medical Research Council. Soon he had his first success by discovering a way of stabilizing thromboplastic activity in plasma and of separating the activity by high-speed centrifugation. The active material appeared to react directly with prothrombin, and Cross showed that the kinetics of the reaction were as might be expected if 'plasma thromboplastin' were an enzyme. For the first time, therefore, it became proper to describe 'plasma thromboplastin' as a substance with the properties of an enzyme and, hence, as plasma prothrombinase. Cross showed further that when centrifuged in the absence of calcium plasma prothrombinase splits into two fractions. One fraction is heat-labile and contains protein: the other resists heating and contains phospholipid. In this way Cross confirmed experimentally the well-known suggestion made by Macfarlane many years ago that 'plasma thromboplastin' consists of two components, one a protein and the other a lipid; and Cross showed that the components are bound together by calcium.

Cross then became involved in a study of the factors that control the adhesiveness of blood platelets. Interest in this had just been stimulated by the discoveries that platelet adhesiveness is greatly and specifically increased by adenosine diphosphate and that this increase is prevented by other, closely related, substances. Cross made important contributions to the clarification of these effects, including the demonstration that they require a plasma protein which is present in the fibrinogen fraction. It now seems that deficiency in this protein causes von Willebrand's disease. Most of Cross's work in this field has been published in *Nature*, in *Thrombosis et Diathesis Haemorrhagica*, and in the *Journal of Physiology*.

From September 1963 until his death Cross and his family had been living in the United States. He went there with a Fullbright Travel Scholarship as Buswell Research Fellow in Biochemistry of New York State University. With Dr. Douglas Surgenor, first in Buffalo and then in Woods Hole, he continued working on the identification of the plasma protein already referred to and on other important subjects. He was invited to contribute to several conferences, including the annual symposium on "Blood" at the Mayo Clinic. Britain could have had no finer representative in the United States than Michael Cross. Although, like other scientists, he must have had tempting offers to stay, there was never any doubt that he wanted to come home. In September he was to have returned to the Royal College of Surgeons to join the new Thrombosis Research Group of the Medical Research Council.

In his work Cross combined enthusiasm with cautiousness in establishing and interpreting his experimental results. He was full of ideas, but his careful chemical training never allowed them to run away with him. He was independent, but very co-operative.

Cross made friends with all who knew him. He was the essence of kindness and helpfulness. He had the ability to get on well with people whose opinions were very different from his; this was important, because he had strong views on social affairs. Almost always he was cheerful, exuberant really; when something went wrong and he became temporarily less than completely happy—one never saw him gloomy—it was still good to be with him because his emotions were so honest, spontaneous, and open.

Cross had a happy and settled family life in a village where he was very much part of the landscape. He played cricket and shove-ha'penny; and quietly he visited the lonely old people to cheer them up. His family, friends and country suffer grievously by his death. Indeed it is almost impossible to believe that this exceptionally lively, happy, very-present figure will not be returning to us. G. V. R. BORN

NEWS and VIEWS

Sprengel's Pump

IT is now almost 100 years since Herman Sprengel developed his mercury air-pump—an invention which he described to the Chemical Society in 1865, and one which proved to have an important influence on the development of both science and industry in the latter part of the nineteenth century, especially in the development of the electric lamp, discharge tubes, the discovery of the so-called 'inert' gases, etc. Sprengel, the man, has gained little notice from biographers, but his mercury pump has been the focus of much attention. Born in Schillerslage, Hanover, on August 29, 1834, Sprengel was educated first at Göttingen and then Heidelberg Universities. Coming to Britain, he worked for three years as an assistant in the chemical laboratory of the University of Oxford, and then as assistant in the laboratories of Guy's and St. Bartholomew's Hospitals. During 1865–70 he was employed in the Farmer's Chemical works and became a naturalized Briton. He devoted much time to detonation and explosives, and was the first to direct attention to the value of picric acid as an explosive when fired by a detonator. For the rest of his life he was engaged on his own inventions and patents, but his *chef-d'œuvre* was his pump, especially once he became one of the eminent men fascinated by the power of the vacuum. Toricelli, Von Guericke with his famous Magdeburg hemispheres, Boyle, Papin, Geissler and Töpler all joined this 'cult of the vacuum', while Röntgen, Crookes, Dewar, Langmuir and Fleming continued the theme with the aid of Sprengel's pump. Sprengel was elected a Fellow of the Royal Society of London in 1878. Mercury with its stable nature, its inertness towards gases and vapours, certainly proved a boon before the advent of high-vacuum pumps, and made it possible for Sprengel and his followers to show up the short-sightedness of Lear's reply, "Why, no, boy; nothing can be made out of nothing" when the Fool questions, "Can you make no use of nothing, nuncle?".

Social Psychology in the University of Sussex: Prof. Marie Jahoda

MARIE JAHODA, who is at present Head of the Department of Psychology and Social Science at Brunel College, studied psychology at the University of Vienna, where she received her Ph.D. in 1932. In Vienna she was assistant director and then director of a research institute. In 1937 she came to England and soon afterwards was awarded the Pinsent-Darwin fellowship at the

University of Cambridge. After studying a subsistence production scheme for unemployed miners in South Wales, and the impact of factory life on the personality of school-leavers, she joined the first team of the War-time Social Survey as assistant editor. In 1945 she went to the United States, where she was first associated with the Research Department of the American Jewish Committee. After spending a year at the Bureau of Applied Social Research at Columbia University she took an appointment at New York University, and she was professor of psychology and director of the Research Center for Human Relations when she left New York in 1958 to return to Great Britain. Joining Brunel College as Nuffield Research Fellow, she played a major part in founding the first Psychology Department in a College of Advanced Technology, and instituted a sandwich course for the education of professional psychologists. Dr. Jahoda is a member of the Council of the British Psychological Society. She takes an active interest in the systematic approach to social issues and is a member of the Home Office Research Advisory Committee, the Television Research Committee and the Scientific Advisory Committee of the National Institute of Industrial Psychology. Dr. Jahoda has written numerous articles, and is co-author of *Die Arbeitslosen von Marienthal*, *Anti-semitism and Emotional Disorder*, and *Research Methods in Social Relations*. Her book *Current Concepts of Mental Health* appeared in 1958; *Race Relations and Mental Health* was brought out by Unesco in 1960; and *The Education of Technologists* was published in the spring of 1963.

Physical Geography in the University of Sheffield: Prof. R. S. Waters

PROF. R. S. WATERS, professor of geography in the University of Canterbury, Christchurch, New Zealand, has been appointed to the chair of physical geography in the University of Sheffield. Prof. Waters is forty-two. He entered the University of Reading in 1940, but his studies were interrupted by war service in the Royal Air Force. After training as a pilot in Canada he was engaged in meteorological flights from Iceland over the North Atlantic. Re-entering the honours school at Reading, Prof. Waters gained a B.A. degree with first-class honours in geography in 1948 and his M.A. in 1951. From 1950 until 1953 he was assistant lecturer in the University of Sheffield and in 1954 went to the University College of the South-West of England (now the University of Exeter) as lecturer. He took up his present chair at Canterbury in August 1962. Prof. Waters was senior geomorphologist in the Spitzbergen Expedition of the Universities of Birmingham and Exeter in 1958, and United Kingdom representative at the International Colloquium on Periglacial Geomorphology at Liège in 1959. He was a member of the New Zealand delegation to the recent International Geographical Congress in London and was organizer of a field symposium held during this Congress on the Pleistocene geomorphology of south-west England. Prof. Waters is secretary of the New Zealand Geographical Society and a member of a technical sub-committee of the New Zealand Committee on the International Hydrological Decade. His publications include papers on geomorphological mapping, periglacial geomorphology and on the geomorphology of south-west England. It is anticipated that Prof. Waters will take up his new appointment in the University of Sheffield early in 1966.

The National Research Development Corporation

IN a written answer in the House of Lords on July 28, the Minister of State, Board of Trade, Lord Drumalbyn, stated that the Government now proposed to seek powers to extend the scale and scope of the work of the National Research Development Corporation to enable it to contribute more effectively to industrial innovation and development, particularly development in which industry shared the risk. For this purpose legislation would be introduced in the next session to raise to £25 million in the first instance the Corporation's present limit of borrowing power from the Board of Trade. The legislation would also provide for a modification and extension of the financial and other conditions under which the Corporation operates so that, in partnership with industry, it would have greater freedom to promote the development and commercial application of new techniques. The Government had also investigated the opportunities presented by the techniques of automatic control of industrial processes and the problems which this might involve. As a result of a recent investigation by the Board of Trade, the Government was satisfied that there was need to co-ordinate, supplement and further stimulate existing activities. This would be the task of a small high-level steering group of officials which was being established under his direction. As a first task this group would urgently review the progress of automation in Britain and other countries and the probable future development of these techniques. In consultation with other interests concerned, it would also draw up a co-ordinated programme for promoting the activities of the various Government, professional, industrial and commercial bodies already at work in this field. With those measures simultaneously announced, arising out of the report of the Trend Committee (*Nature*, 203, 574; 1964). the Government believed that a substantial and practical contribution would be made towards solving the problem of how best to encourage and assist industry to apply commercially the results of research and technological advance.

Nuclear-powered Merchant Vessel

IN answer to a question in the House of Commons on July 30, the Minister of Transport, Mr. E. Marples, said that recently the Furness Shipbuilding Co., Ltd., and the Anglo Norness Shipping Co., Ltd., had approached him and the Secretary of State for Education and Science with the view of building and operating a large merchant vessel powered by a *Vulcain*-type reactor. Imperial Chemical Industries was considering the possibility of using the vessel if suitable terms could be negotiated. Discussions were in progress to see if agreement could be reached on arrangements for financing and organizing such a project. If satisfactory agreement was reached the ship would be built on the North-east Coast by the Furness Shipbuilding Co., and with a quick agreement the ship should be operational by 1968. Mr. Marples added that it was quite clear that the Government would have to play a considerable part in the project, and that the present stage would not have been reached without the research and development on which millions of pounds had already been spent. This information was also given to the House of Lords by the Parliamentary Secretary to the Ministry of Transport, Lord Chesham, on the same day.

Carriage of Dangerous Goods

IN reply to a question in the House of Lords on July 13, the Minister of State, Home Office, Lord Derwent, said that the United Kingdom was a signatory to the European Agreement on the International Carriage of Dangerous Goods, expected to come into operation within the next year. This comprised a detailed and comprehensive code, drawn up by an international group of experts, to ensure that international consignments of dangerous goods were packed and carried safely. Appropriate means for applying similar provisions to domestic traffic were being considered, but while any action should, to some extent, follow the lines of the international agreement, the latter did not bind internal traffic. He did not think that it would be appropriate to modify existing legislation, such as that covering the carriage of explosives, but that any legislation should be based on the international agreement when the final details were settled, probably within six months.

Research at the Home Office Police Research and Planning Branch

In a written answer in the House of Commons on July 24, the Secretary of State, Home Department, Mr. H. Brooke, stated that the principal subjects of research at present being undertaken by the Home Office Police Research and Planning Branch included: research into methods of dealing with serious and unsolved crimes, including an investigation into the possible extension throughout Britain of regional crime squads and criminal intelligence bureaux, and a study of criminal investigation techniques and improved scientific aids; an enquiry into the possible application of computers to fingerprints and other police records; an investigation of the policing of motorways and other through routes, including experiments with the use of a helicopter; investigation of the problem of devising a formula for accurately assessing police establishments.

Scientific and Technological Man-power in Britain

In reply to a question in the House of Lords on July 15, the Earl of Bessborough said that the output of qualified scientists had risen from 6,500 in 1959 to 9,600 in 1964, and that of engineers from 8,800 to 9,900 a year, giving a total of scientific and technological man-power nearly one-third higher than in 1959. This total of 19,500 compared with the 20,000 set as the target for 1970 by the Man-power Committee in its 1956 report.

Employment of Graduates

The report of the University of Glasgow Appointments Committee for the year ended December 31, 1963, records that while competition for appointments was more severe, generally this was met successfully (Pp. 26. Glasgow: The University, 1964). The demand for mathematicians, physicists and electronic engineers continued to be far in excess of supply, but chemists met a more difficult situation, and opportunities for geologists were hard to find outside South Africa. About 53 per cent of honours graduates in science took some form of postgraduate study; only 2 went overseas at that stage. Almost one-third of those with an ordinary degree in science entered the computer field. The number of men with honours degrees in arts or law entering industrial careers increased, but fewer with ordinary degrees did so. Only 39 honours graduates in arts and 50 men with ordinary degrees took up teaching, compared with 41 and 61, respectively, in 1962; for science the corresponding figures for 1963 are 28 and 36, and for 1962, 23 and 37. In November 1963 it was decided to separate the two functions of Appointments Secretary and Adviser of Studies in Science. For women students little change is noted in the general pattern of employment, although the number of openings in Scotland notified to science graduates rose from 34 to 59. Of the 307 graduating in arts, law, pure and applied science, 74 entered direct employment, 177 taking a teacher training course, an increase of 35, but there was a considerable drop in the proportion of honours graduates in science entering teaching, and none of those graduating with honours in mathematics (or mathematics and another subject) did so.

Regional Centres for Radiological Protection Services

The Ministry of Health and the Medical Research Council have jointly set up four Regional Centres of the Radiological Protection Service at Birmingham, Leeds, Manchester and Glasgow. These Centres can now be used in connexion with radiation surveys of radiological establishments and advisory services (including advice on plans of radiological establishments). It is envisaged that, later in the year, the Centres will provide other services, such as personnel film monitoring, calibration of radiation-protection meters and leakage tests of sealed radioactive sources. The Radiological Protection Service, Clifton Avenue, Belmont, Sutton, Surrey, will continue to provide services for establishments in south-east England. All establishments which at present make use of the film-monitoring and other services provided at Sutton will shortly be informed which Centres will provide future services for them.

World Health Organization Reports

The valuable information published from time to time in the Technical Report Series of the World Health Organization is so well known and widely appreciated that it is scarcely necessary to direct the attention of our readers to it. Expert committees of the Organization have recently issued seven Reports, all of which are of great interest. No. 271 (Pp. 18. 1 Sw. fr.; 1*s.* 9*d.*) deals with *Atmospheric Pollutants*. Report No. 272 (Pp. 52. 2 Sw. fr.; 3*s.* 6*d.*) is the tenth report of the Expert Committee on Malaria, and the continued success of the Organization's world-wide campaign against this disease is further discussed in detail in the *W.H.O. Chronicle* (18, No. 6; June 1964) under the title "Malaria Eradication in 1963", together with a map which shows at a glance the astonishing progress that has been made and the hopeful outlook for the future. In 1963 the seventh International Congress on Tropical Medicine and Malaria was held at Rio de Janeiro, and a note on this Congress appears in the *Boletin Chileno de Parasitologia* (19, No. 1; 1964). This Congress was attended by 1,236 experts from 65 countries. Technical Report No. 274 (Pp. 90. 3 Sw. fr.; 5*s.*) deals with *Biological Standardization*; No. 275 (Pp. 27. 1 Sw. fr.; 1*s.* 9*d.*) reports on *Psychosomatic Disorders*; No. 276 (Pp. 53. 1 Sw. fr.; 3*s.* 6*d.*) is on *The Prevention of Cancer*; No. 278 (Pp. 48. 2 Sw. fr.; 3*s.* 6*d.*) is entitled *Postgraduate Preparation of Health Workers for Health Education*. No. 277 (Pp. 69. 3 Sw. fr.; 5*s.*) is a valuable report by the Expert Committee on Helminthiases on *Soil-transmitted Helminths*, which are responsible for much illness, disability and economic loss among millions of human beings and domesticated animals. This Report discusses the extent and geographical distribution of these helminthic diseases, the factors which influence their transmission, their pathogenesis and clinical manifestations, the interrelations between the physiology and biochemistry of the parasites and their hosts, immunity to them, the techniques used to investigate them and methods of controlling them, together with recommendations for research. Copies of the Reports are available from the World Health Organization, Geneva, or H.M. Stationery Office, London.

The International Council of Scientific Unions

The May 1964 issue of the *ICSU Bulletin* includes a preliminary report of the tenth general assembly of the International Council of Scientific Unions, Vienna, November 22–29, 1963, with exerpts from the presidential address (No. 1. Pp. 64. Rome: International Council of Scientific Unions, 1964). There are also brief reports on meetings of the International Unions (Pure and Applied Physics, Geological Sciences, Biological Sciences) as well as notes from various scientific unions, their committees, commissions and permanent services on present activities. A calendar of meetings during 1964 is included. The *Year-book of the International Council of Scientific Unions, 1964*, besides the Calendar of Meetings for 1964, the lists of members of the Executive Committee, Council, and of the various Committees, and Commissions and Executive Bodies of the Permanent Services, includes the statutes and rules of procedure of the Council, and the rules for scientific and special committees and their charters (Rome: International Council of Scientific Unions, 1964). Relations of the International Council with the United Nations Educational, Scientific and Cultural Organization, the World Meteorological Organization, etc., are outlined and, besides a list of addresses of international organizations, there is an alphabetical list of members of the Executive Committee, scientific and special committees, the Council, and officers of the Scientific Unions.

Polish Science Publications

THE *Quarterly Review of Scientific Publications* (No. 1; 1964), issued by the Polish Academy of Sciences, now covers all scientific books published in Poland. There are 439 entries arranged under headings: general; social sciences; biological sciences; pure and technical sciences. There is an index of authors and a subject index.

The *Review of the Polish Academy of Sciences* for January–March (9, No. 1; 1964) includes articles by S. Huechel on the development of research on soil mechanics in Poland, and by J. Dylik on peri-glacial investigations and their significance for palæogeography, as well as an account by P. Kubchowski of the history, development and activities of the Institute of Drugs.

Polish Scientific Periodicals, which will appear ten times a year, and of which Nos. 1 and 2, 1964, have now been published, lists the contents of periodicals published in Poland, arranged under social sciences, biological sciences, pure and technical sciences, and includes an author index. The publication is available from the Polish Academy of Sciences, Palace of Culture and Sciences, Warsaw.

Land Resources in Australia

THE identification of distinctive environments and the pattern of their occurrence are the principal tasks of the Commonwealth Scientific and Industrial Research Organization Division of Land Research and Regional Survey, and the annual report for 1962–63 presents a review of the activities in progress (Pp. 73. Canberra: Division of Land Research and Regional Survey, C.S.I.R.O., 1963). The Land Resources Group has been engaged since 1946 in reconnaissance surveys of large areas, land systems being described to provide basic data for possible land use; productive land use for specific environments is the objective of the Biology Groups; the overall appraisal of the situation with respect to possible commercial production is made by the Agricultural Assessment Group. There is close co-operation throughout with other divisions of the Commonwealth Scientific and Industrial Research Organization. Most of the field work has been carried out in Northern Australia and Papua–New Guinea, with research stations in Northern Territory at Katherine, for crops suitable for monsoon conditions; at Darwin, for rice with seasonal flooding; at Alice Springs, for the problems of arid country; and in Western Australia at Kimberley, for irrigation investigations. Pasture management and the performance of tropical cross-bred cattle, peanut cultivation, selection of cotton varieties and cropping systems with oil seeds, and fluctuations in productivity resulting from erratic distribution of rainfall, are some of the problems under investigation. Water is obviously a dominant factor, and the work is supported by flexibly constituted research units concerned with plant–soil–climate relationships, investigations being directed, for example, to questions of stomatal behaviour in transpiration, transpiration suppressants, diurnal energy and water exchange, nutrient responses, and harmonic analysis of rainfall data. Environmental limitations inevitably impose restrictions on agricultural potential, and the information being collected will be invaluable.

Forest Products Research

THE Forest Products Research Laboratory has compiled a list of its publications which can be obtained free of charge on application to the Laboratory. The list consists of a selection of abstracts of papers published by Laboratory staff during the past few years together with a few Laboratory reports of which stocks of reprints are still available. Topics covered by the reports include: seasoning; woodworking; strength; protections of timber from fungal and insect attack; composite wood; chemistry; general and miscellaneous; industrial surveys. Copies of the list are available from the Director, Forest Products Research Laboratory, Princes Risborough, Aylesbury, Bucks.

Wind and Locust Migration

AN understanding of the factors which determine the course of locust migrations is becoming increasingly important, not only for the purpose of predicting which areas are likely to be invaded but also in order to make the most effective use of aircraft in the application of insecticides. *Meteorology and the Migration of Desert Locusts: Applications of Synoptic Meteorology in Locust Control*, by R. C. Rainey, is an exhaustive technical study of the applications of synoptic meteorology in locust control (*World Meteorological Organization. Technical Note No. 54.* (WMO No. 138. TP. 64. Anti-Locust Memoir 7.) Pp. v+115+3 plates. Geneva: World Meteorological Organization; London: Anti-Locust Research Centre, 1964. 25 Sw. fr.; 42*s*.). Hour-to-hour displacements of individual swarms have been recorded from the air. The direction of displacement follows closely the direction of the winds at swarm level. They may show a steady progressive displacement or there may be repeated reversals, sometimes resulting in closed loops. This down-wind displacement can lead to convergence of swarms or dispersal. A sound basis is thus provided for the use of current synoptic charts and of meso-scale meteorological observations in the interpretation and in the short-term forecasting of the movements of the desert locust.

Radiation Nephritis

DR. JOHN G. MAIER, of the Walter Reed Army Institute of Research, and Dr. George W. Casarett, of the University of Rochester, New York, have investigated the effects of single-exposure localized irradiation of the kidneys in beagle dogs at 500, 1,000 and 2,000 rads, using 220-kV X-rays (*Transactions of the New York Academy of Sciences*, Serial 2, 26, No. 5; March 1964). Both functional and morphological parameters have been investigated in animals killed six months after irradiation. One or both kidneys have been irradiated *in situ*, and single kidneys have been exteriorized and irradiated. From these and earlier experiments, it is concluded that irradiation of the canine kidney produces a progressive arteriolonephrosclerosis. The degree and rate of progression are related to the absorbed dose. At six months following irradiation, minimal arteriolonephrosclerotic changes were seen in the dog kidney receiving 500 rads, severe changes at 2,000 rads, and intermediate changes at 1,000 rads.

Harkness Fellowships of the Commonwealth Fund—New York

THIRTY Harkness fellowships of the Commonwealth Fund–New York are offered each year for study and travel in the United States. Candidates must be British subjects who are citizens of the United Kingdom and present Colonies by birth or by naturalization. Candidates should be between 21 and 32 years of age on September 1, 1965. Recent graduates and graduands who expect to receive their first degree in 1965 may apply, if they do not intend an academic career. Graduates proposing an academic career should have experience of postgraduate research. Other candidates, including non-graduates, should produce evidence of professional qualifications or considerable practical experience. The fellowships are tenable for between twelve and twenty-one months. Round-trip passages to the United States are provided. Emoluments include living and family allowances, travel in the United States, tuition and research expenses, and allowances. Selection of Fellows for nomination to the

Fund is in the sole discretion of the Committee of Award, which will conduct interviews at Harkness House, London, in March 1965. Further information and details of the fellowships can be obtained from the Warden, Harkness House, 38 Upper Brook Street, London, W.1. Applications, on forms provided by the Fund, must reach Harkness House before November 12.

The Paul Instrument Fund Award

THE Paul Instrument Fund Committee has made grants as follows: £1,100 to Dr. P. B. Hirsch, lecturer in physics in the University of Cambridge, as a supplementary grant, for continuation of his work on an electron microscope with velocity analyser. £5,400 to Prof. D. J. E. Ingram, professor of physics, University of Keele, for the design and construction of a millimetre wave-length electron resonance spectrometer to work at zero or near zero magnetic field strengths and with as wide a frequency coverage as possible. £2,200 per annum for two years to Dr. H. Motz, Donald Pollock reader in engineering, University of Oxford, for the construction of a pulsed millimetre wave generator. £3,400 to Dr. E. E. Schneider, reader in solid-state physics, University of Newcastle upon Tyne, as a supplementary grant, for continuation of his work on the application of superconducting cavities to magnetic resonance spectrometers. The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science".

The Australian Association of Clinical Biochemists

THE following have been elected officers of the Australian Association of Clinical Biochemists for the year 1964–65: *President*, Dr. W. Roman; *Secretary*, Dr. D. H. Curnow; *Treasurer*, Dr. D. Watson; *State Representatives on Council*, Mr. M. J. Thomas (Queensland); Dr. F. J. Radcliff (New South Wales); Dr. J. Mackenzie (Victoria); Mr. Z. A. Mejglo (Tasmania); Dr. R. I. Cox (South Australia); and Dr. S. S. Alexander (West Australia).

Institute of Applied Science of Victoria

THE annual report for 1962–63 of the Institute of Applied Science of Victoria records that the new name, "Institute of Applied Science", bestowed by an Act of Parliament in 1961, may now be seen across the outside entablature of the building (Pp. 22. Melbourne: Institute of Applied Science of Victoria, 1964). The post-war years have witnessed the metamorphosis of the original Industrial and Technological Museum into an establishment which now includes a planetarium, a radiocarbon-dating laboratory, evening astronomical telescope demonstrations, special school services, and a fresh approach to applied science display. A large diorama illustrates the Yallourn open-cut works for coal and shows the overburden stratum and various working levels. The model also includes a 1,200-ton coal-winning dredge, belt conveyor, water-spray reticulation, etc. Another display given by Qantas Empire Airways deals with aeroplane engines and summarizes progress from the Wright brothers to turbo-props and turbo-jet propulsion.

University News: London

DR. E. A. BEVAN, University lecturer and demonstrator in the University of Oxford, has been appointed to the chair of botany at Queen Mary College. Dr. James Green, lecturer at Bedford College, has been appointed to the readership in zoology at Westfield College. The following titles have been conferred: professor of sociology (with special reference to the Far East), on Mr. R. P. Dore in respect of his post at the London School of Economics and Political Science and the School of Oriental and African Studies; professor of plant chemistry, on Dr. L. Fowden in respect of his post at University College; professor of geography, on Dr. R. J. Harrison-Church in respect of his post at the London School of Economics and Political Science; reader in geography, on Dr. K. M. Clayton in respect of his post at the London School of Economics and Political Science, and on Dr. J. T. Coppock in respect of his post at University College; reader in chemistry, on Dr. A. G. Davies in respect of his post at University College; reader in forensic psychiatry, on Dr. T. C. N. Gibbens in respect of his post at the Institute of Psychiatry; reader in comparative education, on Dr. B. Holmes in respect of his post at the Institute of Education.

Announcements

THE autumn meeting of the Society for Water Treatment and Examination will be held at Llandudno during September 30–October 2. Further information can be obtained from A. W. H. McCanlis, 41 Carshalton Road, Sutton, Surrey.

A SYMPOSIUM on "Major Elements in Nutrition", organized by the Scottish Group of the Nutrition Society, will be held in Dundee on September 12. Further information can be obtained from Dr. C. F. Mills, Rowett Research Institute, Bucksburn, Aberdeen.

A MEETING of the Biochemical Society will be held in the University of Cambridge during September 17–18. Further information can be obtained from Dr. H. R. V. Arnstein, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7.

A SYMPOSIUM on "The Problems of Food Additives", organized by the Royal Institute of Chemistry, will be held in Manchester on October 1. Further information can be obtained from H. H. Armstrong, Stockport College for Further Education, Wellington Road South, Stockport.

A SUMMER school in "Organic Spectroscopy", arranged by the Royal Institute of Chemistry in collaboration with the Society for Analytical Chemistry, will be held in the University of London School of Pharmacy during September 6–12. Further information can be obtained from D. G. Chisman, Royal Institute of Chemistry, 30 Russell Square, London, W.C.1.

THE fifth international Machine Tool Design and Research Conference will be held in the University of Birmingham during September 14–18. The Conference will include sessions on: international collaboration on machine tool chatter research sponsored by the U.S. Air Force; machine tool design; high-energy rate forming; metal cutting; explosive forming; metal forming; recent developments in machine tool technology. Further information can be obtained from the Organizing Secretary, Department of Mechanical Engineering, University of Birmingham, Edgbaston, Birmingham 15.

THE eighth Conference on Analytical Chemistry in Nuclear Technology on "The Determination of Interstitials and Trace Constituents in Reactor Materials and Products", sponsored by the Analytical Chemistry Division of Oak Ridge National Laboratory, will be held in Gatlinburg, Tennessee, during October 6–8. The Conference will include sessions on: spectrochemical and X-ray methods of analysis; mass spectrometric methods of analysis; nuclear methods of analysis; gas chromatographic methods of analysis; determination of carbon, hydrogen, oxygen and nitrogen in metals; determination of trace constituents by diverse methods. Further information can be obtained from C. D. Susano, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee, 37831.

GOVERNMENT EXPENDITURE ON SCIENTIFIC RESEARCH, 1963

THE report of the Council for Scientific and Industrial Research for the year 1963* records gross expenditure during the year ended December 31, 1963, of £19,479,540, compared with £16,691,172 in the year ended March 31, 1962. The 1963 figure is reduced to £17,231,547 by various receipts from industry and other sources for services rendered, of which £960,142 was from other Government departments and £977,482 from industry—£224,085 being received by the National Physical Laboratory. The net increase on 1962 was £2,853,345, while staff rose from 6,525 to 6,723 during the calendar year. Grants for special researches again rose, to £3,297,186 compared with £2,275,060 in 1962, contributions to the European Organization for Nuclear Research from £1,359,000 to £1,700,000, and receipts of £116,196 reduced the subscriptions to the North Atlantic Treaty Organization and Organization for European Economic Co-operation scientific schemes to £76,436. There is a new item of £28,827 for United Kingdom Space Research Management Services.

Postgraduate training awards amounted to £1,735,067 (£1,289,903 in 1962), and the 1,958 new studentships, an increase of 22 per cent, brought the total current at the end of the year to 4,036. While the value of research fellowships was increased by £100, only 78 new awards were made, compared with 92 in 1962, bringing the total current to 147. Besides 38 studentships and 18 fellowships awarded for study abroad (24 and 28, respectively, in 1962), 20 fellowships and 1 studentship were awarded to assist British scientists and engineers to return from North America. Of the 3,469 current research studentships, 461 were in biology and biological chemistry, 264 in chemical engineering and metallurgy, 979 in chemistry, 161 in electrical and 291 in other fields of engineering, 238 in geology, 76 in the human sciences, 296 in mathematics and 703 in physics. The advanced course studentships were distributed mainly in mathematics (115), physics (91) and engineering, other than electrical (85), with 47 in the human sciences, 44 in chemical engineering and metallurgy, 36 in electrical engineering, 26 in geology, 25 in biology and biochemistry, and 21 in chemistry. All 77 first-year studentships were again in chemistry, and of the 147 research fellowships, 55 were in chemistry, 40 in physics, 25 in biology and biochemistry, and 10 in geology.

Grants for special researches totalled 929, 587 being new, and of the 929 totalling £11,813,668 in value in the year ended March 31, 1963, 194 (valued at £1,308,893) were in chemistry; 149 (£1,334,205) in technology; 147 (£947,245) in biology; 115 (£955,525) in physics; 72 (£601,677) in geology; 66 (£466,881) in the human sciences; 64 (£645,358) in metallurgy and materials; 51 (£3,008,060) in nuclear physics; 33 (£596,668) in space research; 19 (£1,073,275) in astronomy; 9 (£47,311) in mathematics; five each in documentation and in reactors totalled £50,850 and £777,720, respectively. During the academic year 1962–63, the Research Grants Committee considered 639 new applications for grants with a total value of £6,318,000, compared with 487 valued at £4,147,000 in the previous year, and made 513 grants with a total value of £3,596,000 (excluding grants of £100,000 for maintaining existing capital projects, compared with 390 amounting to £2,313,000 in the previous year. The Human Sciences Committee made 31 awards amounting to £150,000. In addition, a grant of £1·1 million was approved for the construction and operation of a 100-MeV electron linear accelerator to enable Prof. P. I. Dee to carry out an extensive programme of investigation into photo-problems in nuclear physics (the accelerator is being built in the grounds of the National Engineering Laboratory, East Kilbride). A further grant of £139,000 was made to the University of Manchester to enable Sir Bernard Lovell to make a survey of the angular diameter of radio stars. A grant of £121,500 was made for Prof. N. J. Petch's work at the University of Newcastle upon Tyne on the development of materials with improved resistance to brittle fracture; smaller grants for work in this field, totalling £126,082, were allocated to other universities. An earmarked grant of £15,300 was offered to the British Welding Association, £100,000 for staff and running costs until 1967 for fundamental research in biosynthetic mechanisms under Prof. E. B. Chain at the Imperial College of Science and Technology, and £142,000 to the University of Southampton to assist the establishment of an Institute of Sound and Vibration Research under Prof. E. J. Richards.

With the exception of fire research and hydraulics research, in which net expenditure decreased slightly, from £57,056 to £39,668 and from £118,152 to £105,489, respectively, expenditure on all branches of the Department's work increased during the year, and in these two fields receipts amounting to £130,937 and £161,379 (both up in 1962) accounted for more than half the gross expenditure. Likewise, a decrease in the net expenditure of the National Lending Library for Science and Technology from £258,922 to £248,024 is accounted for by an increase in receipts from £45,327 to £87,803. While receipts of £399,472 in the previous year gave expenditure on the Tropical Products Institute a credit balance of £169,721, this year they fell to £212,965, leaving the net expenditure at £17,472. There is a new item of £53,215 net on development contracts. The major increases in expenditure during the year are the National Physical Laboratory (£2,164,502 gross; £2,033,109 in 1961–62); road research (£1,086,674; £970,403); the Warren Spring Laboratory (£538,554; £471,181); the National Engineering Laboratory (£1,006,609; £924,978); building research (£858,271; £781,037); radio research (£379,041; £320,163); and the National Chemical Laboratory (£431,416; £409,887). For forest products research the corresponding figures are £216,682 and £194,619; Geological Survey and Museums, £649,807 and £578,919; Laboratory of the Government Chemist, £524,375 and £491,215; Torry Research Station, £252,709 and £226,696; water pollution research, £199,932 and £183,550. Expenditure on headquarters administration increased from £615,536 to £678,775, annual grants to research associations from £2,005,453 to £2,073,565, and special assistance to industry from £59,675 to £81,209.

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* Department of Scientific and Industrial Research. Report of the Research Council for the year 1963. Pp. iv+66. (Cmnd. 2394.) (London: H.M.S.O., 1964.) 4*s.* 6*d.* net. (See also p. 997 of this issue of *Nature*.)

A FUTURE FOR BRITAIN'S WATERWAYS

IN a written answer in the House of Commons on July 31, 1963, the Minister of Transport, Mr. E. Marples, indicated that he expected a preliminary report from the British Waterways Board on its general views as to the future use of the inland waterways, for which it is responsible, about the end of that year. This interim report was issued on December 12, 1963, and published on January 30, 1964*.

While the report emphasizes that the views offered are only provisional, the general approach to new policy outlined is of wide interest. The views raised will need testing by systematic financial investigation not only of the multi-user policy, which the Board now proposes, but also of other courses open to them, as well as by engineering investigations. Interest in the waterways has, however, been accentuated since the report appeared by the growing importance of multi-user approach in other fields, as is to be seen, for example, in the Buchanan Report on road transport (*Nature*, 201, 1059; 1964), in the Manchester proposals for water supply (*Nature*, 202, 1; 1964), and in the proposed scheme for a barrage across Morecambe Bay (*Nature*, 203, 125, 219; 1964). Incidentally, it should be noted that in writing the report, the Board—which was set up by the Transport Act, 1962, and took over the administration of the waterways from the British Transport Commission on January 1, 1963—was by no means clear as to what the impact of the Water Resources Act of 1963 would be on its own function for the supply and distribution of water in England and Wales, or ultimately on water prices.

The report begins by making clear just how far the responsibilities of the Board extend, for there are many inland waterways besides the 2,000 miles vested in the Board, including, as well as the Thames and many rivers, the Manchester Ship Canal (including the Bridgwater Canal), the Rochdale Canal and the Exeter Canal. Those for which the Board is responsible are mainly in England, but they include the Caledonian and four other canals in Scotland, and the Monmouthshire and Brecon Canal and two others in Wales, and before nationalization nearly half of them had been owned or controlled by railway companies. Furthermore, they differed widely in their width, their state of maintenance, and the extent of their use for commercial or other traffic or for other purposes; some 500 miles had been formally closed to traffic, while of a further 600 miles no longer used by commercial traffic some had become unnavigable wholly or in part.

The Board is required, under Section 10 of the Transport Act, 1962: (1) to provide, to such extent as it thinks expedient, services and facilities on these inland waterways and port facilities at any harbour owned or managed by it, having due regard to efficiency, economy and safety in the services provided; (2) to review the manner in which the inland waterways owned or managed by it may be put to the best use, to formulate proposals to that end, and to take all steps open to it to achieve that object. Financially, Section 18 requires the Board to ensure that revenue is sufficient to meet charges properly made to revenue, taking one year with another. Besides the actual waterways, the Board took over responsibility for nearly 100 reservoirs, various docks and warehouses, some estate property, repair yards and workshops, some fleets of carrying craft and some hire-pleasure craft.

This interim report represents the results of the Board's first examination of all aspects of the future use of the inland waterways for which it is responsible, both from the point of view of commercial transport and all the other varied purposes which the waterways could serve. Already the Board is clear that radical departure is necessary from the recommendations of the Bowes Committee of 1956—although the report of that Committee contained pointers to a new and more positive policy. The Board has already completed its appraisal of management organization of the waterways and is altering the general shape and structure of that organization, replacing the geographical divisions by functional divisions, with two Regional Centres, one at Gloucester and one at Leeds, for certain operational purposes. While these changes, to be completed by December 1965, are not expected to cause any significant redundancy among the present 4,000 staff, the Board believes that the new structure will provide the essentials of a sound long-term basis for managing the undertaking and will thus encourage both the present staff and future recruits to make British Waterways their career. In Scotland, while the Caledonian Canal will continue to be run by a manager who will be responsible to the four functional Headquarters officers for those respective aspects of his work and who will utilize the appropriate Headquarters departments for specialized skills for which it would be uneconomic to provide separately, the other canals will for engineering matters be made into one engineering area, with an area engineer reporting direct to the chief engineer.

Before outlining its first general views on policy, the Board directs attention to some basic factors in the financial situation. On present information, maintenance and running costs of a waterway for major transport use are several thousand pounds sterling a mile per annum, but only some £700–£1,400 a mile for canals used mainly by pleasure craft. Here, however, there is need to examine imaginatively the possibilities of savings by new approaches to maintenance which would not lead to long-term deterioration. Conversion of these canals for use mainly as water channels would offer a further saving of 15–20 per cent, and perhaps another 10 per cent saving might be possible if such canals could be reduced to 'mere existence' standards. The true cost of elimination is not accurately known, but is expected to be about £5,000 a mile, with no offsetting income, although maintenance would be saved.

It is against this background that the Board has formed its present opinion that the most workable and defensible basis for future policy is a positive one of cohesion and development of the waterways as an organized entity in the light of all present social and economic conditions. A unified and economical administration should be set up with the prime object of maintaining them for all possible transport and related uses, and also for any and every useful public purpose appropriate to them. Moreover, it should endeavour to make them pay their way as a whole and to the maximum possible extent, by every feasible method of cost recovery, charges and voluntary contributions. The Board believes that it is possible to make them pay much better than in the past when sectional pressures were responsible for some canals being deliberately allowed to fall into disuse and neglect. It regards a policy of run-down and closure as wasteful, frustrating and inefficient, while the third alternative of dismemberment is likely to lead to a long period of uncertainty about individual waterways, in which their condition and prospects would deteriorate, and ultimately to a more expensive and less efficient structure.

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* British Waterways Board. *The Future of the Waterways: Interim Report of the Board.* Pp. vii+52. (London: H.M.S.O., 1964.) 7s. 6d.

The need for early decision is reiterated throughout the report and is one factor responsible for the issue of an interim statement. Particularly in respect of some special cases, such as the Kennet and Avon Canal, the Leeds and Liverpool Canal and the main line of the Grand Union Canal, and some of those used for pleasure craft, the Board stresses the need to provide some reasonable assurance of continuity. The development and use of waterways have for generations been hindered by uncertainty about their future, and this prolonged uncertainty has exacerbated the very difficulties which caused it in the first place. The Waterways Board fully endorses the observations of the Bowes Committee on this point, and this applies to almost every field of the future activity of the waterways.

The Board argues very cogently that this policy of multi-user is the most rational and efficient policy, and it points out that the undertaking for which it has assumed responsibility cannot, as a whole, any longer be regarded solely as a national transport system. As such, some of it is manifestly out of date, though it might well be asked whether the practicability has been sufficiently carefully considered of developing even some sections of the waterways as an alternative means of relieving the congested road system of some of the heavy traffic responsible for delays and damage, or to fill the gaps left by some of the closures of railway lines that have already been made or are in prospect. That, of course, properly lies outside the special responsibility of the Board, but once again it indicates the real danger of departmentalism which the handling so far of the Robbins Report and the Buchanan Report has demonstrated. Apart from that, however, the interim report on the future of the waterways shows clearly the impracticability of neatly and separately arranging waterways as usable and unusable for transport, or even separately labelling individual waters for single particular purposes.

All this is admirably shown on the excellent series of charts with which the report is illustrated. Although, as has already been emphasized, the Board is careful to insist that its present views are subject to modification, the imaginative and constructive approaches it displays in its considerations of commercial transport and related activities, of water supply and channelling, and of the use of the waterways by pleasure craft, for cruising, angling and other purposes are some of the most encouraging and helpful features of the report. There is, for example, the comment that a reasonable transport diversity is a useful support to a healthy economy. Again, the Board notes that many waterways could without major expansion cater for more traffic than they do at the moment—the Lea, going through the industrial East End and the City to the London docks, is one example. It is clear that, given the reasonable support on which it should be able to count, the Board will be prepared to consider in due course with real vision the possibilities of major expansion or improvement or even the building of new waterways.

What does emerge from this interim report is that Britain now has a Waterways Board with a positive policy and one which is imaginatively alive to the many possibilities which such a policy holds for various aspects of public welfare, including the intelligent conservation and use of water resources. Although the revenue for angling is minute compared with the cost of maintaining waterways, that does not mean that angling will be disregarded by the Board, and it recognizes the complexity of the statutory position and is prepared to reconsider this in due course. Meanwhile, in a situation in which the annual rate of loss has been reduced from £1·3 million to less than £1 million and could be reduced by a further £0·75 million if all went well, the Board is concentrating on the general concept of the multi-user system as the best opportunity of attracting new revenue. It is indeed also required by the Transport Act of 1962 to pay interest on a commencing debt, estimated at £19·2 million, retention of which is scarcely a stimulus to sound financial management. Nevertheless, while the Board is not yet ready to make firm proposals regarding the future treatment of this debt, it hopes to do so later. How the engineering survey and the systematic financial and costing investigations to be put in train will affect the tentative views expressed in this report has yet to be seen. It can at least be added, on the evidence of this report, that if the British Waterways Board does not, within the next few years, provide Britain with a waterways system which measures up to the opportunities displayed in this report with insight as well as realism, the responsibility is likely to be that of the Government, and particularly the Minister of Transport, for failing to support it. After this report, the Minister of Transport can have no excuse if he fails to deal urgently and constructively with the problems of administration involved at the central as well as at the regional level when problems have to be handled from the point of view of numerous functions and services, far transcending local, and sometimes regional, needs and interests.

DISEASES OF THE HOP

COMMUNICATION between biologists in different countries, working in different physical and intellectual environments, and publishing their results in different languages, is perhaps nowhere more difficult than in the field of plant virus diseases; the basis for identification of the causal agent still depends on the description of variable and often transient symptoms in one or more species or varieties of host plants, even though these diagnostic techniques are being increasingly supplemented by data from the electron-microscope and the ultracentrifuge, and from serological investigations. Much can be gained by assembling an international group of specialists for a working conference to see and handle diseased material and to discuss common problems. This can be especially valuable if the proceedings of the conference are not published in detail and the participants can therefore feel free to speculate. The conference on hop diseases, sponsored by the East Malling Research Station with the Hop Research Department of Wye College, and held during June 29–July 2 at East Malling and Wye, was organized on this basis. The incentive for arranging the conference came initially from virus workers; but the programme also included discussions on the major fungal diseases of the hop.

The first day of the conference, under the chairmanship of D. Rudd Jones (Agricultural Research Council, U.K.), was devoted entirely to virus diseases. Only one virus, hop mosaic, appears to be recognized in all the major hop-growing areas of the world, though it is not clear how much variation there is between strains of the virus. Papers on this disease dealt with its effect on the yield and quality of early and late cluster hop varieties (C. B. Skotland, U.S.A.) and with changes induced by the virus in the mineral content of the leaves (R. Marocke, France).

Kräuselkrankheit (crinkle) is a disease of economic importance in Germany and eastern Europe, but it is not recognized elsewhere. Its symptomatology and control in Czechoslovakia were discussed by J. Kriz (Czechoslovakia); and P. Chrometzka (Germany) showed how various climatic and nutritional conditions could lead to masking of the symptoms. A striking feature of the disease is the effectiveness with which symptoms can be eliminated by treating affected plants with zinc salts or zinc-containing fungicides; this, together with the somewhat equivocal results of attempts to transmit the disease by grafting, suggests the possibility that zinc deficiency is directly involved.

Kräuselkrankheit symptoms have a number of features in common with those of nettlehead. A paper on the ætiology of this disease by J. T. Legg (U.K.) was presented by K. R. Bock (U.K.), the author being at present in Ghana. This paper also dealt with hop line pattern virus, which can be latent in many varieties but which, when present, either facilitates infection with nettlehead virus or contributes to the development of the nettlehead syndrome. Nettlehead is not recognized in continental Europe, but is probably present in the United States.

Kräuselkrankheit is probably not synonymous with nettlehead; some evidence for this was mentioned in the text of a paper by H. E. Schmidt (D.D.R.). Unfortunately, no member of this author's laboratory, the Institut für Phytopathologie at Aschersleben, was able to attend the conference, and it was not possible to discuss in detail what appeared to be an important contribution to diagnostic procedures for hop viruses. For example, a number of herbaceous indicator plants have been used at Aschersleben to differentiate strains of 'hop ring-and-banding-mosaic virus'. It was not possible to determine whether this virus is related to the hop line pattern virus described by Legg; the latter virus has not been recognized in Germany, Czechoslovakia or Yugoslavia, but is believed to be present in the United States.

Herbaceous indicators have also been used by D. H. Hall (U.S.A.) during investigations of a yellow leaf blotch virus of hops in California, a disease that seems to resemble one previously reported from New Zealand, but is apparently not recognized elsewhere.

Infectious sterility is recognized only in eastern continental Europe. It was discussed by J. Kriz, who indicated that control was likely to be achieved in the near future by the propagation of selected virus-free clones. This preventive approach to the control of virus diseases in general was also stressed in contributions to the discussion by delegates from western continental Europe, Britain and the United States, whereas in southern Germany the curative approach by treatments aimed at ameliorating the effects of the disease on the host plant is predominant.

Split leaf blotch, a disease well known in Britain, is possibly present in Europe and almost certainly present in the United States.

In the second session, under the chairmanship of W. G. Keyworth (U.K.), discussions on hop *Verticillium* wilt diseases were opened by G. W. F. Sewell (U.K.), P. W. Talboys (U.K.) and T. Christie (New Zealand).

The taxonomic position taken by most British workers is that the 'Dauermycelien' and 'microsclerotial' forms of *Verticillium* are referable to the two species *V. albo-atrum* and *V. dahliae*, respectively. Though a majority of American pathologists have regarded these as different forms of *V. albo-atrum*, an increasing number of them are accepting the division into two species as a matter of convenience if not always of conviction.

The major wilt pathogen of hops in Britain and Germany is *V. albo-atrum*, and limited outbreaks caused by this species have also occurred in New Zealand. In the United States relatively minor outbreaks in the past have been caused by *V. dahliae*; but C. E. Horner reported that a new occurrence of the disease in 1964 was caused by *V. albo-atrum*. In other countries hop wilt is of no economic significance and in most areas has not been reported.

The virulent strain of the pathogen causing progressive wilt in Britain does not seem to occur elsewhere, though tests carried out in Germany and New Zealand to discover whether the strains present there correspond with those causing progressive and fluctuating wilt in Britain are difficult to assess because the techniques and environmental conditions of the trials were different from those in Britain. The marked response of *Verticillium* wilts to environmental conditions is an important factor to be considered in investigations both of resistance in the host and of virulence in the parasite. Thus, the initial development of the wilt problem in Germany, and the variable incidence of acute disease from year to year in moderately resistant varieties on certain sites in Britain, were both ascribed, at least in part, to low temperatures during the summer. The increasing prevalence of *V. dahliae* as a wilt pathogen in warmer climates is another aspect of the same problem.

Present investigations of the host–parasite relationship in England continue to lay stress on the importance of the extra-vascular root tissues in determining the severity of the disease.

Research on the status of *V. albo-atrum* in the soil indicates that infection of roots of dicotyledonous plants provides an important means of maintaining soil populations of the pathogen, whereas under a pure grass sward this organism is rapidly eliminated. *V. dahliae*, however, is apparently not amenable to this method of control.

Discussions on downy mildew (*Pseudoperonospora humuli*) fully occupied the third day under the chairmanship of J. E. Crosse (U.K.). The meeting was held at Wye College, and papers were presented by F. Zattler (Germany), J. M. Ogawa (U.S.A.), J. R. Coley-Smith (U.K.), C. E. Horner (U.S.A.), Y. Mori (Japan) and L. Vent on behalf of Z. Petrlik (Czechoslovakia).

Most of the discussion during this session was concerned with over-wintering of hop downy mildew and with the value of streptomycin in early-season control of the disease. In Great Britain, the United States and Japan there is now a great deal of evidence to show that downy mildew over-winters mainly as mycelium in infected hop rootstocks. Oospores of the fungus are very rare in the United States, and although of common occurrence in Britain have never been observed to germinate. On the continent of Europe it is still widely believed that spring infections arise from oospores, and there is certainly evidence that infection of hop rootstocks is less common there than in Britain or the United States.

There was disagreement about the value of streptomycin for early-season control of hop downy mildew. Good control has been achieved in certain areas of the United States but not in others. In Great Britain the antibiotic appears to give slightly better results than conventional protectant fungicides. Mixed results have been obtained in Japan, but in Western Germany the antibiotic has given very poor results.

Control of downy mildew by the use of resistant hop varieties has not reached an advanced stage, except in Western Germany, where two new resistant varieties have been distributed to growers. There is no information regarding physiological specialization in *Pseudoperonospora humuli*, but there appear to be differences in the susceptibility to downy mildew of certain commercial varieties in different countries, and the possibility of physiological specialization should therefore be borne in mind when breeding for disease resistance.

On the final day, with A. F. Posnette (U.K.) in the chair, there were short discussions on miscellaneous diseases, including powdery mildew (*Sphaerotheca humuli*), canker (*Gibberella pulicaris*), *Botrytis* infection of cones, a heptachlor-induced die-out of hop crowns in Washington State, and *Phytophthora* rots in New Zealand and Britain.

The conference was supported by the Agricultural Research Council and the Hops Marketing Board.

P. W. TALBOYS

ARTHRODIRAN ANATOMY

THE classification used by E. Stensiö in his recently published *Anatomical Studies on the Arthrodiran Head*[*] differs from that in present-day text-books. Stensiö, like Jarvik, joins the Placodermata with the Elasmobranchii in a class "Elasmobranchiomorphi". The Placodermata contain the super orders Arthrodira and Holocephali, the former including Gross's Euarthrodira (Arthrodira of Woodward) and the Antiarchi. Stensiö is in agreement with the cogent arguments of Ørvig for relating the Euarthrodiran order of Ptyctodontids with the modern Chimæras—"everything decidedly goes to prove that the Holocephalans are derived from early primitive Ptyctodontids and consequently in all probability are the last survivors of the Arthrodiran stock". Evidently, many general zoologists and palæontologists will be referring to this work to decide how far these opinions are now to be followed.

The figures and plates of Part 1 are excellently set out and there are many beautiful colour-figures. These, the work of a great authority, writing on his subject, enable a more complete picture of the Euarthrodiran fish to be formed. But in the reconstructions, based largely on 'serial grindings', comparison is made predominantly with the cranial anatomy of modern Elasmobranchs rather than with that of Acanthodians, Holocephalans, or early Elasmobranchs: this procedure begs the question of whether the Elasmobranchs are so closely related to the Arthrodires as Stensiö believes. Certainly it is difficult to accept that these early fishes were so advanced as they appear here, for example, in the relations of the profundus nerve to the rest of the trigeminal complex.

The systematic arrangement of the orders is based on the author's views on the evolution of the Arthrodiran pectoral fin, although the Dolichothoraci are treated first in the text. Should these views eventually prove to be mistaken, this would detract little from the value of the book. But many special features of the Arthrodires, such as the strange and probably primitive arrangement of the pineal system, and the evolution, apparently, of autostylic jaw-suspensions from a hyostylic one, really need authoritative explanatory diagrams rather than extensive verbal descriptions. Presumably these will be provided in Part 2, which will cover nine orders, including the crucial Ptyctodontids, and a summary. Part 1 lacks a separate index, so on all counts it is to be hoped that Part 2 will appear soon.

H. P. WHITING

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[*] Kungl. *Svenska Vetenskapsakademiens Handlingar.* Fjarde Serien. Band 9, Nr. 2: *Anatomical Studies on the Arthrodiran Head.* Part 1: Preface, Geological and Geographical Distribution, the Organization of the Arthrodires, the Anatomy of the Head in the Dolichothoraci, Coccosteomorphi and Pachyosteomorphi. Taxonomic Appendix. By Erik Stensiö. Pp. 419 + 62 plates. (Stockholm: Almqvist and Wiksell, 1963.) 220 kr.

INSPECTION OF ALKALI, ETC., WORKS

THE one hundredth report on alkali, etc., works in England and Wales for 1963, issued by the Chief Inspector[*], covers the centenary year of the Alkali Act. An interesting feature of the report is the brief account appended of the early history of the Act and of the Inspectorate. At the end of the year, 2,061 works were registered under the Act involving operation of 3,331, but the number of registered works was 16 fewer than at the end of 1962, and 152 fewer than at the end of 1959.

The Chief Inspector points out that it is often small works, unsuitably placed and surrounded by dwelling-houses, which take up a disproportionately large amount of the time of the Inspectorate, and neither the number of works nor the scheduled processes registered, nor the national statistics for production, can alone provide a yard-stick for the hazards.

During the year, 10,850 visits were paid, including 135 special visits by the Chief or Deputy Chief Inspector, of which 244 were to, or in connexion with, works not registered under the Act, 43 being concerned with the control of radioactive emissions and 43 to, or in connexion with, colliery spoilbanks. During the inspections, 3,433 quantitative analyses were made of gases evolved from the processes, compared with 4,331 in 1962. The decreased number of tests is largely due to the absence of any need for special investigations at the older works. In addition, 615 special samples were taken and submitted for analysis by the Government Chemist.

The specific complaints investigated, largely at the request of the local authorities, concern some 323 works, 256 of which were registered under the Act. Complaints against registered works were most numerous against isotope works, ceramic and electricity works, iron and steel works, cement works, and aluminium works, the volume of complaints against the heavy chemical industry being small.

Commenting on the inclusion in the list of noxious or offensive gases under the control of the Inspectorate of compounds which cannot by any manner of means be held to be toxic, the Chief Inspector points out that toxicity is not the full criterion, and that there is the matter of general nuisance and of interference with amenities. The Chief Inspector also points out that the arrangements for visiting the establishments of the Atomic Energy Authority to consider the best practical means for reducing to a minimum the emission of radioactive gases, mists and dusts, and to make sure that any such discharges are regulated so that public health is in no way in danger, continue to operate adequately. Satisfactory standards continue to be maintained and no public health hazard has arisen. The same satisfactory system is being applied to the nuclear power stations of the Central Electricity Generating Board.

An interesting point noted regarding the cement-production works is that one of the main factors leading to high dust emission when using estuarine clay is the salt content. Moreover, the main alkali metal in the dust is not sodium but potassium, which seems to be present largely as potassium chloride and can at times be as much as half the emission.

Under the same cover is included the report of the Chief Inspector of Alkali, etc., Works (Scotland) for the same year. Under the Scottish Act 275 works operating 375 processes are registered, and some 444 visits were paid, including 44 joint and independent visits by the

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[*] Ministry of Housing and Local Government—Scottish Development Department. Alkali, etc. Works Regulation Act, 1906, and Alkali, etc. Works Orders, 1928–1963. Alkali, etc. Works Regulation (Scotland) Acts, 1906 and 1951, and Alkali, etc. Works (Scotland) Orders, 1952 and 1958. One-Hundredth Annual Report on Alkali, etc. Works by the Chief Inspectors, 1963. Pp. iv + 84. (London: H.M.S.O., 1964.) 5*s*. 6*d*. net.

Chief Inspector, of which about 146 visits were on work not falling directly within the provisions of the Act, but within those of other statutes relating to the prevention of air pollution. The experimental programme of testing dust emissions yielded more useful information in the 30 tests made during the year, but it was again apparent that full observance of the recommended procedure for testing was scarcely practical at most works, although close approximations were made wherever possible. Another markedly defective electrostatic precipitator was found, and again its performance was not suspect by an external instrumental indication of its operation.

To this report also is appended a brief historical account, this time dealing with the operation of the Alkali Act in Scotland during the past hundred years.

BLAST-FURNACE CEMENT IN SOUTH AFRICA

MANY years ago it was established (in Germany) that certain slags, by-product of iron-ore smelting in blast-furnaces, if rapidly quenched from the molten state, then finely ground with an activator such as lime or Portland cement, would set and harden very much like ordinary Portland cement. This resulted in the development of a large cement industry in Europe based on waste slag, which, as a result of considerable research over the years, has greatly expanded in production and economic importance, especially in those countries concerned with an indigenous iron and steel industry.

In the case of South Africa there appears to be a basic difference between the available slag from the iron and steel industry compared with that used for cement-making elsewhere, in that the slag produced in the Republic has a much higher magnesia content than that normally used overseas. For some time cement technologists considered that the use of high magnesia slag was liable to lead to disruptive expansion in Portland cement products made from it; consequently this local slag was looked on with suspicion. However, with continuing expansion of the iron and steel industry and concomitant mounting of slag production (this is at present running at 1,200,000 tons per annum), something drastic had to be done to counteract the accumulation of this unwanted by-product. Research on the problem was initiated at the National Building Research Institute of the Council for Scientific and Industrial Research, Pretoria, and eventually it was demonstrated that this high-magnesia slag was not conducive to unsoundness and that, under prescribed conditions, a good-quality cement could be manufactured from it.

Writing in the *South African Review* (C.S.I.R., ref. No. *R/BOU* 108; 1963/64), Dr. N. Stutterheim, vice-president of the Council, briefly describes the events leading up to the now successful utilization of this slag. He says: "Discussions between the iron and steel industry, which had sponsored the research, the cement companies and the C.S.I.R , resulted in the manufacture of two types of cement: one an interground mixture of granulated slag and Portland cement clinker and the other a finely ground granulated slag which is then mixed with Portland cement in the concrete mixer on the construction site. . . . Last year about 350,000 tons of granulated slag was converted to cement; i.e., about 12 per cent of the country's cement requirements were met from this source. This is only 30 per cent of slag output whereas at least 80 per cent could be so utilized".

It is concluded that it has now been abundantly proved that it is economic to use South African slag for cement manufacture; that by so doing capital and running costs are saved; resources of limestone and coal, otherwise required for equivalent Portland cement production, are extended; a cement possessing properties similar to those of Portland cement, but at lower cost, is being extensively marketed; and it is in the national interest that increasing use be made of this waste slag for cement production.

H. B. MILNER

UPTAKE OF HYDROCARBON CARCINOGENS BY LYSOSOMES

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ALTHOUGH polybenzenoid hydrocarbon carcinogens have been intensively investigated since their discovery more than thirty years ago, remarkably little is known about their intracellular fate and the way by which they bring out malignant changes. In examining the localization of carcinogens, two main approaches have been used: histological examination of the fluorescence of treated tissues and chemical analysis of isolated cell constituents. The first technique has shown that, both *in vivo* and *in vitro*, hydrocarbon carcinogens are quickly taken up into small masses in the cytoplasm of cells and remain demonstrable there for several weeks[1-5]. The second approach has established that if careful fractionations are carried out the carcinogens are not found in nuclei or bound to nucleic acid but are present in cytoplasmic constituents, an appreciable proportion being bound to protein[6-8]. Whether hydrocarbon carcinogens become associated with particular cytoplasmic constituents has never been clear, and our experiments were undertaken to throw light on this problem.

Cell cultures. Cultures of four cell types were made on coverslips. Primary *Macaca* monkey kidney cell cultures were grown in Medium 199 (Glaxo) + 10 per cent calf serum inactivated by heating at 56° for 0·5 h. Chick embryo cell cultures were maintained in *tris*-(hydroxymethyl)-aminomethane–Gey's medium with peptone and lactalbumin hydrolysate[9]. HeLa cells were cultured in Medium 199 + 5 per cent inactivated calf serum. Macrophages were obtained without prior stimulation by intraperitoneal inoculation of 3 ml. of culture medium containing 10 I.U. of heparin per ml. into mice of strain *VSBS/NIMR* weighing 18–20 g. After brief massage the cells were gathered, washed and dispensed in tubes containing small coverslips. The culture medium was solution 199 + 20 per cent inactivated calf serum.

Carcinogens. Hydrocarbon carcinogens (9,10-dimethyl-1 : 2-benzanthracene (DMBA) : 20-methylcholanthrene (MCA); 1 : 2,5 : 6-dibenzanthracene (DBA); and 3 : 4-

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[*] Holder of a NATO fellowship granted by the Italian National Research Council.

benzpyrene (BP), a structurally related non-carcinogenic control (anthracene) and tri*cyclo*quinazoline, a heterocyclic nitrogen carcinogen (kindly provided by Dr. R. W. Baldwin)) were used. Solutions were prepared by adding carcinogens in the form of fine particles to inactivated calf serum (100 µg/ml.) and incubation at 37° for 6 h with continuous gentle stirring. The serum was centrifuged at 13,000g for 0·5 h at 4° and passed through a 'Millipore' filter. Appropriate dilutions of serum were added to the culture media to give a final concentration of carcinogen (confirmed by fluorimetry or radioactivity measurements) of 5–22 µg/ml.

Localization of carcinogens in cells. Cell cultures were incubated for 2–24 h in media containing carcinogen or anthracene, mounted in fresh medium, and examined by fluorescence microscopy with activation from 360 mµ to 400 mµ. The same cells were examined by phase-contrast microscopy and after staining for acid phosphatase by the Gomori technique[10], with or without prior fixation in 1–4 per cent glutaraldehyde in isotonic phosphate buffer, pH 7·0, at 4°, for 2 h.

All the cell types examined after exposure to carcinogens were found to contain brilliant fluorescing granules in the cytoplasm (Figs. 1–3). The granules concerned appeared dense by phase-contrast microscopy and their distribution in individual cells and in the different cell types corresponded with that of lysosomes. Careful examination and photography of the same groups of cells by fluorescence microscopy and light microscopy after acid phosphatase staining showed that identical organelles contained the fluorescing carcinogens and lead sulphide deposit. No fluorescence was seen in background cytoplasm, mitochondria or nuclei. All the carcinogens examined, and anthracene, showed a similar intracellular distribution. With the hydrocarbons fluorescence was light blue or blue-violet; with tri*cyclo*quinazoline it was yellow.

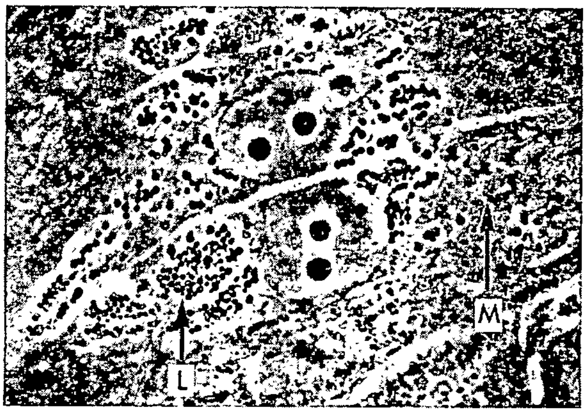

Fig. 1. Monkey kidney cells in culture, unfixed and examined by phase-contrast microscopy. The dense lysosomes (*L*), which are mainly in a perinuclear position, are readily distinguished from the less dense, filamentous mitochondria (*M*), most of which lie in the peripheral cytoplasm (× *c*. 870)

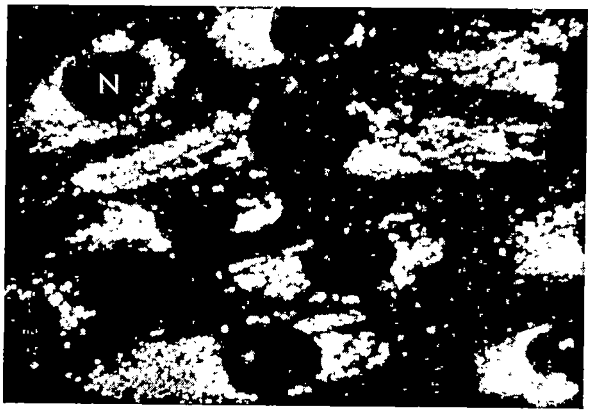

Fig. 2. Monkey kidney cells in culture, 24 h after exposure to 20-methylcholanthrene, examined without fixation by fluorescence microscopy. Lysosomes containing carcinogen fluoresce light blue, while nuclei (*N*) and peripheral cytoplasm containing mitochrondria show no fluorescence (× *c*. 390)

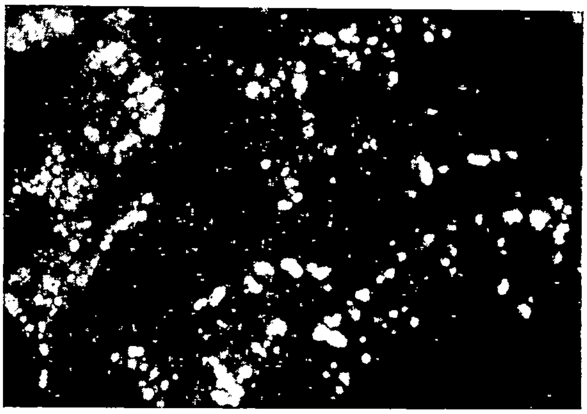

Fig. 3. Mouse macrophages in culture, 24 h after exposure to, 9,10-dimethyl-1 : 2-benzanthracene, unfixed but counterstained with euchrysine, showing light blue fluorescence of lysosomes in the cytoplasm (× *c*. 870)

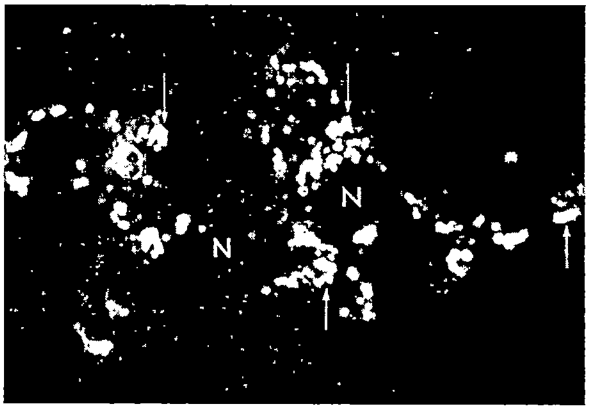

Fig. 4. HeLa cells in culture exposed 24 h previously to 3 : 4-benzpyrene, unfixed and showing fluorescence of lysosomes, many of which, as indicated by the arrows, tend to be concentrated as caps over one pole of the unstained nuclei (*N*) (× *c*. 390)

We conclude that the hydrocarbon carcinogens are taken up by cells and selectively concentrated in lysosomes. A possible objection to this interpretation is that the carcinogen is also present elsewhere in the cells but with its fluorescence quenched. Although quenching of fluorescence of aromatic hydrocarbons is less likely to occur than that of compounds with more reactive chemical groups, it was thought necessary to check this possibility. Cells were incubated for 24 h with medium containing 5 µg/ml. of 1 : 2,5 : 6-dibenzanthracene uniformly labelled with tritium (Radiochemical Centre, Amersham). After incubation for a further 2 h in medium without carcinogen, the cells were fixed with 1 per cent acetic acid (v/v) and coated with Kodak '*AR* 10' emulsion for autoradiography. Exposures of 8 days gave abundant granules, the distribution of which corresponded exactly with that of the lysosomes, none being present in any part of the nuclei except immediately adjacent to the lysosomes and none in areas of cytoplasm rich in mitochondria but without lysosomes. It is concluded that if any carcinogen is present in parts of the cells other than the lysosomes the amount is too small to detect by the methods used in our investigations.

In order to obtain information on the duration of fluorescence, monkey kidney cells were incubated with all four carcinogens and anthracene for 24 h and then incubated in fresh medium without carcinogens for varying periods before examination by fluorescence microscopy. Incubation for 24 h in fresh medium greatly reduced the intensity of anthracene fluorescence, which was sometimes yellowish rather than blue. After 48 h the anthracene fluorescence had virtually disappeared, but the carcinogen fluorescence was little changed, apart from the presence in some cells in addition to the typical lysosomal fluorescence of a weaker more diffuse area of fluorescence in a paranuclear zone. The only other change noted was that the fluorescence of cells just exposed to BP was violet or blue-violet, whereas that of cells exposed 24 h previously was blue. These preliminary observations suggest that the non-carcinogenic anthracene may be more rapidly metabolized or lost from lysosomes than the carcinogens, and further investigations on more closely related carcinogenic and non-carcinogenic compounds are in progress.

In vivo *experiments*. The objection could also be raised that the behaviour of cells cultured *in vitro* might not be representative of what happens *in vivo*. Experiments were therefore carried out on mice. DMBA was dissolved in paraffin (m.p. 45°) and 0·2 ml. containing 100 μg carcinogen introduced subcutaneously in adult male *VSBS/NIMR* mice. After 1 week the animals were killed, the paraffin removed and impressions and spreads of connective tissue cells examined. Although the cytological detail was less well shown than in cultured cells, in all but a few apparently dead cells the fluorescence of the carcinogen was confined to cytoplasmic granules having the distribution of lysosomes.

As others had previously observed[2,4,5] polybenzenoid hydrocarbon carcinogens are taken up into the cytoplasm of cells. We have demonstrated, further, that the hydrocarbons we have used are specifically concentrated in lysosomes, and not in mitochondria as Graffi[2] supposed. De Duve[11] and his colleagues have shown that lysosomes contain hydrolytic and other enzymes, including acid phosphatase, acid DNase, acid RNase and acid protease (cathepsin). The properties of lysosomes have been conveniently summarized at a recent Ciba Foundation symposium[12]. Evidence is accumulating that lysosomes of living cells take up neutral red[12], and we have shown that they also selectively concentrate a number of basic dyes and drugs, including several aminoacridines, chloroquine, quinine, stilbamidine, antrycide and ethidium. Since the hydrocarbon carcinogens are uncharged, it is clear that basicity is not a necessary requirement for a compound to be taken up into lysosomes. From repeated observation it seems that the carcinogens are concentrated in preformed intracellular organelles (primary lysosomes) rather than phagocytic vacuoles (phagosomes), although the possibility cannot be excluded that carcinogens enter the cells in micropinocytotic vacuoles which eventually fuse with the primary lysosomes. On the other hand, if any crystals of carcinogen are present (as in some of our preliminary experiments not involving serum solubilization) these are incorporated into typical phagosomes.

The concentration of carcinogens in lysosomes has a number of interesting implications. First, it is likely that for some time no metabolic conversion takes place. The observations of Sloane and his colleagues[5] on fluorescence spectroscopy *in vivo* in rabbit ear chambers, and after uptake of hydrocarbon carcinogens by cells *in vitro*, suggest an initial solution of the carcinogens similar to that produced in serum. With BP, emission at short wave-lengths decreased and that at long wave-lengths increased quite rapidly. This parallels the observations of Doniach *et al.*[3] and ourselves that the initial fluorescence with BP is blue-violet whereas after 24 h it is blue. With the other carcinogens no change in fluorescence is detectable. Our observations indicate that fluorescence of anthracene disappears much more rapidly, presumably owing to loss from lysosomes or metabolic conversion, than that of the carcinogens. Whether conversion by hydroxylation and coupling with glucuronic acid occurs in the lysosomes themselves cannot be decided on available evidence. The characteristic proteins to which carcinogens become bound[7] may well be lysosomal. However, it seems unlikely that protein binding is a necessary event in carcinogenesis since the stable compound tri*cyclo*quinazoline, which is a potent carcinogen, is not bound to protein[13], although we have found it to be concentrated in lysosomes like the polybenzenoid hydrocarbons.

Secondly, in at least some cells the carcinogens appear to decrease the stability of lysosomal membranes, as shown by acid phosphatase staining of the lysosomes of unfixed macrophages when they have taken up carcinogens. This occurs to a much smaller extent in kidney cells, and Mr. J. Dingle kindly allows us to quote his observation that hydrocarbon carcinogens have much less dramatic effects on the integrity of isolated kidney lysosomes than comparable concentrations of vitamin A. Nevertheless, high concentrations of carcinogens in lysosomes could well bring about considerable release into the cytoplasm of lysosomal enzymes, and this provides an explanation for the well-known cytotoxicity of hydrocarbon carcinogens both *in vivo* and *in vitro*. Attention was directed to this phenomenon long ago by Haddow[14] and others, and the evidence has recently been reviewed by Vasiliev and Guelstein[15].

Lower concentrations of carcinogens could bring about gradual release into the cytoplasm over long periods of enzyme such as DNase, RNase and protease, which might well damage the mechanisms controlling cell division—whatever these may be—without impairing the capacity of the cells to divide. Since the carcinogens have marked electron-donating and accepting properties[16], charge transfer with constituents of the lysosomal membranes could be involved in the enzyme activation.

Deckers-Passau *et al.*[17] had previously demonstrated activation of lysosomal enzymes in livers of rats treated with dimethylaminoazobenzene; these changes were more marked in animals with riboflavin deficiency, which enhances the carcinogenic effect of the dye. More recently, Nodes and Reid[18] observed within one week of feeding rats 3′-methyl-4-dimethylaminoazobenzene a marked rise in the acid RNase activity in the supernatant fraction, indicating activation of the lysosomal enzyme. Carbon tetrachloride is also known both to activate lysosomal enzymes of liver[19] and to be carcinogenic in some species[20].

The hypothesis that lysosomes are involved in carcinogenesis might provide a simple explanation for co-carcinogenesis, that is, the enhancement of the effects of small doses of carcinogens by subsequent treatment with unrelated chemicals. Three of the most effective co-carcinogenic agents for hydrocarbon carcinogens are croton oil[21], non-ionic detergents of the 'Tween' group[22] and hyperoxia[23]. All these affect lysosomes. We have observed that treatment of cultured macrophages and monkey kidney cells with croton oil (10 μg per ml. in serum added to medium), 'Tween 60' (0·001 per cent w/v, in culture medium) or 95 per cent oxygen + 5 per cent carbon dioxide for 12 h allows lysosomal enzymes to be stained in unfixed preparations, indicating that the permeability of the lysosomal membranes has been increased. Effects of detergents and the role of lysosomes in oxygen toxicity have been investigated in some detail and the results will be presented elsewhere. All the co-carcinogens could enhance effects of small amounts of carcinogen in the lysosomal membrane. Since virus infections often cause activation of lysosomal enzymes[24], interaction with carcinogens is also possible at the level of the lysosomes. Likewise, the decreased interferon production in cells after treatment with hydrocarbon carcinogens[25] may be attributable to release of lysosomal enzymes which inhibit interferon synthesis or bring about its intracellular destruction.

The toxicity of DMBA to adrenal and testicular cells of treated rats[26,27] may also be due to lysosomal damage. From the experiments of Richter and Saini[4] it is clear that spermatogonia and spermatocytes accumulate large amounts of hydrocarbon carcinogens in cytoplasmic organelles, which from their distribution appear to be lysosomes. The selective toxicity of DMBA for these cells, not shown by other hydrocarbon carcinogens, is presumably due to the particular configuration of the former, perhaps its non-planar structure[28].

One of the factors making polybenzenoid hydrocarbons so efficient as carcinogens could be that they act as selecting as well as inducing agents. The importance of selection at the cellular level has already been discussed[29]. The results summarized by Vasiliev and Guelstein[15] show that in many instances growth of normal cells is inhibited by concentrations of hydrocarbons that are well tolerated by malignant cells. It follows that in tissues where relatively high concentrations of carcinogens are present malignant cells will be at a competitive advantage. Selective inhibition of normal cells could favour emergence of malignant cell clones in several ways. One is purely mechanical: in skin and other epithelia multiplication of cells in the basal layer produces a steady movement of cells towards the surface: potentially malignant cells could be eliminated by this rising tide, but selective inhibition of normal cell division would give the malignant cells a greater chance of securing a 'foothold' from which to multiply out. A second possible selective mechanism which has recently attracted attention is the partial suppression by carcinogens of the immune cellular response which is concerned in the elimination of tumour cells differing antigenically from host cells[30].

Our results provide no evidence for direct interaction of hydrocarbon carcinogens with genetic material. Earlier experiments of others indicating interaction of carcinogens with DNA have recently been criticized by Giovanella *et al.*[8], who conclude that the likelihood of such an interaction producing a cancer-specific mutation is extremely remote. Many powerful carcinogens are weakly, if at all, mutagenic, and many efficient mutagens are non-carcinogenic in standard test situations. Giovanella and his colleagues reiterate the earlier conclusion of the Wisconsin group that carcinogenic hydrocarbons initiate the cancerous transformation through specific binding to key proteins. However, as already stated here, examples such as tri*cyclo*quinazoline suggest that protein binding is not a necessary property of a carcinogen. Pitot and Heidelberger[31] explain the apparently genetic perpetuation of carcinogen-induced metabolic changes on the basis of specific disruptions in metabolic regulatory circuits. We would offer an alternative hypothesis not dependent on analogies with control mechanisms in micro-organisms: that the primary target of carcinogens in cells is the lysosomes, and that secondary effects (probably due to released lysosomal enzymes, and perhaps involving DNA or RNA) are perpetuated when the cells continue to divide. Chromosome breaks and other changes sometimes observed in carcinogen-induced tumour cells, and in virus-infected cells, could also be produced by lysosomal enzymes. Whatever hypothesis is adopted, the fact that carcinogens are selectively concentrated in lysosomes and remain there for relatively long periods has to be taken into account.

We thank Mr. M. R. Young for help with the microscopy and photomicrography.

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EXTRACTION AND PURIFICATION OF CALCITONIN

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Dr. H. A. SOLIMAN and Dr. I. MacINTYRE

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COPP *et al.*[1] postulated the existence of a plasma-calcium lowering hormone on the basis of perfusion experiments in dogs. We confirmed the existence of this new hormone, calcitonin[2]. Copp *et al.*[1] had concluded that the parathyroid glands were the source. However, our recent investigations have shown that calcitonin is a thyroid hormone secreted in response to a high plasma calcium[3].

Presumably due to their calcitonin content, thyroid extracts from dog, rat, ox, pig, monkey and goat lower plasma calcium when injected into rats[3-5]. Hirsch *et al.*[6] first reported 50- to 100-fold purification of these extracts by centrifugation at 100,000*g* followed by gel-filtration on 'Sephadex *G*-50'.

Methods of extraction and purification of calcitonin and some investigations of its nature and properties are described here.

A method of calcitonin bio-assay was developed in which the material to be tested is infused for 1 hr into the tail-vein of a 150-g rat of the Hammersmith piebald strain (Kumar, Foster and MacIntyre, unpublished results). The rats were killed immediately after the infusion and the plasma calcium measured by flame spectrophotometry[7]. Until a standard preparation was produced, one unit of activity was defined as one-tenth of the amount of calcitonin necessary to produce a fall of 0·5 m.equiv. calcium/l.

Pig thyroid was found to be a richer source than ox. It also had the theoretical advantage that the pig thyroid does not contain parathyroid glands[8]. Fresh pig glands were defatted by repeated washing with acetone and the dry powder taken as the starting material.

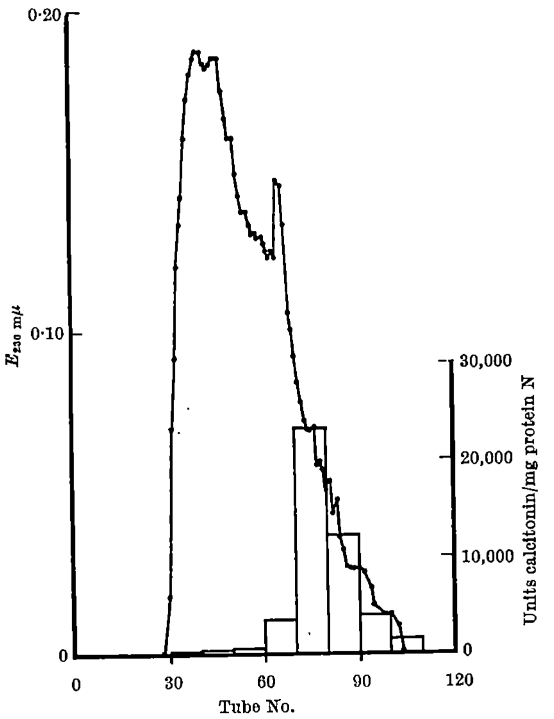

Fig. 1. Gel-filtration of calcitonin on 'Sephadex *G*-100'. 0·1 M acetate, *p*H 4·6, containing 0·2 M sodium chloride was used as eluant and 5-ml. fractions were collected. Activities reported are the average of assays done in triplicate as described in the text. In three consecutive runs values of 23,000, 11,000 and 15,000 units/mg protein nitrogen were obtained in the most active fractions. Minor peaks and variations occurred in the inactive region of the curve with different batches

Crude extract. 100 g of acetone-dried thyroid tissue was stirred with 1 l. of 0·2 N hydrochloric acid at 60°–70° for 5 min. The mixture was allowed to stand at room temperature for 1 h and then filtered through muslin, and the residue was washed with 1 l. of water and the washings added to the filtrate. This will be referred to as crude extract. The potency at this stage was about 180 units/mg protein nitrogen estimated by the Lowry method[9]. When dialysed at 4° for up to 48 h against 0·1 M acetate buffer at *p*H 4·6 or 0·1 N hydrochloric acid in 24/32 'Visking' tubing all the activity remained with the dialysis residue. Extract dialysed against 0·2 N hydrochloric acid was dried from the frozen state and taken up in 0·1 M buffers of *p*H 2–9. No activity was lost below *p*H 4·6 after 2 weeks at 4°. Above *p*H 7·2 a precipitate formed in the crude extract at 4° and some activity was lost with the precipitate. Other samples of dried dialysed crude extract were taken up in 0·1 M acetate buffer at *p*H 4·6. 1·0 M sodium chloride precipitated most of the inactive protein. When the supernatant from such a precipitate was increased to 2·0 M with sodium chloride a further precipitate formed which contained most of the activity.

Salt fractionation. These observations were used for purification as follows. 1 l. of crude extract was dialysed at 4° overnight in 24/32 'Visking' tubing against 0·1 M acetate buffer at 4°. 1 l. of 2 M sodium chloride was added gradually with stirring and the solution left for 12 h at 4°. The resulting precipitate was separated by centrifugation and discarded and an equal volume of 3 M sodium chloride was added to the supernate and allowed to stand for 12 h at 4°. The active precipitate was isolated by centrifugation and dissolved in 0·1 N hydrochloric acid or 0·1 M acetate buffer. The resulting solution had a potency of about 1,200 units/mg protein nitrogen. It was relatively stable to boiling and lost 12 per cent activity in 15 min, 46 per cent in 30 min and 77 per cent in 2 h in 0·1 M acetate buffer at *p*H 4·6. Incubation of the extract with pepsin or trypsin at a *p*H of 3 and 7, respectively, resulted in complete loss of activity after 6 h. Appropriate controls were carried out. Boiling with 1·0 N hydrochloric acid or 1·0 N sodium hydroxide for 1 h resulted in complete loss of activity.

Gel-filtration. The salt-precipitated extract was further purified by gel-filtration. 20 ml. of extract in 0·1 M acetate buffer *p*H 4·6, containing also 0·2 M sodium chloride, was applied to a column of 'Sephadex *G*-100' (82 cm × 2·5 cm) equilibrated with the same solution. 5-ml. fractions were collected at a flow rate of 30 ml./h and the extinction at 230 mμ measured in each tube. Groups of 10 tubes were pooled for assay. Fig. 1 shows a typical result. The main activity was eluted in tubes 70–90.

The most active fraction had a potency of 23,000 units/mg protein nitrogen: infusion of only 0·4 μg protein nitrogen lowered rat plasma calcium by 10 per cent. At this stage of purification the activity passed through the 'Visking' membranes (24/32) which retained the activity of crude extracts. This suggests that calcitonin was associated with larger molecules in the crude extract.

Discussion

The thyroid calcium-lowering hormone, calcitonin, is a polypeptide or low molecular weight protein. Its solubility at acid *p*H and loss of activity on incubation with trypsin and pepsin excludes thyroxin as the active principle. Further, Hirsch *et al.*[4] found no reduction in calcium in the rat when thyroxin was injected. The calcium-lowering activity penetrated the gel matrix of 'Sephadex *G*-100'. Calcitonin is therefore distinct from thyroglobulin, which has a molecular weight of 600,000 (ref. 10), far above the exclusion limit of 100,000.

The potency of calcitonin and its wide distribution in mammalian thyroids suggests that it is of major importance in calcium homœostasis. This may entail considerable revision of present concepts.

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CALCITONIN PRODUCTION AND THE MITOCHONDRION-RICH CELLS OF THE DOG THYROID

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THE possibility of a thyroid origin for calcitonin was suggested by Hirsch *et al.*[1], who extracted a hypocalcæmic substance from the thyroid glands of a number of mammalian species. More recent investigations by Foster *et al.*[2] in the goat have established the thyroid origin of calcitonin in that animal, and we are reporting here the results of histochemical investigations carried out on dog thyroid glands perfused with high and low calcium blood. Earlier investigations (unpublished) in parallel with the perfusion experiments of Kumar *et al.*[3], using histochemical techniques and electron microscopy, failed to show any change in the cells of parathyroids subjected to high (7 m.equiv./l.) or low (4 m.equiv./l.) calcium-levels for short periods (3 h).

In the dog thyroid, as in the human gland, two types of cell can be found in the acini. These are the preponderant principal cells and the so-called mitochondrion-rich cells[4]. The latter are present not only in the acini, but also in groups or nodes which lie between adjacent acini, a position which has been described as 'parafollicular' (Fig. 1). Cells in this position are usually regarded as tangentially cut normal acinar cells. Histochemical methods show that they have an entirely different enzyme pattern and must be regarded as metabolically distinct even though they are clearly acinar in origin (that is, not truly interfollicular).

Investigations carried out on the human thyroid by Tremblay and Pearse[5] showed that the mitochondrion-rich cells possessed an oxidative enzyme activity many times higher for most systems than the adjacent principal cells. In the latter the distribution of mitochondria was predominantly on the luminal side of the nucleus while in the mitochondrion-rich cells it was uniform throughout. This observation is true also for the parafollicular cells in the dog thyroid. As can be seen from Fig. 2, they contain a much higher level of mitochondrial α-glycerophosphate dehydrogenase than the acinar (principal) cells. Levels of this enzyme are comparable to those found in the principal cells of the parathyroid and in the β-cells of the pancreatic islets (Fig. 3).

Control thyroid-parathyroid glands (calcium-level, 5·5 m.equiv./l.) were obtained from 4 animals at the beginning of the perfusion experiments. The remaining thyroid-parathyroid glands were then perfused with high (7 m.equiv./l.) or low (4 m.equiv./l.) calcium blood for 3 h by the technique of Kumar *et al.*[3].

Thin slices of all the glands were quenched, immediately after removal, in liquid air (− 185°) and stored at −90° until ready for cutting. Cryostat sections (4–8μ) of all glands were simultaneously incubated for demonstration of the following enzymes: acid phosphatase, alkaline phosphatase, α-glycerophosphate dehydrogenase (menadione), NADH-diaphorase and non-specific (α-naphthyl and indoxyl) esterases. Techniques used were those given by Pearse[6]. Selected blocks were freeze-dried in a thermoelectrically controlled apparatus[7] and subjected to formalin

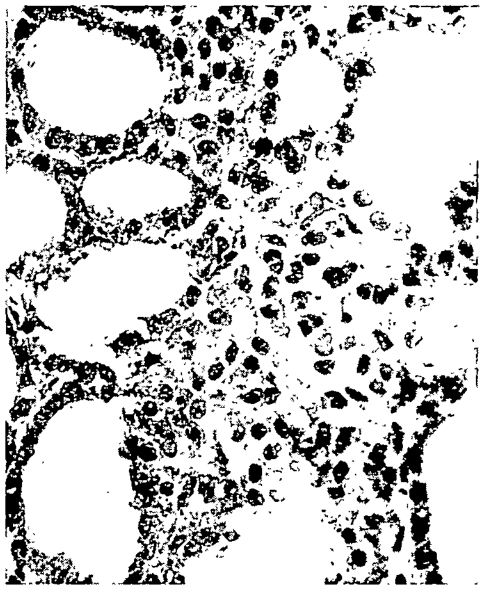

Fig. 1. 4μ Cryostat section. Shows distribution of parafollicular cells in normal dog thyroid (hæmatoxylin and eosin, × 370)

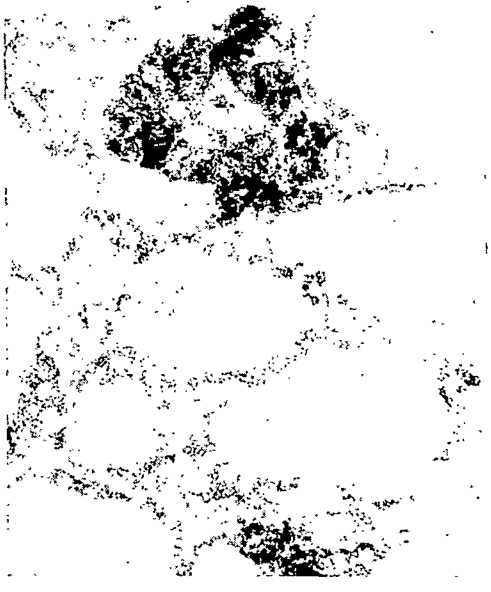

Fig. 2. 8μ Cryostat section. Shows high mitochondrial α-glycerophosphate dehydrogenase in the mitochondrion-rich cells. Normal dog thyroid (× 370)

vapour treatment for 1 h at 80° (ref. 8). These blocks were used to examine the distribution and activity of adrenergic nerves and of fluorescent material within the cells. The position of the parafollicular cells relative to the basement membrane was established and confirmed in periodic acid–Schiff-stained sections.

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In the glands perfused with high calcium blood a definite increase in acid phosphatase was noted in the mitochondrion-rich cells (Fig. 4), and to a lesser extent in the principal cells also. The normal level of acid phosphatase in the thyroid gland is moderate, though much higher than in the parathyroid gland. There was no difference between control glands and those perfused with low calcium blood.

No difference could be detected with NADH-diaphorase, but α-glycerophosphate levels were lower in the mitochondrion-rich cells of the high calcium glands.

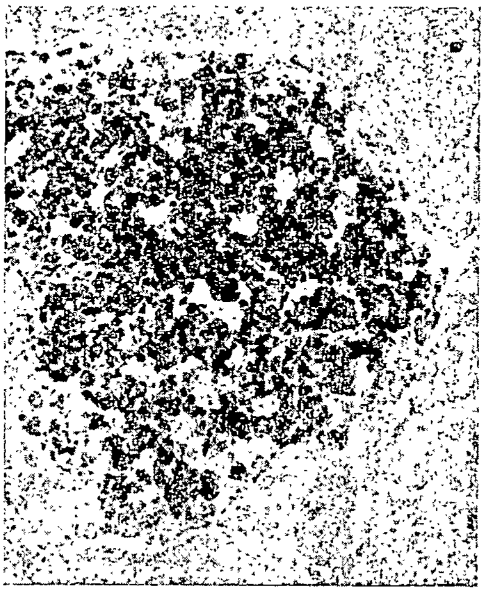

Fig. 3. 4μ Cryostat section. Rat pancreas. Very high α-glycerophosphate dehydrogenase levels in the β cells of an islet of Langerhans (× 150)

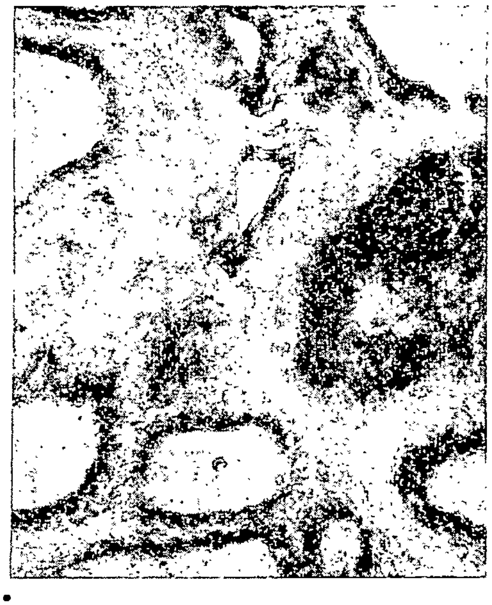

Fig. 4. 8μ Cryostat section. Acid phosphatase in parafollicular cells. High calcium perfused gland (× 370)

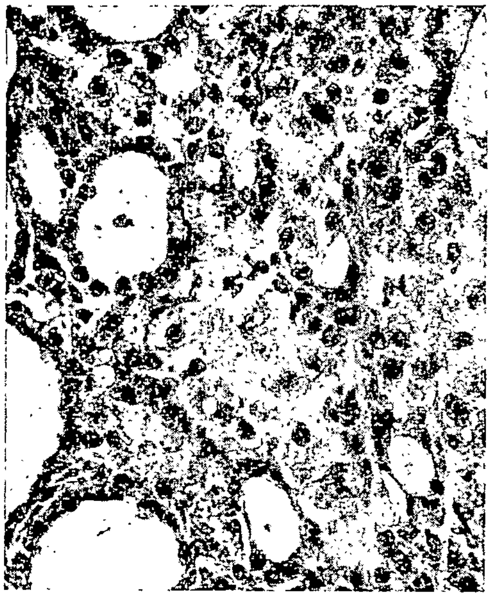

Fig. 5. 4μ Cryostat section. Extensive vacuolation in intra- and para-follicular cells. Low calcium perfused gland (hæmatoxylin and eosin, × 370)

The levels of non-specific (indoxyl) esterase were distinctly lower after high calcium perfusion than in controls from the same animals. No corresponding elevation could be seen in the low calcium-treated glands.

Cryostat sections of the low-calcium glands stained with hæmatoxylin and eosin revealed an unusual sensitivity of the intra- and para-follicular mitochondrion-rich cells to low calcium-levels. Extensive vacuolation was present in the cells (Fig. 5). Although this may well be regarded as an artefact there is no reason to ignore its significance on that account (it was not present in sections incubated for various enzymes, in which the cells were presumably protected by the more or less isotonic media used).

Discussion

The results reported here indicate very strongly that it is the mitochondrion-rich cells which are responsible for the secretion of calcitonin. It is interesting to note that Seecof[4] regarded the two thyroid cell types described in his paper as comparable to the α and β cells of the pituitary or pancreatic islets. Tremblay and Pearse[5] suggested that the mitochondrion-rich cells, and the closely related Askanazay or Hürthle cells, "might conceivably be carrying out some function other than the production of thyroglobulin and thyroxin".

Further work is necessary to confirm our results. Morphological and cytochemical investigations at the electron microscope level are in progress to this end. As soon as calcitonin samples of sufficient purity are obtained attempts will be made to produce antibodies to the hormone. If successful, immunofluorescent investigations will then be carried out.

We thank Mr. J. Bancroft for his assistance and Mr. W. Brackenbury for the photomicrographs.

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L FORM INDUCTION OF NATURALLY OCCURRING METHICILLIN-RESISTANT STRAINS OF *Staphylococcus aureus*

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RECURRENCE of clinical staphylococcal infections occurs in some patients who have been treated with penicillin or methicillin until they are clinically well and cultures are negative for staphylococci. Although the 'persister' state is well known, it is not well understood. This phenomenon, as described by Kagan *et al.*[1], may be due to the capacity of staphylococci to convert to and revert from *L* forms. Staphylococcal *L* forms can be maintained on media containing up to 5,000 μg/ml. of methicillin or penicillin. This high degree of resistance carries serious implications if similar *L* forms occur *in vivo*.

The induction of staphylococcal *L* forms in the presence of methicillin was reported by Kagan *et al.*[1]. The methods used were similar to those adopted by Dienes and Sharp[2], Marston[3], and Prozorovskii[4]. These investigators induced staphylococcal *L* forms from penicillin *G*-sensitive strains in a medium containing penicillin *G* and a high concentration of sodium chloride.

Work reported by Kagan *et al.*[1] described *L* form induction in methicillin-resistant staphylococci. However, these methicillin-resistant strains of staphylococci were laboratory induced.

The appearance of 71 isolates of *Staphylococcus aureus*, which were resistant to all available therapeutic penicillins including methicillin, was reported by Stewart and Holt[5]. These strains were resistant to 20 μg/ml. methicillin or more. The methicillin-resistant organism first appeared, passively, in a nasal carrier; it spread into 8 out of 48 wards in Queen Mary's Hospital for Children at Carshalton, Surrey, England. It spread eventually to fifty patients, most of whom developed no active infection. However, there were two deaths. One of these was a child who died from septicæmia after acquiring the organism in a surgical wound. This patient acquired the organism while receiving methicillin and streptomycin. Despite a change in therapy to erythromycin after positive blood cultures were obtained, the child died. At necropsy the same resistant strain was recovered from the spleen, right ventricle and lung; as in previous instances it was of phage type 75/77.

The appearance of naturally occurring methicillin-resistant staphylococci led to the work reported here of *L* form induction of naturally occurring methicillin-resistant staphylococci.

Five strains (1–5) of naturally occurring methicillin-resistant staphylococci from Carshalton, England, were investigated.

The medium used was brain heart infusion agar ('Difco') with 5 per cent sodium chloride and 20 per cent heat inactivated filtered human serum. Some plates contained this same medium and up to 500 μg/ml. methicillin. Other plates were used with the same medium without antibiotic but with a central well in which was placed 500 or 1,000 μg methicillin in 0·05 ml. distilled water.

The *Staphylococcus aureus* cultures were seeded to 2·5 per cent rabbit blood brain heart infusion agar ('Difco') for isolation of pure cultures. Single hæmolytic colonies were transferred to brain heart infusion broth and incubated approximately 18 h. Cultures were then transferred by cotton swab to the methicillin *L* form inducing media. Cultures were then incubated at 37° C and kept from dehydrating by the use of rubber bands to seal the Petri dishes.

Table 1 shows the results of culturing the five strains of *Staphylococcus aureus* on medium containing 500 μg/ml. methicillin. There were no granular colonies and *L* forms predominated in the medium containing 500 μg/ml. methicillin.

Table 1. *L* Form Induction of Naturally Occurring Methicillin-resistant *Staphylococcus aureus* Strains

	Staphylococcus aureus strain numbers 1	2	3	4	5
Numbers of *L* form colonies	20	75	98	128	76
Numbers of normal colonies	0	3	0	15	0

The central well method with 500–1,000 μg/ml. methicillin also yielded staphylococcal *L* forms. In general, as the concentration of methicillin increased in the serum-containing medium, the confluent bacterial growth was replaced by discrete bacterial colonies mixed with a few

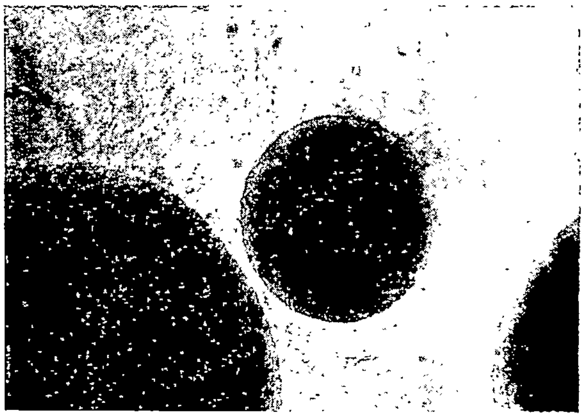

Fig. 1. Appearance of normal staphylococcal colonies (× c. 66)

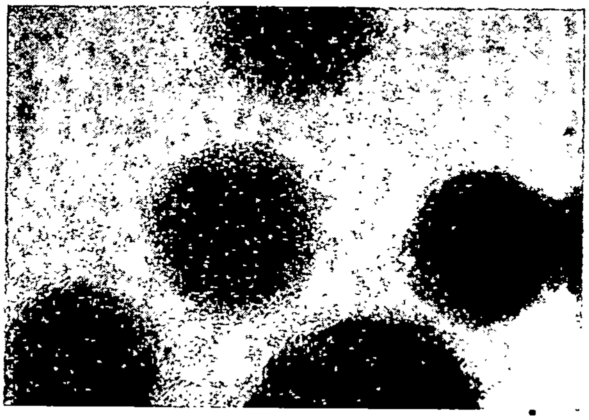

Fig. 2. A group of granular colonies × c. 66)

L colonies and some small as well as large granular colonies, and, finally, only *L* forms (Figs. 1–7).

L colony formation was readily obtained from all five strains of methicillin-resistant *Staphylococcus aureus* by methicillin induction. Some reversion to parent methicillin-resistant staphylococcal forms occurred on subsequent transfer of *L* forms to media containing 500 µg/ml. forms obtained from parent *Staphylococcus aureus* strains that were sensitive to methicillin.

Granular colonies appearing in close proximity to the *L* forms of staphylococci (Figs. 4 and 5) when transferred to media containing up to 500 µg/ml. of methicillin reproduced identical granular type colonies. These granular colonies were medium large and opaque to white in colour; their cells were Gram-positive and swollen. On the same medium, but lacking methicillin, seedings from the granular colony reverted to normal pigmented *Staphylococcus aureus* colonies. Cells from the latter colonies were normal in appearance. The granular, as well as *L*, forms were both maintained successfully in repeated transfers on medium containing 500 µg/ml. methicillin. Unlike the parent forms, when granular colonies were cultured continuously in medium containing methicillin, *L* phase variants did not appear.

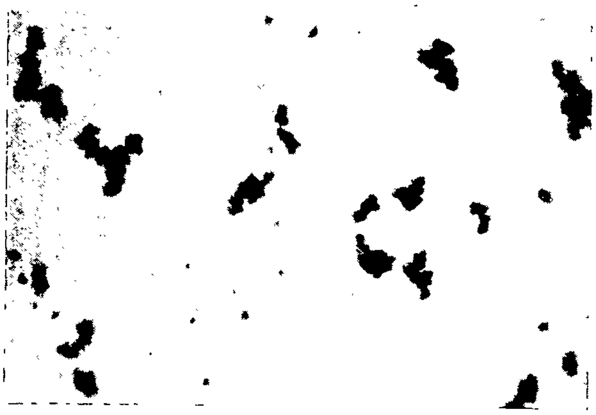

Fig. 3. A Gram stain of swollen cocci from the granular colonies (× *c*. 660)

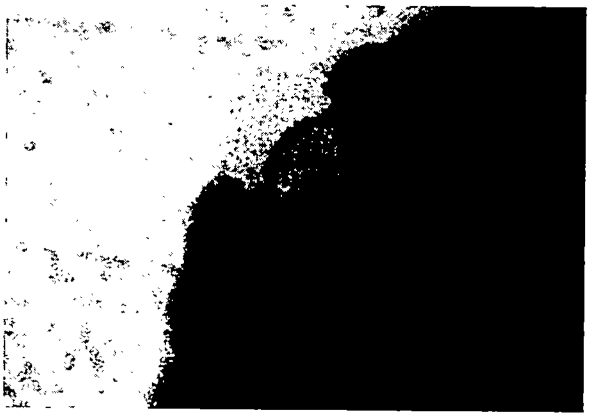

Fig. 4. *L* colony being overgrown by a large granular type colony (× *c*. 66)

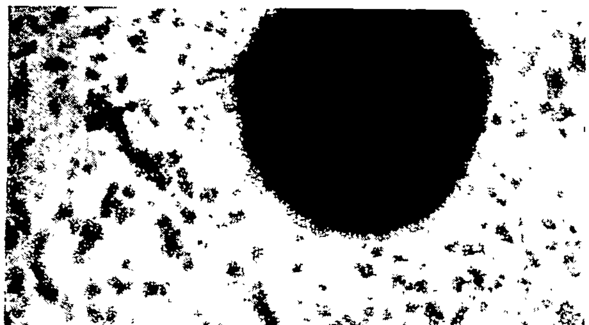

Fig. 5. *L* colony and a granular colony growing in close proximity to each other (× *c*. 66)

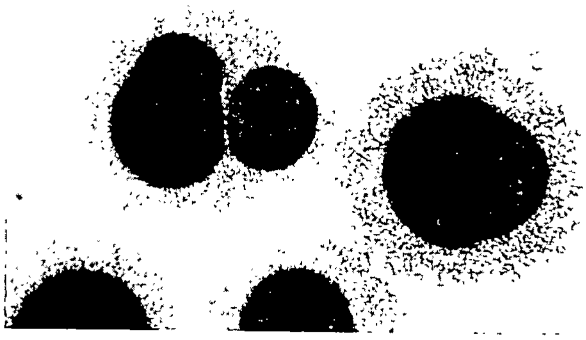

Fig. 6. A pure culture *L* form of *S. aureus* strain No. 3 after 60 h incubation (× *c*. 66)

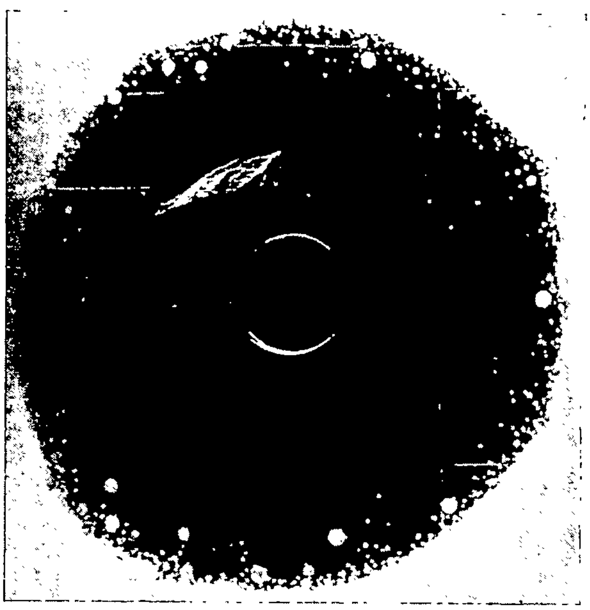

Fig. 7. Centre well method with 500 µg methicillin added to well. Small pin point *L* forms and larger granular colonies are visible

The significance or relation between the granular and *L* forms is not clear, especially since the granular forms failed to produce *L* forms on subsequent cultures in the presence of increased concentration of methicillin. The reversion from granular to normal pigmented *Staphylococcus aureus* colonies in the absence of methicillin resembles the reversion of *G* forms or small colony penicillin variants of *Staphylococcus aureus* reported by Youmans *et al.*[6].

Some have considered the swollen or granular type cells to be intermediate between *L* forms and normal forms of staphylococci[2,3,7]. However, this granular type of growth could be a mutation entirely unrelated to the *L* form. From the fact that the granular forms of these particular strains could not be induced to *L* forms, it would appear that at least under these conditions they are not a stage in the development of *L* forms. Simultaneous occurrence of granular and *L* forms in close proximity may not be limited to conditions *in vitro* but also may appear *in vivo* in the 'persister' state. To understand more about the 'persister' state of staphylococcal infections, further investigations with both forms may prove important.

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ML: A NEW ANTIGEN FOUND IN LEUKÆMIAS AND MAMMARY TUMOURS OF THE MOUSE

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CYTOTOXIC antibodies with specific activity against leukæmia cells can be prepared in mice by immunization with leukæmias of various types. Antisera of this sort have been used to define three distinct antigenic systems in mouse leukæmias: (1) The *G* antigen[1] of leukæmias induced by Gross virus[2]; (2) the *FMR* antigen[3] of leukæmias induced by Friend[4], Moloney[5] or Rauscher viruses[6]; (3) the *TL* (thymus leukæmia) antigen[7] of spontaneous leukæmias, leukæmias induced by a variety of agents, and normal thymus of certain strains.

A fourth antigenic system associated with mouse leukæmia is described here. The antigen has been detected in several leukæmias of the *DBA*/2 strain and in the mammary tissue and mammary tumours of mouse strains which are infected with the mammary tumour agent. In view of its tissue distribution, the appropriate designation for this antigen is *ML* (mammary leukæmia).

The antigen was first detected by iso-immunization of *C57BL*/6 mice with a *DBA*/2 spontaneous leukæmia, *DBA*/2 ♀ *SL*1. Antiserum from these mice was absorbed *in vivo* in normal *DBA*/2 mice in order to remove cytotoxic isoantibodies. This absorbed serum was cytotoxic for *DBA*/2 ♀ *SL*1, another spontaneous leukæmia, *DBA*/2 ♂ *SL*2, and a urethan-induced leukæmia, *DBA*/2 ♂ *U*12; there was no cytotoxic effect on several transplanted leukæmias induced in adult *DBA*/2 mice by percutaneous application of 7,12-dimethylbenzanthracene (DMBA) (Table 1).

Antisera with *ML* specificity have been prepared also by immunization of *C57BL*/6 mice with *DBA*/2 ♂ *SL*2,

Table 1. Cytotoxic Tests* on *DBA*/2 Leukæmias with the Serum *C57BL*/6 ♀ anti *DBA*/2 ♀ *SL*1 absorbed *in vivo*† in Normal *DBA*/2 Mice

Test cells from leukæmia	Trans- plant generation	Antiserum dilution									
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1,024
		% dead cells (stained by trypan blue)									
DBA/2 ♀ *SL*1	25	72	73	60	56	46	32	28	14	<10	<10
DBA/2 ♂ *SL*2	16	75	76	70	64	57	51	46	41	26	<10
DBA/2 ♂ *U*12	13	48	38	34	28	18	<10	<10			
DBA/2 ♀ or ♂ DMBA-induced leukæmias Nos. 2, 3, 4, 5, 6, 12, 15 and 16	13–40	<10	<10	<10	<10	<10	<10				

* Equal volumes (0·03 ml.) antiserum dilution, cell suspension (2×10^6/ml.) and pooled absorbed guinea pig serum (diluted 1/3) incubated for 45 min at 37° C. Diluent: Medium 199. Controls (omitted from Table): cells incubated with either antiserum or guinea-pig serum alone showed <10 per cent stained. For full details of technique see refs. 7 and 8.

† Absorption performed by injecting 0·5 ml. pooled undiluted antiserum intravenously. The serum was recovered 4 h later by cardiac puncture.

Table 2. Cytotoxic Tests with the Serum *C57BL*/6 ♂ anti *DBA*/2 ♂ *SL*2 absorbed *in vivo* in Normal *DBA*/2 Mice
Results of absorption with various *DBA*/2 leukæmias

Leukæmia cells used for absorption	Transplant generation	Dilution of absorbed antiserum*					
		1/15	1/30	1/60	1/120	1/240	1/480
		% *DBA*/2 ♂ *SL*2 cells stained by trypan blue					
None (control)		55	47	37	27	14	<10
DBA/2 ♀ *SL*1	27	<10	<10	<10	<10	<10	<10
DBA/2 ♂ *SL*2	20	<10	<10	<10	<10	<10	<10
DBA/2 ♂ *U*12	8	19	16	11	<10	<10	<10
DBA/2 ♀ DMBA 1	42	48	49	39	30	11	<10
DBA/2 ♀ DMBA 2	17	51	44	32	29	14	<10
DBA/2 ♀ DMBA 5	29	57	47	37	22	<10	<10
DBA/2 ♀ DMBA 9	33	49	45	37	28	11	<10
DBA/2 ♀ DMBA 12	38	22	<10	<10	<10	<10	<10
DBA/2 ♂ DMBA 17	2	—	43	34	26	16	<10
DBA/2 ♂ DMBA 18	Primary	49	42	36	23	19	<10
DBA/2 ♀ *L* 1210	>100	<10	<10	<10	<10	<10	<10
DBA/2 ♂ *P* 1534	>100	<10	<10	<10	<10	<10	<10

See footnotes to Table 1.

* Serum diluted as indicated in first column and absorbed with an equal volume of packed cells for 45 min at room temperature and 45 min at 4° C.

Table 3. Cytotoxic Tests with the Serum *C57BL/6* ♀ anti *DBA/2* ♀ *SL*1 Absorbed *in vivo* in Normal *DBA/2* Mice

Results of absorption with various *DBA/2* normal tissues

Normal tissue used for absorption	Dilution of absorbed antiserum 1/16	1/32	1/64	1/128	1/256	1/512
	% *DBA/2* ♂ *SL*2 cells stained by trypan blue					
None (control)	71	62	43	37	16	<10
DBA/2 ♂ *SL*2 (positive control)	<10	<10	<10	<10	<10	<10
Brain	66	56	42	31	<10	<10
Thymus	68	60	44	33	15	<10
Heart	65	59	43	32	14	<10
Lung	69	61	41	37	19	12
Liver	69	55	41	31	11	<10
Spleen	65	52	41	30	12	<10
Kidney	67	55	41	35	14	<10
Erythrocytes	65	59	43	35	15	<10

See footnotes to Tables 1 and 2.

Table 4. Leukæmias of Strains other than *DBA/2* that did not absorb Activity from *ML* Antisera

	A	*C57BL/6*	*BALB/c*	*AKR*	*C3H/An*	*C3Hf/Bi*	*C58*	I	$(C57BL/6 \times A)F_1$
Spontaneous	2	4	2	2			1	1	
Chemical carcinogen									
DMBA				2	2		3		
Urethan			1	2					
X-radiation	3	5	3						1
Virus									
Gross		1	1			1			
Moloney		2							
Rauscher		2							

and by immunization of strains other than *C57BL/6* [*BALB/c*, *I*, $(C3H/An \times I)F_1$ and $(C57BL/6 \times A)F_1$] with either *DBA/2* ♀ *SL*1 or *DBA/2* ♂ *SL*2. Six antisera prepared by prolonged immunization of strains *C57BL/6*, *I* or $(C3H/An \times I)F_1$ with five individual DMBA-induced *DBA/2* leukæmias, and absorbed *in vivo* in *DBA/2*, contained no *ML* antibody. Eight antisera prepared in *DBA/2* against eight histo-incompatible leukæmias of various types showed no cytotoxic activity against *DBA/2* ♀ *SL*1 or *DBA/2* ♂ *SL*2.

The distribution of *ML* antigen was investigated by the more sensitive technique of absorption of specific activity from *ML* antisera. The three leukæmias which were sensitive in the direct cytotoxic test removed *ML* activity (Table 2). Absorption was also observed with three leukæmias that showed little or no sensitivity in the direct cytotoxic test—*DBA/2* ♀ DMBA 12, and the two long-transplanted *DBA/2* ascites leukæmias *P* 1534 (spontaneous) and *L* 1210 (induced by 20-methylcholanthrene). The other DMBA-induced leukæmias, including two (Nos. 17 and 18) which were induced by neonatal injection of DMBA, were uniformly *ML*−. Table 3 shows that absorption of *ML* antiserum with a variety of tissues of normal *DBA/2* mice was entirely negative. No leukæmia of any strain other than *DBA/2* has absorbed activity from *ML* antiserum (Table 4). The absorption data clearly distinguish *ML* from the *G*, *FMR* and the *TL* systems. Both *DBA/2* ♀ *SL*1 and *DBA/2* ♂ *SL*2 carry the *TL* antigen as well as *ML* specificity (both absorb *TL* activity from the serum *C57BL/6 anti ASL*1 (ref. 7)). Absorption of *ML* antisera with the prototype *TL*+ leukæmias *ERLD* and *ASL*1 (ref. 7) does not reduce *ML* activity.

A variety of chemically induced sarcomas of strains *A*, *C3H*, *BALB/c* and $(C3H/An \times I)F_1$, including both ascites and solid forms, did not absorb *ML* antibody. In contrast, *ML* activity was removed by spontaneous mammary tumours from mice of strains carrying the mammary tumour agent. Twenty-seven primary mammary tumours were examined, of which 11 are shown in Table 5. Twenty tumours, including 8 of those listed in Table 5, gave clear-cut absorption. The remaining 7 tumours showed incomplete absorption under the conditions of the test. The mammary tumours were obtained from strains *C3H/An* (5 tumours), *DBA/2* (8 tumours), *A* (2 tumours) and $(C3H/An \times I)F_1$ (12 tumours). The specificity of absorption of *ML* activity by mammary tumours was confirmed by tests with *H*-2 and *TL* antisera; non-specific absorption of these antibodies by mammary tumour cells did not occur. Mammary tissue obtained from lactating mice 5–10 days *post partum* has also been used in absorption tests. *ML* antibody was absorbed by mammary tissue from strains *DBA/2* and *C3H/An*, which have the mammary tumour agent, but not by mammary tissue from the agent-free *C57BL/6* and *BALB/c* strains. No tissue from strains *C3H/An* and $(C3H/An \times I)F_1$ other than mammary gland and mammary tumour removed *ML* activity.

The *ML* antisera used in this work contained an unidentified antibody with cytotoxic activity for *DBA/2* thymus. This antibody was not absorbed by *ML*+ mammary tumour tissue.

Table 5. Cytotoxic Tests with the Serum *C57BL/6* ♂ anti *DBA/2* ♂ *SL*2 absorbed *in vivo* in Normal *DBA/2* Mice

Absorption of *ML* antibody by primary spontaneous mammary tumours*

Strain of tumour used for absorption	Dilution of absorbed antiserum 1/20	1/40	1/80	1/160	1/320	1/640
	% DBA/2 ♂ *SL*2 cells stained by trypan blue					
None (control)	55	47	31	26	17	<10
DBA/2 ♂ spleen (negative control)	52	37	31	25	16	<10
DBA/2	<10	<10	<10	<10	<10	
DBA/2	<10	<10	<10	<10	<10	
DBA/2	33	20	12	10	<10	
C3H/An	<10	<10	<10	<10	<10	
C3H/An	16	<10	<10	<10	<10	
C3H/An	32	24	14	11	<10	
$(C3H/An \times I)F_1$	<10	<10	<10	<10	<10	
$(C3H/An \times I)F_1$	<10	<10	<10	<10	<10	
$(C3H/An \times I)F_1$	24	12	<10	<10		
A	11	<10	<10	<10	<10	
A	30	22	16	<10	<10	

See footnotes to Tables 1 and 2.

* Mammary tumour cells were obtained by mincing the solid tumour with scissors and washing the suspended cells three times. These preparations contained 50–80 per cent cells stained by trypan blue.

The finding of an antigen that is shared by leukæmias and tissues from normal mice of certain strains suggests a parallel with the *TL* system[7]. In the case of *ML*, however, there are clear indications that the antigen is associated with presence of a known virus, the mammary tumour agent. If indeed *ML* is determined by the mammary tumour agent, this may indicate either that this virus can be leukæmogenic in some circumstances or that the presence of *ML* antigen in *DBA/2* leukæmias is an example of antigenic conversion[8] brought about by the infection of leukæmia cells with an unrelated virus.

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USE OF SIMPLE GEL-DIFFUSION TECHNIQUES TO ASSIGN ANTIGENIC MARKERS TO NATIVE PROTEINS

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MACROMOLECULES have been characterized with precision in terms of amino-acid sequences in proteins, sugar sequences of polysaccharides and, less successfully, nucleotide sequences of nucleic acids. These achievements still have not left the biologist with practical means of examining many aspects of biological control of macromolecule synthesis and function. So far, detailed analyses of the fate of individual sequences of the macromolecules understandably have been neglected in investigations of such problems as complementation and isozyme formation. Instead, reliance has been placed on indicators of overall structural changes, such as changes in net charge, enzymatic efficiences, etc.[1]. This article suggests an approach to identification of distinct sites on macromolecules which combines simplicity of apparatus with rapidity and ease of analysis. By gel-diffusion methods macromolecules can be characterized in their native, undegraded state provided that they are antigenic. Less than 1 mg of material is needed, and this need not be entirely pure. A requirement, however, is that a supply of a number of related antigens be available; a readily available source would be the products of mutants, although in theory any cross-reacting substances would suffice.

The surface antigens of *Paramecium* can serve as a prototype system and meet all these requisites. The class dealt with is that of the *C* serotypes and the five used were all naturally occurring variants at the same locus[2]. These antigens are large, of approximately 250,000 molecular weight, almost exclusively protein, with a minor carbohydrate constituent, and are soluble in saline[3]. The antigenic structure in terms of operationally defined determinants has been examined in detail. In addition, the specificities of antibodies possessed by individual sera have been assigned, and the determinants possessed by an antigen have been classified into those that are unique and those that are shared with other antigens. Finally, procedures for tagging individual 'determinants and their possible utility in the investigation of several biological problems are described.

A combination of two gel-diffusion methods is used: double diffusion in tubes[4], and double diffusion on microscope slides (modified from Ouchterlony)[5]. Preer tubes are used primarily to determine approximate equivalence ratios of antigen and sera: those concentrations yielding an intense, sharp band of precipitate, a band that does not change location in the agar column from its position when first visible. For most of the sera used, this was the amount of antigen extracted from 10^7 cells and suspended in 1 ml.

Once equivalence concentrations are assigned, the Ouchterlony technique is the sole gel-diffusion method used. Behind this method lies a simple principle: lines of antigen–antibody precipitate meet and fuse when there are no antibodies diffusing from the serum well that can distinguish the two antigens being compared. If the antigens share no determinants—with reference to the antibodies available—the bands will cross, with neither precipitate forming an apparent barrier to the diffusion of unrelated antigen or antibody molecules. However, if two antigens are related, although not identical, a spur may form near the point of fusion as an extension of the precipitate. This spur is made up of the antigen that reacts exclusively with at least one fraction of the population of antibody molecules[6].

Equivalence or near-equivalence concentrations of reactants are chosen for use in Ouchterlony patterns because the band formed is dense and narrow and because artefactual spurs tend to form when either reactant is in extreme antigen or antibody excess. Tests for genuineness of spurs observed are described here. In essence, spur formation in gels is analogous to a liquid precipitin test for the presence in the supernatant of antibody specific for homologous antigen, following maximal precipitation by a cross-reacting antigen. In gel-diffusion the presence of unprecipitated antibody after interaction of serum with one antigen solution is detected by the formation of a precipitate trapped in the agar. The free antibodies diffusing unimpeded through the original band are precipitated by the second antigen preparation. Thus, a spur is both indicative of multiple antibodies in a serum and of distinctive antigens. We may therefore say that, operationally, one determinant difference can enable two antigens to be discriminated.

Assigning Determinants to Each Antigen

When any two *C* antigens were compared side by side with serum prepared against one of the antigens, in most instances a spur formed with the immunizing (homologous) antigen. In the few instances when a serum did not give rise to a spur, it could be shown by other methods, for example, the ability to immobilize differentially living cells of the two strains from which the antigens were extracted, that the serum lacked the ability to discriminate between the antigens. The significant observation, from the point of view of antigenic identity, is that if any serum makes distinctions between two antigens, regardless of the rarity of such a serum, the two antigens must be considered different in some respect.

However, when two antigens are compared using as a source of antibodies a serum prepared against a third cross-reacting antigen, no particular pattern should be anticipated other than a band fronting each well; complete fusion, spurs or crosses are all apt to occur. Using several *C* antisera, all non-homologous antigens can be compared with each other. The complete listing of spurs observed can be viewed in a relatively simple framework formed by a single set of premises: (*a*) Any antigen that gives a spur with another antigen possesses a determinant that is missing in the second antigen. (*b*) This same determinant is shared by the antigen used in eliciting the antibodies, the immunizing antigen.

An illustration of spur and fusion patterns observed with one anti-*C* serum is shown in Fig. 1. This is a photograph of a microscope slide stained for protein in which the serum used was prepared against 197 *C* antigen.

The spurs observed with several antisera are presented in Table 1. Each number represents a separate *C* antigen; each isolated from a genetically distinct stock. In the first column are listed all the possible pairings. To facilitate analysis, a certain amount of redundancy is incorporated into the presentation of the results. Thus, each antigen is represented several times: once when paired with the homologous immunizing antigen, again when each non-homologous antigen is compared with it, and a third time when it is compared in turn with each non-homologous antigen. The antigen forming a definite spur as a continuation of the precipitate appearing in front of its well is recorded as 'direction of spur'. Blank

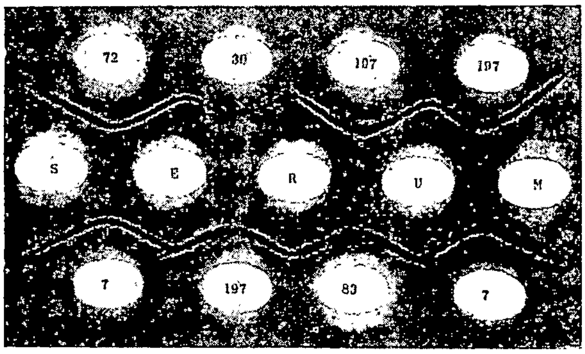


Fig. 1. Gel-diffusion reactions of purified homologous and heterologous immobilization antigens with an anti-197 serum. Antigen from stock 197 (the homologous strain) forms spurs with 7 and 30 antigens; 83 antigen forms a spur with 7 antigen. All other antigen combinations fuse in 'reactions of identity'

spaces represent complete crosses, or poorly defined spurs.

Three categories are of significance. These classes of reactions and the interpretations ascribed to them are:

(1) An antigen forms a spur with every other antigen, with the exception of the immunizing antigen. This antigen shares with the antigen originally used to elicit the antibodies a determinant missing in all the other antigens.

(2) One antigen, regardless of the antigen it is compared with, fails to form a spur. This antigen is missing at least one determinant that is possessed by all the other antigens.

(3) Two antigens form bands that fuse, but with every other antigen (except the immunizing antigen) each of these antigens forms a spur. These two antigens together with the immunizing antigen share a determinant lacking in all other antigens. There obviously are several variations on this particular set of relationships especially as the number of antigens being compared becomes larger than the five used here.

How these interpretations have been applied to the *C* serotypes can be seen by comparing the reactions actually observed (Table 1) with a matrix of determinants (Table 2) derived from these patterns. Each letter in the matrix represents a determinant; a determinant is defined simply as an antigenic entity recognized by an operation in gel diffusion, that is, inability or ability to form a spur. This operational term undoubtedly has an intramolecular counterpart, but whether it is an amino-acid substitution, a folding pattern, etc., of course cannot be stated from the gel-diffusion information alone.

Examples of each of these three principles will serve to illustrate the actual mechanics for evolving the matrix. A letter, *G*, was placed in the 30 and 7 rows because, with anti-7 serum, 30 and 7 gave a spur when tested with each of the other antigens: 197, 72 and 83. Thus, following the first principle, it can be said that 30 shares with the antigen used to elicit the antibodies, 7, a determinant missing in every other antigen. This determinant is represented by the letter *G*.

Table 1. DIRECTION OF SPURS FORMED BY CROSS-REACTING ANTIGENS IN OUCHTERLONY GEL-DIFFUSION PATTERNS

Test sera Anti-7		Anti-83		Anti-197	
Antigen* neighbours	Direction of spur	Antigen neighbours	Direction of spur	Antigen neighbours	Direction of spur
7–30	7	83–30		197–30	197
7–197	7	83–197		197–7	197
7–72	7	83–72	83	197–72	197
7–83	7	83–7	83	197–83	197
30–197	30	30–197	*f*	30–7	
30–72	30	30–72	30	30–72	30
30–83	30	30–7	30	30–83	83
197–30	30	197–30	*f*	7–30	
197–72	72	197–72	197	7–72	
197–83	197	197–7	197	7–83	83
72–30	30	72–30	30	72–30	30
72–197	72	72–197	197	72–7	
72–83	72	72–7		72–83	83
83–30	30	7–30	30	83–30	83
83–197	197	7–197	197	83–7	83
83–72	72	7–72		83–72	83

* Antigens placed in adjacent wells. *f*, fusion.

Table 2. MATRIX OF ANTIGENIC DETERMINANTS FOR *C* SEROTYPES AS DETERMINED BY GEL DIFFUSION

Serotype	Determinants										
30*C*		*F*	*G*	*H*	*I*	*J*		*L*	*M*		*O*
7*C*	*C*	*F*	*G*		*I*	*J*			*M*		
72*C*		*F*		*H*		*J*	*K*	*L*	*M*		
83*C*				*H*	*I*				*M*	*N*	*O*
197*C*		*F*		*H*	*I*		*K*	*L*		*N*	*O*

The determinant *F* in the matrix is present in every antigen save 83. Assignment of this determinant was based on the observation that, with anti-7 serum, 83 failed to form a spur when paired with either 30, 197 or 72. According to the second principle, these three antigens share a determinant, together with the immunizing antigen, that is missing in 83. This is not the only possible explanation, but the simplest, and one not contradicted by any other reaction.

An example of the third principle would be the reactions of 197 and 30 antigens when tested with anti-83 serum. Both these antigens form a spur with 7 and 72, but fuse with each other. Therefore, we can conclude that 197, 30 and 83 (the immunizing antigen) share a common determinant denoted here by the letter *O*. Incidentally, this particular 83 antiserum apparently did not contain antibodies against the *M* and *N* determinants. Otherwise, 83, in comparisons with 30 and 197, would have formed a spur. The 7 and 197 antisera, on the other hand, were able to distinguish their respective homologous antigens from all the cross-reacting antigens.

The completed matrix represents the fewest number of determinants that are required to account for the antigenic specificities observed. When as many determinants as possible have been assigned, the final step is to check whether: (*a*) each antigen has at least one determinant that is missing from a second antigen; (*b*) each antigen shares at least one determinant with a second antigen.

The first requirement stems from the observation that the immunizing antigen generally forms a spur with every other antigen (Table 1). If the determinants assigned thus far fail to fulfil this requirement, then a unique determinant is added to the matrix. For example, *C* had to be tacked on to the determinants for 7 because 7 had no other determinant to distinguish it from 30. On the other hand, 30 can be distinguished from, say, 72 by the determinants *G*, *I* and *O*, from 7 by the *H*, *L* and *O* determinants, etc.

On the basis of analysis with only a few sera, initially each antigen was arbitrarily assigned a unique determinant to account for the spur observed with the immunizing antigen. It was only as more sera became available, and additional determinants were recognized, that it was seen that for most antigens the homologous serum reaction was also consistent with a unique distribution of the shared determinants such as described previously for 30. This finding also suggested the possibility that as more sera were prepared, the number of determinants might correspondingly increase *ad infinitum*. However, with more than twice the number of sera utilized in setting up the matrix in Table 1 as was available in the original investigations (18 compared with 8)[7], only three determinants have had to be added, suggesting that a limit was being approached.

The second requirement for shared determinants stems from the fact that all the antigens form a precipitate with serum prepared against any one of them. This requirement is easily met with the *C* serotypes. However, were this not the case, an additional common determinant would have to be added to all antigens, for example, *X*. Should a serum be found that is unable to distinguish any of the antigens, every antigen pairing giving rise to a reaction of identity (fusion), then the existence of a common determinant could be assumed.

Assigning Antibodies to Individual Antisera

Given an antigen with its set of distinguishing determinants, certain rather limited predictions can be made concerning the kinds of antibodies that should be elicited by injection of this antigen. Because some of the *C* antigens have as many as eight determinants, it would appear that some anti-*C* sera might have as many as eight kinds of antibodies. However, it is well known that rabbits vary considerably in their ability to respond to an antigen, and some antigens are more effective immunizing agents than others. The fact that so many pairings—even between antigens with many determinants—gave single spurs or complete fusion indicates that most, if not all, sera have relatively few detectable kinds of antibodies. With the information from the matrix, it now became possible to describe in detail the antibody content of any particular serum in qualitative terms.

For example, in a 197 antiserum, one may expect to find at the most antibodies against *F*, *H*, *I*, *K*, *L*, *N* and *O* determinants. Knowing the determinant constitution of every antigen, each kind of antibody can be identified by noting whether a particular pattern of spurs is observed, assuming the presence of each antibody singly. The predicted reactions in Table 3 for antibody against each determinant were derived from a comparison of the sets of determinants comprising each antigen (Table 2). For example, 72 possesses *H* which 7 lacks. A spur with 72 is therefore to be expected when it is paired with 7, if *H* antibodies are present. To determine whether *H* antibodies are present, the column headed *H* is scanned. The expectation of fusion, of course, can be ignored in most cases because few sera have just one kind of antibody. The crucial prediction is the kinds of spur anticipated, which are expected to occur among four pairings. Reading across the row from each pairing, it can be seen that with one pairing, 7–83, other antibodies that might be present in the serum (such as anti-*F*) could give rise to spurs also, but these would be in opposite directions and the double spurs would appear as a cross. Thus, this particular pairing might give ambiguous results. Again, if this serum had both *I* and *K* antibodies, then when 30 and 72 are compared a double spur might form. However, were only *I* antibodies present, a single spur alongside the 30 well would appear. In this manner, by checking the reactions observed with those predicted for each column, the presence of antibodies specific for each determinant can often be determined for every *C* antiserum. In those instances where unique determinants are assigned an antigen (such as *C* in 7), it can be assumed that antibody specific for this determinant will probably be found in the homologous serum.

Obtaining Determinant-specific Reactions

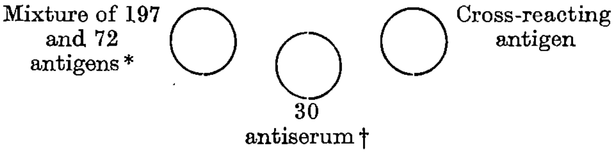
When the procedures already outlined here were followed, it proved possible to demonstrate that several antisera had but two or three kinds of antibodies defined in terms of specificity towards operationally distinguishable determinants. Consequently, a particular determinant generally could be assigned to a new antigen on the basis of whether it reacted to form characteristic patterns of spurs when compared with each antigen. For example, if a serum were shown to contain anti-*F* and anti-*K* antibodies exclusively, an antigen could be tested for the presence of *F* and *K* by comparing each antigen with it and checking for the reactions predicted by the matrix, as was done in making up Table 3.

Table 3. Reactions of Antibodies against Specific Determinants in 197 Antiserum

Antigen neighbours	Expected antibody specificities *F*	*H*	*I*	*K*	*L*	*N*	*O*
30–197	*f*	*f*	*f*	197	*f*	*f*	*f*
30–7	*f*	30	*f*	—	30	—	30
30–72	*f*	*f*	30	72	*f*	—	30
30–83	30	*f*	*f*	—	30	83	*f*
7–197	*f*	197	*f*	197	197	197	197
7–72	*f*	72	7	72	72	—	—
77–83	7	83	*f*	—	—	83	83
72–197	*f*	*f*	197	*f*	*f*	197	197
72–83	72	*f*	83	72	72	83	83
197–83	197	*f*	*f*	197	197	*f*	*f*

f, fusion. –, no band. Numbers in body of Table indicate direction of spur.

The usual method for securing specific antibody is to absorb selectively all antibodies but those having the desired specificity. Any absorption can also be carried out directly in gel by choosing appropriate patterns. Indeed, as has been noted here, spur formation itself is a consequence of selective absorption. Thus, if one wanted to determine whether a cross-reacting antigen possessed determinant *G*, the following pattern could be set up to obtain a reaction with antibody specific to *G*:



* Possesses *F*, *H*, *I*, *J*, *K*, *L*, *M*, *N* and *O* determinants.
† Containing several kinds of antibodies, including *G* specific ones.

Those antibodies with *G* specificity would be free to diffuse through the 197–72 precipitate and form a spur with the cross-reacting antigen. If *G* were absent in the cross-reacting antigen, complete fusion of the two bands would be evident were all other determinants possessed by the 30 antigen (the immunizing antigen) also shared by the cross-reacting antigen.

In sum, the presence of individual determinants on an antigen can be recognized either by assaying sera for antibody content and selecting the most specific or by selectively absorbing in gel.

Spurs as a Criterion for Partial Identity

It is critical for the success of the proposed analysis that spurs observed truly represent the reactions of a single antigenic species of molecules with specific antibodies. It is as a consequence of this interaction that a band of precipitate forms, the major portion of the length of which is a complex of antigen and cross-reacting antibody, and a smaller segment of which is a complex with specific antibody. In our experience the most common source of misinterpretation has been the presence in the system of a second unrelated antigen as an impurity in one antigen preparation. By a coincidence of concentrations, the band formed by this antigen may precisely overlie the original precipitate.

Fortunately, there are several tests for distinguishing pseudo from true spurs. In one, the serum is absorbed with the antigen solution not giving rise to the spur, and then the supernatant is allowed to diffuse against the suspect antigen solution. Antibody to impurity would have been left untouched by the absorbent, so that the position of the band formed with the test antigen would be the same as initially observed, allowing for dilution by absorbent. However, were the spur genuine, the band would shift in its position away from the antigen well since at least some cross-reacting antibody would have been removed. This test is best carried out in Preer double diffusion tubes where displacements in band positions can more accurately be measured.

In the second test, mixtures of the two antigen solutions in varying proportions are allowed to diffuse in separate Preer tubes against unabsorbed serum. If two bands are formed only when the suspect antigen solution is not in excess, then the spur is genuine. The appearance of two bands when either antigen solution is in excess speaks for an impurity. These predictions are based on the observations on a system comprised solely of homologous and cross-reacting antigen[4]. With the latter in excess, antibody capable of precipitating only homologous antigen is free to diffuse through the leading heterologous antigen–

antibody precipitate and form a second band as the antibody is precipitated by the trailing front of homologous antigen. With homologous antigen in excess, only one band was seen as all antibodies were precipitated by the leading front. An impurity might be expected to form a second band (or possibly even a third) independent of which antigen solution was in excess, since neither major antigen–antibody precipitate would act as a barrier to the passage of antibody directed against impurity.

A third check on the genuineness of a spur is the simplest. The antigen solution in question is placed in the central well of an Ouchterlony slide surrounded by a circle of wells, each well filled with a different cross-reacting serum. With a true spur situation a single continuous band should be found bordering the central well. The presence of an unrelated antigen would be revealed by the formation of a second band or a continuation of the precipitate in the regions of those sera with antibody directed against the impurity. Fig. 2 illustrates this for two preparations of *C* antigens. In *A* no impurities were present, and a spur formed by this antigen when compared with a heterologous antigen was apparently *bona fide*. Another antigen preparation that had an overlapping contaminant presented the pattern shown by *B*.

Procedures similar to those outlined here can also be used for discriminating between spurs due to cross-reactivity and those due to overlapping of two related antigens, such as might occur with a mixture of native and partially denatured antigens.

Limitations of Method

From the way in which data are accumulated, certain limitations inhere in this approach. For example, if all antisera available possess antibody in high titre directed against many determinants, then individual, discrete spurs may be replaced by crosses resulting from double spurs. This would effectively eliminate the possibility of arranging an unambiguous matrix. Also, if the antigens are not soluble, difficulties in free diffusion might arise, although particulate antigens have been successfully investigated in agar[8].

Should the concentrations of antigens be less than 10 γ/ml. or the sera be low in antibody content, precipitates, and especially spurs, might not be easily visible. With such systems, the approach to structural analysis of antigens suggested here can still be applied, substituting Preer tubes for Ouchterlony slides. In lieu of spur formation, the criterion for partial and complete identity becomes the number of bands formed subsequent to mixtures of antigens in varying proportions.

Other requirements for utilization of the procedures include at least partial purification of the antigen so that multiple bands are not a frequent occurrence, and the presence of precipitating as contrasted with univalent antibodies. However, these are requirements shared by most immunological methods and so cannot be considered uniquely restrictive.

Discussion

A relatively simple method has been outlined for providing antigenic macromolecules with multiple identifying characters. Such landmarks can be recognized individually without destroying the integrity of the antigen. The conclusion that a single species of molecules may possess many antigenic determinants is not unexpected in the light of several investigations on partially degraded antigens[9]. Intact antigens, however, hitherto have not readily lent themselves to such analysis, although there have been some exceptions[10]. A suggestion, perhaps unique, derived from this investigation is that an antigen which can elicit antibodies capable of distinguishing it from all cross-reacting antigens need not possess a unique determinant.

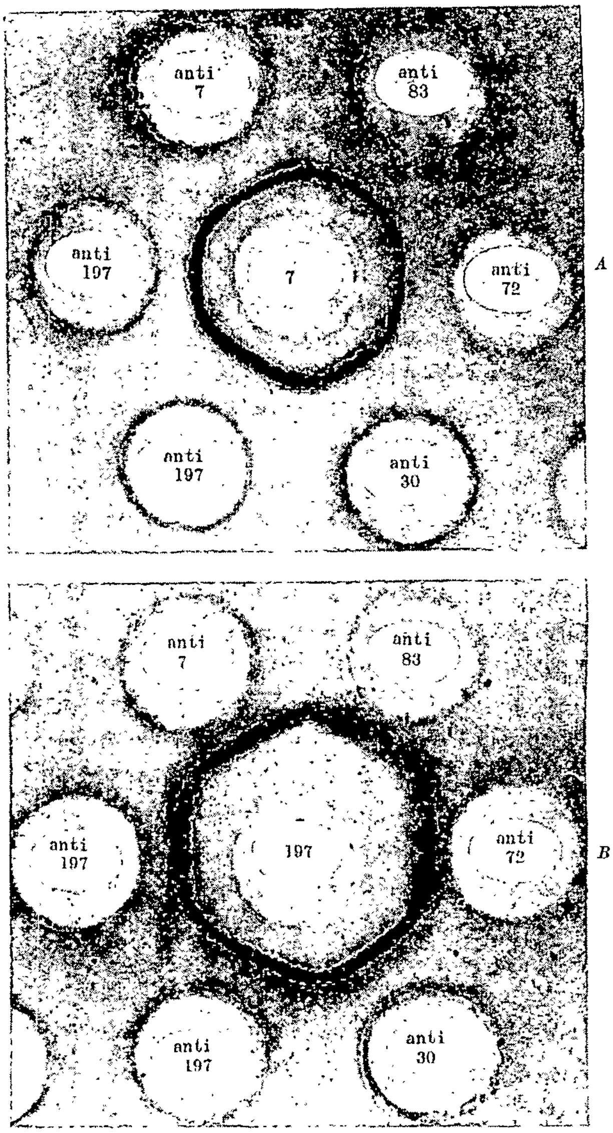


Fig. 2. A, Purified *C* antigen from stock 7 surrounded by six different anti-C sera in wells punched in agar on a microscope slide. The slide was dried, washed in saline and stained with aniline blue. *B*, Partially purified 197*C* antigen solution containing at least one other antigen. The two antigen–antibody precipitates with one of the anti-197 sera tend to overlap and could give the illusion of a spur when compared with a single antigen

Although naturally occurring mutants have provided the antigens used here, for certain purposes partially denatured or disrupted molecules that retain antigenicity may also serve as a collection of cross-reacting substances for determinant analysis. However, restraints might then be placed on interpretations of the role these determinants can assume in the native molecule where they may be hidden. Conceivably, relatively few sera may be needed for an analysis of antigens. The typical heterogeneity in the kinds of antibodies induced by immunization of different rabbits with the same antigen may provide in itself a source of antibodies capable of distinguishing antigens. Thus, a chronic barrier to the examination of many immunological phenomena is here turned to advantage.

Once a mosaic of determinants is assigned to a group of proteins, the fate of sub-units can be followed during the course of chemical manipulations or in their native state in living systems. For example, the method should prove helpful in complementation investigations where it would be highly desirable to compare the reconstituted products of mixtures of different mutants with wild-type protein, without being forced to rely on a single criterion. Wild-type and non-mutant protein could be compared to see whether all determinants of both parents are possessed by the new protein. Also, were the molecules capable of dissociation into sub-units retaining their antigenicity, specific sub-units might be recognized by their determinant characteristics. The time of appearance, localization and concentration changes of sub-units could then be followed. A description of the course of assembly of native proteins during development might also prove practicable at a finer level than previously attempted. A related area of considerable interest which would probably benefit from detailed analysis of sub-unit reassortment and aggregation is that of the control of the synthesis of isozymes[11].

In conjunction with other methods, it may also be possible for this technique to be useful in resolving certain problems of protein structure not otherwise easily attacked. For example, *Neurospora* adenylsuccinate synthetase mutants located at certain points within a cistron tend to restore enzymatic activity to a degree greater or less than would be expected based on their position within the linkage group[12]. Were a refolding of a polypeptide chain or a juxtaposition of sub-units responsible for a return to a near-native configuration[13], then conceivably the determinant construction of heterokaryons might be more like the wild-type than either parent separately or in combination—that is, a 'new' antigenic specificity might be found. The lack of agreement between the linearity of linkage and complementation maps possibly could be resolved were concomitant changes in determinant composition also known. Since antigenic specificity itself is known to be highly dependent on folding, conclusions about tertiary structure might also be drawn from determinant analysis.

The probability of success in extending this approach to systems other than the *Paramecium* immobilization antigens appears to be high. The *Paramecium* antigens that have been successfully used here are not particularly effective as immunizing agents, rarely eliciting sera with antibody concentrations as high as 200γ per ml. Particularly suitable would be an examination of evolutionary relationships among species and genera through a comparison of individual serum proteins, such as albumins. This, of course, would provide but a single criterion of ancestry and might in itself be misleading. Thus, one determinant difference between two albumins, and two differences between either of these and a third albumin, need not mean that the original albumins are more 'closely' related than is either to the third. The single determinant difference may be a consequence of several amino-acid substitutions, and each of the other determinant differences result from a single amino-acid difference.

The major advantage offered by this immunological approach is that a single parameter is not relied on to compare proteins. For example, a change in net charge that is a reflexion of a single amino-acid substitution may also be mimicked by the sum of several amino-acid changes. However, using the suggested antigenic analysis, the number of parameters is greatly increased as each determinant may be followed separately, and a single net effect is less likely to obscure several independent alterations.

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RETENTION OF IMMUNOLOGICAL INFORMATION

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(I) By Syngeneic Radiation Chimæras

By Dr. A. J. S. DAVIES, BARBARA DOE,
A. MARJORIE CROSS and E. V. ELLIOTT

It is recognized that immunological information developed in an adult mouse in response to antigenic challenge may be retained when the animal is irradiated and given syngeneic bone marrow but is not retained to the same extent after irradiation and injection of allogeneic bone marrow[1,2].

A further series of experiments concerned with the immune responses of syngeneic and allogeneic radiation chimæras to sheep red cells is here described. The results are examined in order to determine whether residual immunological activity after irradiation and bone marrow therapy must necessarily be attributed to persisting host cells.

Mice

Male mice of the Chester Beatty *CBA* and *BALB/c* and of the Harwell *CBA/T6T6* strains were used throughout. Host (*CBA*) mice were 10–15 weeks of age at the time of irradiation. Donor mice (*BALB/c* or *CBA/T6T6*) were 8–10 weeks of age when killed. The *CBA/T6T6* mice have marker chromosomes[3].

Immunization

(*a*) *Active.* Mice were immunized by intraperitoneal injection of 0·2 ml. of a 20 per cent suspension of sheep red blood cells in Alsever's solution, 20 and 13 days before irradiation and 60 days after irradiation.

Immune mice were bled from the retro-orbital sinus. Serial doubling dilutions were made of the serum in saline in 'Perspex' hæmagglutination tiles. Aliquots of thrice-washed sheep red cells were added to the tiles, which were kept at 37° C for not less than 2 h. The total titration volume was 0·05 ml. Titres were expressed as logarithms to the base two of the reciprocal of the final dilution showing macroscopically visible agglutination.

(*b*) *Passive.* Eighty inbred male *BALB/c* mice were immunized with sheep red cells on each of three occasions separated by a fortnight. Seven days after the third injection blood was taken from the mice, pooled and after 1 h at 4° C, to facilitate retraction of the clot, the immune serum was taken off.

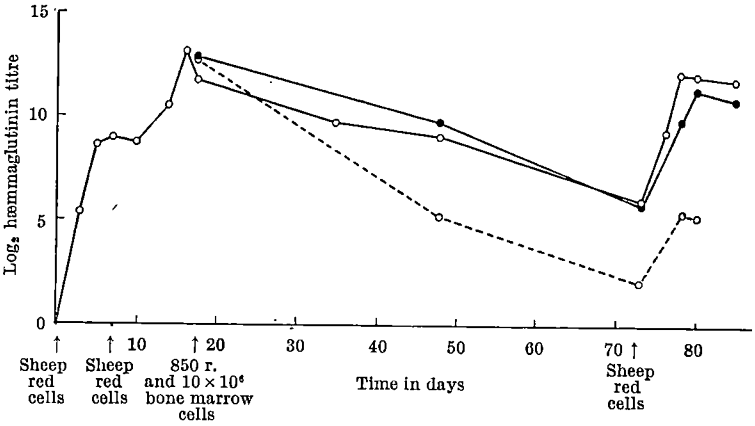

Fig. 1. The hæmagglutinin titres of various kinds of *BALB/c* mice in relation to time. (The normal mice were not irradiated)
—○—, Normal mice; —●—, *CBA/T6T6→CBA* syngeneic chimæras;
---○---, *BALB/c→CBA* allogeneic chimæras

0·4 ml. of this serum was injected intraperitoneally into each of 48 male *BALB/c* mice. Titrations were carried out on individual sera from all passively immunized mice 36 h, 6·5, 13·5, 20·5 and 27·5 days after their injection with immune serum. In all instances about 0·05 ml. of blood was withdrawn from the retro-orbital sinus. This afforded the minimum amount of serum that could be used in our titration system and represented about one-fortieth of the total blood volume of the mice. In these circumstances as only five bleedings were made on any one mouse it was felt that any effect of bleeding *per se* on serum titre could be ignored. The passively immunized mice were split into three groups of of 16 (four boxes with four mice in each). Two of these groups were subjected to 850-r. X-irradiation three days after passive immunization. One group of irradiated mice was injected intravenously with 10×10^6 cells of syngeneic (*BALB/c* bone marrow, the other with the same number of allogeneic (*CBA*) bone marrow cells, within 4 h of irradiation. The third, unirradiated group of mice was not subjected to further treatment. The irradiated mice would have died had they not been injected with bone marrow.

Irradiation and Bone Marrow Therapy

All mice received 850-r. total body dose at LD_{99}, 30 days and within 5 h were given, intravenously, 10×10^6 cells of either *BALB/c* (allogeneic) or *CBA/T6T6* (syngeneic) bone marrow in 0·4 ml. medium 199. Mice were maintained for 5 days before irradiation and 15 afterwards on a 20 per cent protein diet containing aureomycin (in the form of 'Aurofac' kindly supplied by the Cyanamid Chemical Corp., Ltd.; estimated dose 1 mg/mouse/day).

Cytological Check on Chimærism

Three and five days after receiving their third injection of sheep red cells (that is, 63 and 65 days after irradiation and bone marrow injection) groups of chimæras were injected with 'Colcemid' (4 mg/kg body-wt.) 1½ h prior to removal of half the spleens under ether anæsthesia. The piece of spleen removed was divided into two, half being retained for histological examination and half being made into a cell suspension in hypotonic sodium citrate preparatory to cytological examination[4] (syngeneic chimæras only).

Serological Check on Chimærism

Sixty days after irradiation red cells were removed from putative allogeneic chimæras and tested for their identity with appropriate iso-antisera[5].

Tests for Chimærism

(1) *Allogeneic chimæras.* All 20 animals surviving until 60 days after irradiation were found to have exclusively *BALB/c* (donor) red cells.

(2) *Syngeneic chimæras.* 289 cells in nine mice engaged in a response to a third injection of antigen were examined and were found all to be of donor (*CBA/T6T6*) type. This result agrees with the larger series of experiments[6] showing that host cells are rarely found 30, 50 or 100 days after 850-r. irradiated *CBA* mice have been injected with *CBA/T6T6* bone marrow.

Histology

Portions of the spleen removed on the third or fourth day after injection of sheep red cells (63 or 64 days after irradiation) were fixed and prepared for histological analysis. It was not possible to distinguish on the grounds of mitotic frequency between animals undergoing a primary and a secondary response to sheep red cells.

Titrations

(*a*) *Active immunity.* The titration results are given in Table 1 and Fig. 1. It can be seen that the response of normal *CBA* mice to a single injection of sheep red cells is rapid, giving a peak titre within 5–7 days. The primary response of syngeneic chimæras 60 days after irradiation (not shown in Fig. 1) was very similar and the peak titre was not significantly different from that of normal mice. The primary response of the allogeneic chimæras 60 days after irradiation was, however, significantly less than either normal mice or syngeneic chimæras.

The response of normal mice to a second injection of sheep red cells was also found to be rapid, giving a peak titre about 7 days after injection which was about 3–4 logs above the peak following the first injection. The titration values of the mice which were to be irradiated showed that they were behaving as normal before irradiation and could be regarded as satisfactory experimental material. It is noteworthy that titres observed in the syngeneic chimæras 41 and 66 days following the last contact with sheep red cells almost exactly paralleled those found in normal mice over the same period. In contrast the titres in the allogeneic chimæras were significantly lower than those of normal mice and of syngeneic chimæras.

The response of normal mice to a third injection of sheep cells was more rapid than that of the syngeneic

Table 1. LOG_2 HÆMAGGLUTININ TITRES (±*S.D.*) OF VARIOUS KINDS OF *CBA* MALE MICE

Response to injection No.	Days after last injection of antigen	Normal mice	*CBA/T6T6* ⟶*CBA* chimæras	*BALB/c* ⟶*CBA* chimæras
1	3	5·43 ± 1·25	—	—
	5	8·75 ± 0·83	9·67 ± 0·92*	—
	7	9·00 ± 1·00	9·33 ± 0·95	5·14 ± 1·63*
	12	—	9·00 ± 1·0	—
2†	3	8·75 ± 0·97	—	—
	5	10·50 ± 1·80	—	—
	7	13·06 ± 2·92	—	—
	12	11·71 ± 0·71	(12·75 ± 0·76)‡	(12·75 ± 0·76)‡
	28	9·73 ± 1·62	—	—
	41	9·00 ± 0·50	9·78 ± 1·44	5·13 ± 1·03
	66	5·87 ± 0·35	5·67 ± 1·28	2·00 ± 0·73
3§	3	9·25 ± 0·66	—	—
	5	12·12 ± 0·65	9·83 ± 1·75	5·36 ± 1·90
	7	12·00 ± 0·50	11·33 ± 3·30	5·23 ± 1·48
	12	11·75 ± 0·66	10·78 ± 1·77	—

* Injection 60 days after irradiation.

† Injection seven days after first injection.

‡ Titres two days before irradiation.

§ Injection 73 days after second injection, 60 days after irradiation in the case of chimæras.

Table 2. DECLINE IN PASSIVE IMMUNITY OF THREE CLASSES OF *BALB/c* MICE EXPRESSED AS MEAN FALLS IN TITRE AND CALCULATED HALF-LIVES BETWEEN THE TIMES INDICATED AFTER PASSIVE IMMUNIZATION

Mice	Mean drop in titre and calculated half-lives during period indicated (see Fig. 2)							
	Period 1		Period 2		Period 3		Period 4	
	Mean drop in titre	Half-life	Mean drop in titre	Half-life	Mean drop in titre	Half-life	Mean drop in titre	Half-life
Normal	2·38 ± 1·45	2·10	1·50 ± 1·06	4·67	1·47 ± 0·89	4·76	1·20 ± 0·72	5·83
Syngeneic chimæras	2·86 ± 0·63	1·75	1·64 ± 0·48	4·27	2·00 ± 0·71	3·50	1·00 ± 0·93	7·00
Allogeneic chimæras	3·25 ± 1·09	1·54	2·88 ± 0·77	2·43	1·63 ± 0·85	4·29	1·50 ± 0·50	4·67

chimæras, but the peak titres were not significantly higher. In both cases the peaks reached about the level attained during a response of normal mice to a second injection of red cells. At twelve days the response of the syngeneic chimæras to a third injection was significantly higher than the response of a similar group of mice to a first injection of red cells.

The response of the allogeneic chimæras to a third injection was indistinguishable from a primary response and was in any case drastically reduced in comparison with either normal mice or syngeneic chimæras.

(*b*) *Passive immunity.* The results of the passive immunity experiment which are summarized in Fig. 2 and Table 2 can be interpreted as follows: the titre of the pooled immune serum was $\log_2 13$ before its injection into the experimental mice. Within 36 h the serum titres of the injected mice were about $\log_2 11$. This drop in titre could be attributed largely to dilution, but perhaps also to retention of antibody in extravascular sites and in part to decay or destruction of the antibody molecules. In normal mice the rate of decline of amount of antibody does not vary significantly after 36 h and follows an exponential curve which defines the half-life as about 5 days. Both groups of irradiated mice show a significantly more prolonged period of accelerated decay than normal mice, but stability is achieved in the syngeneic chimæras during period one (top of Fig. 2) and in period two in the allogeneic chimæras.

Statistical analysis showed that there were no significant differences between the decay rates of antibody in period one taken by itself. During period two, however, the two groups of chimæras differed from each other, the allogeneic having a higher decay rate than the syngeneic chimæras. Further, the allogeneic chimæras had a higher decay rate than did the normal mice. Within period three the decay rates were similar for all groups of mice giving an antibody half-life of almost 5 days.

The results of Garver *et al.*[1] and of Hollingsworth[2] taken in conjunction with the present findings demonstrate that active immunity to red cell antigens can persist in heavily irradiated animals that have received syngeneic bone marrow as a therapeutic measure. The additional information provided by the present findings can be assessed after consideration of the results of the subsidiary experiment on passive immunity, which showed that in normal mice and irradiated animals the biological half-life of mouse anti-sheep-red-cell antibody was about 5 days. This value is similar to that of 4·5 days quoted by Humphrey (personal communication), rather higher than that which can be calculated from the results of Mitchison[7] and lower than the 8 days quoted by Dresser[8]. On the basis of this result, Fig. 1 needs the added interpretation that actively immune normal mice and syngeneic chimæras synthesize antibody during the period from day 20 to day 70, as the rate of decline of amount of antibody (apparently half-life 9 days) is too slow to be accounted for simply by passive loss.

The rate of passive decline when allogeneic bone marrow is injected after irradiation indicates an antibody half-life of 2 days for about 8–10 days, after which it approximate to the normal passive decay rate. It is not possible to say whether synthesis of antibody stops or is reduced in the allogeneic chimæras, but it is possible to say that by 20 days after irradiation and probably less it is continuing at approximately the same (declining) rate as was observed in normal mice and syngeneic chimæras, assuming that the antibody present is qualitatively unchanged.

For the present purpose, immunity may be considered under two headings: (*a*) synthesis of antibody; (*b*) the anamnestic response. The synthesizing component is apparently insensitive to radiation and the injection of bone marrow. The anamnestic component is apparently destroyed by irradiation and injection of allogeneic bone marrow but is maintained, albeit slightly reduced, after irradiation and the injection of syngeneic bone marrow.

The most plausible explanation of the results is "that treatment of severe irradiation injury with bone marrow cells does not supplant the humoral antibody production mechanisms of the host with those of the donor"[1]. This may be extended to cover skin homograft immunity on the basis of the results of Cross *et al.*[9]. The question to decide is, first, whether host cell persistence is likely and, secondly, if host cells do persist, what type of cell is required for the maintenance of immunity as considered here.

Early in the history of the investigation of radiation chimæras Ford and his colleagues[10] showed that damaged host cells could be found dividing in the spleen, thymus,

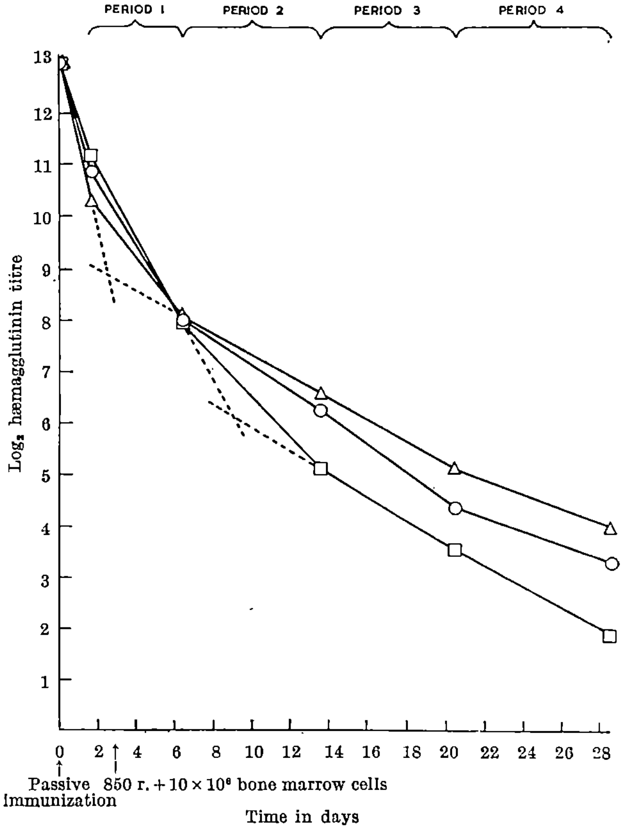

Fig. 2. The hæmagglutinin titres of various kinds of passively immunized *BALB/c* mice in relation to time (normal mice were not irradiated) △, normal mice; ○, *BALB/c→BALB/c* syngeneic chimæras; □, *CBA→BALB/c* allogeneic chimæras

bone marrow and lymph nodes of irradiated animals injected with bone marrow. These cells were found in diminishing numbers with increasing time after irradiation and could only rarely be found after 30 days. Dividing cells present at later times in all hæmatopoietic and lymphoid organs were almost always donor cells. Since then many methods, other than cytological ones, have shown that chimærism established after radiation is often permanent and complete.

The cytological and serological checks on chimærism used in the present experiments, 60 days after irradiation, indicated that the erythropoietic system of the allogeneic chimæras and the dividing cells in the spleens of the immunologically stimulated syngeneic chimæras were donor type. Thus the chimæras the immune response of which is under consideration were typical, but neither the tests performed on them nor the data about chimæras adduced here are sufficient evidence that no host cells were present. All that can be said is that they were not discovered.

The problem can be approached from another aspect. If the immune response observed is a property of the host, which cells are involved and would they be detectable in tests for chimærism ?

The antibody synthesized by the chimæras and by the normal mice during the decline phase of the immune response is likely to have derived largely if not wholly from plasma cells, as few other cells have been shown regularly to synthesize antibody. It is well known that plasma cells appear after irradiation, though it is not clear whether they simply become obvious because other cells have been destroyed and phagocytozed or whether they are produced in response to an immunological stimulus[11]. Nossal[12] maintains that at least some plasma cells have a relatively short functional life and cell division is required to ensure their replacement. If such divisions of host cells occur in order to provide antibody-producing cells, why have they not been seen ? Alternatively, the plasma cells under consideration are long-lived and radiation-resistant. No conclusion can at present be arrived at except to say that the mechanism for the continuation of antibody synthesis may in part survive irradiation, but even if the host cells observable early after irradiation are part of the mechanism, its cellular components cannot be found to divide in appreciable numbers subsequent to 30 days after irradiation. It is tempting to wonder whether the continuation of antibody synthesis becomes a property of the donor cells, which are present in abundance in radiation chimæras, rather than remaining a property of host cells the very existence of which may be a chimæra.

Nossal[12] concludes from an extensive and elegant series of experiments that "the cell type (in resting primarily immunized nodes) capable of responding instantly to antigenic stimulation, and thus presumably carrying specific immunological memory, is a rapidly proliferating primitive lymphocyte". The experiments of Makinodan *et al.*[13] indicate that the D_{37} of the appropriate cells, primitive lymphocytes or not, is 70 r. The dose of radiation used in the present experiments was 850 r. and it must be concluded that very few of the relevant cells could have survived such irradiation. If, however, sufficient cells did survive and, by their active proliferation on antigenic stimulation, 60 days after irradiation, initiated the response to a third injection of sheep cells observed in the syngeneic chimæras, why were not their divisions observed ?

The allogeneic chimæra proved to be incapable of effecting a recognizable anamnestic response which could either mean that the relevant host cells had been destroyed by a graft-versus-host reaction or that part of the immunological system necessary for the receipt and processing of antigenic material fails to develop. The authors incline to this latter view and are attempting to disprove it.

Over all, the evidence for the persistence of sufficient host cells to maintain immunity in chimæras as observed is not good. The alternative hypothesis, that it is the donor cells which do the work, requires that the capacity to do so is in some way transferred from the irradiated host cells to the incoming donor tissues. This possibility will be considered more extensively elsewhere. It must suffice to say here that we can find no evidence, after irradiation of immune mice, for massive release of antigen, complete in that it simulates injection of sheep red cells (unpublished observations). The finding discussed by Campbell[14], that immunity to protein antigens may be maintained by the persistence of haptenic polypeptides strongly bound to low molecular weight RNA, which, it is not inconceivable, could be liberated on irradiation, is perhaps relevant. Further, there is an accumulation of largely circumstantial evidence that transfer of immunological information between cells is a very real possibility[15-22].

The problem raised here from the very artificial situation existing in radiation chimæras relates to the general problem of the persistence of immunological information in immunized mice. However, until a clear description of the cells involved in an immune response can be made any approach is bound to have a large speculative component. This matter has been thoroughly discussed by Humphrey[23] and it should be noted that he says "we cannot rule out the possibility that the precursors of antibody-producing cells never actually ingest antigen directly, but are stimulated by nucleoproteins, derived from phagocytic cells".

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(II) By Thymectomized Syngeneic Radiation Chimæras

By A. MARJORIE CROSS, ELIZABETH LEUCHARS and DR. A. J. S. DAVIES

IT has been shown[1] that mice which were immunized to sheep erythrocytes, before exposure to a potentially lethal dose of irradiation followed by syngeneic bone marrow therapy, continued to synthesize hæmagglutinins after irradiation. When a further injection of sheep erythrocytes was given there was a weak but significant anamnestic response. The data presented here concern similar experiments in which immunized mice were thymectomized before irradiation and syngeneic bone marrow therapy. It has been shown[2] that such animals are unable to respond to a primary challenge with certain antigens and it was considered to be of interest to determine whether they would respond to secondary challenge with such antigens. In a further experiment bone marrow was taken from neonatally thymectomized donors to see whether immunological incompetence of the donor would affect the result obtained.

Male mice of the *CBA* strain were used as irradiated hosts and as donors of bone marrow cells. In addition skin was taken for grafting from *Ak* mice which have the same *H*-2 antigens as *CBA*, and *C57BL* mice which have different *H*-2 antigens. All mice were from highly inbred Chester Beatty strains.

Thymectomy or sham operation was performed as described by Miller[3]. At the end of the experiment all thymectomized mice were checked for thymic remnants, none of which was found. The radiation dose used was 850 r. After irradiation the mice were injected with 5 million syngeneic bone marrow cells.

Mice were immunized with *Ak* skin grafts using the method of Billingham and Medawar[4] and by intraperitoneal injections of 0·2 ml. of a 20 per cent suspension of sheep erythrocytes in Alsever's solution. The time schedule is shown in Table 1. Hæmagglutinins were estimated as before[1].

The survival times of *Ak* and *C57BL* skin grafts on normal mice are shown in Table 2. *Ak* skin grafts on pre-immunized mice showed an accelerated second-set type of rejection. The skin grafting results in the thymectomized irradiated mice are shown in Table 3. Neither the thymectomized nor the irradiated control groups showed a typical second-set rejection, but the mean survival time on irradiated mice was significantly shorter than that of a first graft of *Ak* skin ($P < 0·01$). The mean survival time on pre-immunized thymectomized mice (17·2 days) did not differ significantly from the mean survival time for a first graft of *Ak* skin on thymectomized control mice (16·6 days). In the experimental groups most of the *Ak* grafts showed longer survival times and were rejected between 20 and 50 days. However, the number of grafts surviving until 50 days (9 out of 64) is considerably less than that found by Miller, Doak and Cross[2] in thymectomized, irradiated mice which had not been pre-immunized (36 out of 39).

A surprising finding was that the thymectomized irradiated mice which had been pre-immunized to *Ak* also rejected their *C57BL* skin grafts. However, the specificity of the immunization to *Ak* antigens was shown by the fact that, although the *C57BL* grafts were more foreign to the *CBA* hosts than the *Ak* grafts, their survival time was longer than that of the *Ak*. Twice as many *C57BL* as *Ak* grafts were still intact at 50 days. In view of this finding experiments were carried out with normal mice to see whether immunization with *Ak* skin had any effect on the survival of subsequent *C57BL* skin grafts (Table 2). It was found that there was in fact a highly significant reduction in the mean survival time of the *C57BL* grafts ($P < 0·001$), indicating that mice immunized to *Ak* had also been made immune to some of the antigens possessed by *C57BL*.

The results of the hæmagglutinin titrations are shown in Table 4. It can be seen that the titres of antibody remaining from the immunization procedure were similar in all groups. Normal pre-immunized mice showed a $\log_2$ titre of 11·71 at the time at which the experimental groups were irradiated. Experiments with mice passively immunized against sheep erythrocytes[1] indicated a half-life of 4–5 days for these antibodies. If antibody synthesis had been completely stopped in the experimental groups by thymectomy and irradiation, antibodies should not have been detectable by day 60.

After a further injection of sheep erythrocytes the mean titres of all groups showed an increase which was statistically significant. In only 3 of the 31 individual mice in experimental group I, and 3 of the 15 mice in experimental group II, was the increase in titre less than fourfold.

The increase in titre is shown graphically in Fig. 1. The thymectomized control group showed a rapid increase to day 5, similar to that in normal mice. The increase in the irradiated controls was slower, and in these mice the survival times of skin grafts were longer than in the other controls. The increase in titre of the two experimental groups was very similar although the starting titre of the group given bone marrow from neonatally thymectomized donors was considerably lower. In thymectomized irradiated mice which had not been pre-immunized the mean titre had increased to only $\log_2$ 0·25 by day 7 (ref. 2).

It is now clear that the immunological response of thymectomized *CBA* syngeneic radiation chimæras to a variety of primary antigenic stimuli is reduced or absent[2]. The results of the present experiments show that established immunity can persist despite removal of the thymus and irradiation. The responses are, however, weak and can only be defined as secondary by virtue of the fact that control animals show no primary response.

Interpretation of the results of the present experiments is complicated because the control group of mice which were simply thymectomized showed no secondary response to skin grafts, although their secondary response to sheep erythrocytes was enhanced. Thymectomy of the adult

Table 1. EXPERIMENTAL SCHEDULE

	Day 0	Day 8	Day 12	Day 15	Day 20	Day 28			
Group						Day 0	Day 28	Day 60	Day 65 Day 67 Day 69
Normal	First graft of *Ak* skin	First injection of sheep erythrocytes	Second graft of *Ak* skin	Second injection of sheep erythrocytes	—	—	*Ak* and *C57BL* skin grafts	Titration of residual hæmagglutinins Injection of sheep erythrocytes	Titration of hæmagglutinins
Control I					Sham thymectomy	Irradiation and syngeneic bone marrow			
Control II					Thymectomy	Sham irradiation and syngeneic bone marrow			
Experimental I					Thymectomy	Irradiation and syngeneic bone marrow			
Experimental II					Thymectomy	Irradiation and syngeneic bone marrow from neonatally thymectomized donors			

Table 2. SKIN GRAFT SURVIVAL TIMES IN NORMAL *CBA* MICE

Treatment	Donor skin	No. of mice in group	No. of mice showing skin-graft survival for: less than 20 days	20-30 days	M.S.T. ± S.D.
Non-immunized	*Ak*	20	6	14	21·7 ± 2·50
	C57BL	11	11	0	14·6 ± 2·13
Immunized with *Ak* skin	*Ak*	10	10	0	9·9 ± 0·94
Immunized with *Ak* skin	*C57BL*	9	9	0	10·5 ± 2·77

Table 3. Skin Graft Survival Times in *CBA* Mice Pre-immunized with *Ak* Skin

Treatment	Donor skin	No. of mice in group	No. of mice showing skin-graft survival for:			M.S.T. ± *S.D.*
			< 20 days	20–50 days	> 50 days	
Control I Sham-thymectomized 850 r. and syngeneic bone marrow	*Ak* *C57BL*	20	18 20	2 0	0 0	19·1 ±2·3 18·3 ±0·33
Control II Thymectomized sham-irradiated syngeneic bone marrow	*Ak* *C57BL*	20	20 20	0 0	0 0	17·2 ±1·16 16·97 ±0·21
Experimental I Thymectomized 850 r. and syngeneic bone marrow	*Ak* *C57BL*	42	4 4	32 29	6 9	29·7 ±4·9* 31·3 ±6·7*
Experimental II Thymectomized 850 r. and syngeneic bone marrow from neonatally thymectomized donors	*Ak* *C57BL*	22	6 1	13 12	3 9	24·8 ±4·5* 35·0 ±8·56*

* Mean survival time for grafts rejected before 50 days.

appears to have little effect on immune responses, except those to closely related antigens[5,6]. It does, however, result in a diminution of the number of lymphocytes in the peripheral blood, and it has been suggested that this is due to sequestration of lymphocytes in the lymph nodes (Gowans, personal communication). If this is the case it is possible that an insufficient number of cells would be released on second challenge with antigen to cause accelerated rejection of skin grafts, although the production of antibodies, when cell release is not necessary, would be unaffected. The response of thymectomized mice to a second skin homograft, then, would be slow and indistinguishable in timing from the primary response, but the lymph nodes would contain cells committed to respond to the antigens concerned. This point is at present being investigated in collaboration with Dr. P. Dukor.

The results with thymectomized and irradiated mice indicate that once the primary response has been initiated the thymus is no longer necessary for: (*a*) continuation of the primary response—otherwise synthesis of hæmagglutinins would not have continued; (*b*) initiation of a secondary response.

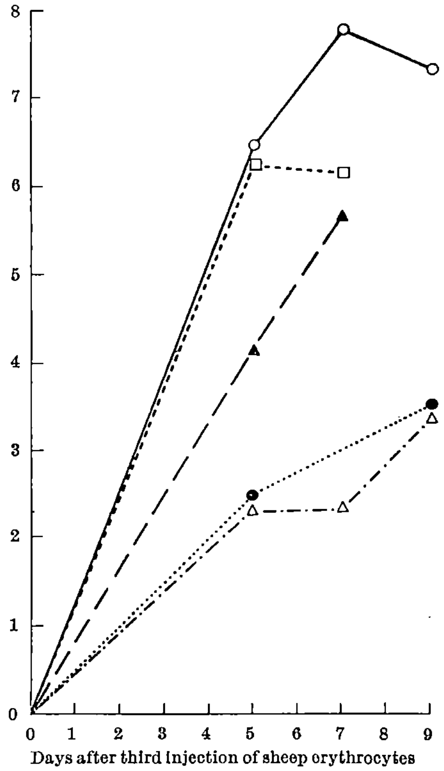

Fig. 1. Increase in hæmagglutinin titre after a third injection of sheep erythrocytes 80 days after the first
□, normal mice; ▲, control I, irradiated; ○, control II (thymectomized); △, experimental I; ●, experimental II. Bone marrow from neonatally thymectomized donors

Table 4. Hæmagglutinin Titres in Mice Pre-immunized to Sheep Erythrocytes after a further Injection of Antigen 80 Days after the First

Treatment	Mean $\log_2$ titres and *S.D.* at following times after antigen injection			
	Day 0	Day 5	Day 7	Day 9
Normal	5·87 ±0·35	12·12 ±0·65	12·0 ±0·5	
Control I 850 r. and syngeneic bone marrow	5·67 ±1·28	9·83 ±1·75	11·33 ±3·3	
Control II Thymectomized, sham irradiated, and syngeneic bone marrow	6·8 ±1·211	13·27 ±1·977	14·53 ±1·154	14·13 ±2·25
Experimental I Thymectomized, 850 r. and syngeneic bone marrow	7·16 ±3·037	9·471 ±3·027	9·50 ±2·693	10·53 ±3·54
Experimental II Thymectomized, 850 r. and syngeneic bone marrow from neonatally thymectomized donors	4·331 ±2·463	6·801 ±2·033		7·867 ±3·759

The secondary response is weak in thymectomized syngeneic chimæras as it is in allogeneic chimæras. Both groups show lymphoid atrophy and weakening of response may be due to faults in the afferent or efferent immunological pathways.

The secondary response in the experimental mice survives the effects not only of thymectomy but also of irradiation. Davies *et al.*[1] showed that in syngeneic radiation chimæras the secondary response to antigens encountered prior to irradiation was not lost. They considered whether this response was mediated by surviving host cells or by activation of injected bone marrow cells, either directly by antigen, or indirectly by transfer of some kind of 'information'. The same arguments apply to thymectomized irradiated mice, but a further interesting point arises from the present results. The thymectomized mice of experimental group II were injected, after irradiation, with bone marrow from neonatally thymectomized mice. The cells of the donors *in situ* could react only weakly or not at all to sheep erythrocytes or skin homografts and are unlikely to have had the ability to be sensitized with antigen when transferred to a new host. If transfer of information does occur, the cells capable of picking it up are present in the bone marrow of neonatally thymectomized as well as normal mice.

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ENTHALPY OF BINDING OF AROMATIC AMINES TO BOVINE SERUM ALBUMIN

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SERUM albumin is physiologically important for its transport function[1]. It binds numerous otherwise insoluble or only slightly soluble substances and transports them to the site of their physiological activity. Laurence[2] has interpreted this binding in terms of absorption of the small molecule on the surface of the albumin molecule. In order to gain further insight into the mechanism by which albumin effects this binding, other workers[3,4] have attempted to characterize the process in terms of thermodynamic parameters derived from the binding dissociation equilibrium at two temperatures. Both groups of workers concluded that two types of sites were involved in the binding of a variety of molecules, both neutral and ionic. Laurence[2], however, required only one type of binding site in his interpretation. Before mechanistic inferences could be induced from the thermodynamic data the latter had to be corrected for the fact that the equilibrium is between states in which the bound molecules possess differing numbers of degrees of freedom[4,5]. These corrections were actually derived from theoretical approximations and introduced an additional uncertainty into the data. The present work was undertaken to obtain meaningful thermodynamic data without the uncertainty inherent in the comparison of the bound state with the dissolved state.

One possible approach would correct the thermodynamic parameters derived from the dissociation equilibrium by the corresponding empirical quantities describing the dissolved state. The thermodynamic parameters for solution of solids are determined, usually, from solubility data, that is, by comparing the dissolved state with the crystalline state of the compound. Consequently, this methodology simplifies the comparison of the bound and crystalline states of the bound compound. It parallels the approach of solution thermodynamics; indeed, Everett[6] has pointed out the analogy between the thermodynamic treatments of solution and adsorption on theoretical grounds. The bound molecules should possess no rotational freedom either in the bound or crystalline state, so no disorientation entropy correction is necessary in comparing the bound and crystalline states.

The experimental method contingent on the above approach was as follows. Six aromatic amines were selected for investigation; *p*-aminobiphenyl, *p*-phenylacetanilide, *N*-acetyl-2-aminofluorene, *p,p'-bis*(*N,N*-dimethylaminophenyl)methane and *p,p'-bis*(*N,N*-dimethylamino)benzophenone (Michler's 'ketone') are all crystalline solids which are easily assayable by ultra-violet spectrophotometry. The pertinent data are given in Table 1. The solubility of each of these compounds in aqueous bovine serum albumin buffered at *p*H 7·5 and 10·5 with phosphate and carbonate buffers, respectively, was determined by shaking at 9·0 ± 0·2° C, 17·0 ± 0·5° C and 25·0 ± 0·5° C for 16 h. Experiments showed shorter shaking times to be inadequate to maximize amine solubilization; longer times resulted in no further change in the extent of the solubilization. Rapid filtration of the mixtures, during which the solution temperatures did not change, gave solid-free solutions. These were analysed by taking their spectra at appropriate dilutions against the buffered protein solutions as blanks. No change in the spectra of the protein or the compounds was noted. The amount of amine in solution was then the sum of the amine dissolved in the buffer solution independently of the protein and that bound to the albumin. The former quantity was assumed to be equal to the solubility of the amine in the buffer analogously determined in the absence of protein. The amount of amine bound to protein was then divided by the albumin concentration (based on a molecular weight of 64,500 (ref. 1)) to give a ratio, K, which was constant for 0·5, 2·5, 5·0 and 7·5 per cent bovine serum albumin solution at each temperature and *p*H. These data are reported in Table 2. It is interesting that the binding power of the serum albumin, measured in terms of K, is higher in solutions of higher *p*H; this result apparently contradicts the observation of Klotz[7].

Table 1

Compound	Melting point (°C)	Absorption maximum (mμ)	ε
p-Aminobiphenyl	52–53	271	$1·9 \times 10^4$
p-Phenylacetanilide	177–178	268	$2·5 \times 10^4$
2-Aminofluorene	129–130	278	$1·5 \times 10^4$
N-Acetyl-2-aminofluorene	203–205	276	$2·5 \times 10^4$
p,p'-bis(*N,N*-Dimethylaminophenyl)methane	91–92	252	$1·6 \times 10^4$
p,p'-bis(*N,N*-Dimethylamino)benzophenone	178–179	375	$3·3 \times 10^4$
Bovine serum albumin		279	$3·6 \times 10^3$

Table 2. BINDING OF AROMATIC AMINES BY BOVINE SERUM ALBUMIN

	*p*H	K_9	K_{17}	K_{25}
p-Aminobiphenyl				
	7·5	3·6 ± 0·4	7·0 ± 0·9	8·5 ± 0·4
	10·5	3·9 ± 1·4	8·3 ± 0·5	7·8 ± 0·2
p-Phenylacetanilide				
	7·5	0·65	1·0 ± 0·1	1·2 ± 0·1
	10·5	0·85 ± 0·08	1·35 ± 0·18	1·2 ± 0·1
2-Aminofluorene				
	7·5	1·8 ± 0·2	2·1 ± 0·1	1·7 ± 0·2
	10·5	2·4 ± 0·1	3·6 ± 0·1	2·6 ± 0·1
N-Acetyl-2-aminofluorene				
	7·5	0·55 ± 0·08	1·0	1·1 ± 0·2
	10·5	0·76 ± 0·03	1·0 ± 0·1	0·90 ± 0·08
p,p'-bis(*N,N*-Dimethylaminophenyl)methane				
	7·5	0·22 ± 0·02	0·42 ± 0·02	0·47 ± 0·02
	10·5	0·35 ± 0·04	0·60 ± 0·02	0·85 ± 0·07
p,p'-bis(*N,N*-Dimethylamino)benzophenone				
	7·5	0·082 ± 0·009	0·20 ± 0·02	0·26 ± 0·05
	10·5	0·125 ± 0·004	0·26 ± 0·02	0·33 ± 0·02

From the change in K with temperature it was possible to calculate an enthalpy change ΔH, supposedly characteristic of the binding process for each temperature increment. These parameters are reported in Table 3. Most significantly, the observed enthalpies over the lower temperature increment appear to be larger than those for the higher one by, on the average, 11 ± 4 kcal/mole. Despite the uncertainties in the values of ΔH, this difference is statistically real. The melting-points of the compounds indicate that the temperature variation of the activity of the solid state, a, is negligible (< ± 3 per cent) according to the usual approximation[8]:

$$R \ln a = \Delta S_f \ln(T/T_m)$$

where ΔS_f is the entropy of fusion at the melting-point, T is the temperature at which the activity is a, and T_m is the melting-point of the compound. Therefore, the source of the variation of enthalpy change with temperature must lie in the character of the bound state.

Table 3. ENTHALPY OF BINDING BY BOVINE SERUM ALBUMIN

	pH	ΔH (kcal/mole) at: 9–17° C	17–25° C
p-Aminobiphenyl	7·5	14 ± 6	4 ± 4
	10·5	17 ± 7	−1 ± 2
p-Phenylacetanilide	7·5	10 ± 2	4 ± 4
	10·5	10 ± 6	−2 ± 6
2-Aminofluorene	7·5	3 ± 4	−4 ± 3
	10·5	9 ± 2	−7 ± 1
N-Acetyl-2-aminofluorene	7·5	13 ± 4	2 ± 2
	10·5	6 ± 3	2 ± 4
p,p′-*bis*(*N*,*N*-Dimethylaminophenyl)methane	7·5	13 ± 4	2 ± 2
	10·5	17 ± 4	2 ± 3
p,p′-*bis*(*N*,*N*-Dimethylamino)benzophenone	7·5	20 ± 5	6 ± 6
	10·5	16 ± 3	5 ± 3

In the description of the bound state, two types of binding sites have been postulated[3,4] (*vide supra*). If these two types are each characterized by different thermodynamic parameters, then that on which the binding process has a larger ΔH value will become more important with increasing temperature. The enthalpy change calculated as above will then appear to increase. Exactly the opposite effect has actually been observed. The temperature dependence of the enthalpy change cannot reasonably be attributed to destruction of the binding sites by thermal denaturation involving gross changes in the structure, for example, helix-coil transformations, either. The optical rotation of bovine serum albumin at temperatures of 0°–40° C does not support such a speculation[9]. If a change in protein structure is involved in the apparent temperature dependence of enthalpy, it is something more subtle. Yet it is realistic to assume that the enthalpy temperature dependence is an artifice generated by temperature dependence of the relation of the activity of the bound amine to K. From Laurence's[2] absorption model, this relationship should be a function of the active surface area of the albumin molecule available for binding.

Weber[10] has demonstrated reversible thermal dissociation of the albumin into sub-units. Such dissociation could either (*a*) generate additional active surface area for binding, or (*b*) destroy the molecular geometry necessary for binding. The observations obtained here could then be explained in terms of this dissociation hypothesis by postulating the generation of greater binding area between 9° C and 17° C than between 17° C and 25° C (mechanism *a*), by greater destruction of active sites between 17° C and 25° C than between 9° C and 17° C (mechanism *b*), or by a combination of these mechanisms. Intuitively, one expects a small positive ΔH between the absorbed and crystalline states. The experimental values obtained at the higher temperature range therefore would seem less likely to be artificial than those at the lower range. On these grounds, mechanism *a* is more favourable than mechanism *b*; however, a combination of mechanisms *a* and *b*, implying an artificiality of the values at both temperature ranges, is certainly not excluded. As an alkaline medium enhances dissociation of serum albumin[10], the increased binding at higher pH is in accord with this interpretation.

An ambiguity as to the exact origin of the apparent temperature dependence of ΔH, however, remains. The thermodynamic parameters, by themselves, are of little diagnostic value in elucidating the adsorption mechanism, as the apparent temperature dependence of binding may not characterize the binding process *per se*. The temperature-dependence of the extent of binding irrespective of origin is significant, however; the physiological consequences, in terms of temperature-dependence of transport phenomena, for example, appear to be unexplored.

This work was carried out in the laboratory of Dr. W. C. Hueper, chief of the Environmental Cancer Section.

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DIRECT DEMONSTRATION OF REAGIN ACTIVITY IN PURIFIED γ_{1A}-GLOBULIN

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ALTHOUGH many precipitating and agglutinating antibodies have been demonstrated in isolated, purified human 7 *S* (γ_{ss}, γ_2) and 19 *S* (γ_{1M}) immunoglobulins[1], antibody activity in the third class of human immunoglobulins (γ_{1A}-globulins) has been reported only recently[2–5]. Indirect evidence suggests that reagins, the spontaneously produced skin-sensitizing antibodies found in the serum of allergic human subjects, are present in the γ_{1A}-globulin. This evidence consists of the presence of skin-sensitizing activity in impure immune globulin preparations rich in γ_{1A}-globulin[6], the inhibition of reaginic activity by local injection of γ_{1A}-globulin at the sensitized site[7], the inhibition of positive sensitization reactions by addition of anti-γ_{1A} antisera to the sera or fractions under investigation[8], and the fixation of ragweed antigen labelled with iodine-131 after immunoelectrophoresis[9]. However, since "reagin still represents a nebulous concept to many immunologists, some doubting the legitimacy of its classification as an antibody"[10], attempts at direct demonstration of reagin activity in purified γ_{1A}-globulin seemed warranted.

The γ_{1A}, γ_{ss} and γ_{1M}-globulin fractions of the serum of three persons highly allergic to ragweed but not allergic to *Aspergillus* were isolated and purified by a slight modification of a previously described method[11]. Each γ_{1A}-preparation gave one band when tested with anti-human serum and unispecific anti-γ_{1A} antisera by immunoelectrophoretic and gel-diffusion techniques, and did not precipitate with antisera specific for λ_{ss} and γ_{1M} globulins (Fig. 1). An 0·05 ml. sample of each of the purified γ_{ss}, γ_{1M} and γ_{1A}-globulin preparations from each serum was injected intradermally into three normal recipients, and the test sites were challenged 24 h later by either scratch test or intradermal injection of test antigens[12]. All sites challenged with the control *Aspergillus* extract were negative. When challenged with 2 protein nitrogen units (PNU) of ragweed extract, all sites of injections of γ_{1M} and γ_{ss} fractions, as well as of buffered saline (control), were negative, whereas the sites injected with γ_{1A}-globulin gave strongly positive reactions (Fig. 2). As little as 0·05 μg N of the γ_{1A} of one subject produced positive Prausnitz-Küstner (P-K) reactions (8 mm diameter weal and 35-mm diameter erythema) when challenged similarly by intradermal injection of ragweed extract; 0·017 mg N of the γ_{1A} of another donor produced positive reactions

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* Parts of this work were carried out while one of us (K. I.) was visiting assistant professor, Department of Medicine (Hæmatology Unit), University of California School of Medicine.

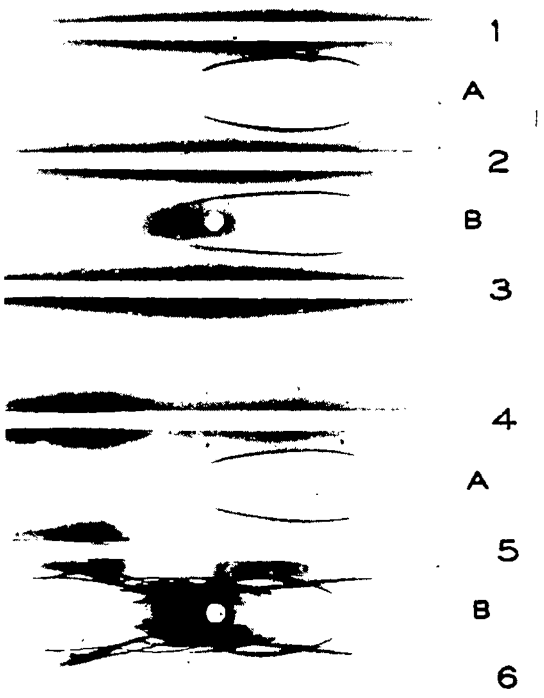

Fig. 1. Immunoelectrophoretic pattern of γ_{1A}-preparations. Numbers indicate antiserum troughs containing anti-human γ_{1A} (1, 2 and 3) and anti-whole human serum (4, 5 and 6). Letters indicate antigen wells containing (A) γ_{1A} from allergic serum and (B) whole serum

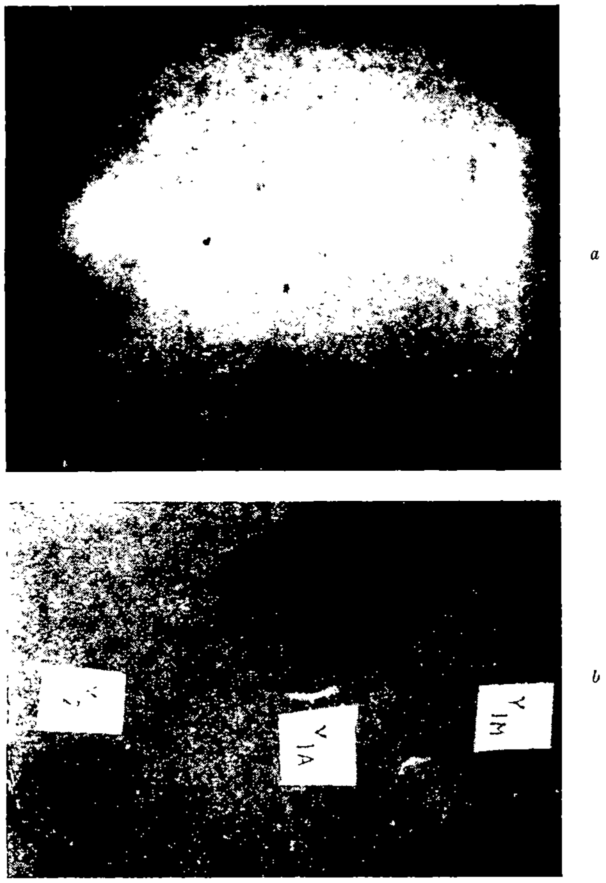

Fig. 2. a, Sites of injection of purified immunoglobulin (γ_{1A}) preparations, showing skin reactions (Prausnitz-Küstner) to ragweed extract (positive, left side) and to *Aspergillus* extract (negative, right side); b shows reactions with ragweed extract that were positive with purified (γ_{1A}) and negative with (γ_2) and (γ_{1M})

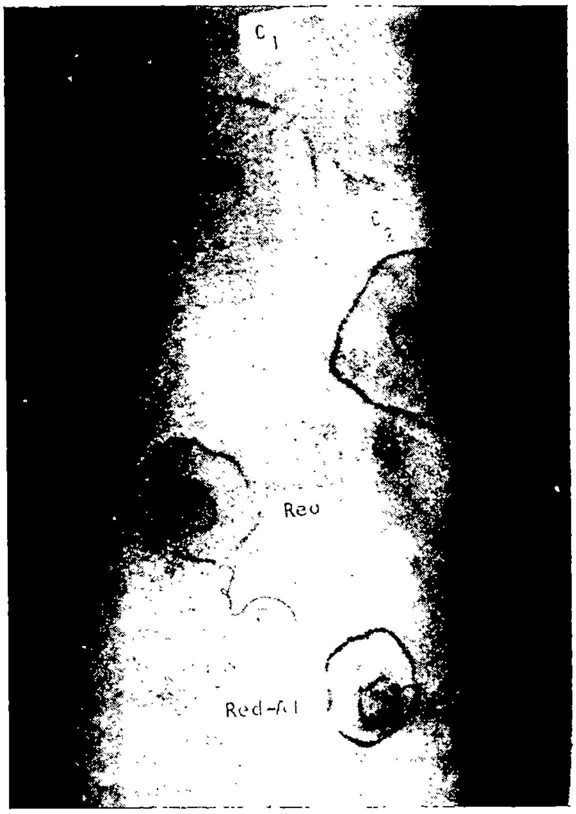

Fig. 3. Skin reactions (Prausnitz-Küstner) to control (C_1, 0·14 μg N. C_2, 0·07 μg N), reduced (Red) and reduced alkylated (*Red-A1*) (0·7 μg N) γ_{1A}-globulin preparations containing reagin to ragweed. Definite erythema and weal reactions to the untreated (control) γ_{1A}-preparations, not clearly visible in the photograph, were present

by scratch test in three normal recipients (7–13 mm weal and 24–46 mm erythema).

Results of additional experiments also supported the conclusion that the anti-ragweed reagin in the sera of the three allergic subjects were γ_{1A}-globulins. (1) Reagin to ragweed was demonstrated by P-K reaction with concentrated parotid saliva (rich in γ_{1A}-globulin and without detectable 7 S γ-globulin[13–15]) from the three allergic persons. The concentrated salivas contained 1 mg/ml. of γ_{1A}, but were devoid of detectable γ_{ss}-globulin by immunoelectrophoresis. (2) In fractions obtained by 'Sephadex G-200' gel-filtration of the sera from the three allergic donors, γ_{1A}-globulin localization and reagin activity were parallel. Reagin and γ_{1A} were eluted from the 'Sephadex' columns simultaneously and appeared before antibodies of the γ_{ss} (γ_2) type, although there was considerable overlap[8,16]. (3) As with whole sera, reaginic activity disappeared when the γ_{1A}-preparations were exposed to heat (56° C for 4 h).

In further experiments, the γ_{1A}-globulin fraction of a reaginic serum was treated with 0·1 M mercaptoethanol for 4 h at room temperature at pH 8·0. One portion of the reduced protein was then dialysed against borate-buffered saline; another was dialysed against iodoacetate (0·02 M) for 24 h and against borate-buffered saline for another 48 h. The original γ_{1A}-fraction (0·05–0·07 μg N), the reduced material (0·7 μg N), and the reduced and alkylated preparation (0·7 μg N) were injected intradermally into a normal subject. When the sites of injection were challenged by intradermal injection of 2 PNU of ragweed pollen extract, the original γ_{1A}-globulin fraction produced a definite erythema and weal reaction (Fig. 3), the reduced material (without alkylation) gave a ± erythema (no weal), and the reduced and alkylated material gave essentially no reaction. Similar results were obtained on reduction-alkylation of the γ_{1A} of the other allergic donors.

These observations demonstrate that 'spontaneously-induced' reaginic antibodies are present in purified γ_{1A}-globulin; however, the possibility that reagins to at least some allergens may also be present in the γ_{ss} (γ_2) or γ_{1M} globulins of some individuals (especially in individuals deliberately immunized or 'desensitized' to specific allergens) is not excluded[9,16]. It is evident that the reaginic activity of the pure γ_{1A}-globulin fraction of an allergic serum, like that of the whole serum[17], is inactivated by reduction of disulphide bonds. This procedure has been shown to split the disulphide bonds of a 9·55 γ_{1A}-myeloma protein[18]. In contrast, there is retention of activity when γ_{ss} (γ_2)-globulin antibodies are treated similarly[19,20]. Results published elsewhere indicate that polymer-type heterogeneity exists in the γ_{1A}-globulin of single individuals[21]. On analytical ultracentrifugation of one of the purified γ_{1A}-globulins with reagenic activity, similar heterogeneity was noted, in that two peaks (6·7 *S*, 91 per cent; 9·7 *S*, 9 per cent) were present. Experiments designed to localize reagin activity to the 6·7 or 9·7 γ_{1A}-globulin of allergic individuals are currently in progress.

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A preliminary report of these experiments has been presented elsewhere[22].

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SOME SIMILARITIES BETWEEN A VIRAL INHIBITOR OF PLANT ORIGIN AND CHICK INTERFERON

By DR. K. H. FANTES and C. F. O'NEILL

Virus Unit, Glaxo Laboratories Ltd., Sefton Park, Stoke Poges, Bucks

INTERFERONS have been produced in mammalian and avian cells of a great variety.

Delbruck and Luria[1,2] have described interference in *E. coli* between ultra-violet-inactivated bacteriophage and live phage of the same or a different variety, and Mercer and Mills[3] have produced an interferon-like substance in *Pseudomonas aeruginosa* on interaction with inactivated bacteriophage *Pc*.

Viral interference in plants has recently been reviewed by Rageti[4] and by Atanasoff[5], and properties of antiviral substances of plant origin have been described in the past few years by several authors[4,6–9]. The material isolated by Ragetli and Weintraub from apparently healthy carnation plants seemed to possess such marked similarities to chick interferon that we decided to examine these in some more detail. The few properties mentioned of the substance obtained by Loebenstein and Ross[8], after infecting *Datura stramonium* plants with tobacco mosaic virus, also resembled those of chick interferon, but the material produced by Sela and colleagues[9] with tobacco mosaic virus in *Nicotiana glutinosa* L. plants appeared to be entirely different.

The known properties of the antiviral carnation factor of Ragetli and Weintraub and those of chick interferon are summarized in Table 1.

Ragetli and Weintraub[7] subjected their purified carnation factor to a number of chemical reactions to ascertain which chemical groups were essential for antiviral activity. We applied the same reactions to partly purified chick interferon (material before the DEAE chromatography step)[17], diluted to about 200 interferon units (chick embryo fibroblasts/Semliki Forest virus system) and 50 µg protein[24] per ml. The results obtained by Ragetli and Weintraub[7] with carnation factor are compared with our chick interferon results in Table 2. For all the experiments mentioned in Tables 2 and 3, reagent control experiments without interferon were run in parallel to make sure that no cell toxicity resulted from the treatments. Interferon control experiments were also always carried out; in these, interferon was subjected to the

Table 1. PROPERTIES OF CARNATION FACTOR AND CHICK INTERFERON

	Carnation factor	Chick interferon
Chemical nature	Protein (refs. 4, 6, 7)	Protein (refs. 10–12)
Molecular weight	At least 10,000 (ref. 4)	13,000–38,000 (refs. 12–14, 32)
Sedimentation constant	1·4 (ref. 4)	1·9–3·06 (refs. 12–15)
Precipitated by ammonium sulphate at	50% saturation (ref. 4)	60% saturation (ref. 11)
Heat stability	Stable at 60°, gradual inactivation at higher temperatures (ref. 7)	Similar (refs. 12, 13, 16)
Behaviour towards DEAE-cellulose	Not absorbed at *p*H 7·8 (ref. 6)	Similar (ref. 17)
Behaviour towards *CM*-cellulose	Is adsorbed at *p*H 6·0 and eluted by raising *p*H and ionic concentration	Similar (refs. 12, 17)
Antiviral spectrum	Active against wide variety of viruses and also against viral nucleic acid (refs. 4, 7)	Similar (refs. 10, 18, 19) 39)
Mode of antiviral action	Indirect, via the host (ref. 4)	Similar (refs. 10, 36)
Optimum application time for antiviral activity	Before or with virus, not after (ref. 4)	Similar (refs. 10, 18, 36,
Species specificity	Activity is influenced by the species of the host cell (ref. 4)	Similar (refs. 20, 21, 35)
Effect of EDTA on activity	None (ref. 7)	None (ref. 22)
RNase-activity	None (ref. 7)	None (ref. 17)
Sugar content	None (ref. 4)	Trace carbohydrate (ref. 12)
Electrical charge	Neutral (ref. 4)	Slightly basic (refs. 11, 12) Slightly acidic (refs. 14, 23)
Effect of papain on activity	None (ref. 7)	None (ref. 11) Near total inactivation (refs. 12, 22)
Effect of trypsin on activity	None (ref. 7)	Partial inactivation (refs. 10, 12)
Effect of leucine aminopeptidase on activity	None (ref. 7)	None (ref. 12)
Effect of carboxypeptidase on activity	None (ref. 7)	None (ref. 12)

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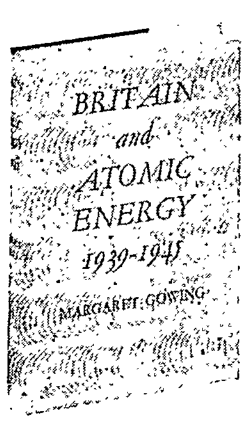

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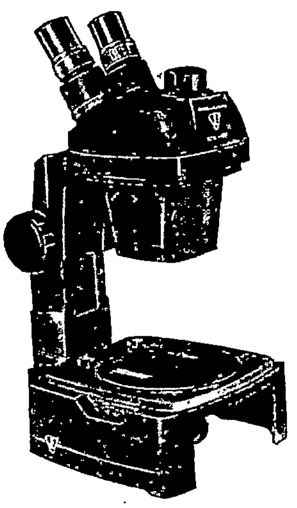

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conditions summarized in Tables 1–3, but in the absence of the chemical reagents, so as to ensure that any loss of activity was due to the latter and not to damage caused by adverse *p*H or temperature. All samples were thoroughly dialysed against Earle's buffer before assay.

Ragetli and Weintraub[7] concluded from their experiments that an amino-group, probably an ε-amino-group of lysine, is essential for biological activity of their factor. The argument in favour of an ε-amino-group of lysine being involved was based mainly on the different results which were obtained with the two concentrations of acetic anhydride. Since neither acetic anhydride nor benzoylchloride completely abolished antiviral activity, it was further argued that the essential amino-groups were not readily available for acylation; on the other hand, the possibility of groups other than amino groups playing an additional part in the antiviral mechanism could not be dismissed. They, however, think it unlikely that sulphydryl, disulphide or hydroxyl groups are important for the biological action.

Our results obtained with chick interferon again show that an amino-group is needed for biological activity, but this activity is completely destroyed by either acetic anhydride at either level, or by benzoylchloride. Sulphydryl or hydroxyl groups, if present, seem to be, as in the carnation factor, of little importance. (Phenyl isocyanate at *p*H 5·8 gave fluctuating results, even after the buffer concentration was increased to minimize the possibility of an upward *p*H drift.)

One striking difference between the two molecules was revealed by their behaviour towards performic acid. This had no effect on the activity of the carnation factor, but (at the higher level) invariably destroyed the potency of chick interferon. Neither formic acid nor hydrogen peroxide, when used separately under otherwise identical conditions, impaired the biological activity of chick interferon. Performic acid is known to oxidize disulphide bonds[25,40]. In order to get more evidence for the presence of such a bond, we subjected chick interferon to a number of the treatments used in the reductive fission of disulphide links. The results of these and of some other treatments are given in Table 3.

The results show that those reductive treatments known to break disulphide bonds always reduced or abolished interferon activity. Further attempts to inactivate interferon with chemicals that react with sulphydryl groups again failed. Reduced activity observed after treatment with cysteine and bromoacetate[29] together did not lead to greater inactivation than that caused by cysteine alone—support for the view that the chemical attack is directed towards a disulphide and not a sulphydryl group.

An attempt was made to restore antiviral activity lost after 8 h treatment at *p*H 5 with benzyl mercaptan (100 mM), by bubbling air through such a solution for 16 h at room temperature, having first removed the reducing agent by dialysis against phosphate buffer (M/50, *p*H 6·5). No activity was thereby restored; the material possibly behaves like insulin under this treatment[33]. Similar aeration of an untreated interferon solution had no effect on its activity. According to Sun Yu-Kun and Tsou Chen-Lu[29], bromoacetate at *p*H 5 had no effect on sulphydryl groups (of papain), but it attacked the imidazole group of histidine; interferon activity, however, stayed intact at this *p*H and also at *p*H 8·2, at which *p*H sulphydryl groups are methylcarboxylated. Cyanogen bromide at *p*H 2 completely inactivated interferon. Such a reaction was shown by Gross and Witkop[41] to cleave ribonuclease and by Hofmann[31] to cleave the peptide chain of trypsinogen at the carboxyl end of methionine residues. The question whether

Table 2. EFFECT OF SOME REAGENTS ON ANTIVIRAL ACTIVITY OF CARNATION FACTOR AND CHICK INTERFERON

Reagent	Concentration (mM)	*p*H	Duration of reaction (h)	Temperature of reaction (°C)	Residual inhibitor activity (%)		Protein groups primarily attacked (ref. 7)
					Carnation factor (ref. 7)	Chick interferon	
Acetic anhydride	5·5	7·5	2	0°–4°	100	30, <5, <10	—NH₂ (α), —OH
	55·0	7·5	2	0°–4°	5	20, <5, <10	—NH₂ (α and ε), —OH
Benzoylchloride	25·0	7·5	2	0°–4°	10	<5, <10	—NH₂
Benzylchloride	25·0	7·5	2	0°–4°	100	100, 100	—NH₂ (at elevated temp. only)
Fluoro-dinitro-benzene	8·1	8·0	2	0°–4°	30	65, 35	—NH₂, —OH*, —SH
	81·4	8·0	2	0°–4°	10	35, 25	—NH₂, —OH*, —SH
Nitrous acid	1,000	4·0	0·5–0·75	0°–4°	20	15	—NH₂, —SH, —OH*
	1,000	4·0	1·5	0°–4°	5	10	—NH₂, —SH, —OH*
	1,000	4·0	3	0°–4°	0	5	—NH₂, —SH, —OH*
p-Cl-Mercuribenzoate	0·0735	6·8	0·33	*R*°	100	100, 100, 100, 100	—SH
Performic acid	0·44	7·0	7	0°–4°	100	100, 100, >75, 67	—S—S—
	4·4	7·0	7	0°–4°	100	<5, <5, <5, <5	—S—S—
Formic acid	170	7·0	7	0°–4°		100	
Hydrogen peroxide	4·4	7·0	7	0°–4°		100	
Phenyl-isocyanate	9·3	8·1	0·25	0°–4°	0	10, <5	—NH₂, —OH*
	9·3	5·8	0·25	0°–4°	100	100, 50, 100, 50, 100, 35, 35, 50	—SH

* —OH from tyrosine. *R*° = Room temperature.

Table 3. EFFECT OF REDUCING AND OTHER REAGENTS ON ANTIVIRAL ACTIVITY OF CHICK INTERFERON

Reagent	Concentration (mM)	*p*H	Duration of reaction (h)	Temperature of reaction (°C)	Residual inhibitor activity (%)	Protein groups primarily attacked
Benzyl mercaptan (ref. 26)	100	5·0	8	*R*°	<5, <10	—S—S—
Benzyl mercaptan	100	5·0	72	*R*°	<5, <10	—S—S—
Sodium-thioglycollate (ref. 27)	1,000	5·0	1	*R*°	10, 10	—S—S—
Sodium-thioglycollate	1,000	5·0	18	*R*°	5, <5	—S—S—
2-Mercaptoethanol (ref. 28)	200	7·2	24	4°	25, 50	—S—S—
2-Mercaptoethanol (ref. 28) followed by iodoacetate	200 10	7·2 7·2	24 24	4° 4°	20, 35	—S—S— then —SH
Iodoacetate (ref. 28)	10	7·2	24	4°	65, 100	—SH
Bromoacetate (ref. 29)	5	5·0	3	37°	100, 65	Imidazole (histidine)
Bromoacetate (ref. 29)	5	8·2	3	37°	100, 100	—SH
Cysteine (ref. 33)	25	5·0	3	37°	50, 50	—S—S—
Cysteine (ref. 33)	25	8·2	3	37°	50, 50	—S—S—
Bromoacetate + cysteine (ref. 29)	5 25	5·0	3	37°	70, 50	Imidazole histidine/ —S—S—
Bromoacetate + cysteine (ref. 29)	5 25	8·2	3	37°	40, 50	—SH/—S—S—
K-Cyanate (ref. 30)	0·5	6·0	24	*R*°	100, 100	—SH
K-Cyanate (ref. 30)	5	6·0	24	*R*°	100, 100	—SH
K-Cyanate (ref. 30)	50	6·0	24	*R*°	100, 100	—SH
Cyanogenbromide (refs. 31, 41)	16·5	2·0	24	*R*°	<5, 10	γ—S—methyl of methionine

R° = Room temperature.

a similar reaction occurred in the interferon molecule will not be answered with certainty until sufficiently large amounts of pure chick interferon become available for more detailed investigations. The postulated presence of one or more essential disulphide groups in the molecule is clearly also hypothetical at present, since it is not even known that chick interferon contains any sulphur. No sulphur-containing amino-acid was found in the hydrolysates of the carnation factor[7].

Although chick and other interferons show species specificity, such specificity is not absolute[20,21,34,35]. We therefore investigated whether the carnation factor possessed antiviral activity against Semliki Forest virus in chick embryo fibroblasts, the system used by us for the assay of chick interferon. Several carnation plants (*Dianthus caryophyllus*) were homogenized in the presence of 0·15 M sodium chloride (0·5 v/w), the brei was filtered through coarse sintered glass and the filtrate clarified by low- and high-speed centrifugation as described by Ragetli and Weintraub[6]. The supernatant layer, after dialysis against Earle's buffer, was found to be cytotoxic in the assay system, even at high dilution. Portions of the fluid were therefore purified further by the method of Ragetli and Weintraub[6] and also by the method used by us for chick interferon (without DEAE cellulose chromatography)[17]. Two- to ten-fold concentrates (by volume) of juice, purified by either scheme, were no longer cytotoxic, but they were also unable to protect chick fibroblasts against viral attack.

This failure to protect chick cells was in fact not really surprising. Nevertheless there appear to be many chemical, physical and biological similarities between the plant factor and chick interferon, which make one wonder whether the production of the two materials is stimulated by related agents (viruses, foreign nucleic acids[37]) for similar defence purposes. Although the carnation factor was obtained from apparently healthy plants, it is conceivable that they were carriers of a so far undetected virus. Alternatively, by analogy with animal cells[38] even normal uninfected plant cells might perhaps carry low levels of an interferon-like substance.

On the other hand, there are examples to show that antiviral activity in some plants is induced or increased by viral infection. Loebenstein and Ross[8] have shown that the concentration of an extractable antiviral factor (resembling interferon) in the leaves of *Datura stramonium* was much higher after than before infection with tobacco mosaic virus. Sela *et al.*[9] found that the juice of uninfected *Nicotiana glutinosa* L. leaves contained antiviral activity, but that an additional new antiviral substance was produced when the plants became infected with tobacco mosaic virus. This newly formed substance is a nucleoprotein and differs from chick interferon in its behaviour towards ion-exchange celluloses.

It thus appears that plants contain a number of naturally occurring antiviral substances resembling avian, and probably mammalian, interferons to varying degrees. Atanasoff[5] pointed out that resistance to virus disease in plants has been known for a much longer time than resistance in animals. Plant strains, thus protected, are of great importance, and one cannot but speculate whether the principle underlying that resistance is the same as the more recently discovered interferon-mediated viral immunity of mammalian and avian cells.

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FORMATION OF TRIPLET STATES OF SOLUTES IN THE RADIOLYSIS OF ORGANIC LIQUIDS

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AND
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WE have investigated the transient absorption spectra produced by short pulses (dose $\simeq$ 6,500 rads in 2 μsec) of 4-MeV electrons incident on dilute solutions, generally about 10^{-2} M in concentration, of naphthalene or benzophenone or diphenyl in various aprotic organic liquids at room temperature using techniques previously described[1]. The results are summarized here.

(1) 9·8 *mM solutions of naphthalene* (*N*). This solute has a singlet ground state and a triplet excited state, N^T, at about 21,000 cm^{-1} higher energy, and can capture

Table 1. TRANSIENT SPECIES IN THE PULSE RADIOLYSIS OF 9·8 mM NAPHTHALENE SOLUTIONS

Species	N^T			$\dot{N}$H		
Solvent	λ_{max} (mμ)	G ε_{max}	Decay half-life (μsec)	λ_{max}	$G \times$ ε_{max}	Second-order decay k/ε (cm sec^{-1})
Benzene	420	15,100	1·59	←absent→		
*Cyclo*hexane	415	10,200	5·85	330	2,900	$2·5 \times 10^6$
Ether	←absent→			325	13,600	Not measured
Tetrahydrofuran	←absent→			330	11,050	$4·5 \times 10^6$
Methanol (ref. 2)	←absent→			330	20,200	$1·05 \times 10^6$

electrons to form the anion, N^-, and hydrogen atoms to form the $\dot{N}$H radical. Each of these species has a distinct absorption spectrum by which its presence can be detected. N^- was not observed in any of the five solvents listed in Table 1; instead, either N^T or $\dot{N}$H or a mixture of the two was usually detected. The spectrum of N in *cyclo*hexane is shown in Fig. 1. It has two peaks at 390 and 415 mμ which are almost identical with those previously observed for N^T (ref. 2) and an absorption band at 330 mμ assigned to N.H (ref. 3). In confirmation of these assignments it was found that the decay of the N^T band is always first order and that of the $\dot{N}$H band is second order. The relevant results are given in Table 1.

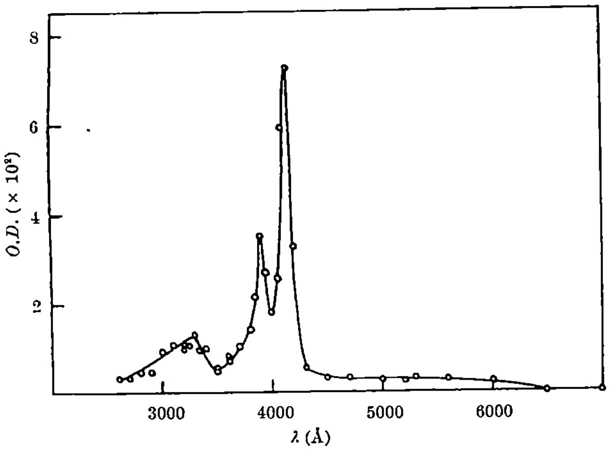

Fig. 1. Absorption spectrum of 9·8 mM naphthalene in *cyclo*hexane taken at the end of a 2-μsec pulse of 4-MeV electrons

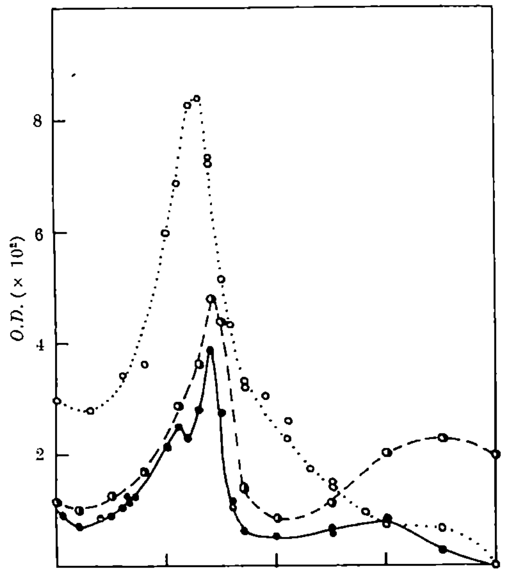

Fig. 2. Absorption spectra of solutions of benzophenone in benzene (···O···), *cyclo*hexane (—●—) and tetrahydrofuran (--◐--) taken at the end of a pulse of 4 MeV electrons

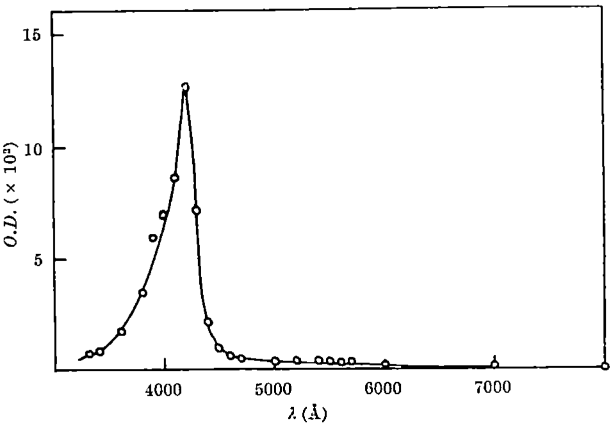

Fig. 3. Absorption spectrum of pulsed benzene solution containing 15 mM benzophenone and 9·7 mM naphthalene

The triplet state of the solute may be formed either by a spin-permitted energy transfer from a high-energy triplet state of the solvent, S, (equation (1)) or by electron exchange (equation (2)) with a secondary electron or

$$S^T + N \rightarrow S + N^T \qquad (1)$$

$$e^-(\uparrow) + N(\uparrow\downarrow) \rightarrow N^T(\uparrow\uparrow) + e^-(\downarrow) \qquad (2)$$

possibly as the result of a collision of N^- with a positive ion. The first of these is obviously appropriate to benzene as a solvent but cannot be true for *cyclo*hexane unless a higher excited state of this solvent decomposes forming a molecule in a suitable triplet state, for example, as in equation (3):

$$C_6H_{12} \rightsquigarrow C_6H_{12}^*;\ C_6H_{12}^* \rightarrow H_2 + C_6H_{10}^T;$$
$$C_6H_{10}^T + N \rightarrow C_6H_{10} + N^T \qquad (3)$$

The last mechanism is unlikely to be important in non-polar media such as C_6H_6 and C_6H_{12}.

The NH radical may arise from (*a*) electron capture by N followed by proton transfer from a protic solvent molecule or a molecular ion to N^- (equation (4)) or (*b*) hydrogen atom scavenging by the solute (equation ((5))

$$e^- + N \rightarrow \dot{N}^-;\ N^- + RH \text{ or } RH^+ \rightarrow \dot{N}H + R^- \text{ or } R \qquad (4)$$

$$H\cdot + N \rightarrow \dot{N}H \qquad (5)$$

or (*c*) hydrogen atom abstraction from the solvent by the triplet state (equation (6)):

$$N^T + RH \rightarrow \dot{N}H + R\cdot \qquad (6)$$

Mechanism (*a*) will only be possible in polar media where charge separation can occur and is the probable mechanism in methanol (protic solvent in which the electron can be solvated) and tetrahydrofuran (aprotic but may well solvate the electron). Mechanism (*b*) is likely in *cyclo*hexane, where scavengeable hydrogen atoms are known to form part of the primary radiolysis products, but may be less likely in benzene which shows marked auto-scavenging power[4]. Mechanism (*c*) is possible for *cyclo*hexane but is known to be impossible for benzene[2a]. The pattern discernible in Table 1 is thus broadly that expected, but we cannot be sure whether mechanism (*b*) or

(c) is predominant in the case of *cyclo*hexane. Since the NH band did not perceptibly increase as the N^T band decayed in this solvent we think that (b) is the more likely origin of NH in this liquid.

(2) *Solutions of benzophenone* (BP). Fig. 2 shows the spectra obtained in benzene, *cyclo*hexane and tetrahydrofuran, and Table 2 summarizes the data. The spectra of the ketyl, $\dot{B}PH = \varphi_2\dot{C}OH$, and its anion, $BP^- = \varphi_2\dot{C}O^-$, have been shown to have $\lambda_{max} = 540$ and 610 mμ in water–isopropanol mixtures[2a], and $\varepsilon^{545 m\mu}$ for $\dot{B}HP$ is $5{\cdot}1 \times 10^3$ M^{-1} cm^{-1} (ref. 6), and since the spectral characteristics of BP^T are also well known and this decays by a first-order law, whereas $\dot{B}PH$ decays bimolecularly, the assignments in Table 2 can be made with some confidence. The pattern is evidently similar to that for naphthalene solutions in that BP^T is formed in a non-polar solvent, but since benzene is an auto-scavenging solvent for H atoms and cannot transfer a hydrogen atom to BP^T no $\dot{B}PH$ is formed. BP^T is not observed in *cyclo*hexane in accordance with expectation because it readily abstracts a hydrogen atom from this solvent. A slight difference exists in that BP^- is formed but not N^- in tetrahydrofuran.

Table 2. TRANSIENT SPECIES IN THE PULSE RADIOLYSIS OF BENZOPHENONE SOLUTIONS

Solute concentration (mM)	Species Solvent	BP^T			$\dot{B}PH$			BP^-
		λ_{max} (mμ)	G ε_{max}	$t_{1/2}$ (μsec)	λ_{max} (mμ)	G ε_{max}	k/ε (cm sec^{-1})	λ_{max} = 700
12·1	Benzene	530	11,700	2·11 (ref. 4)	←Probably absent→ or small amounts			Absent
7·9	*Cyclo*-hexane	←Absent or only minor→			540	6,300	$1{\cdot}74 \times 10^6$	Possibly trace*
13·8	Tetrahydrofuran	←Absent or only minor→			545	6,200	$1{\cdot}76 \times 10^6$	Present

Porter and colleagues[2,6] have shown that energy transfer from BP^T to N (equation (7)) occurs readily and when a benzene solution containing 15·5 mM BP and 9·7 mM N was irradiated only the N^T spectrum was observed (Fig. 3).

$$BP^T + N \rightarrow BP + N^T \qquad (7)$$

It is interesting that $G\ \varepsilon^{420}$ is in this case 17,850, whereas its value in the absence of BP but at the same naphthalene concentration was 15,100 (Table 1), which has implications for the mechanism of formation of triplet states of solutes which we propose to investigate.

(3) *Solutions of diphenyl* (φ_2). Only a limited number of experiments were made with this solute but they sufficed to indicate: (i) that no φ_2^- is formed in 8·1 mM solution in 3-methylpentane, although Hamill *et al.*[7] find that this is produced by γ-irradiation of this solution in the glassy state at 77° K; (ii) in 3-methyl pentane only a single sharp peak is formed at $\lambda_{max} = 360$ mμ, $G\ \varepsilon^{360} = 16{,}600$, which decays unimolecularly with $t_{1/2} = 3{\cdot}4$ μsec, and this we assign to the triplet $\varphi_2{}^T$ (this assignment is in accord with that of Porter and Windsor[8] from investigations of the flash photolysis of φ_2 in liquid paraffin); (iii) pulsed 10·7 mM φ_2 in tetrahydrofuran gives a more complex spectrum with three peaks, one at 360 mμ (which we tentatively assign to $\varphi_2{}^T$), one at 305 mμ, which might be φ_2H, and one at ~400 mμ which might be part of the φ^{2-} band system.

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* Dr. E. J. Land informs us he has definitely observed BP- in this system.

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INTERFEROMETRIC RAMAN SPECTROSCOPY USING INFRA-RED EXCITATION

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WE are reporting here the first results of the researches in the Basic Physics Division of the National Physical Laboratory, aimed at extending the usefulness of Raman spectroscopy by developing methods of infra-red excitation. Many pure substances and industrial intermediates are strongly coloured and their Raman spectra cannot be recorded using ultra-violet and visible lines, but nearly all compounds have a region of transparency in the near infra-red. If, therefore, methods could be developed in which a selection of infra-red exciting lines were used, Raman spectroscopy could be more widely applied and could become a valuable complement to standard infra-red analysis.

The basic difficulty comes from the fact that as the wavelength of the exciting line is increased, the scattered intensity falls off rapidly. It is therefore important to seek a spectroscopic technique which makes best use of the reduced amount of scattered radiation, and in this respect the virtues of the Michelson interferometer plus Fourier transform method are now well known. An interferometer for the near infra-red region which uses a strip-hinge mirror movement has already been described[1]. The large intensity ratio between direct and Raman scattered radiation poses technical problems of maintaining linearity in the amplifying and detecting systems. It is therefore an important consideration in choosing the source that there should be means of discriminating one from the other. In this respect the gallium arsenide injection laser has the great advantage that in any experiment requiring the elimination of the exciting line from the scattered radiation, a gallium arsenide filter may be placed in front of the photomultiplier. Gallium arsenide has a very steep absorption edge the wave-length of which can be changed by varying the temperature. Thus the filter can be set to attenuate strongly the exciting line and transmit any Raman lines of longer wave-length

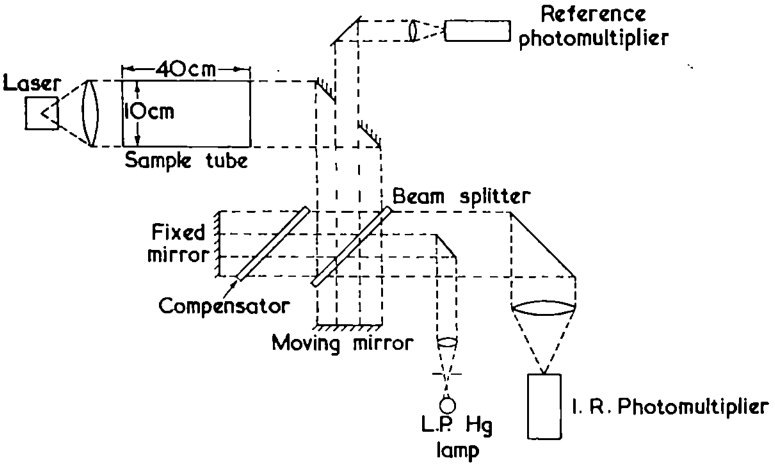

Fig. 1. Experimental arrangements for near infra-red Raman spectroscopy

that may be present. Though the use of such a filter prevents the observation of the anti-Stokes lines and has not been used in these first observations reported here, we believe that in less-favourable cases where absorption in the sample itself does not provide the discrimination, this property of gallium arsenide will be of value.

Our sample tube used with the laser differs from conventional Raman sources in having plain glass windows at each end so that the exciting radiation passes through the sample and directly into the interferometer. The laser is pulsed and the photomultiplier voltage pulses are 'gated' to remove the noise between pulses. The output is recorded digitally at intervals of mirror movement equal to the wave-length of the mercury violet line *Hg* 4047 Å and spectra are obtained by Fourier transformation in a digital computer. The experimental arrangement is shown in Fig. 1.

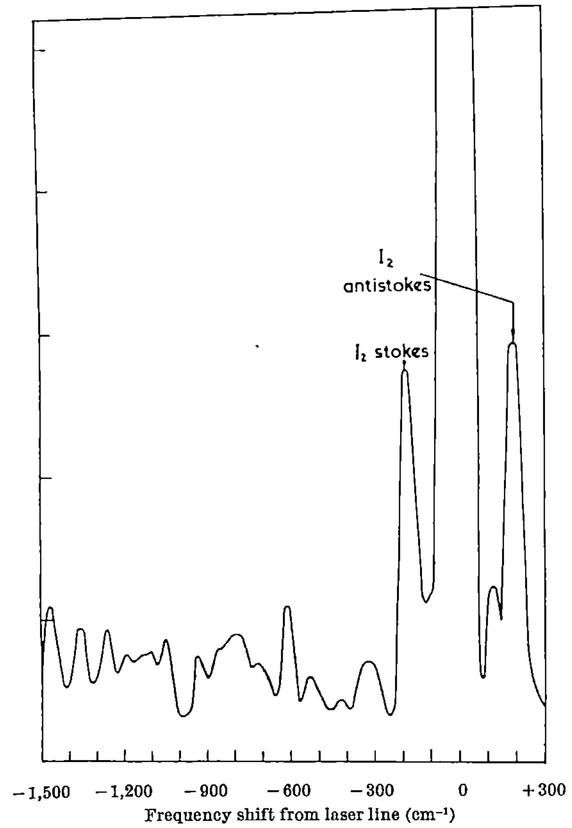

Fig. 2. Raman spectrum of iodine dissolved in carbon tetrachloride

Using this apparatus we have observed the Raman spectrum of iodine dissolved in carbon tetrachloride at a molar ratio of 1 : 1,000. The spectrum is shown in Fig. 2. The Stokes and anti-Stokes lines of iodine show up clearly at ± 200 cm^{-1} with an intensity of about 1 per cent of the laser-line intensity. Such a high value, together with the approximate equality of the Stokes and anti-Stokes intensities, indicates that the emission is stimulated. Further evidence has been obtained by sending the beam into the cell at an angle and showing that the ratio of Raman intensity to laser intensity remains unchanged. This proves that the Raman emission is angularly correlated with the laser beam—as expected for stimulated emission. The spectrum obtained with skew illumination also shows the Raman line ν_1, of carbon tetrachloride at 460 cm^{-1}.

We have also observed strong Raman emission from very dilute (M/800) solutions of the cyanine dye 1,1′-diethyl thia-tricarbocyanine iodide in methanol and the spectrum is shown in Fig. 3. The absorption spectrum of this dye shows an intense ($f \approx 1\cdot0$) band peaked at 13,200 cm^{-1}. The structure of this absorption suggests that the compound will show a strong Raman spectrum and consequently a stimulated Raman effect is to be expected. The Raman features are probably unresolved multiplets and their intensity is such (10 per cent for the 300 cm^{-1} line) as to suggest that these may in fact be stimulated. The anti-Stokes lines are not observed because of strong absorption by the liquid.

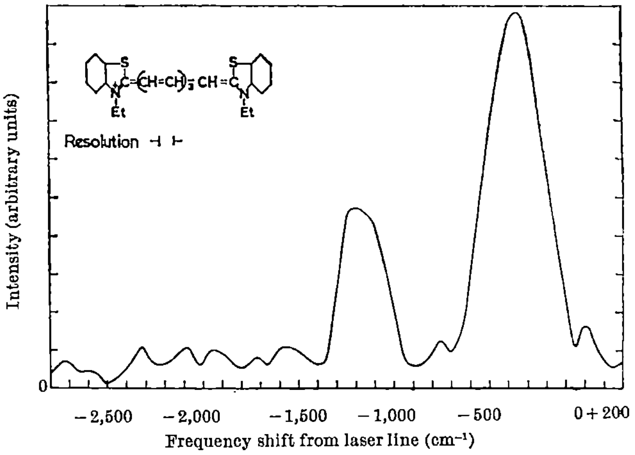

Fig. 3. Raman spectrum of 1,1′-diethyl thia-tricarbocyanine iodide dissolved in methanol

The possibilities of Raman measurements with near infra-red exciting lines have thus been demonstrated. We believe, therefore, that technical Raman spectroscopy with a wide field of application could be achieved with a combination of interferometer and stimulated emission sources. Such sources will be available in an increasing range of wave-length and power as time goes on, and should permit most substances to be investigated by choosing the source wave-length to lie in a region of low absorption.

We thank M. J. Coupland, Services Electronics Research Laboratory, for supplying the laser.

This work forms part of the research programme of the Basic Physics Division of the National Physical Laboratory and is published by permission of the Director.

[1] Gebbie, H. A., Habell, K. J., and Middleton, S. P., *Proceedings of the Conference on Optical Instruments 1961* (Chapman and Hall, Ltd., London, 1962).

SCHEELITE STRUCTURES: SINGLE CRYSTAL GROWTH AND TRANSMISSION DATA

By Dr. B. COCKAYNE and J. D. RIDLEY

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SEVERAL of the scheelite structures are now of interest as possible host lattices for laser materials. It is already reported in the literature[1-3] that many of these structures have been grown from the melt in single crystal form using the Czochralski technique[4] but, with the exception of $CaWO_4$ (refs. 5 and 6), little information has been published on the transmission properties of this group of materials. The results are now reported for the single crystal scheelites $SrWO_4$, $BaWO_4$, $CaMoO_4$ and $SrMoO_4$, and are compared with similar data for $CaWO_4$. Some aspects of the growth process are also described.

The technique of crystal growth for all the crystals was identical to that described fully elsewhere for $CaWO_4$ (ref. 6), in which the starting material, prepared as a powder by Levy-West Laboratories Ltd., is melted in an iridium crucible using radio-frequency induction heating. The crystals were pulled from the melt in an argon/oxygen atmosphere. For $SrWO_4$ and $BaWO_4$, only 4 vol. per cent of oxygen was required in the atmosphere above the melt surface to prevent reduction of the material, which imparts a dark-brown colour to the crystal; these conditions are this identical to those reported for $CaWO_4$ (ref. 6). A similar volume of oxygen in the atmosphere was, however, insufficient to suppress the reduction of $CaMoO_4$ and $SrMoO_4$ crystals, which exhibit a blue colour characteristic of the lower oxides of molybdenum. The reduction can be suppressed to a certain extent by increasing the oxygen content of the atmosphere to 10 vol. per cent but is not entirely removed by this proportion. Single crystals of $BaMoO_4$ were also grown, but these cleaved catastrophically on cooling and contained a precipitate which rendered the material opaque, thus suggesting that the starting material was impure.

During the course of the work the melting-points of this group of materials were measured and are given in

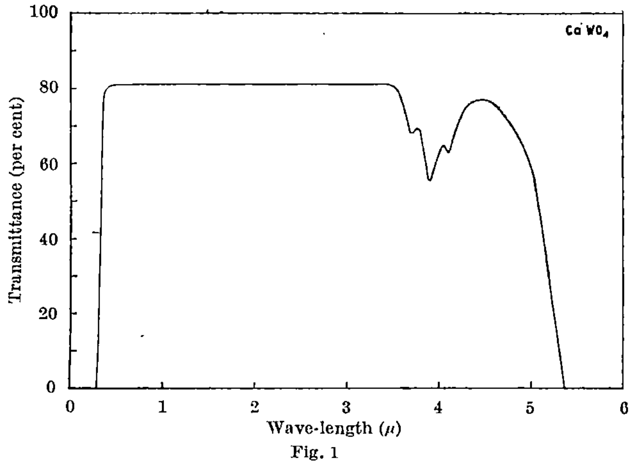

Fig. 1

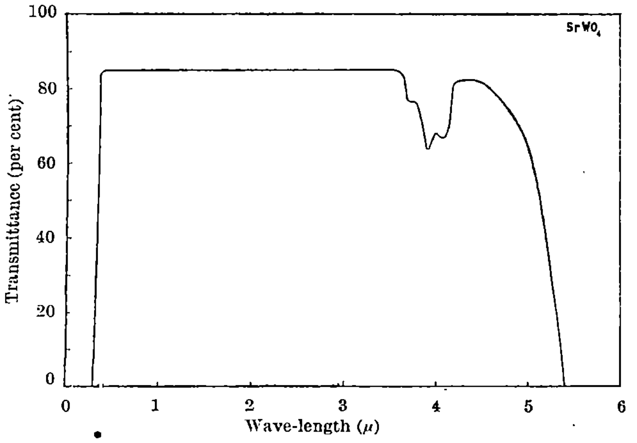

Fig. 2

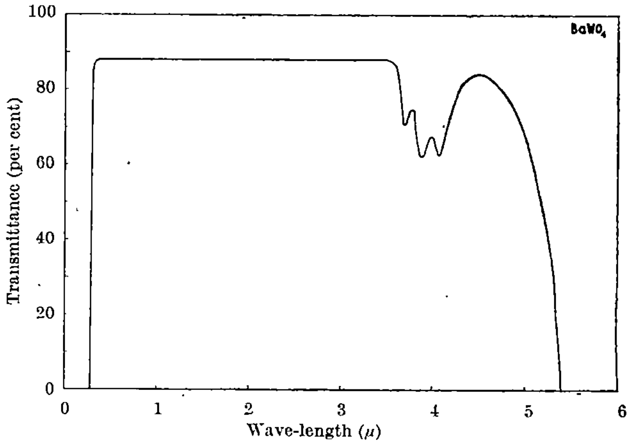

Fig. 3

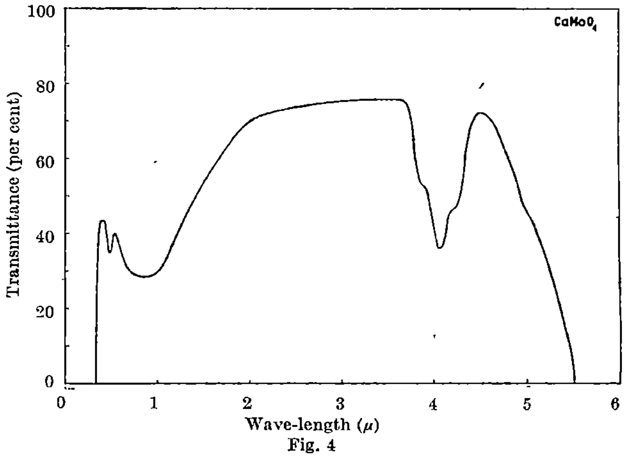

Fig. 4

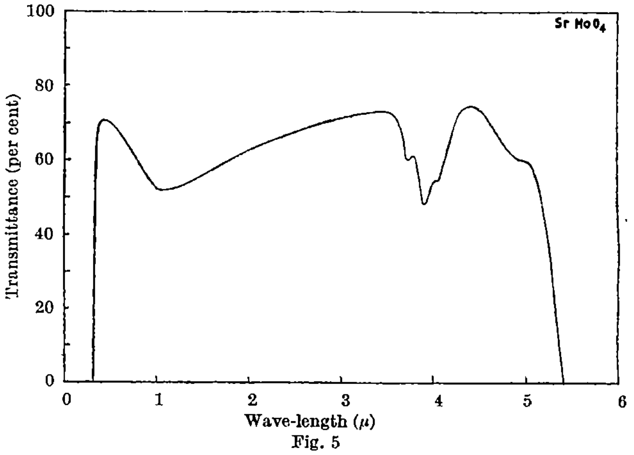

Fig. 5

Table 1

Scheelite compound	Melting point (°C)
$CaWO_4$	1,566
$SrWO_4$	1,566
$BaWO_4$	1,485
$CaMoO_4$	1,449
$SrMoO_4$	1,468
$BaMoO_4$	1,459

Table 1. These were determined using a thermal arrest method and by measuring the temperature at which an 'island' of solid, lifted just above the melt on a thermocouple, was observed to melt and fall back into the crucible. The latter method was found to be the most reproducible and gave results repeatable to ± 3° C.

The specimens for transmission studies were prepared as 5-mm thick sections by cutting the crystals with a wire saw and subsequent polishing with successively finer grades of diamond paste. The variation of transmittance with wave-length for the scheelites examined is shown in Figs. 1–5; the measurements were made at room temperature using standard Perkin–Elmer spectrophotometers. The transmission properties of these materials are very similar apart from the absorption in the molybdates at wave-lengths up to 2μ, which is most probably due to the particles imparting the blue colour to the crystals.

[1] Nassau, K., and Van Uitert, L. G., *J. App. Phys.*, **31**, 1508 (1960).

[2] Van Uitert, L. G., Swanekamp, F. W., and Preziosi, S., *J. App. Phys.*, 32, 1176 (1961).

[3] Preziosi, S., Soden, R. R., and Van Uitert, L. G., *J. App. Phys.*, 33, 1893 (1962).

[4] Lawson, W. D., and Nielsen, S., *Preparation of Single Crystals* (Academic Press, Inc., New York, 1958).

[5] Nassau, K., and Broyer, A. M., *J. App. Phys.*, **33**, 3064 (1962).

[6] Cockayne, B., Robertson, D. S., and Bardsley, W., *Brit. J. App. Phys.* (in the press).

A RECENT VOLCANIC ERUPTION ON BOUVETOYA, SOUTH ATLANTIC OCEAN

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THE Norwegian island of Bouvetøya (54° 26′ S., 3° 24′ E.) is the southernmost of the volcanic islands rising from the Mid-Atlantic Ridge. About 9·5 km from east to west and 7 km from north to south, it reaches a height of 935 m above sea-level. The gently domed upper part of the island ends abruptly in rock cliffs up to 335 m high along the western side, but to the east it is bounded by lower ice cliffs. Except for the steep sea cliffs the entire island is covered by ice which has a maximum thickness on the coast of about 60 m.

An account of the physiography and structure of Bouvetøya by Holtedahl[1] was based largely on information and photographs supplied by the *Norvegia* expeditions 1927–28 and 1928–29. Rock specimens collected during these expeditions were later described by Broch[2], who also presented five chemical analyses.

On January 1, 1958, during an aerial reconnaissance made at the request of Rear-Admiral H. H. Biermann, observers from U.S.C.G.C. *Westwind* obtained photographs of Bouvetøya showing a low platform at the foot of the

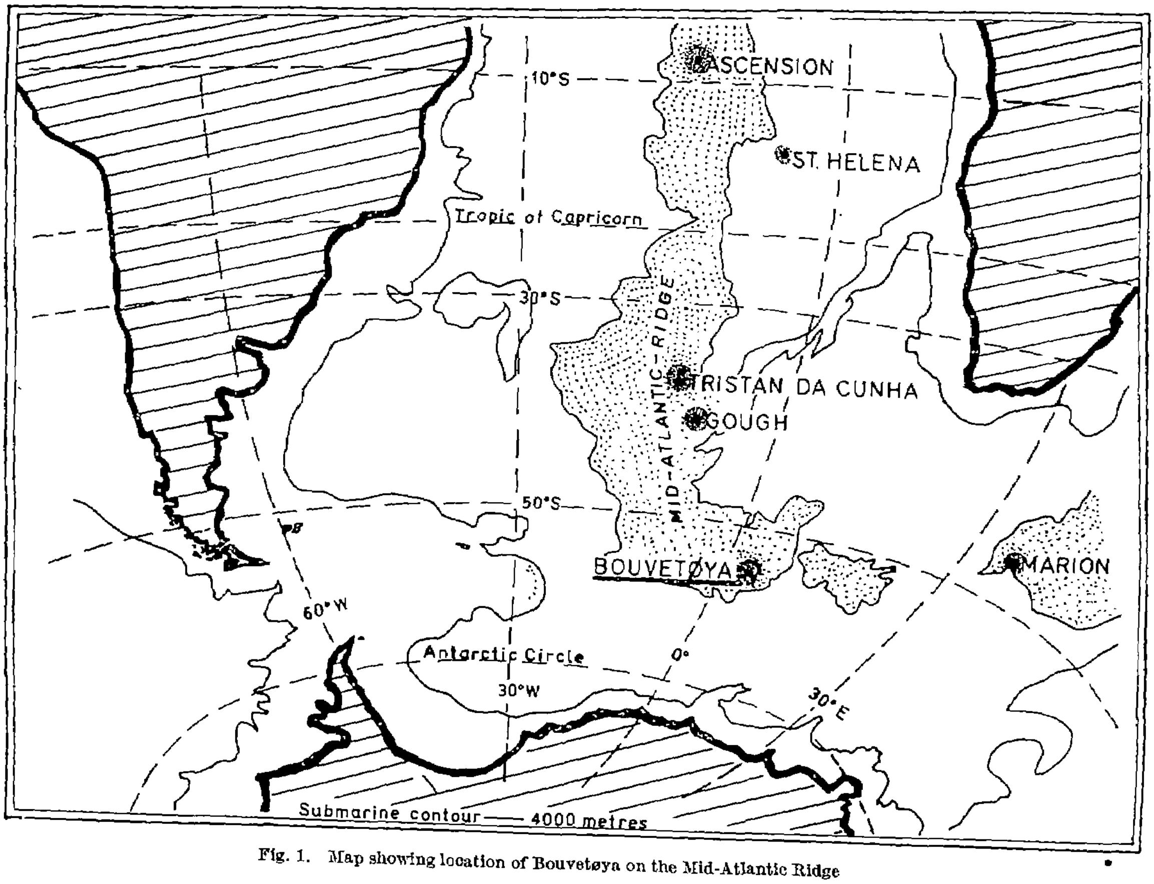

Fig. 1. Map showing location of Bouvetøya on the Mid-Atlantic Ridge

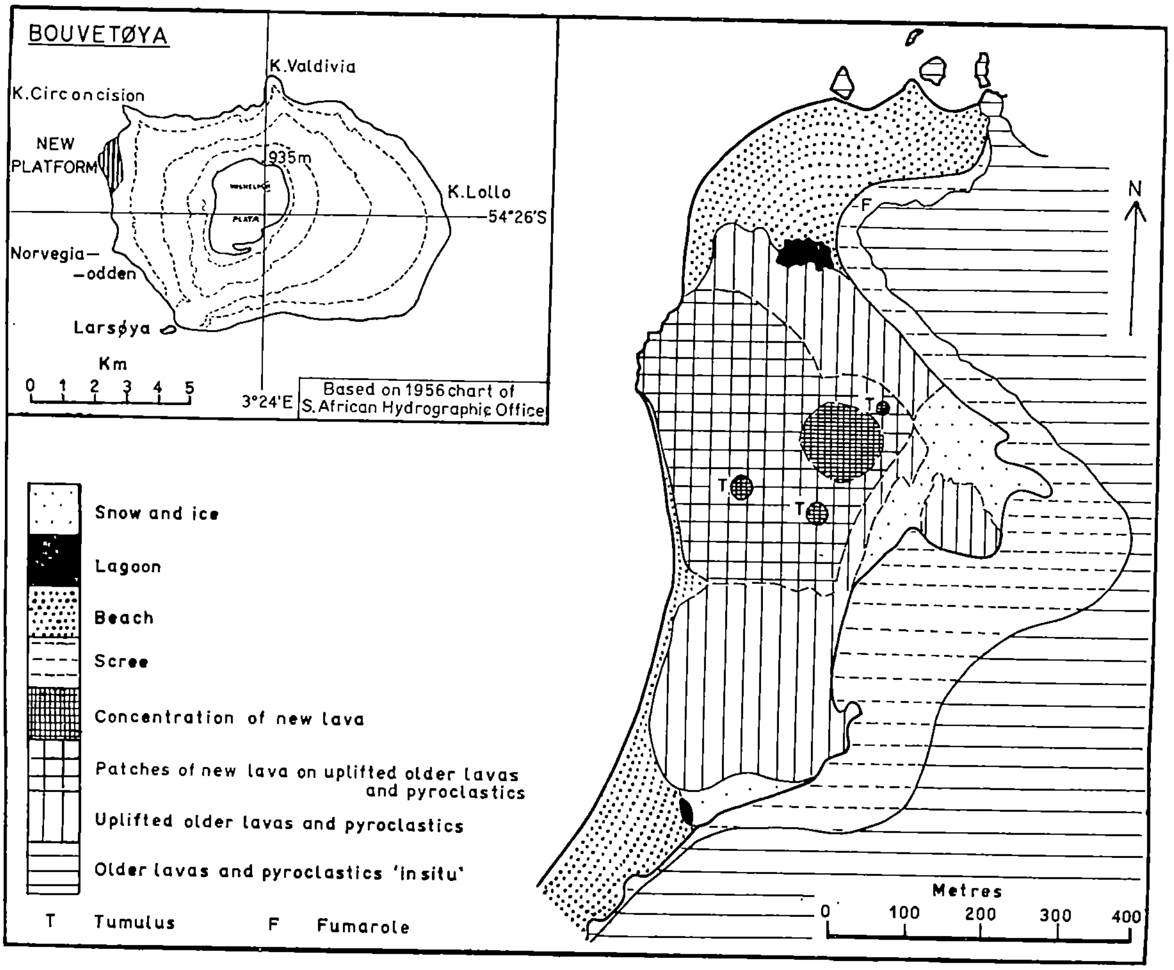
BOUVETØYA
K.Valdivia
K.Circoncision
NEW PLATFORM
935m
K.Lollo
54°26'S
Norvegia-odden
Larsøya
Km
0 1 2 3 4 5
3°24'E
Based on 1956 chart of S.African Hydrographic Office
Snow and ice
Lagoon
Beach
Scree
Concentration of new lava
Patches of new lava on uplifted older lavas and pyroclastics
Uplifted older lavas and pyroclastics
Older lavas and pyroclastics 'in situ'
T Tumulus
F Fumarole
N
F
T
T
T
Metres
0 100 200 300 400

Fig. 2. Geological sketch map of new platform. Inset shows position of platform on the west coast of Bouvetøya

cliffs on the north-western side of the island, 1 km to the south of Kapp Circoncision. Lieut.-Commander A. B. Crawford, who afterwards examined the photographs, concluded that the platform did not exist in February 1955 when he landed on Kapp Circoncision with a party from S.A.S. *Transvaal*. This was finally confirmed on December 10, 1959, when Lieut.-Commander Crawford, aboard M.V. *Polarbjørn*, observed the new feature for the first time. On March 31 and again on April 2, 1964, British Antarctic Survey scientists were landed by helicopter from H.M.S. *Protector* to investigate the new platform. On the second occasion they were accompanied by scientists from the South African Research Ship *R.S.A.*

Extending for about 650 m from north to south and for 365 m at its widest point from east to west, the new platform has a hummocky surface rising to an average height of 25 m and a maximum of 40 m above sea-level. To the east it is flanked by talus fans derived from the cliffs behind, to the west it terminates in low sea cliffs, and to both north and south it is adjoined by broad beaches. The feature is evidently the result of a volcanic eruption which involved the extrusion of a small volume of new lava and the uplift of a considerably larger volume of older lavas and pyroclastics: the magma was apparently highly viscous at the time of eruption. No crater is visible and there are no distinct lava flows. The central part of the platform consists of irregular patches of a new block lava resting on disturbed older material; the new lava appears to be concentrated on the higher ground towards the north-east, but it also forms well-developed tumuli about 12 m in diameter and 5 m high a short distance to the south-west of the highest point. Flaggy slabs of lava, often showing flow-banding, are breaking away from the tumuli along prominent concentric joint surfaces. The northern and southern parts of the new platform appear to be composed entirely of upheaved debris including boulders and fragments of old lava and hillocks of a greenish tuff and ash, similar to that which crops out in the cliffs behind.

Individual blocks of the new lava have reddish-brown surfaces but are greenish-grey in the interior. Pyroxene phenocrysts are conspicuous in the hand specimen. In thin section, phenocrysts of plagioclase, zoned chiefly within the andesine range, clinopyroxene, ore and occasionally olivine are seen in a fine-grained matrix of plagioclase, alkali feldspar, pyroxene and ore. Both apatite and calcite are abundant accessory constituents. The mineralogy of the new lava suggests a trachyandesite composition.

This small eruption on Bouvetøya, which evidently occurred between February 1, 1955, and January 1, 1958, is another instance of volcanic activity along the Mid-Atlantic Ridge within the past decade, other eruptions having occurred in the Azores (1957), Tristan da Cunha (1961) and Iceland (1961 and 1963).

We thank the Norwegian Government for permission to land on Bouvetøya; also the captain, officers and ship's company of H.M.S. *Protector* for their assistance, and Lieut.-Commander A. B. Crawford for supplying details of the discovery of the new platform. The investigation was carried out as part of a British Antarctic Survey project and during the tenure of Department of Scientific and Industrial Research/NATO awards.

[1] Holtedahl, O., *Det. Norske Videnskaps Akademi, Oslo* (1929).

[2] Broch, O. A., *Det. Norske Videnskaps Akademi, Oslo* (1946).

LETTERS TO THE EDITOR

PHYSICS

Distribution of Particle Size in Dust

DAVIES[1] says that when the power law is used in the form:

$$dN/dD = KD^{-\gamma} \qquad (1)$$

where N is the number of particles below diameter D, then K is not just an arbitrary concentration factor. He then states that the smallest particle size (D_S) is a function of K, a statement that appears to me to be just the opposite of the real situation.

In practice both D_S and D_L are fixed by factors such as the resolution limit of the microscope being used and the largest particle that the sampling instrument can sample, and K will be a function of both these quantities. Solving for K in equation (3) of Davies's communication we get:

$$K = N_T(\gamma - 1)/[D_S{}^{1-\gamma} - D_L{}^{1-\gamma}] \qquad (2)$$

From equation (2) it is apparent that K is proportional to N_T, but as it also depends on γ and D_S it is not, as Davies pointed out, merely a concentration factor. The dependence of K on γ and D_S is due to the fact that we are dealing with a probability density function and not an ordinary regression situation.

The appropriate probability density function is:

$$P(D) = (\gamma - 1)D^{-\gamma}/(D^{1-\gamma} - D_L{}^{1-\gamma}) \qquad (3)$$
$$\text{for } D_S \leqslant D \leqslant D_L$$

and the cumulative probability for particles of diameter less than D is:

$$(D_S{}^{1-\gamma} - D^{1-\gamma})/(D_S{}^{1-\gamma} - D_L{}^{1-\gamma}) \qquad (4)$$

and hence:

$$P(D) = \frac{dP(D)}{dD} = (\gamma - 1)D^{-\gamma}/(D_S{}^{1-\gamma} - D_L{}^{1-\gamma}) \qquad (5)$$

$$\text{and} \quad \frac{dN}{dD} = N_T \frac{dP(D)}{dD} = KD^{-\gamma} \quad \text{(as it should)} \qquad (6)$$

If $dP(D)/dD$ is plotted against D on log–log paper the result will be a straight line; however, I do not see why $P(D)$ (or N per cent) when plotted against D on log-probability paper can be expected to yield a straight line as we are not dealing with the log normal distribution.

If D_L is much larger than D_S ($D_L = 100\mu$ is large), then:

$$1 - P(D) = (D^{1-\gamma} - D_L{}^{1-\gamma}) / (D_S{}^{1-\gamma} - D_L{}^{1-\gamma})$$
$$\approx (D/D_S)^{1-\gamma} \qquad (7)$$

and plotting results of equation (7) on log–log paper gives a linear relationship of slope ($1-\gamma$). In practice one plots $1 - N/N_T$ against D and obtains γ from the slope of the line.

A final comment is that when drawing graphs of any distribution it is often very misleading to omit parts of the graph and, if possible, this should be avoided (for example, in Fig. 1 of Davies's paper for $K = 10$, $D_S = 0{\cdot}1\mu$, and hence the curve for the interval $D_S \leqslant D \leqslant 1\mu$ has been omitted although a number of particles would occur in this range).

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University of New South Wales,
Sydney.

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[1] Davies, C. N., *Nature*, **201**, 172 (1964).

DR. DAVIES[1] refers to the significance of the multiplier K in the 'power law' frequency function $dN = KD^{-\gamma}\,dD$ describing the size distribution of particles. His remarks, however, could apply to any function of the general form $Kf(D)$. The multiplier K does not in any way define the relative frequencies with variation of D; this is completely defined by $f(D)$. If, however, two or more distributions were combined, it would be necessary to specify individual values of K in order to define the contributions of the different components:

$$K_1f_1(D) + K_2f_2(D), \text{ etc.}$$

Apart from its use with a multi-term distribution the multiplier K could be used to adjust the value of a definite integral to a specific total, although it is rarely used for this purpose. In the examples shown by Davies the total is fixed at 100 per cent and the lower limit of the integral is indirectly varied by varying K. His curves express the cumulative per cent below size where 100 per cent corresponds to:

$$K\int_{D_S}^{D_L} D^{-\gamma}\,dD$$

The four curves correspond to different values of K but have the same upper size limit of $D_L = 100\mu$. The lower size limit D_S is not directly specified, but since the total of the integral is fixed at 100 it follows that D_S is completely determined by the value of K. The curves, therefore, demonstrate only the change of visual shape (that is, not mathematical shape) that must result from plotting any given function over a progressively wider range of D while maintaining the total unchanged.

Thus, although the distribution is completely specified by $D^{-\gamma}$, the visual shape of the curves calculated from it can be varied according to the way the results are calculated and displayed.

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Ministry of Power, Sheffield.

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[1] Davies, C. N., *Nature*, **201**, 172 (1964).

Light from Shocked and Detonating Powders

Blackburn and Seely[1] have published evidence that the light from strongly shocked granular salt was largely independent of the interstitial gas. They inferred that it did not (as I had thought[2,3]) arise from compression of the gas, and assumed that the same conclusion would apply to detonating explosive powders. While this extrapolation is plausible, it would be advantageous to have it confirmed.

In a recent communication[4], however, Blackburn and Seely refer to a side-on still photograph taken by Cumming in this laboratory[5], which shows a marked dependence on the included gas (butane, air, argon) for PETN detonating in a water-sheathed plastic tube. Against this, they present an end-on time-resolved record, in which the first flash of light from the free surface of PETN is equally intense in butane, methane and air. As they remark, it is difficult to reconcile these results.

The discrepancy is not due to the fact that Cumming's published photograph was a still one. This photograph was one of a set of five, the other four time-resolved and all showing the same effect. We have recently taken side-on streak records of a further five PETN detonations in polyvinyl chloride, cellulose acetate and 'Perspex' tubes, with and without water sheaths. These records show in general an unmistakable difference between butane, air and argon: Fig. 1 is an example. It is true that our time-resolution is moderate (1 mm/μsec) and primary light from the detonation front may therefore conceivably be masked by later light which would be separated under high resolution. However, even if this is so, it does not alter the fact that the total light output depends very much on the nature of the gas.

To assess the effect of the confining tubes, and for comparison with Blackburn and Seely's end-on free-surface photographs, we have taken numerous streak records of PETN lying in the lower part of a sealed 'Perspex' trough, divided into three compartments in tandem, through which butane, air and argon were circulated. The camera viewed the PETN surface from above. The contrast between light intensities in the three gases is so great as to present a problem in printing from the negative. In Fig. 2 the print exposure has been lengthened to accentuate the air line. The argon line is still submerged in a cloud of intense scattered and secondary light, while the butane line has already almost disappeared, although quite distinct on the negative. Longer print exposure suppresses the air line also and isolates the argon line.

We have repeated Blackburn and Seely's end-on experiment, using butane and air only. Although the record shows a more intense primary line from the air, the difference is much less pronounced than in Fig. 1. Even a single free-surface test of this kind can be regarded as significant, since the absence of atmospheric shock light from the butane proves that the gas was successfully circulated, which is not so easy to guarantee with confined charges.

It is generally thought that the lateral surface layer of a detonating explosive column may react less quickly and completely than elsewhere. When poorly confined, the surface layer may even survive unreacted. It can therefore be suggested that what we record in a side-on shot is a rather muted version of affairs inside the mass of explosive. If end-on shots report more extreme conditions, the similar behaviour of air and butane in such shots may be due to the greater shock intensity. One would then expect the differences to reappear with a milder explosive. We have carried out a few experiments similar to Blackburn and Seely's with nitroguanidine at loading densities of 0·2, 0·185 and 0·17 g/cm^3. The charges were 1 in. in diameter, 3 in. long, and simultaneous records showed steady speeds between 2 and 3 km/s, according to density. At 0·20 g/cm^3, there was rather little difference between air and butane, though the air was somewhat brighter.

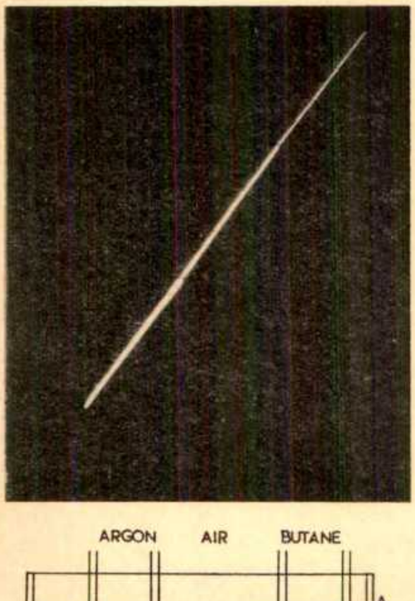

Fig. 1. Streak photograph of PETN detonating in a 0·5 in. internal diam., 0·625 in. outer diam. polyvinyl chloride tube within a tank of water. Butane and argon were passed continuously through the first and third sections, while the second contained air. *A*, initiator

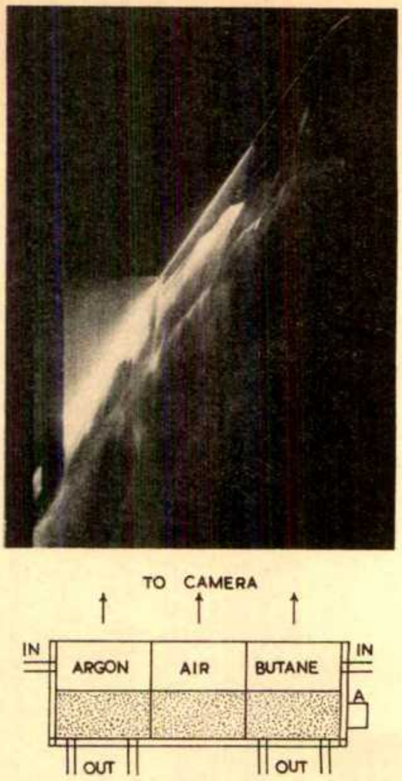

Fig. 2. Streak photograph of PETN detonating in a partially filled trough. The camera slit image lay along the median line of the powder surface. *A*, initiator

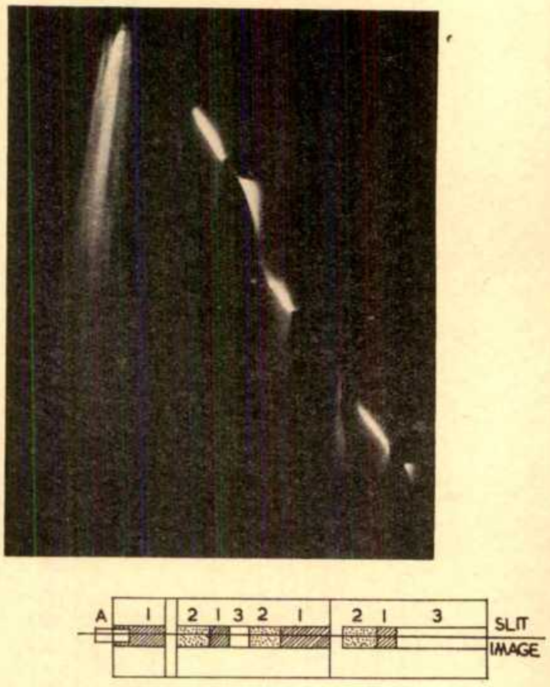

Fig. 3. Streak photograph of detonations and shocks at 1 atm. and 200 atm. initial pressure. 1, Plastic explosive; 2, PETN; 3, sodium chloride, in 0·375 in. diam. axial holes in square-sectioned 'Perspex' blocks. *A*, initiator

As density decreased, both primary lines became less bright, and the contrast between them increased. At 0·17 g/cm^3, the air line was still distinct; but the butane line had almost disappeared. One should not attach great weight at the moment to this slender evidence; but it is at least consistent with the foregoing ideas, which may also be relevant in comparing explosive and inert powders.

An important part of the earlier evidence collected by Blackburn and Seely was the persistence of light in a shocked inert powder which had been highly evacuated. We have tried to supplement this by experiments on both inert and explosive powders in which the initial pressure was raised to 100 or 200 atm. The argument here is that one would expect the shock pressure ratio and thus the gas pocket temperatures to be much reduced[3]. Fig. 3 shows the experimental arrangement and a typical trace. The first plastic charge transmitted detonation through the 'Perspex' barrier to the first PETN primer in the 1-atm. section, which initiated the second plastic charge, and this delivered a standard blow to the sodium chloride. The transmitted shock set off the second PETN primer and then the third plastic charge, so that the process was repeated symmetrically in the high-pressure section. It seems therefore permissible to compare the behaviour of the first PETN and the sodium chloride charges at 1-atm. and at high initial pressure.

One can see a definite reduction in light output from the PETN at 200 atm., and more conspicuously from the salt, in which the trace dies out very quickly. Similar, but rather less marked, effects were found at 100 atm. However, it is perhaps more significant that so much light persists at all at these high initial pressures.

I thank Dr. Seely for sending me advance details of his experiments, and for his comments. I thank also Mr. Cumming for his advice, and Mr. H. Andrew and Mr. J. Logan for assistance in the experiments.

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[1] Blackburn, J. H., and Seely, L. B., *Nature*, **194**, 370 (1962).
[2] Paterson, S., *Nature*, **167**, 479 (1951).
[3] Paterson, S., *Fifth Intern. Symp. Combustion*, 672 (Reinhold, 1955).
[4] Blackburn, J. H., and Seely, L. B., *Nature*, **202**, 382 (1964).
[5] Taylor, J., *Detonation in Condensed Explosives*, plate 10 (Oxford, 1952).

Resistance Inequalities

IT is well known that the electrical resistance tensor r is symmetric in the absence of a magnetic field. It is the purpose of this communication to point out that this implies that certain inequalities exist between the diagonal and off-diagonal elements of r. To obtain these we note that since r is symmetric it can always be diagonalized by a rotation of co-ordinate axes, and the resulting diagonal tensor will have all its diagonal elements positive, since these will be the resistances along the 'new' axes. Hence r, considered as a matrix, is positive-definite and this implies that inequalities between its elements must exist of the form:

$$r_{xx}r_{yy} \geqslant r^2_{xy}$$

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GEOLOGY

The British Mesozoic Committee

LAST year, a note was published in *Nature*[1] which summarized the recommendations of an international colloquium at Luxemburg on the definition of Jurassic stages and added the views of the British Mesozoic Committee on these recommendations. The stated object of this note was to give other British Mesozoic specialists the opportunity of considering the recommendations and of expressing their opinions about them. As a result of this and of further recommendations from other national committees brought forward at a meeting of the international Mediterranean Mesozoic Committee in September 1963, the British Committee decided that further discussions were necessary. A general meeting was, therefore, arranged at the Geological Society of London on February 26. This meeting, which was attended by 65 geologists who were specializing in the Mesozoic, was intended to guide the Committee in framing its further recommendations to the international organization.

Immediately after the general meeting, the Committee agreed on the following recommendations: (1) The British Mesozoic Committee considers that, not withstanding the Statement of Principles embodied in Part 25 of the twenty-first Session of the International Geological Congress, Mesozoic stages must in practice be defined in terms of faunal zones (that is, "belts of strata, each of which is characterized by an assemblage of organic remains, of which one abundant and characteristic form is chosen as index" Marr 1898, Arkell 1933). (2) As a general principle, only the base of each stage should be regarded as fixed, preferably by reference to a specified point in a type section for the lowest zone. (3) The Rhaetian should be regarded as the topmost stage of the Triassic System, and no part of it should be included in the Jurassic. (4) The terms Lias, Dogger and Malm should be rejected as international stratigraphical units. (5) The Jurassic should be divided into Lower, Middle and Upper divisions. (6) The British Committee does not recommend the definition of named substages. (7) The British Committee does not consider that a case has been made for the recognition of the Aalenian as a separate stage. (8) The Bajocian Stage and the Middle Jurassic should be regarded as beginning with the *opalinum* Zone. (9) There exists a difference of opinion among British specialists, but a majority favour the inclusion of the Callovian Stage in the Upper Jurassic. (10) In the interests of achieving international stability and uniformity of nomenclature, British specialists have no objection to the eventual dropping of the term 'Portlandian' as an international stage name. (11) Many British specialists favour the retention of the Kimmeridgian Stage in its broader sense, up to and including the *pallasioides* Zone. However, the British Committee recommends that only the base of this stage should be defined (as the *baylei* Zone) and are prepared to accept the next successive stage as the Volgian, as at present defined by the Jurassic Commission of the Stratigraphical Committee of the U.S.S.R. (12) The British Committee prefers the retention of both the Volgian and Tithonian Stages at the top of the Jurassic, and both the Ryazanian and Berriasian Stages at the bottom of the Cretaceous until such time as the Boreal and Tethyan provinces have been adequately correlated.

Postscript. The above recommendations were presented at a meeting of the International Committee near Marseille in May. They were all accepted, except that a majority favoured the retention of the Aalenian as a stage and the placing of the Callovian in the Middle Jurassic (though the objections of the British and Russian committees to this were to be forwarded to the International Congress). Also the Volgian Stage was preferred as the top division of the Jurassic, with the Tithonic (*sic*) to be retained only as a facies name, since it contravenes the rules of stratigraphical nomenclature.

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Marine Levels in South Italy

AFTER a personal discussion with Mr. A. E. Gunther, I am writing with his encouragement to comment on his communication in *Nature*[1] and with reference also to an earlier article in the *Illustrated London News*.

In those publications Mr. Gunther suggested that the supposed presence of *Lithodomus* borings in the columns of the temples at Paestum, and the presence of marine erosion terraces at a similar height of about 20 m in various parts of Italy and Sicily, combine to indicate an extensive rise and fall of relative sea-level in the whole of this area between about A.D. 500 and A.D. 1500.

I feel that it is necessary to comment on this hypothesis since the literature shows that geologists and archæologists tend to accept uncritically publications on this topic, where they would be far more cautious in the mainstream of their respective disciplines. In the past this has resulted in misleading papers having a long-standing effect on the literature.

This is not the place to produce a massive list of authorities, but I wish simply to outline a point of view which can be checked by reference to recent authors on Pleistocene sea-levels, and regional archæology.

First, there has been no eustatic change of sea-level greater than 2 m in the past 2,000 years, and probably none more than 1 m. During the past million years there have been frequent large oscillations of sea-level due to glacial control, resulting in the sea passing through the present level many times. There are thus many Pleistocene erosion traces within 20 m of present sea-level, but all older than 2,000 years.

Owing to the seismicity of the west Italian coast it is possible for local earth movements, such as those which have occurred in the north Bay of Naples, to lower and raise archæological remains in such a way that erosion marks on the buildings are aligned with nearby Pleistocene traces of much greater age.

Many of the terraces at or near 20 m in south Italy are of such a nature as to have required hundreds, if not thousands, of years of erosion, while a postulated 20 m sea-level between A.D. 500 and A.D. 1500 can only have remained at its maximum altitude for one century at the outside. If such a widespread submersion and uplift had occurred, 53 major coastal cities would have been inundated at a rate of 5–6 m per century, including Pisa, Civitavechia, much of Rome, Naples, Messina and Syracuse, as well as about 2,300 km² of land. Apart from minor references to the spread of malarial swamps, which can result from silting with no change of level, there is no literary evidence to support such an event, nor does archæological evidence support it.

Most of the sites in the area have been thoroughly excavated and there are no signs of general submersion, apart from a few scattered sites partly submerged to-day as a result of local earth movements. At Civitavechia, Rome, Naples, Salerno and Syracuse, there were buildings erected during the medieval period at water-level or within a few metres of it, and there is no evidence that marine sediments overlie these sites. Furthermore, the rapid accumulation of sediments at the mouths of many small rivers on the west coast of Italy implies a more or less constant relative sea-level, while if there had been a change much of this sediment would have been deposited farther inland and over archæological remains.

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[1] Gunther, A. E., *Nature*, **201**, 909 (1964).

A. E. GUNTHER describes relatively large movements of uplift and subsidence of the land with respect to the sea in historical times in southern Italy. The *Lithodomus* borings at Paestum indicate a relative subsidence of the land, followed by an uplift of 18·5 m, in the time that has elapsed since the construction of the temples. If Gunther's contention that the uplift has occurred since the Middle Ages is correct, then an average rate of uplift of approximately 3–4 cm per annum is implied. In Calabria, however, where a number of colleagues and I have recently completed a geological survey of the whole province, at a scale of 1 : 25,000, for the Cassa per il Mezzogiorno, uplift has occurred at an appreciably slower rate.

A magnificent series of erosional levels occur round the coasts of Calabria, the highest reaching a height of 1,000 m above present sea-level. There is good evidence that the uplift movements, which raised the erosional surfaces to their present position, began during the Calabrian Stage of the Pleistocene, and, therefore, there has been approximately 1,000 m of uplift during the Quaternary. Assuming a duration of 10^6 years for the Quaternary, the average rate of uplift during this time was approximately 1 mm per annum.

Variations in the rate of movement can be demonstrated by considering the ages of the various levels. The lowest, extending to a height of 120 m above sea-level, is of Tyrrhenian age, as shown by the *Strombus bubonius* fauna present in the vicinity of Reggio di Calabria. The next higher terrace has been attributed to the Sicilian, and the level above that, with a lower limit of about 300 m, is probably of Calabrian age. The higher terraces, with the probable exception of the highest, 1,000 m level, can also be attributed to the Calabrian. It appears, therefore, that more than half the Quaternary uplift occurred during the Calabrian Stage, and that in post-Calabrian times the movement slowed down. Assuming the duration of the Calabrian was $3{\cdot}5 \times 10^5$ years, then the average rate of uplift during that time was approximately 2 mm per annum. Again, assuming an age of 4×10^6 years for the Tyrrhenian terrace, the average rate of uplift since that time has been about 0·3 mm per annum.

The variations discussed here are all relatively long-term. Geological evidence for shorter-term variations is lacking. Coming to modern times, Omori[1] has estimated that the average rate of uplift for the Calabro–Sicilian area, for for the period 1900–08, was 2–4 mm per annum. This figure is of the same order as those deduced above from the total Quaternary, the Calabrian and the post-Tyrrhenian uplifts, and leads one to assume that the rate of uplift in Calabria has been fairly constant throughout the Quaternary up to the present day. This rate of movement is significantly less than that quoted above for Paestum. The probable reason for this difference is that Paestum is located in a particularly active geological zone.

During the survey of Calabria no evidence for a modern reversal of the uplift movement was observed. The Holocene sediments of the coastal plain, extending into the river valleys, indicate that there has been a relative subsidence of the land since the end of the last glaciation, but this was probably caused by the return of glacial melt water to the oceans. In all probability the uplift movement continued during the Holocene, but the rise in sea-level was greater than the uplift of the land and therefore a relative subsidence resulted. The most recent evidence, provided by remnants of modern raised beaches, indicates that uplift is again dominant.

Two conclusions can be drawn from the foregoing discussion. First, modern movements of uplift and subsidence in southern Italy have not been uniform over the whole region and therefore correlation of levels based on height above sea-level cannot be relied on except over relatively short distances. Secondly, the hypothesis of a widespread 18-m historical level cannot be sustained.

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Stream Deposition in the Mediterranean Area in Historical Times

C. Vita-Finzi[1] has indicated that stream action during the Middle Ages caused rapid changes synchronously over large areas in the Mediterranean, producing in the valleys a flat-topped alluvial fill into which the present river channels were later cut. During field-work in western Turkey, I noticed similar deposits in most of the basins even as far north as Gordion (Polatli) on the Sakarya. Up to now these deposits have not been dated, but a detailed examination of the Küçük Menderes delta (near Ephesus) showed that accumulation of sediment in the delta mainly occurred during the Hellenistic period (about 300–100 B.C.)[2]. The sequence of events in the delta could be dated by historical evidence; for a detailed discussion the reader is referred to the original paper. After a decrease in accumulation during the Roman period, almost no changes occurred from about A.D. 800 up to about 1935, when a canal was cut which shortened the river considerably and caused some new accumulation near the canal mouth. There is, of course, always the possibility that some time during the Middle Ages accumulation increased again and was followed by a period of severe marine erosion that obliterated all trace of the medieval accumulation, but this possibility seems to be rather remote, considering the regularity of the beach ridges that form the present coast.

The findings at the Küçük Menderes delta suggest that the river carried much more sediment seaward during the Hellenistic period than before and after. To explain the increase in sediment load an increase in soil erosion is suggested, due to an increase in deforestation and cultivation on the hill slopes during Hellenistic times when Asia Minor became Hellenized. The decrease in delta growth and the incision of the streams can be due to an after-effect of soil erosion: when most of the soil has been removed, there is little left on the nearly bare slopes to be transported any more, while the process of soil formation is far too slow to supply as much sediment to the streams as was transported when the old soils were eroded. The result is a decrease in sediment transport by the streams which leads, presumably, to incision and lowering of the stream-bed.

A certain measure of synchronicity in soil erosion, as suggested by Vita-Finzi for the Middle Ages, should not be surprising, as soil erosion may have increased more or less simultaneously in many regions in the Mediterranean due to disorganization and neglect after the break-up of the Roman empire. On the other hand, severe soil erosion may have occurred much earlier than the Middle Ages especially in the eastern Mediterranean. At one locality at least accumulation did not occur mainly after the Roman period but before.

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MINERALOGY

An Occurrence of Görgeyite in Greece

The new mineral, görgeyite, was described in 1953 by Mayrhofer[1] from salt beds at Ischl, in Upper Austria, as $K_25Ca(SO_4)_6.1–1\frac{1}{2}H_2O$. In the same year, Mokievsky[2] announced the discovery by Nefedov of a sulphate mineral of comparable composition in salt deposits of Lake Inder, Kazakhstan S.S.R., to which the name mikheevite was given. That the two were identical was suspected by Fleischer[3], and after Nefedov[4] published further data on mikheevite, Meixner[5] showed that this was indeed the case. The name görgeyite has priority. We wish to record a third occurrence of the mineral.

A bore-hole, Astakós No. 1, was drilled recently by BP Exploration Co. (Greece), Ltd., about 9 km north-north-east of the town of Astakós in western Greece (approximate co-ordinates of the site: 38° 36′ 30″ N., 21° 07′ 35″ E.; elevation, 250 m). It encountered a thick sequence of Triassic evaporites, in which halite and anhydrite are dominant minerals, with polyhalite and dolomite present in smaller amounts. A sample of drill-cuttings from 1,544 m depth yielded a few anhedra, 2–4 mm in diameter, of a grey-white mineral which broke with a hackly fracture to show a glassy, colourless interior and one very poor cleavage. Refractive indices in sodium-light are: α 1·560, β 1·570, γ 1·584. By direct measurement with a spindle-stage, $2V\gamma = 81°$. One optic axis is nearly perpendicular to the poor cleavage. These optic data agree closely with Meixner's values for görgeyite. There was insufficient material for a chemical analysis, but X-ray fluorescence indicated sulphur, calcium, potassium and small amounts of strontium.

The X-ray powder pattern agreed only poorly with A.S.T.M. card 2–551 for $K_25Ca(SO_4)_6.H_2O$. Weissenberg photographs led initially to the following triclinic unit cell: *a*, 6·822 Å, *b*, 17·134 Å, *c*, 9·390 Å, α 90° 36′, β 111° 16′, γ 101° 24′, cell volume, 998·4 Å^3. The shortest axis showed two-fold symmetry in the zero- and upper-layer equi-inclination photographs, and oscillation photographs indicated a mirror plane perpendicular to it. The diffraction symmetry is hence $2/m$, and the true monoclinic unit cell has twice the volume of the triclinic cell above: *a*, 17·509 Å, *b*, 6·822 Å, *c*, 18·208 Å (all ± 0·008 Å), β 113° 16′, $a:b:c$ 2·567 : 1 : 2·669, cell volume, 1998 Å^3. Nefedov's first choice of unit cell was monoclinic, with *a*, 17·14 Å, *b*, 6·71 Å, *c*, 18·24 Å (from his *kX* values), β 114°, but he concluded that the symmetry arose from twinning and adopted a triclinic cell. We have seen no evidence of twinning.

Systematic extinctions observed were $(h + k)$ odd for the general reflexions hkl and l odd for the $h0l$ zone. The space group is therefore either Cc or $C2/c$, and as Mayrhofer determined the crystal class as $2/m$, the centrosymmetric space group may be assumed to be the true one. The statistical $N(z)$ test of Howells[6], which was made on the $h0l$ intensities, yielded a curve close to the hypercentric distribution and provides support for this choice.

The specific gravity of several clear fragments of the mineral was found by a flotation method to be 2·91 ± 0·01, which is close to the calculated value of 2·901 for four molecules of $K_25Ca(SO_4)_6.H_2O$ in our monoclinic cell, and to Nefedov's value of 2·93. Meixner's figure of 2·77 can be explained only by the presence of inclusions or extensive substitution by light cations.

The principal discrepancy between our own and the published data occurs in the cell dimensions. Although Nefedov's axial ratios are close to ours, we believe that the absolute values of the sides of his unit cell are in error, as the cell volume, chemical formula, and calculated density are discordant. There is some similarity between the two triclinic cells which were chosen, and their relationship to our monoclinic cell has been determined from a stereographic projection:

Astakós		Nefedov		
Triclinic	Monoclinic	Triclinic	Monoclinic	
a	$[0\bar{1}0]$	*a*	$[010]$	
b	$[1\bar{1}2]$	*b*	$[\bar{1}10]$	
c	$[\bar{1}10]$	*c*	$[\bar{1}12]$	probably
100	$\bar{1}11$	100	$\bar{1}10$	,,
010	001	010	$\bar{2}01$	,,
001	$\bar{2}01$	001	001	,,

As a check, particularly on the water-content, the salt $K_25Ca(SO_4)_6.H_2O$ ('pentasalt') was synthesized by Hill's[7] method and a thermogravimetric analysis confirmed that it contains only one H_2O per molecule. The powder pattern of the synthetic salt is identical with that of the

Astakós görgeyite, and hence the uncertainty in the water-content shown in the published data is resolved. Further, powder diffraction data were calculated from our single-crystal results of the monoclinic and triclinic cells, using an Elliott 803 computer with a programme written by Mr. D. J. Smith, and excellent agreement with the observed $\sin^2 \theta$ values was obtained. Neither of Nefedov's cells fitted our data. We are submitting our findings to the A.S.T.M. Powder Data File.

We thank the Chairman and Directors of British Petroleum Company, Ltd., for permission to publish this communication.

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GEOCHEMISTRY

Relative Distribution of Thorium, Uranium and Lead estimated from the Isotopic Composition of Lead in Ores

IF the isotopic ratios $[^{206}\text{Pb}/^{204}\text{Pb}]_0$, $[^{207}\text{Pb}/^{204}\text{Pb}]_0$, and $[^{208}\text{Pb}/^{204}\text{Pb}]_0$ of primeval lead are known, the time t of formation of ore of lead (the present time = 0) and the present-day atomic ratios $[^{238}\text{U}/^{204}\text{Pb}]$ and $[^{232}\text{Th}/^{204}\text{Pb}]$ which would be observed in the original source of lead in ores can be estimated[1,2] from the measured isotopic ratios $[^{206}\text{Pb}/^{204}\text{Pb}]$, $[^{207}\text{Pb}/^{204}\text{Pb}]$ and $[^{208}\text{Pb}/^{204}\text{Pb}]$ by:

$$\frac{[^{206}\text{Pb}/^{204}\text{Pb}] - [^{206}\text{Pb}/^{204}\text{Pb}]_0}{[^{207}\text{Pb}/^{204}\text{Pb}] - [^{207}\text{Pb}/^{204}\text{Pb}]_0} = 138\,\frac{e^{\lambda_{238}T_0} - e^{\lambda_{238}t}}{e^{\lambda_{235}T_0} - e^{\lambda_{235}t}} \qquad (1)$$

$$[^{238}\text{U}/^{204}\text{Pb}] = \frac{[^{206}\text{Pb}/^{204}\text{Pb}] - [^{206}\text{Pb}/^{204}\text{Pb}]_0}{e^{\lambda_{238}T_0} - e^{\lambda_{238}t}} \qquad (2)$$

$$[^{232}\text{Th}/^{204}\text{Pb}] = \frac{[^{208}\text{Pb}/^{204}\text{Pb}] - [^{208}\text{Pb}/^{204}\text{Pb}]}{e^{\lambda_{232}T_0} - e^{\lambda_{232}t}} \qquad (3)$$

where λ_{232}, λ_{238} and λ_{235} are the decay constants of thorium-232 ($0{\cdot}499 \times 10^{-10}$/yr.), uranium-238 ($1{\cdot}54 \times 10^{-10}$/yr.) and uranium-235 ($9{\cdot}78 \times 10^{-10}$/yr.), 138 the isotopic ratio of uranium-238 to uranium-235 at present, and T_0 the age of the Earth ($4{\cdot}55 \times 10^9$ yr.). However, no attempt has been made to use these equations in order to determine the relationship, so far as distribution is concerned, of thorium, uranium and lead.

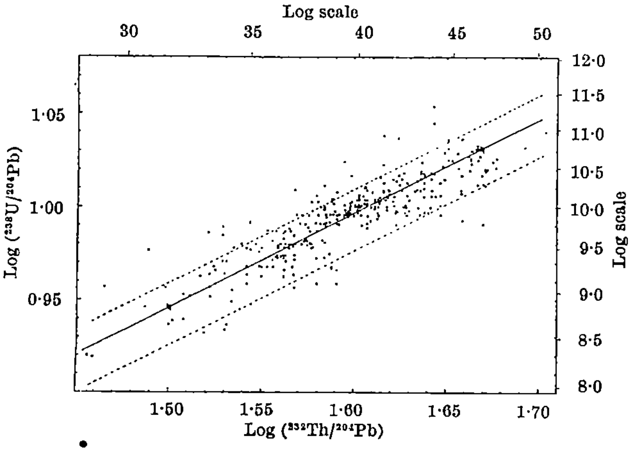

Fig. 1

In this communication the values estimated by me were used as the primeval lead, and the same data on common leads as used in the previous paper[3] were taken up again. The result of calculation is shown in Fig. 1. In this diagram, both co-ordinates are set on logarithmic scales, for a logarithmic scale rather than a direct scale seems to be tenable in general with the theoretical consideration[4,5].

As exemplified before[5,6], it will be of great value to quantitative or theoretical consideration to express the abundance relationship in a mathematical form. The application of least square method[3] to Fig. 1 yields the empirical formula:

$$\log\,[^{238}\text{U}/^{204}\text{Pb}] = 0{\cdot}503 \log\,[^{232}\text{Th}/^{204}\text{Pb}] + 0{\cdot}192 \qquad (4)$$

If the atomic ratios $[^{238}\text{U}/^{204}\text{Pb}]$ and $[^{232}\text{Th}/^{204}\text{Pb}]$ are reduced to the weight concentration ratios, we get:

$$\log\,(C_{\text{U}}/C_{204}) = 0{\cdot}503 \log\,(C_{\text{Th}}/C_{204}) + 0{\cdot}231 \qquad (5)$$

where C_{U}, C_{Th} and C_{204} stand for the weight concentrations of uranium, thorium and lead-204.

The distributional relationship with small deviations as seen in Fig. 1 is of much geochemical importance, because it appears to suggest that the abundances of minor elements in the material in which lead of ores originated have simple aspects at large. The empirical formula obtained here will be scrutinized in mathematical terms separately.

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CHEMISTRY

Fluorescence of Organic Mixed Excimers

Birks and Christophorou[1], examining mixed excimers, reported that the sum of the fluorescence intensities from two solutions, one containing c moles of pyrene (component A) and the other c moles of 1-methylpyrene (B). is equal to the fluorescence intensity from a mixed solution containing c moles each of A and B.

The relation between the fluorescence intensity F of an excited dimer and the bulk concentration c is given (in the absence of other quenching processes) by the Stern–Volmer relation $Fc = F_\infty/(1 + c_h/c)$, where c_h is the mean self-quenching concentration[2].

If dimers are formed indiscriminately between compounds A and B, then F should follow the foregoing equation where c is now the total concentration of the solution. The direct proportionality between the intensity and the concentration as reported in ref. 1 is only possible when $c \ll c_h$. For $c = 5 \times 10^{-3}$ M, this requires $c_h = 0{\cdot}1$ M.

In oxygen-free *cyclo*hexane, c_h for pyrene is $7{\cdot}5 \times 10^{-4}$ or less[3]. In the presumably aerated solutions used in ref. 1, c_h would have been greater[4], though an increase to 0·1 M would be remarkable. Accordingly, we have investigated a similar system (pyrene and 4-methylpyrene) in aerated solution using the same concentrations as in ref. 1. We find the dimer fluorescence intensities of the mixed solution (c of A, and c of B) equal the average of the intensities of solutions of the pure substances at concentration $2c$, which in turn equals only 0·7 times the sum of the intensities of solutions of the pure substances at concentration c.

The inference is that mixed dimers are formed with the same probability and quantum efficiency as the pure dimers and that c_h in the presence of air is only about 8×10^{-3} M.

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Dr. Selinger has directed attention to an unintentional ambiguity in our original communication. In comparing the fluorescence spectrum Z of the mixed solution, containing equimolar concentrations of A and B, with the sum $(A + B)$ of the fluorescence spectra of the two separate solutions of A and B, the relative quantum intensities of the monomer components of Z and $(A + B)$ were normalized. Reference to this standard procedure, which has been followed in other reports[1] of excimer fluorescence spectra from these laboratories, was unfortunately omitted. The ratio of the quantum yield of excimer fluorescence I_D to that of monomer fluorescence I_M is given by[2]:

$$\frac{I_D}{I_M} = \frac{q_D c}{q_M c_h}$$

where c is the molar concentration, c_h is the 'half-value' concentration, and q_M and q_D are the monomer and excimer fluorescence quantum efficiencies at $c = 0$ and $c = \infty$, respectively. For the normalized spectra, the excimer fluorescence intensity is proportional to I_D/I_M and hence to c. For the unnormalized spectra, I_D and I_M vary non-linearly with c, according to the Stern–Volmer relations[2]. Our original data[3], prior to normalization, agree closely with those reported by Selinger. They substantiate the conclusion, implicit in our previous communication, that mixed dimers of similar compounds are formed with the same probability and fluorescence quantum efficiency as the pure dimers.

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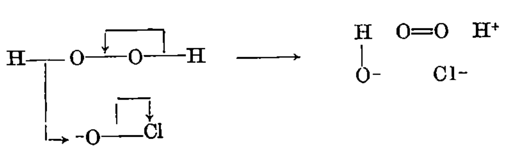
Chemi-luminescence as a Diagnostic Feature of Heterolytic Reactions which Produce Oxygen

The recent suggestions by Khan and Kasha[1] and by Ogryzlo *et al.*[2] that the emission of light which occurs during the interaction of hydrogen peroxide and sodium hypochlorite might be ascribed to singlet–triplet transitions of molecular oxygen have led us to examine the possibility that among those chemical reactions which yield molecular oxygen the heterolytic processes alone might prove to be chemi-luminescent.

By electron-pair movements, for example:



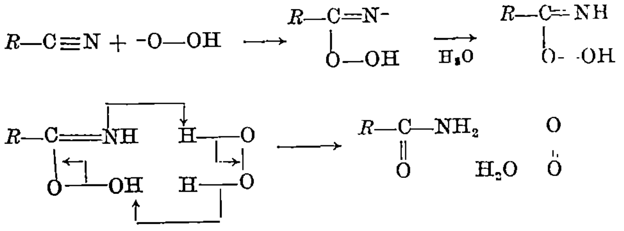
oxygen should initially be formed in a diamagnetic singlet state, whereas in reactions involving one-electron movements, for example:

$$\{Fe(CN)_6\}^{3-} + (:O—O\text{–}H)^- \longrightarrow \{Fe(CN)_6\}^{4-} + \cdot O—O\text{–}H$$
$$\{Fe(CN)_6\}^{3-} + \cdot O—O\text{–}H \longrightarrow \{Fe(CN)_6\}^{4-} + \cdot O—O\cdot + H^+$$

the oxygen should be formed in a di-radical, or triplet, state. Suggestions that heterolytic reactions might initially give activated forms of singlet oxygen, or that singlet–triplet transitions of molecular oxygen may need two oxygen molecules, are both irrelevant to this simple mechanistic differentiation between the two contrasting types of oxygen-producing reactions.

With the help of Dr. E. J. Bowen, who has put a sensitive photomultiplier at our disposal, we have found that other heterolytic oxygen-producing reactions are indeed chemi-luminescent.

While from the NaOCl—H_2O_2 reaction there is a short-lived emission of red light, from admixture of chloramine-*T* with neutral hydrogen peroxide the emitted light has both red and blue components. Singlet oxygen is formed in the reaction between alkaline hydrogen peroxide and nitriles; the mechanism of this[3] can be represented as:



When benzyl cyanide was used with 30 per cent hydrogen peroxide in alcoholic potassium hydroxide a bluish light, strong enough for visual observation in a darkened room, was emitted for 5–10 min. Examination with a photomultiplier and a red filter (Ilford 204) showed that this had a small red component. Acrylonitrile also gave strong emission of blue light, but with benzonitrile the emission was just detectable with the photomultiplier. A transient emission of light was also observed during the interaction of hydroxylamine with alkaline hydrogen peroxide.

No emission of radiation could be detected during the oxygen-producing reactions between hydrogen peroxide and $KMnO_4$, MnO_2, $K_3Fe(CN)_6$ and $Ce(SO_4)_2$, which are all one-electron-transfer processes.

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Temperature Variation of β-Type Interlamellar Sorption Complexes of Graphitic Acid with Alcohols

Further investigations have been made of the β-type interlamellar sorption complexes formed by graphitic acid with normal alcohols[1-3]. We find that the formation of β-complexes (chain perpendicular to layer) or α-complexes (chain parallel to layer) with any given alcohol depends on the method of formation and pretreatment of the graphitic acid[4]. We have evidence that this statement may be true also for the α- and β-complexes of montmorillonite.

For the work recorded here, we selected one of the 'best' acids (that is, forming β-complexes most easily). We used a sample of graphitic acid dried at ambient temperature, and the same sample dried at 65° C. The complexes formed were examined by X-ray diffraction at ambient temperature, at +70° C and at −50° C. Results, in the form of a graph of basal spacing versus chain-length, are shown in Fig. 1.

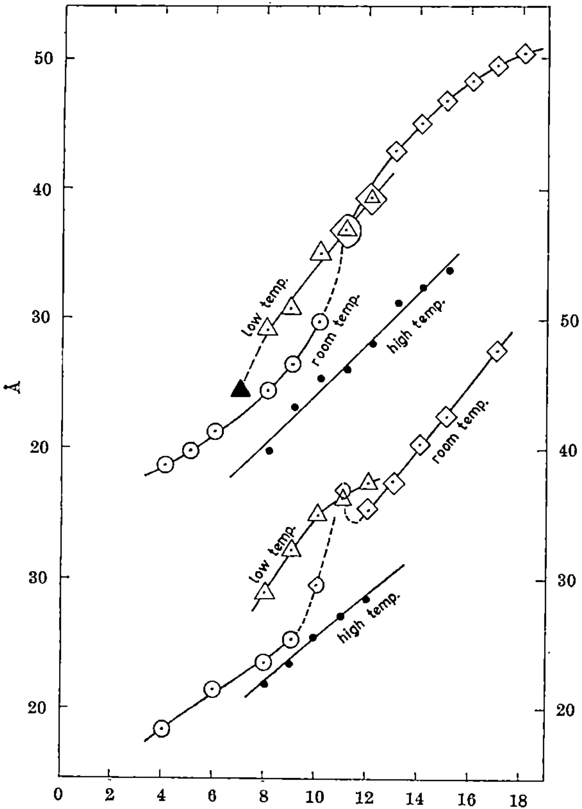

Fig. 1. Graphs of spacing versus chain-length for alcohols with graphitic acid. The upper series of graphs (left-hand scale) is for acid dried at room temp.; the lower series (right-hand scale) is for acid dried at 65° C. The type of plot indicates whether the sorbed alcohol is in the solid or liquid state, as indicated by the side-spacing line at about 4·3 Å. △, Solid, at −50° C; ◇, solid, at room temperature (18°–22° C); ▲, liquid, at −50° C; ○, liquid, at room temperature (18°–22° C); <), partly solid, partly liquid (or mixed), room temperature (18°–22° C); ●, liquid, at 70° C

From the presence of a side spacing at about 4·3 Å, in the form of a line or band, it can be determined whether the sorbed alcohol is in the solid or liquid form[5]. This information is indicated in Fig. 1. However, we suspect the solid–liquid transition is not a sharp one, and that intermediate forms exist.

It will be seen that at −50° C, the alcohols are generally in the solid form, and the observed spacing corresponds to that calculated for a fully extended chain. At +70° C, they are liquid, and give a lower spacing, presumably due to thermal motion. At ambient temperature a transition occurs, which takes different forms depending on the pre-treatment of the graphitic acid. The transition occupies a range and does not necessarily occur at the solidification point of the free alcohol. The 9-, 10- and 11-C alcohols at ambient temperature show anomalous diffuseness of the basal spacing, indicating interstratification, or other irregularities. We have evidence that at 70° C, the transition probably occurs in the region of octadecanol (normal m.p. 58·5° C).

The spacings for the pre-heated graphitic acid are generally lower than those for the acid dried at ambient temperature. The samples give only fully extended chains at low temperatures.

The slope of the graph for complexes with liquid alcohols is lower than that for the solid, presumably due to the influence of thermal motion. We have evidence (not all of which is presented here) that the solid–liquid transition becomes more marked as the temperature increases.

It should be noted that the formation of β-complexes is never complete, and that a proportion of unchanged graphitic acid exists in every case.

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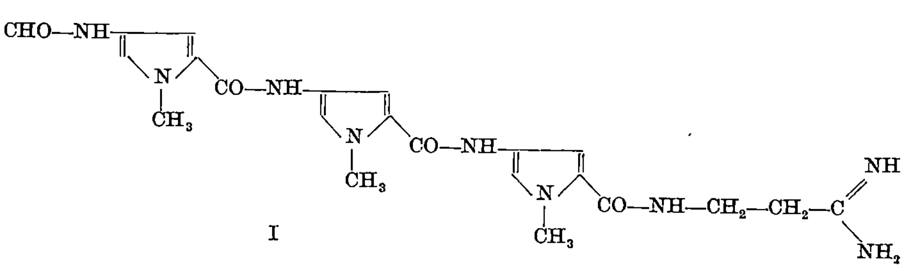
Structure and Synthesis of Distamycin *A*

Distamycin, a mixture of antibiotic substances exhibiting predominantly antifungal activity, was obtained by submerged fermentation and butanol extraction of the mycelial mass of a *Streptomyces* sp. An amorphous product (fraction *A*), showing a positive Ehrlich reaction and a typical ultra-violet spectrum, was separated from the other active components by solvent fractionation and chromatography on aluminium oxide[1,2]. Partially purified preparations of this fraction, obtained during isolation investigations, were found to inhibit in various degrees some experimental tumours in mice[3] and to interfere with the process of cell division *in vitro*[4]. The ultra-violet and infra-red spectra of these preparations showed some similarities with the antibiotic netropsin[5] (synonyms are antibiotic *T*–1384 (ref. 6) and congocidin[7]). Presence of the latter antibiotic in fraction *A* of distamycin could, however, be readily excluded by the lack of a positive Sakaguchi reaction and by direct chromatographic and spectroscopic comparison with an authentic sample.

The new basic antibiotic distamycin *A* is obtained in good yield as the hydrochloride (white to pale yellow crystals) when a few drops of 6 N hydrochloric acid are added to an aqueous solution of fraction *A*. The compound inhibits selectively the multiplication of phage *T*1 in *E. coli* K_{12} (ref. 8) and exhibits interesting antiviral and antimitotic properties. In this communication it is shown that distamycin *A* is β-{1-methyl-4-[1-methyl-4-(1-methyl-4-formylaminopyrrole-2-carboxamido)-pyrrole-2-carboxamido]-pyrrole-2-carboxamido}-propionamidine, corresponding to structure I.

Distamycin *A* hydrochloride, recrystallized from dilute-hydrochloric acid, melts at 184°–187° on the Kofler microscopic hot stage and is optically inactive. The crystals are firmly solvated; their composition, as deduced by elemental analyses after exhaustive drying, is $C_{22}H_{27}O_4N_9$.HCl. The ultra-violet spectrum in 96 per cent ethanol has maxima of absorption at 237 mμ (ε = 3 × 10⁴) and at 303 mμ (ε = 3·7 × 10⁴). The infra-red spectrum is consistent with the presence of an amidinium group (broad absorption band in the region 2,500–3,500 cm⁻¹ and peak at 1,690 cm⁻¹) and shows secondary amide bands at 1,660 and 1,630 cm⁻¹ indicative of a peptide-type structure.

Distamycin *A* was degraded by N hydrochloric acid in aqueous methanol to a diacidic base, crystallized as the

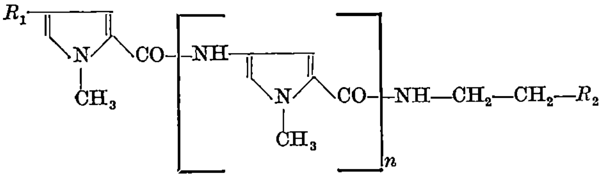


I

dihydrochloride $C_{21}H_{27}O_3N_9.2HCl$ (dec. slowly above 150°). On treatment with 0·2 N sodium hydroxide at room temperature the antibiotic gave one mole of ammonia and a neutral compound $C_{22}H_{26}O_5N_8$ (dec. 246°), with 95 per cent weight yield. Degradation of an amidine to an amide group was confirmed by the changes in the infra-red spectrum (the neutral compound does not show, *inter alia*, the 1,690 cm^{-1} band but exhibits a new amide band at 1,670 cm^{-1}, in addition to the secondary amide bands). Mild acid treatment of this neutral compound afforded a monoacidic base $C_{21}H_{26}O_4N_8$, isolated as its crystalline hydrochloride (m.p. 208°–210°). The new base shows an ultra-violet spectrum very similar to that of the antibiotic itself and to netropsinin (III, $R_1=NH_2$, $R_2=CONH_2$), the main degradation product of netropsin[6,9]. The infra-red spectrum shows absorption bands indicative of amine salt and of amide groups. Hydrolysis of the antibiotic with N sodium hydroxide in aqueous methanol gave two moles of ammonia and an amphoteric degradation product, isolated as its crystalline sulphate $C_{21}H_{25}O_5N_7 \cdot \frac{1}{2}H_2SO_4 \cdot 2H_2O$ (dec. 200°). Its infra-red spectrum exhibits absorption at 1,710 cm^{-1}, indicative of a carboxyl group.

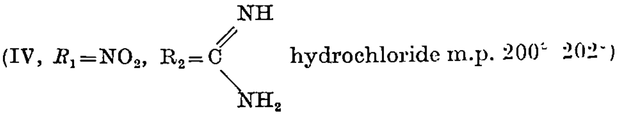
Prolonged treatment of distamycin *A* with 6 N hydrochloric acid at 120° and paper or thin-layer chromatography of the hydrolysate revealed the formation of β-alanine together with an unidentified ninhydrin reactive component. This component was also obtained when 1-methyl-4-aminopyrrole-2-carboxylic acid was subjected to the same acid treatment.

The C_{21}-monoacidic base was immediately related to the known netropsinin as follows. When netropsinin, obtained either by synthesis[10] or by degradation of the antibiotic netropsin, was condensed with 1-methyl-4-nitropyrrole-2-carboxylic acid chloride[10] in presence of aqueous sodium bicarbonate, and the resulting nitroamide (IV, $n=R_1=NO_2$, $R_2=CONH_2$; m.p. 260° dec.) hydrogenated catalytically, compound of structure IV where $R_1=NH_2$, $R_2=CONH_2$ was obtained and isolated as the hydrochloride, identified with the hydrochloride of the C_{21}-monoacidic base (identical melting point, infra-red and ultra-violet spectra and chromatographic behaviour). Formylation of the latter yielded a compound identical in all respects with the neutral degradation product. Therefore this substance has structure IV, where $R_1=CHO—NH$, $R_2=CONH_2$. Structure IV, where $R_1=NH_2$, $R_2=COOH$, is consistent with the properties of the amphoteric degradation product. From these investigations structure I was tentatively assigned to the antibiotic distamycin *A*. The nuclear magnetic resonance spectrum in deuterium oxide shows 20 protons as expected. A singlet at $\delta=7·76$ can be associated to the formamide group, a hypothesis in agreement with the fact that formic acid is liberated after alkaline hydrolysis of the antibiotic.



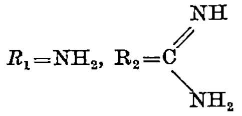
II, $n=0$; III, $n=1$; IV, $n=2$.

Structure I has been confirmed by synthesis. 1-Methyl-4-nitropyrrole-2-carboxylic acid chloride was allowed to react with β-aminopropionitrile in presence of aqueous sodium bicarbonate to give the nitronitrile of structure II, $R_1=NO_2$, $R_2=CN$ (m.p. 135°). This compound was then hydrogenated with palladium on charcoal in dimethylformamide to the corresponding aminonitrile which was condensed directly with the acid chloride to yield the nitronitrile of structure III, $R_1=NO_2$, $R_2=CN$ (m.p. 248°–250°). Catalytic hydrogenation to the corresponding amino derivative followed by condensation of the latter with the acid chloride gave the nitronitrile of structure II, $n=2$, $R_1=NO_2$, $R_2=CN$ (dec. above 270°). This compound was converted through the imidoester hydrochloride to the corresponding nitroamidine:



$$(\mathrm{IV},\ R_1=\mathrm{NO_2},\ \mathrm{R_2}=\mathrm{C}\begin{array}{l}{=}\mathrm{NH}\\ -\mathrm{NH_2}\end{array}\quad \text{hydrochloride m.p. } 200^\circ\text{–}202^\circ)$$

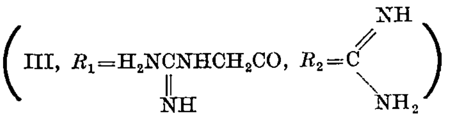
which, on hydrogenation with Adams catalyst in acetic acid, yielded the amino-amidine represented by IV where:



$$R_1=\mathrm{NH_2},\ \mathrm{R_2}=\mathrm{C}\begin{array}{l}{=}\mathrm{NH}\\ -\mathrm{NH_2}\end{array}$$

identical in all respects with the C_{21}-diacidic base obtained by mild acid treatment of the antibiotic. Formylation[11] of this compound gave the formylaminoamidine I. Its hydrochloride was identical by melting point, infra-red, ultra-violet and nuclear magnetic resonance spectra, chromatographic and electrophoretic behaviour, and degradation products, as well as by the biological properties with the antibiotic distamycin *A* hydrochloride.

Recently a similar synthesis of the antibiotic congocidin:



$$\left(\mathrm{III},\ R_1=\mathrm{H_2N}\underset{\underset{\mathrm{NH}}{\|}}{\mathrm{C}}\mathrm{NHCH_2CO},\ R_2=\mathrm{C}\begin{array}{l}{=}\mathrm{NH}\\ -\mathrm{NH_2}\end{array}\right)$$

has been described[12].

All compounds described in this communication gave correct elemental analyses and showed satisfactory spectroscopic properties.

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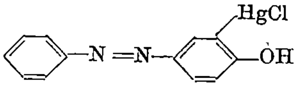
BIOCHEMISTRY

New Chromophoric Reagents for Labelling the Thiol Groups of Proteins

MANY methods have been proposed for the determination of thiol groups in proteins involving the direct attachment of a coloured moiety to the sulphydryl groups[1–7]. In one case at least[5], such a reaction has been used to introduce a coloured label on the SH group of a protein in order to facilitate the isolation of cysteine peptides and the determination of their amino-acid sequence. In this communication we report the synthesis and potential usefulness of two new azomercurials, 4-phenylazo-2-chloromercuriphenol (I) and 4-(*p*-nitro-



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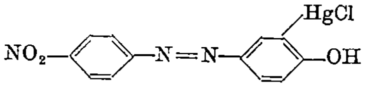

II

phenylazo)-2-chloromercuriphenol (II), which appear to offer certain advantages over previously reported chromophoric reagents of this type.

Compound I was prepared as follows. To 1·14 g of *o*-chloromercuriphenol[8], dissolved in 30 ml. of 15 per cent potassium hydroxide, were added 2·5 g sodium acetate, and the mixture was cooled to − 2°. This was followed by the dropwise addition of a solution of diazotized aniline composed of 0·32 g of freshly distilled aniline, 4 g of crushed ice, 1·7 ml. of concentrated hydrochloric acid and 0·25 g sodium nitrite. An orange-yellow precipitate was collected by filtration, thoroughly washed with distilled water, and finally dried over calcium chloride (yield 1·13 g). Further purification was achieved by passing a hot benzene solution of this product through a column of acid-washed alumina (Merck). After elution of a yellow impurity with benzene, compound I could be eluted with a mixture of benzene and ethanol (100 : 4). After evaporation of the solvent, the residue was recrystallized from hot benzene to give orange-yellow crystals (m.p. 146°–147°, uncorr.) anal.: calculated for $C_{12}H_9N_2OClHg$: C 33·2 per cent, H 2·1 per cent, Hg 46·4 per cent; found: C 32·3 per cent, H 2·2 per cent, Hg 45·7 per cent.

Compound II was prepared in essentially the same manner as compound I except that *p*-nitroaniline was used in place of aniline. Compound II was isolated in the form of orange-red crystals (m.p. 218°–220°) anal.: calculated for $C_{12}H_8N_3O_3Hg$: C 30·1 per cent, H 1·7 per cent, Hg 42·0 per cent; found: C 30·9 per cent, H 1·7 per cent, Hg 41·4 per cent.

Fig. 1 shows the reaction of various levels of cysteine with a constant level of compound I added in excess.

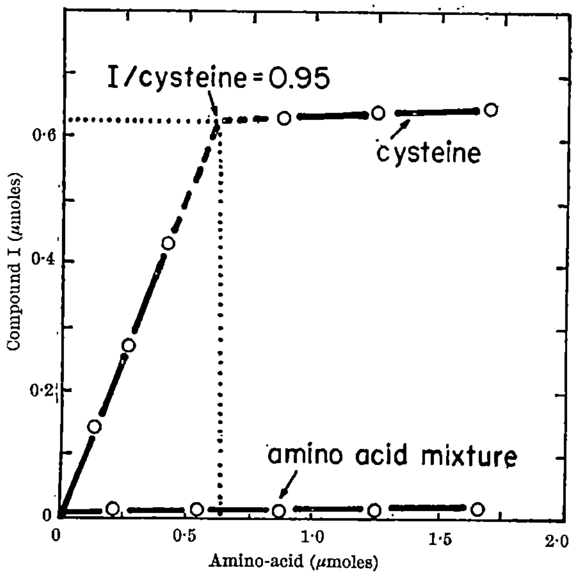

Fig. 1. Reaction of cysteine with compound I. To graded levels of cysteine, or a mixture of 18 amino-acids not containing cysteine, in 4 ml. 0·1 M phosphate buffer, *p*H 6·2, were added 6 ml. toluene containing 10 μmoles of compound I. After shaking for 1 min, absorbance of toluene layer was read at 350 mμ. Amount of compound I reacting with cysteine was calculated from difference in absorbance from control without cysteine and by reference to a standard calibration curve of compound I in toluene

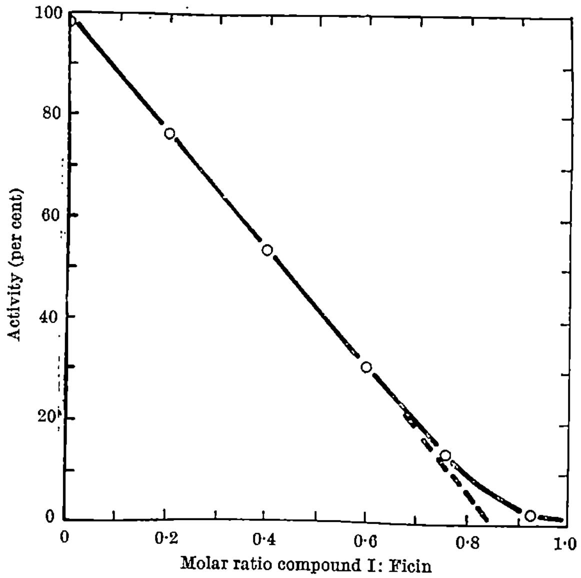

Fig. 2. Inhibition of ficin by compound I. To increasing amounts of compound I, dissolved in 1 ml. 0·085 M phosphate buffer, *p*H 6·2, containing 15 per cent ethyl alcohol, was added 1 ml. of the same buffer containing 0·08 μmoles of ficin. Activity was measured on casein (ref. 9) after incubation for 10 min at 37°. Activity in absence of inhibitor was taken as 100 per cent

The equivalence or breaking point of this curve indicates that compound I had reacted with cysteine in the expected molar ratio of 1 : 1. Similar results were obtained with compound II. As shown in Fig. 1, compound I did not react with any other amino-acid, thus establishing its specificity for the sulphydryl group.

The inhibitory effect of compound I on the activity of the sulphydryl enzyme, ficin[9], is shown in Fig. 2, where it is evident that the activity of ficin was completely lost at a level of 0·86 moles of compound I per mole of enzyme. This value corresponds quite closely to the expected value of 1 thiol group per molecule of ficin[9,10]. A similar experiment with compound II gave a value of 0·98. The reaction mixture from each of these experiments was subjected to dialysis and gel filtration on 'Sephadex *G*-50' in order to remove the last traces of the azomercurials, and then analysed for mercury[11]. These data showed that 0·65–0·70 mole of mercury per mole of protein had been incorporated, thus providing strong evidence for the formation of a mercaptide.

Apart from their ease of preparation, compounds I and II would appear to offer several additional advantages over the reagent, *N*-(4-dimethylamino-3,5-dinitrophenyl) maleimide (DDPM), proposed by Witter and Tuppy[5] for the labelling of SH proteins. Unlike DDPM, which is sparingly soluble in water, the azomercurials described here are quite soluble in aqueous media provided ethanol is present

Table 1. COMPARISON OF MOLECULAR EXTINCTION COEFFICIENTS (ε_M) OF CHROMOPHORIC REAGENTS FOR SULPHYDRYL PROTEINS

Compound	Solvent	λ_{max}	ε_M
4-Phenylazo-2-chloromercuriphenol (I)	15% ethanol	350	25,900
	15% ethanol–0·05 M NaOH	410	22,800
	15% ethanol–6 N HCl	470	40,402
4-(*p*-Nitrophenylazo)-2-chloromercuriphenol (II)	15% ethanol	385	23,300
	15% ethanol–0·05 M NaOH	505	30,900
	15% ethanol–6 N HCl	480	28,200
N-(4-Dimethylamino-3,5-dinitrophenyl) maleimide (DDPM)	Dichloroethane	425	2,380*
Na 4-(4-Acetoxy mercuriphenylazo)-1,7-Cleve's acid	0·1 M glycine–acetate buffer, *p*H 8	450	17,300†

* Determined in this laboratory on compound prepared according to Witter and Tuppy (ref. 5).

† Reported by Nosoh (ref. 7).

at a final concentration of 15 per cent, a condition not likely to precipitate or denature most proteins. Compounds I and II are also much more intensely coloured, especially in acid or base respectively, than DDPM or Nosoh's water-soluble azomercurial[7] (see extinction coefficients in Table 1) and hence should provide a much more sensitive indicator of any labelled peptides which may be isolated from derivatized proteins. The colour of peptides labelled with I or II separated by paper chromatography or electrophoresis could be considerably intensified by exposure to the fumes of concentrated hydrochloric acid or ammonium hydroxide.

Work is now in progress to test the applicability of compounds I and II for the labelling of SH proteins for the purpose of isolating cysteine-containing peptides.

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L-Cysteine as Inhibitor of Potassium Retention by Brain Slices

L-Cysteine, in common with several other amino-acids, is able to inhibit the uptake of L-histidine by brain slices, but the inhibition which it produces, unlike that produced by the other amino-acids, is non-competitive[1]. It therefore appeared possible that cysteine might also have an inhibitory action on other transport phenomena. Its effect on the transport system for potassium, as indicated by alteration in the level of potassium in brain slices (normally maintained well above that of a suitable environment[2]), has therefore been investigated, and compared with the effect of other amino-acids.

The technique used was essentially similar to that described elsewhere, with similar precautions taken[1]. Slices of rat brain were incubated with agitation for 1 h at 37° C in Krebs's bicarbonate saline which contained the amino-acid under investigation. The tissue was then treated as described elsewhere[3] and the potassium estimated in tissue fluid by means of a flame photometer. There was no detectable alteration in the amount of any of the amino-acids in the suspending medium during the period of incubation, as judged by visual inspection of paper chromatograms[1]. (Reduced glutathione, which might be expected from its structure to have effects somewhat similar to those of cysteine, did show evidence of alteration in its nature and so has not been included in the series.) Cysteine did not interfere with the estimation of potassium when present at ten times the concentration of the latter. The *p*H of the suspending medium was measured at the end of all experiments and lay within the range 7·3–7·5.

The concentration of potassium in isolated tissue suspended in a suitable medium may be altered by the presence of amino-acids[2,4,5], but, as shown in Table 1, cysteine produced a greater reduction in the concentration of potassium in brain slices than did any of the other amino-acids investigated.

Table 1. Effect of Amino-Acids on Potassium in Brain Slices

Amino-acid added to suspending medium	No. of tissue samples for each amino-acid	Potassium concentration in tissue m.equiv./l.
None	24	46 ± 4
Glutamic acid	8	50 ± 3*
Aspartic acid	8	50 ± 4*
Taurine	8	49 ± 2
Tyrosine	8	48 ± 2
Alanine, glycine, proline, valine	6	47 ± 2 to ± 3
S-Ethyl-cysteine, histidine hydrochloride, methionine, phenylalanine	6 or 8	46 ± 1 to ± 5
S-Methyl-cysteine	10	44 ± 4
β-Alanine, leucine, 2-thiolhistidine, ornithine hydrochloride	8	44 ± 3
γ-Aminobutyric acid, cystine, tryptophan	8	44 ± 2
Lysine hydrochloride	10	43 ± 4
Arginine hydrochloride	8	43 ± 3
Cysteic acid	10	43 ± 3*
Cysteine hydrochloride	16	38 ± 3‡†

Amino-acids were of the L-form where applicable. Initial concentration of each amino-acid in suspending medium 2 mM. Figures shown for potassium concentration are mean and standard deviation. Incubation 1 h 37° C.

*$P < 0·05$, †$P < 0·001$: significance of difference from concentration of potassium with no added amino-acid. ‡$P < 0·01$: significance of difference from concentration of potassium in the presence of any of the other amino-acids used.

The effect of cysteine appears to be dependent on the presence of a free thiol group not attached to a resonating system. Those amino-acids containing a blocked thiol group (*S*-ethyl-cysteine, *S*-methyl-cysteine, cysteic acid, cystine and methionine) or in which the thiol group was attached to a resonating system (2-thiolhistidine) altered the concentration of potassium in the tissue relatively slightly or not at all.

Cysteine has been found to have a toxic effect on the intact animal[6] and on cultured tissue[7] and also to alter the activity of certain intracellular enzymes *in vivo*[8]. It is therefore likely, particularly in view of the non-competitive nature of its effect on L-histidine uptake, that the inhibitory effect of cysteine on the transport of both L-histidine and potassium is the result of interference with intracellular processes on which both types of transport are dependent.

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Changes in the Fatty Acid Composition of Sebum associated with High Carbohydrate Diets

Excess of carbohydrate in the diet has been considered to be of significance in the ætiology of certain types of skin disorder[1]. With this in mind the opportunity was taken to examine the fatty acid composition of the sebum in subjects who were on experimental diets containing a relative excess of carbohydrate.

The subjects, seven adult males, were given an experimental diet consisting of 500 g of either sucrose or raw maize starch daily with *ad libitum* quantities of lean meat and green vegetables with vitamin supplements. The experimental diet was given for 25 days followed by 25 days on a normal free-choice diet, with a final 25 days on a diet which contained the alternative carbohydrate. Full details of the dietary intake are given elsewhere[2].

Samples of sebum were obtained on the 4, 11, 18 and 25 days of the diet by 'washing' the face at the end of a day with some cotton wool at the end of a wooden stick. The cotton wool was frequently dipped in a solution of equal parts of iso-propyl alcohol and petroleum ether (b.p. 40°–60° C). The lipid was extracted from the solution by the method of Hirsch *et al.*[3] and the proportion of each fatty acid was determined by gas-chromatography. Identification of all the peaks in the record was not attempted because some difficulty would have arisen in obtaining pure samples of each methyl ester. Presumptive identification only was made, based on the knowledge that the retention time in the gas-chromatograph is related logarithmically to the chain-length for each acid of the same degree of unsaturation.

The results (Table 1) showed first that a high carbohydrate diet altered the fatty acid pattern of the sebum, and secondly, that the nature of this alteration was dependent on the type of carbohydrate eaten. This was seen in the differences in the response between the starch and sucrose diets in the C16 : 0, C16 : 1 and C17 : 0 fatty acids in the sebum.

Table 1. PERCENTAGES OF THE FATTY ACIDS IN THE SEBUM ON CONTROL, STARCH AND SUCROSE DIETS

Fatty	Control		Starch diet			Sucrose diet		
acid	Mean	*S.E.* ±	Mean	*b*	*S.E.* ±	Mean	*b*	*S.E.* ±
12 : 0	0·7	0·09	0·7		0·05	0·6		0·07
14 : 0	5·3	0·28	4·6		0·25	4·9		0·24
14 : 1	3·5	0·19	3·1		0·14	2·9		0·27
15 : 0	5·4	0·26		−0·09	0·02		−0·07	0·03
15 : 1	2·7	0·23		−0·08	0·02		−0·06	0·02
16 : 0	22·7	1·20		+0·15	0·07	24·2		0·95
16 : 1	43·2	1·67	49·4		1·12		+0·31	0·14
17 : 0	3·6	0·37		−0·07	0·03	2·5		0·32
18 : 0	1·5	0·17	1·3		0·12	1·7		0·22
18 : 1	9·4	0·51	8·5		0·55	9·4		0·51
18 : 2	2·0	0·23	2·0		0·31	1·7		0·16

b, significant ($P < 0·25$) slope of line-percentage of fatty acid against time. *S.E.*, standard error.

It was expected that a relative excess of carbohydrate in the diet might be reflected in the composition of the sebum, but the finding that the type of dietary carbohydrate eaten exerts a qualitative influence on the sebum was not expected.

These changes in the fatty acid pattern of the sebum raise one point of biochemical interest, namely, the presence in sebum of appreciable amounts of fatty acids found only in low concentrations in the serum or adipose tissue, and support the findings of Weitkamp *et al.*[4], who reported odd-carbon numbered fatty acids in hair.

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Reduction of Dehydroascorbic Acid by Animal Tissues

IT has long been known that an enzyme catalysing the reduction of dehydro-L-ascorbic acid (DHA) by glutathione to ascorbic acid occurs in plant tissues[1–3]. It can also be shown that DHA is in part reduced to ascorbic acid by animal tissues, although until recently no clear evidence was available to suggest that this is an enzymatic reaction. Recently, however, it has become clear that the reduction of DHA by hæmolysates of human erythrocytes occurs at a rate greater than can be accounted for in terms of a purely chemical reduction by glutathione[4,5].

Hæmolysates of erythrocytes from different species have now been examined for their ability to catalyse the reduction. The rate of reaction was measured in terms of the amount of ascorbic acid formed from dehydroascorbic acid in the presence of a standard amount of carbon monoxide-saturated hæmolysate. The preparation of the hæmolysate and the measurement of the ascorbic acid formed were carried out as previously described[5,6]. The catalytic effect of erythrocytes from the different species examined is given in Table 1, the µg of ascorbic acid formed in 30 min at pH 7·0 from 0·5 mg DHA in the presence of 1 mg of glutathione being taken as an index of the activity. At these concentrations of glutathione and DHA the chemical (that is, non-catalysed) reaction is virtually non-existent. The increased activity of the factor in cases of pernicious anæmia is of interest. It is now known that the activity of a number of enzymes in pernicious anæmia erythrocytes is increased in this way and to a comparable extent[5].

Table 1. FORMATION OF ASCORBIC ACID FROM DHA IN THE PRESENCE OF DIFFERENT HAEMOLYSATES

	Ascorbic acid formed (µg) (mean value for each species)
Human, normal (5)	31
Human, pernicious anæmia (5)	75
Kitten (2)	12
Bullock (2)	25
Sheep (2)	0
Mouse (4)	75
Rat (5)	252
Guinea pig (6)	270

µg ascorbic acid formed in 30 min at room temperature from 0·5 mg of DHA and 1 mg of glutathione in the presence of 12·5 ml. of carbon monoxide-saturated hæmolysate (≡2·5 ml. erythrocytes). The figures in brackets refer to the number of determinations made for each species.

Extracts of the liver of three species examined (rabbit, rat, guinea pig) also catalysed the reaction. *Active*, partially purified preparations have been made from guinea-pig liver. The fresh liver was ground with acid-washed sand in the presence of 0·9 per cent saline containing 0·001 per cent 'Tween 80' (50 ml. saline to each 20 g liver). The extract was centrifuged and the two semi-solid lower layers discarded. The reddish-brown upper layer was fractionated with ammonium sulphate at 10° C and the fraction separating between 52 and 66 per cent saturation collected. This was dissolved in the minimal quantity of 0·1 M phosphate buffer (pH 6·8) and dialysed overnight at 5° C against 0·01 M phosphate buffer (pH 6·8). Any solid matter separating out was removed by centrifugation and discarded. The preparation was stored at −20° C. In some cases the fractionation was repeated. The active fraction was retained by '*CM*-Sephadex *C*50' (a cation exchange material) but not by anion-exchangers such as DEAE-'Sephadex', thus suggesting its association

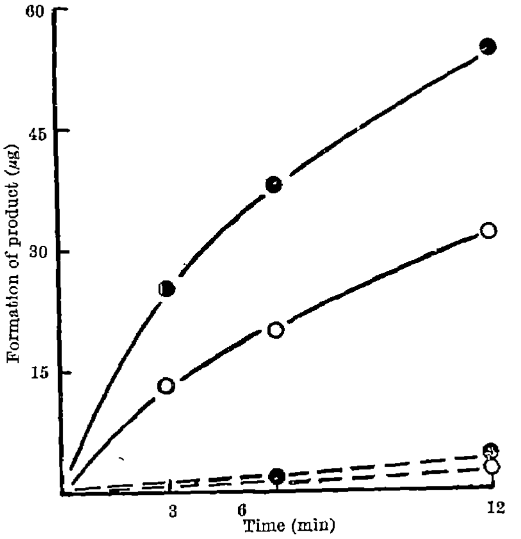

Fig. 1. Effect of the liver preparation on the formation of L-ascorbic acid (○) and D-isoascorbic acid (●) in the presence of glutathione. 1 ml. (2 mg) of glutathione and 0·6 ml. (1·2 mg) of DHA or DHisoA were allowed to react at room temperature in the presence of 2 ml. 0·2 M phosphate buffer, pH 6·3. ——, with 2 ml. of liver preparation; — —, with 2 ml. of water in place of the liver preparation

with a basic protein. Some preparations were further purified by running through DEAE-'Sephadex' columns buffered to *p*H 8·0; this removed a certain amount of non-active protein.

The preparation catalysed the reduction of DHA to ascorbic acid by glutathione. No reduction occurred in the absence of glutathione and the amount of ascorbic acid formed was directly proportional to the amount of liver preparation added. Dehydro-D-isoascorbic (dehydro-D-araboascorbic) acid was reduced more rapidly than DHA (Fig. 1). The preparation was inactive at *p*H values below 6·0 and appeared to have a maximum activity at about *p*H 7·0; activity measurements above *p*H 7·0 were difficult because of the increasing rapidity of the chemical (that is, non-catalysed) reaction with increase in *p*H above 7·0.

Azide, carbon monoxide and fluoride were without effect on the reaction at M^{-2} concentration. It was difficult to assess the effect of sulphydryl inhibitors because of their inhibition of the non-catalysed reaction. Dialysis for 20 h against 0·01 M phosphate buffer (*p*H 6·8) at 5° C increased the activity by 20 per cent; 0·1 per cent EDTA had a similar activating effect. Heating the preparation for 6 min at 98° C resulted in a 50 per cent loss of activity.

It would therefore appear that there is present in animal tissues a factor catalysing the reduction of DHA to ascorbic acid in the presence of glutathione. Its comparative resistance to destruction by heat is in contrast with results obtained for the corresponding plant enzyme[2]; it is, of course, known that certain partially-purified enzymes are markedly thermostable[7,8].

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Bone Growth and Uptake of Radiocalcium in Tetracycline-treated Rats

THE selective localization of tetracyclines in mineralizing tissues has prompted consideration of possible untoward skeletal effects associated with their use in medical practice. Commanding attention in this respect are investigations in human beings showing that exposure to these antibiotics during tooth formation produces hypoplasia of the dental enamel[1–5]. It has also been reported that tetracycline can interfere with the growth of the long bones in infants[6,7]. Moreover, there is evidence that these agents can impair fracture healing in dogs[8] and can adversely affect mineralization in a wide range of animal forms including the larval sand dollar[9], a teleost fish[10], the chick embryo[11], a marine mollusc[12] and the rat[13,14].

This communication reports the results of work designed to investigate the effect of graded doses of tetracycline on bone growth and on the skeletal and dental uptake of radiocalcium in rats.

Twenty-one day-old male Sprague–Dawley rats were randomly assigned to groups (I–IV) and given intragastrically 1·0 ml. of distilled water (I) or 1·0 ml. of an aqueous solution containing either 1·0 mg (II), 5·0 mg (III), or 20·0 mg (IV) of oxytetracycline hydrochloride (courtesy of Chas. Pfizer and Co., Inc., New York) on each of ten consecutive days. All received intraperitoneally 1·0 ml. of a solution (*p*H 6·8) containing 5 μc. of calcium-45 and 10 μg of calcium (as the chloride) 1 h after each intubation. The rats were housed individually and fed a commercial laboratory chow.

Four hours after the final injection the rats were killed and one femur, the lower incisors, and the third molars were excised from each. This molar was selected since the bulk of the enamel and crown dentine is deposited between 20 and 25 days of age[15]). The femurs were measured proximally–distally with a micrometer, then extracted in alcohol (4 h) and in ether (8 h). All samples were incinerated overnight at 550° C and the ash of each dissolved in hydrochloric acid and analysed for calcium-45[16].

Gain in body-weight (mean g ± *S.E.*) was: I, 40·8 ± 2·4 (10 rats); II, 38·6 ± 1·9 (11 rats); III, 44·3 ± 3·1 (11 rats); and IV, 45·5 ± 2·9 (10 rats). None of these values differs significantly ($P > 0.05$) from one another. The mean length (mm) of the femurs of these same animals at termination was: I, 21·76 ± 0·21; II, 21·41 ± 0·26; III, 21·80 ± 0·25; and IV, 21·80 ± 0·17. Again, these values were statistically indistinguishable. The mean length of the femur at 21 days of age was 16·99 ± 0·11 mm based on measurements obtained with a group of 15 comparable rats. This lack of effect of tetracycline on the growth of long bones in rats is in contrast to the results of work on a human being cited earlier[6], where radiographic estimation of fibular growth in normal premature infants receiving tetracycline indicated a 40 per cent mean inhibition during the period of administration (9–12 days). It is noteworthy that Group IV rats received two and a half to five times more tetracycline per kilogram of body-weight (approximately 0·50 g/day at the start to 0·25 g/day at death) than did these same children (0·1 g/day).

Comparison of femur ash weights (Table 1) by analysis of variance also gives no indication that tetracycline affected bone mass or, for that matter, the ash weight of the incisor and molar teeth. Indeed, previously unreported findings from another experiment[17] indicated that the femurs of rats fed a diet containing 0·01 per cent of oxytetracycline hydrochloride for 100 days weighed more ($P < 0.01$) than those of the controls, namely, 432·5 ± 10·1 mg (13 rats) as compared to 362·7 ± 13·6 mg (13 rats) expressed on the dry, fat-free basis. The ash content (per cent) of these same bones was also higher ($P < 0.01$), being 57·68 ± 0·33 and 55·53 ± 0·73 for the tetracycline-supplemented and control rats, respectively. Of interest in this connexion are reports that the administration of chlortetracycline increased skeletal growth in young calves[18]. In rachitic rats, an increased calcification response to vitamin D[19] and a higher bone ash concentration[20] were reported. In the work recorded here the ash per cent of the femurs, incisors, and molars (Table 1) was unaffected by tetracycline. These different responses to tetracycline administration may possibly be related to nutritional status since the incorporation of chlortetracycline into purified diets adequate in all known dietary essentials except calcium caused no increase in body-calcium retention by growing rats[21].

Table 1. EFFECT OF TETRACYCLINE ON ASH CONTENT AND RADIOCALCIUM UPTAKE OF RAT FEMURS, INCISORS, AND MOLAR TEETH *

Sample	Tetracycline (mg/day)	No. of samples	Ash weight (mg)	Ash (%)	Calcium-45 (total % dose)
Femur	0·0	10	52·20 ± 1·88	42·40 ± 0·56	2·60 ± 0·084
	1·0	10	56·87 ± 1·25	44·94 ± 0·38	2·37 ± 0·096
	5·0	11	55·82 ± 2·17	44·32 ± 0·45	2·69 ± 0·057
	20·0	10	53·50 ± 1·86	43·84 ± 0·94	2·41 ± 0·082
Lower incisors	0·0	10	28·21 ± 0·59	66·94 ± 0·70	1·06 ± 0·044
	1·0	9	30·16 ± 0·60	68·99 ± 0·34	0·90 ± 0·031
	5·0	11	29·01 ± 0·64	67·89 ± 0·45	1·02 ± 0·061
	20·0	10	27·79 ± 0·64	66·72 ± 0·41	1·04 ± 0·029
Third molars	0·0	10	9·59 ± 0·38	74·22 ± 1·23	0·50 ± 0·029
	1·0	10	10·54 ± 0·33	73·44 ± 0·65	0·45 ± 0·046
	5·0	11	9·41 ± 0·37	70·71 ± 0·80	0·50 ± 0·031
	20·0	11	9·81 ± 0·28	75·31 ± 0·85	0·48 ± 0·011

* All values expressed as mean ± *S.E.*

Radiocalcium uptake by the different samples is shown in Table 1. (Data are expressed as percentage of total dose retained since comparison of total ash weight with total dose showed no correlation within groups.) In each case examination of the results by analysis of variance revealed no significant differences ($P > 0 \cdot 05$) attributable to tetracycline administration. There is general agreement that the fixation of radiocalcium by the mineral phase of calcified tissues occurs through its incorporation in forming crystals and by isoionic surface exchange with pre-existent crystals; the amount of isotope retained is the result of these processes and that of resorption. It has previously been shown that, in young growing rats, resorption associated with skeletal remodelling of the long bones has little or no effect on the retention of previously fixed radiocalcium for a period of time in excess of that used here[22]. It would follow from this that the radiocalcium content of the femur reflects the processes of mineral accretion and exchange and not resorption. Certainly this is the case with the incisor and molar teeth since these structures are not subject to resorption. One must conclude that under the present experimental conditions, at least, tetracycline administration had no effect on the uptake of radiocalcium by either bone or teeth. Considering the pharmacological importance of these agents there is, we believe, an important need for further assessment of their role in calcification.

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Location of Glyceraldehyde-3-phosphate Dehydrogenase in Starch-gels

SPECIFIC staining reactions have been used to locate several enzymes in starch-gels after the electrophoresis of crude enzyme extracts, and these techniques have been used to detect molecular variants of these enzymes[1–3]. This communication reports a technique for staining glyceraldehyde-3-phosphate dehydrogenase, an important enzyme in intermediary metabolism, and some results obtained in the electrophoresis of preparations from strains of streptococci.

The micro-organisms were grown either in a synthetic medium[4], or in a broth containing 1 per cent glucose, 0·4 per cent sodium chloride, 0·5 per cent yeast extract (Difco), and 1 per cent proteose peptone (Oxoid). The cultures were incubated at 37° C for 24 h, and the cells gathered by centrifugation at 2,000*g* for 15 min, re-suspended in an equal volume of water and re-centrifuged. The bacterial pellets were then suspended in distilled water (about 200 mg wet weight/ml.) containing 1 mg of cysteine hydrochloride. Disintegration of the cell-walls was effected by shaking this suspension with an equal volume of glass ballotini[5] (Jencons, grade 11) for 20 min using a Griffin flask shaker at its maximum rate of oscillation. The beads were filtered using a sintered-glass funnel (porosity No. 2), and the slightly opalescent filtrate centrifuged at 10,000*g* at 0° C for 30 min. Electrophoresis of this extract was carried out as described by Fildes and Parr[3], using about 10 V/cm for 1 h, in a gel prepared in 0·01 M phosphate buffer of *p*H 7·0. The gels were sliced horizontally and stained on the cut surface. The staining mixture was prepared by incubating 1 mg of aldolase (Sigma Co.) with 50 mg of fructose-1 : 6-diphosphate disodium salt in 1 ml. of 0·1 M *p*H 7·5 *tris* chloride buffer for 30 min at 37° C, and then mixing this preparation with 9 ml of 0·1 M *p*H 8·5 *tris* chloride buffer in which was dissolved 100 mg of agar, 2 mg of NAD, 2 mg of the tetrazolium salt 'MTT', 0·4 mg of phenazine methosulphate, and 15 mg of sodium arsenate. The temperature was about 45° C when this mixing was carried out, and the whole was immediately poured on to the starch-gel when it set as a clear pale yellow layer. After incubation at 37° C for about 0·5 h the positions of the enzymes were revealed by dark-blue bands.

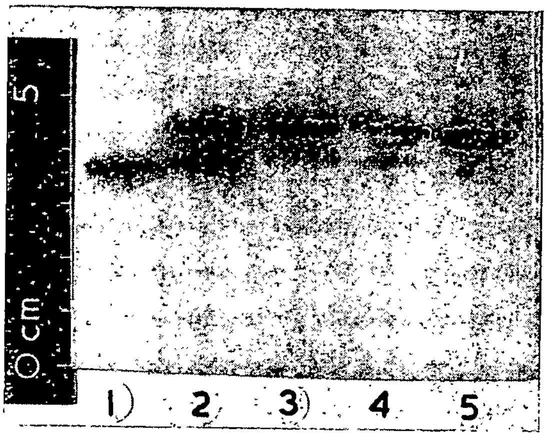

Fig. 1. Starch-gel electrophoresis of glyceraldehyde-3-phosphate dehydrogenase of five Group *D Streptococci*

Fig. 1 shows the results of applying this method to *Streptococcus faecalis HT*25 isolated from human dental plaque (sample 1), *Streptococcus faecalis* N.C.T.C. 775 (2), N.C.D.O. 580 (3), and N.C.D.O. 582 (4), and *Streptococcus zymogenes* N.C.I.B. 8886 (5). All strains show two isoenzyme fractions, those of *HT*25 being so close together as to appear as one band in some instances. In the other four strains the two components are more widely separated. The stain is equally intense in both bands of N.C.T.C. 775, but the other three strains show a higher activity in the faster moving fraction. Hence there are strain differences in the isoenzymatic forms of this enzyme among the group *D Streptococci*.

Similar results have been obtained by using glyceraldehyde-3-phosphate derived from its diethylacetal, and also by using a different enzymatic preparation of this material[6]. The method described gave a staining reaction with a commercial preparation of glyceraldehyde-3-phosphate dehydrogenase.

There have been several attempts to subdivide the streptococci on the basis of various biochemical reactions, but such classifications have evident shortcomings[7]. Recently it has been shown[8,9] that the *faecalis* group comprises a continuous spectrum of biotypes, and that the named subdivisions, *zymogenes*, *liquefaciens*, etc., are by no means species, but represent types which have the more common combinations of the biochemical properties used in the attempted classifications. The results reported here, and those of the electrophoresis of other enzymes, indicate that besides the variation in constitutive enzymes implied above, there is also heterogeneity of isoenzymatic forms among various strains of this group of micro-organisms.

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PHYSIOLOGY

Potassium Effects on Cæsium – Choline Interactions in Muscle Membrane

In an earlier paper it was shown that addition of small concentrations of choline ion to bathing solutions which already contain cæsium significantly reduces the resting membrane potential of frog sartorius muscles[1]. The work recorded here is an extension of this investigation, and is concerned with the following questions : (*a*) Are the combined effects of cæsium and choline on the membrane potential reversible ? (*b*) To what extent does potassium affect the cæsium–choline interaction ? (*c*) Does the entire muscle cell surface participate in the cæsium–choline interaction ?

Experimental procedures were similar to those already described in ref. 2. Sartorius muscles from *Rana pipiens* were dissected so as to leave the lower side practically free of connective tissue, and the nerves were cut as close to the muscle surface as possible. Individual muscles were mounted in a 500-ml. 'Lucite' chamber at 120 per cent of resting length by means of a special holder and micrometer system. Bathing solutions were made up by adding cæsium, potassium or choline chloride in appropriate amounts to standard potassium-free Ringer's formula (112 mM NaCl, 1·89 mM $CaCl_2$, 2·5 mM Na_2HPO_4, 0·5 mM NaH_2PO_4). In some experiments, 300 units/ml. of hyaluronidase were added to the indicated solution. All experiments were carried out at 25° C. Twenty pairs of muscles were used in each experimental solution. Only potentials which developed instantaneously with microelectrode penetration were recorded and used in the data. Each point shown in Figs. 1 and 2 is the average of potential measurements on 180 cells.

Muscles were scanned by mounting the microelectrode assembly in a modified *X–Y* plotter which laid out a pattern of electrode positions in steps of 200μ.

The results are summarized in Figs. 1 and 2. It is clear from Fig. 1 that the depolarizing effect of 10 mM choline in 2·5 mM cæsium–Ringer is *not* reversed when the muscle is washed with choline-free cæsium–Ringer, but is reversed when the muscle is washed with normal (2·5 mM potassium) Ringer. In the absence of cæsium, choline produces no depolarization, in agreement with earlier work (Fig. 2*A*). In the presence of both potassium and cæsium, the depolarization by choline is less than in cæsium without potassium (Fig. 3). The fractional depolarization (ratio of resting potential before addition of choline to resting potential in presence of choline) produced by 10 mM choline is 1·27 in 2·5 mM cæsium–Ringer, but only 1·15 in Ringer containing both 1·25 mM cæsium and 1·25 mM potassium. Treatment with hyaluronidase does not alter the depolarization produced by 10 mM cæsium–Ringer, and the fractional depolarization produced by 10 mM choline under these conditions is still 1·27 (Fig. 2*B*). Although not shown in each figure, all these effects are reversible and repeatable. The lack of time dependence of all the potentials over periods of several hours is also noteworthy.

The results of the scanning studies were negative : no significant differences were obtained in potential measurements from different regions of a single muscle fibre. End-plate regions could be identified visually under the microscope, and several electrode penetrations were achieved in each such region.

The interactions between cæsium, potassium and choline suggest the following working hypothesis. Suppose that receptor 'sites' or 'configurations' to which choline can attach in some way are present on the cell surface, and that the result of the attachment of choline to these sites is a decrease in measured membrane potentional. Potassium interferes with this choline-receptor interaction, and cæsium enhances it. The stability of the choline depolarization in cæsium–Ringer, especially after washing off excess choline, indicates that choline reacts in an all-or-none fashion when cæsium is present. The potassium effects also require us to conclude that the sites are on the 'outside', since choline on the 'inside' would be prevented from binding by the high levels of intracellular

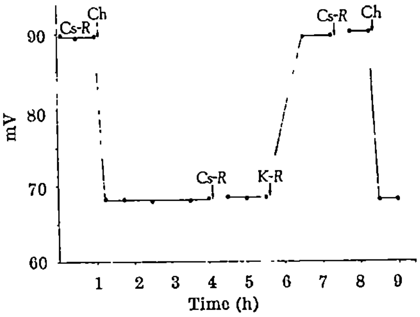

Fig. 1

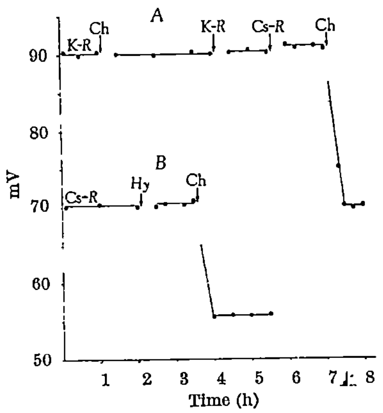

Fig. 2

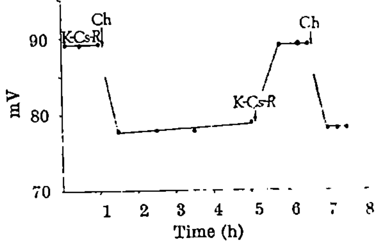

Fig. 3

Figs. 1, 2 and 3. Measured membrane potentials in millivolts plotted as a function of time, with the muscles bathed in solutions indicated by the letter symbols as follows: Cs-*R*, 2·5 mM cæsium–Ringer, except in Fig. 2*B* where the Cs concentration was 10 mM; K–*R*, 2·5 mM potassium Ringer; Ch, indicated solution made up to 10 mM in choline chloride, and applied at times indicated by vertical arrows; K–Cs–*R*, potassium-cæsium–Ringer, 1·25 mM K and 1·25 mM Cs in the same solution. *Hy*, 300 units of hyaluronidase per ml. added to the indicated solution. Times at which solutions were changed are indicated by vertical arrows potassium. Both cæsium and choline have been shown to enter the cell under our experimental conditions[1,3].

Are the postulated receptors for choline identical with the widely accepted acetylcholine receptors implicated in motor end-plate function[4,5]? Much more work is needed to clarify this situation, but the fact that excess external potassium results in liberation of acetylcholine from nerve endings is suggestive[6,7]. However, the apparent independence of the cæsium–choline depolarization from external sodium concentration, at least over the range 56–112 mM sodium chloride[1], is puzzling, since it prevents us from explaining the measured potential decrease as a result simply of increased sodium permeability. Sensitivity to acetylcholine is normally restricted to the motor end-plate region, but spreads over the whole muscle surface after denervation[8,9]. This spreading out process does not begin for at least 8 h after denervation, however (15 h under our laboratory conditions), so it is not likely to be involved in these choline effects. The scanning results indicate that the ability to react with choline is distributed over the entire muscle cell surface, including the motor end-plate region, but not limited to it.

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Lack of Artificial Acclimatization to Heat in Physically Fit Subjects

DURING investigations of artificial acclimatization to heat on three physically fit subjects, the usual changes in sweat rates, rectal temperatures and pulse rates were not observed. The acclimatization procedure consisted of five 2-h walks on an every-other-day sequence. The daily protocol was 50 min walking at 6·4 km/h on a level treadmill and 10 min rest each hour. The environmental conditions were dry bulb weight = 49° C, wet bulb weight = 28° C, and globe temperature = 49° C. Comparing the results of walk 1 with walk 5, the average pulse rate decreased 1 beat/min; the sweat rate decreased 66 g/h, and the rectal temperature decreased 0·3° C.

Other workers have shown that the major physiological changes occur after four or five exposures[1–3] and the optimal exposure time was 100 min[4]. It was hypothesized that the physiological changes induced in the subjects as a result of their high level of physical fitness precluded any heat effects. Thus the subjects were able to perform as though they were acclimatized to heat.

The question then arises when conducting heat acclimatization investigations that utilize physical exercise: which changes are due to environmental stimuli and which are due to the exercise? A further complication is that, since exercise also produces heat, what is the relationship between exercise heat and environmental heat on the response of the subject. Fox *et al.*[5] have recognized the possible separate effects of environment and exercise and commented that "a part of the large decrease in pulse rates normally observed when using the conventional technique (of heat acclimatization) may be due to the improved cardiovascular efficiency consequent on the repeated performance of a particular physical exercise. . . ."

In understanding the mechanisms of heat acclimatization it seems imperative that cognizance be taken of the level of physical fitness of the subjects as well as the relative contributions of the separate effects of the particular exercise and environment.

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Direct Evidence for an Action of Acetylcholine on Motor-nerve Terminals

SOME twenty-five years ago Masland and Wigton[1] showed that antidromic action potentials could be recorded from the ventral roots of cats during the muscular contraction provoked by the intra-arterial injection of acetylcholine. As a result of these experiments they suggested that acetylcholine might have an excitant effect on motor-nerve terminals. Later work[2] has shown that many other quaternary ammonium compounds have the same ability to provoke antidromic action potentials in motor nerves. In particular Werner[2] has shown, by ingenious but indirect means, that these drug effects are not due to excitation of the terminals by synchronous muscle action potentials. Despite these experiments which seem most satisfactorily explained by the presence of some cholinoceptive sites on the nerve terminals[3], it has recently been claimed that at the present time there is no direct evidence for an action of acetylcholine on motor-nerve terminals[4]. This problem is of special interest, for an excitant action of the acetylcholine released by nerve impulses on nerve terminals is the central feature of a new hypothesis of transmitter release at ganglionic and neuromuscular junctions put forward by Koelle[5]. This excitant action is thought to release further acetylcholine in a sort of positive feed-back action.

In the investigation recorded here the effect of muscle activity was eliminated by using rat diaphragm-phrenic nerve preparations *in vitro*, paralysed with either *d*-tubocurarine (Burroughs Wellcome) or an excess of magnesium chloride in the bathing Krebs–Ringer solution. The problem of acetylcholine action was attacked directly, using the methods developed by Hubbard and Schmidt[6] for stimulating nerve terminals. As in that investigation the terminals were located by the preliminary recording of extracellular miniature end-plate potentials in magnesium-poisoned preparations or by the recording of focal end-plate potentials in curarized preparations. The recording microelectrodes were filled with 4 M sodium chloride and had 1·5–3μ tips and 1–3 MΩ resistance. Stimulating pulses of 0·1 msec duration from a Grass isolation unit were then applied through the same electrodes and the single all-or-nothing antidromic impulses set up by the stimulation were recorded in the phrenic

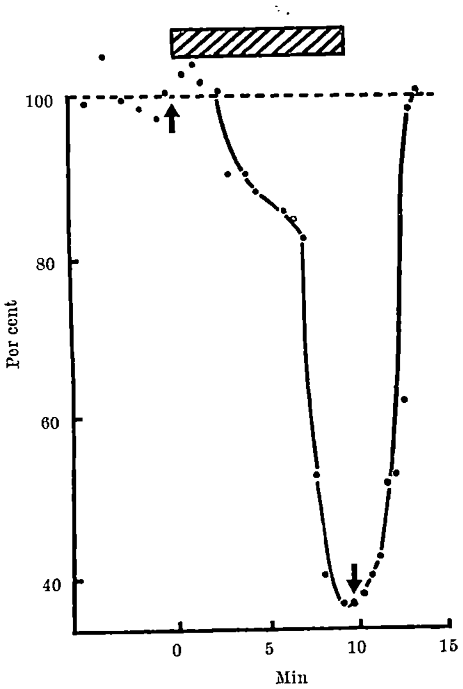

Fig. 1. The effect of acetylcholine on the threshold of motor-nerve terminals in a curarized preparation. The dots indicate threshold, that is the amplitude of current pulses adequate to evoke antidromic action potentials in 2–4 of six trials when applied in the vicinity of motor-nerve terminals. Threshold (ordinate) is expressed as a percentage of the average threshold before the introduction of acetylcholine (dotted line, 100 per cent). At time 0 (abscissæ) acetylcholine 10^{-7} g/ml. was introduced (marked by first arrow and the beginning of the hatched bar). After a big fall in threshold the acetylcholine was washed out (arrow and end of hatched bar). The threshold then progressively returned to the control value. The temperature was 35° C and all solutions contained *d*-tubocurarine chloride in a concentration of $1\cdot8 \times 10^{-6}$ g/ml. and prostigmine chloride in a concentration of 10^{-6} g/ml.

nerve with a pair of platinum electrodes. The threshold of the terminals was assessed by recording the stimulating pulses across a 50Ω resistance in the stimulating circuit and measuring, from filmed records, the pulse amplitude necessary for setting up antidromic impulses. Under the experimental conditions a 95 per cent exchange of solutions took 3–4 min.

Fig. 1 illustrates a typical experiment in which the control solution contained *d*-tubocurarine chloride ($1\cdot8 \times 10^{-6}$ g/ml.) and prostigmine chloride (10^{-6} g/ml.). The average pulse amplitude required to elicit antidromic action potentials is shown as 100 per cent and indicated by the dotted line in the graph. At the time marked by the arrow and the beginning of the hatched bar the perfusing fluid was changed to a solution differing only in that it contained acetylcholine chloride (A. G. Fluka) in a concentration of 10^{-7} g/ml. Three min later there was an abrupt fall in the pulse amplitude required to set up antidromic impulses. By 9 min effective pulses were only 37 per cent of the control strength, and after 9·5 min the acetylcholine was washed out with control solution (arrow and end of hatched bar). Within 30 sec of the change of solution the threshold had risen and after 4·5 min of washing the threshold had returned to the control value. Similar results have been observed in many other curarized and magnesium-paralysed preparations. Preliminary experiments indicate that this fall in threshold is the result of the stimulation of a typical cholinoceptive system, for the effect of acetylcholine is antagonized by curare and increased by prostigmine.

These results thus provide direct evidence for a depolarizing action[7] of acetylcholine on nerve terminals. This depolarization could generate action potentials by a local circuit action involving nearby nodes of Ranvier. At first sight these results also support Koelle's hypothesis. However, the experiments of Ciani and Edwards[8] indicate that perfusion with acetylcholine reduces the number of quanta of transmitter released by nerve stimulation at frog neuromuscular junctions *in vitro*. We have made similar observations at junctions in the rat diaphragm. This reduction in transmitter output is presumably a consequence of the depolarization of the terminals. Action potentials in the depolarized terminals would be reduced in size and thus have a reduced ability to liberate transmitter[9]. It would appear therefore that the physiological action of acetylcholine on nerve terminals is a negative feed-back on further acetylcholine release rather than the positive feed-back postulated in Koelle's hypothesis.

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Concentration of Ammonia in the Gastric Venous Blood of Dogs during Acid Secretion

AMMONIA has been detected in gastric juice during acid secretion in response to parenteral histamine in many species, including man and dog[1–4]. This has been attributed to the action of urease in the gastric mucosa on endogenous urea[4,5]. Conflicting observations have been made as to whether ammonia is also released into gastric venous blood during acid secretion[6,7]. Turner[6] found a

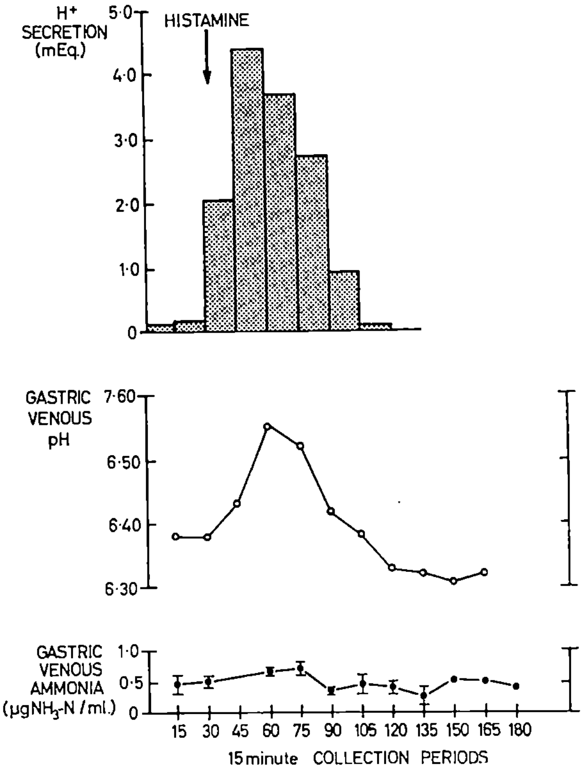

Fig. 1. Gastric venous ammonia concentration following subcutaneous histamine. The range of duplicate estimations of ammonia concentration is indicated

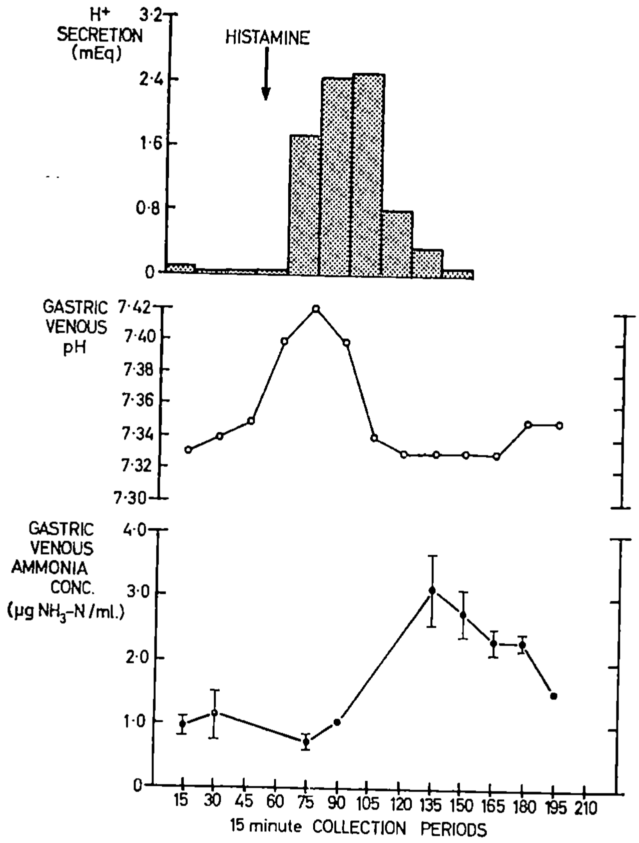

Fig. 2. Gastric venous ammonia concentration following subcutaneous histamine. The range of duplicate estimations of ammonia concentration is indicated

rise in arterial ammonia concentration 30–60 min after histamine in those cirrhotic patients with a previous history of hepatic coma or pre-coma, but not in cirrhotic patients without such a history. He suggested that the origin of this rise might possibly be related to gastric secretion. A further attempt has therefore been made to determine whether ammonia is released into the venous blood leaving the stomach during, or after, histamine stimulated acid secretion in dogs.

At laparotomy under pentobarbitone anæsthesia in eight mongrel dogs, a cannula was inserted into the splenic vein distal to the entry of the gastric veins, and splenectomy was performed. A fine nylon snare was passed around the splenic vein proximal to the entry of the left gastric vein, so that the venous drainage from the stomach could be obstructed temporarily at will while blood samples were taken[9]. A Thomas cannula was inserted into the stomach for collection of gastric juice. Blood ammonia was measured by a modification of Seligson's method. Gastric juice was titrated using Topfer's solution.

24–48 h later, control specimens of gastric secretion and gastric venous blood were taken, and 0·04 mg/kg histamine was injected subcutaneously. Gastric acid secretion and gastric venous *p*H and ammonia concentration were then monitored for 1·5–2 h.

In nine experiments on eight dogs we observed no increase in the ammonia-levels in gastric venous blood during the first 75 min after histamine (the period of gastric acid secretion). There was, however, a rise in the venous *p*H during this period.

75–150 min after histamine (the period following gastric acid secretion), we found a definite increase in the ammonia concentration of the gastric venous blood in one out of six experiments. Fig. 1 is a typical example of the findings in five of these animals. Fig. 2 shows details of the experiment in which an increase in the ammonia concentration was observed.

With this exception, therefore, we have been unable to demonstrate a rise in the ammonia concentration of gastric venous blood during, or after, histamine induced acid secretion. The rise in *p*H of the venous blood samples during acid secretion suggests that these consisted mainly of gastric venous effluent.

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Iontophoretic Application of Gamma-Aminobutyric Acid on Crayfish Muscle

It has been observed that γ-aminobutyric acid (GABA) mimics the action of inhibitory transmitter in several crustacean muscles[1–5]. In these investigations the action of GABA was investigated by perfusing the preparation with solutions containing the drug, and iontophoretic micro-application of GABA to these preparations was not performed. The latter has advantages over the perfusion experiment in providing localized application of the drug and more precise control of its duration.

In the present experiment, single- or double-barrelled microcapillaries were loaded with 1 M GABA (*p*H adjusted to about 4 by hydrochloric acid) or with 1 M L-glutamate (*p*H 8). Under these conditions GABA is cation and L-glutamate is anion. Thus GABA was injected by cathodic (outward through the capillary) and L-glutamate by anodic current. The abductor muscle of the dactyl in the 1st or 2nd walking leg of *Cambarus clarkii* was used. The potential changes were recorded intracellularly with a capillary filled with 3 M potassium chloride and the membrane potential was changed by the current through a 3 M potassium citrate-filled microcapillary.

One barrel of a double-barrelled capillary was filled with GABA and the other with L-glutamate. Thus GABA and L-glutamate could be applied to the same spot. The capillary was moved along the surface of a muscle fibre while passing a strong cathodic current through a GABA-loaded barrel. Several spots were found on a muscle fibre where GABA produced a potential change, usually in a depolarizing direction at resting potential. Although more detailed investigations were undertaken with smaller injection currents, both rising and falling phases of the GABA-induced potential were found to be several times slower than those of the glutamate-induced depolarization[6]. Fig. 1 shows an example of the GABA-induced depolarization. The GABA-induced potential was obtained from a rather broad area, compared with that for the glutamate potential, and it was rather difficult to locate the GABA-sensitive spot critically. This is probably due to the rather large dose of GABA required to obtain an appreciable potential change and to its slow time course.

When the membrane potential was altered by polarizing current through a current electrode inserted in the same fibre, the GABA-induced depolarization reversed its sign at about −60 mV (Fig. 2). On the other hand, the glutamate potential had its reversal potential near zero membrane potential. In most cases the GABA-sensitive area overlapped with the glutamate-sensitive spot. How-

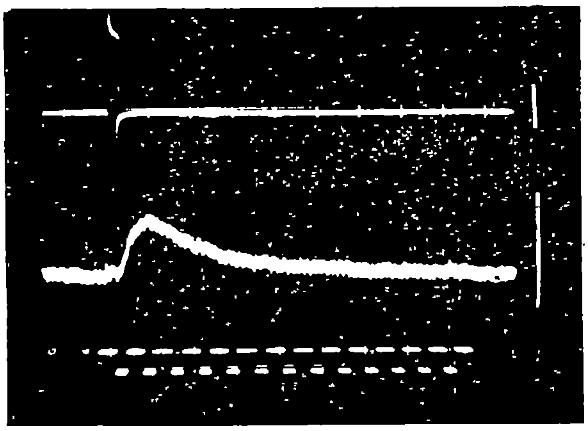

Fig. 1. Depolarization produced by iontophoretic injection of GABA (middle trace): upper trace, monitored injection current; lower trace, 10 c/s. Calibrations: current, 5×10^{-7} amp; potential, 5 mV

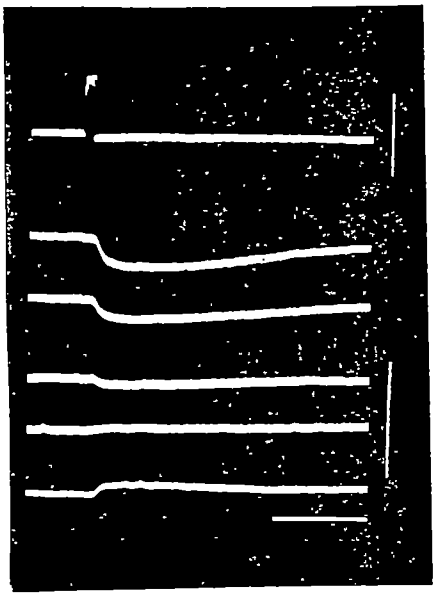

Fig. 2. GABA-induced potentials at varied membrane potentials. The bottom trace was obtained at the resting potential (−70 mV). Uppermost trace, monitored injection current. Calibrations: current, 1×10^{-7} amp; potential, 5 mV; time, 1 sec

ever, at some spots the injection of GABA produced a potential change, but there was no response to the injection of L-glutamate and vice versa. Prolonged application of GABA produced a maintained potential change, in contrast to the glutamate potential which showed marked desensitization. It was shown that L-glutamate mimics the excitatory transmitter[6]. Thus, an interaction between GABA and L-glutamate might be expected, as is observed between the inhibitory and the excitatory transmitters. During and after conditioning doses of GABA, injection of L-glutamate produced less depolarization than when unconditioned.

We thank Prof. C. C. Hunt for his advice.

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HÆMATOLOGY

Interaction of Glutathione and Epinephrine within the Human Red Blood Cell

REACTION of the sulphydryl group of glutathione with oxidation products of epinephrine, norepinephrine and β-3,4-dihydroxyphenyl-l-alanine *in vitro*[1-4] suggested a search for similar reactions involving the glutathione in intact red cells. The importance of such chemical behaviour arose from the fact that the fate of epinephrine and norepinephrine during the relatively brief period of their physiological action remained unknown[5]. During incubation of freshly drawn human blood with a catecholamine in the work recorded here, the total sulphydryl group of the glutathione within the red cells rapidly decreased. This observation suggested an interrelationship between the catecholamines and glutathione *in vivo*.

Human blood was obtained in random fashion from 45 healthy male and female volunteers aged 22–42 years, with use of aqueous heparin sodium obtained from the Riker Co. of Los Angeles, California, in a concentration of 5,000 units per ml. The bitartrate forms of the catecholamines were supplied by the Winthrop Co. of New York. Separate solutions containing 10 mg of epinephrine or norepinephrine or 8 mg of ascorbic acid per ml. of water were formed immediately before use. All chemicals were of reagent grade. Trichloroacetic acid and sodium hydroxide were kept in 5 per cent and 5 N solutions, respectively. After solution of 46·6 g of the tetrasodium salt of (ethylenedinitrilo)-tetraacetic acid (EDTA), obtained from the Matheson Co., in 800 ml. of water, 5 ml. of glacial acetic acid were added to adjust the *p*H to 7·5 and the volume was adjusted to 1 l. with water to form a 0·11 M solution of EDTA. A 0·0308 per cent solution of *bis* (*p*-nitrophenyl) disulphide (Eastman Kodak Co.) in acetone was formed (PNPD).

At zero time, the indicated amount of epinephrine in 0·1 ml. of water was added to 1 ml. of freshly drawn blood in a number of test-tubes. Each test-tube was incubated for the designated period of time in a water bath maintained at 37° C without evidence of hæmolysis. Then, for deproteinization, 9 ml. of trichloroacetic acid were added. Ten minutes later, the solution was filtered through Whatman No. 1 filter paper. To 1 ml. of filtrate was added a solution containing 4 ml. of EDTA, 4 ml. of acetone and 0·5 ml. of PNPD, in compliance with the method of Ellman[6] for measurement of sulphydryl groups. The absorbancy of the yellow colour was measured within 1 min thereafter at 412 mμ in a Beckman *DU* spectrophotometer, with water as the blank.

The decrease in the blood non-protein sulphydryl group which resulted from the presence of epinephrine in two different blood specimens is shown in Fig. 1. Essentially similar results were obtained in every case. Duplicate specimens in 15 experiments yielded results within 9 per cent of each other during all the experimental steps. The absorbancy of the residual sulphydryl group decreased rapidly within the first 5 min and reached an essentially stable value by 15 min. In the specimens tested, the initial absorbancy of the sulphydryl group varied, without apparent pattern, between 108 and 165, while the final levels for 1 mg of epinephrine ranged between 60 and 75 per cent of the initial levels. Increase in epinephrine up to 300 μg resulted in a corresponding decrease in the plateau level of the —SH group, while increase above 500 μg had little additional effect. The absence of bitartrate in epinephrine U.S.P. did not affect the results. Such easily oxidizable substances as l-norepinephrine bitartrate, isoproterenol hydrochloride (Winthrop Co.) and l-dihydroxyphenylalanine yielded essentially the same results as l-epinephrine bitartrate. D-Amphetamine sulphate (Smith, Kline and French), l-phenylephrine hydrochloride (Winthrop Co.) and methoxamine hydrochloride (Burroughs Wellcome) were ineffective. Use of

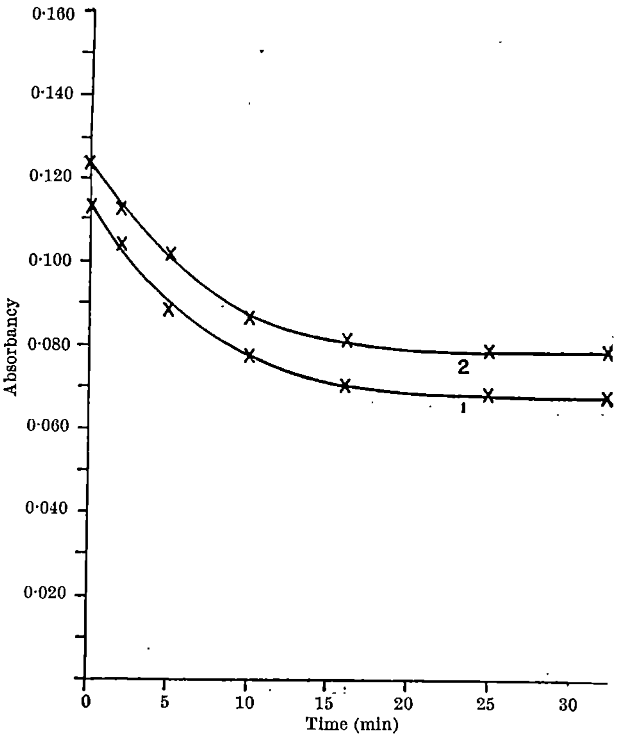

Fig. 1. Absorbancy as a function of time for the sulphydryl group remaining in 0·1 ml. of human blood on incubation of 1 ml. of blood at 37° C with 1 mg of epinephrine

EDTA as an anticoagulant yielded the same results as heparin. When 20 μg of glutathione in either 1 ml. of trichloroacetic acid or 1 ml. of water was added to Ellman's reagent, an average net absorbancy reading of 99, with a standard deviation of 7, was obtained in six determinations, the absorbancy of the PNPD blank without glutathione being six. During 30 min of incubation at 37° C in the absence of epinephrine, the non-protein sulphydryl group of blood did not change. Plasma did not effect the disappearance of the sulphydryl group significantly. Indeed, the net absorbancy of the —SH group in 1 ml. of the trichloroacetic acid filtrate formed from 1 ml. of plasma was only six. Furthermore, in contrast to the behaviour in blood, half of 200 μg of glutathione added to 1 ml. of plasma disappeared in 20 min, and 1 mg of epinephrine inhibited the decrease appreciably.

In investigations concerning the mechanism of the glutathione reaction, the epinephrine was added not to blood but rather to 1 ml. of blood trichloroacetic acid filtrate the *p*H of which had been adjusted to 7·5 by addition of 0·3 ml. of a 1·1 M EDTA solution. The results were essentially the same as in Fig. 1. Epinephrine did not cause disappearance of the —SH group below *p*H 5·2 within 30 min, while at *p*H 5·8 the disappearance was the same as at *p*H 7·5. The pink colour and the increased absorbancy at 480 mμ produced by as little as 4 μg of adrenochrome failed to appear as the —SH group decreased. Ascorbic acid had a marked effect on the disappearance of the —SH group in blood and filtrate. Addition of 3 mg of ascorbic acid to 1 ml. of blood immediately before 1 mg of epinephrine prevented disappearance of the —SH group for 30 min. One mg of ascorbic acid prevented the disappearance for 15 min; but nearly the full decrease in the —SH group occurred after 15–30 min. Similar results were obtained in the filtrate.

The non-protein sulphydryl group of the red cell is largely glutathione[7]. The recovery of glutathione added to blood was nearly 100 per cent in the Ellman technique, while added cysteine and ergothionine were not at all recovered[7]. Since the sulphydryl group of blood is present almost solely within the red cell, the progressive decrease in its concentration during incubation with epinephrine must have resulted from intracellular reactions. Only a small portion of the added epinephrine actually affected the sulphydryl group. Use of relatively large amounts of epinephrine was necessary to permit reasonably large changes in the absorbancy produced from the sulphydryl group. Sensitive methods, such as those dependent on radioactivity, should enable the results of the present investigation to be extended without difficulty to physiologically significant levels of epinephrine.

The inhibitory effect of ascorbic acid suggested that the disappearance of the —SH group involved oxidation of the epinephrine. Decrease in the —SH group on incubation of the protein-free filtrate with epinephrine indicated that the oxidizing agent was not a protein. It required a *p*H higher than 5·8 for full effectiveness. Its action was rapid but inadequately strong to produce any adrenochrome from the large excess of epinephrine, in contrast to manganese dioxide. The disappearance of erythrocyte glutathione in the presence of epinephrine in the present investigation was compatible with similar behaviour in the intact living organism. These observations might equally be applicable to the cells of such organs as the liver and kidney, in agreement with the finding that repeated injection of epinephrine into rats over 4·5 h led to a decrease in the glutathione —SH group within these organs[8]. The mechanism of the sulphydryl disappearance, including its relationship to the formation of presently known metabolic products of the catecholamines, such as metanephrine and normetanephrine[5], remained to be determined.

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Panagglutination of Erythrocytes coupled with Diazotized *p*-Nitroaniline

MODIFICATION of erythrocytes *in vitro* by various enzymatic or chemical treatments renders them agglutinable by normal sera from mature donors. This agglutination reaction is called 'panagglutination'[1,2]. In the course of our investigation on the specific reaction of azohapten-erythrocytes with antisera against azohaptens[3], we observed that after coupling erythrocytes with diazotized *p*-nitroaniline (PNA), they were agglutinated by normal rabbit serum absorbed with untreated erythrocytes[3]. This communication deals with the finding of a similar phenomenon with human serum.

PNA-erythrocytes were prepared by adding 0·5 ml. of 0·005 M *p*-nitrobenzenediazonium chloride to a suspension of 0·05 ml. three-times washed packed human erythrocytes in 4·5 ml. phosphate buffer (0·15 M, *p*H 7·3) with immediate mixing. After standing for 15 min at room temperature, the coupled cells were washed twice with 10 ml. physiological saline, containing 1 per cent decomplemented rabbit serum absorbed with untreated human red cells, or 0·06 per cent human serum albumin, and then resuspended in 2·5 ml. of the same solution. To obtain consistent results, the erythrocytes used for coupling should be freshly drawn, and tested within 0·5–1 h after coupling. Normal and pathological human sera and plasma were obtained by venipuncture and used immediately, or stored at $-25°$ C. The sera used were either blood group compatible, or absorbed with a sample of the same cells which were afterwards coupled with PNA. Titration was performed on a glass plate as already described[3]. Controls with untreated erythrocytes, as well as controls of the coupled cells with saline only, were always included. Serum titres are expressed as the reciprocal of the highest dilution giving agglutination.

The results obtained with 235 human sera are summarized in Table 1. From the pattern of agglutination observed, it appears that the titre is raised in patients with hepatitis and anæmia as compared with sera from healthy donors. The titre is also raised in pregnant women. It is lowered, on the other hand, in patients with leukæmia. However, the number of sera is too limited to allow any definite conclusions. Inhibition tests with PNA-tyrosin and absorptions with insoluble PNA-γ-globulin as described in ref. 3 were performed and induced no appreciable reduction of the agglutination titres. This indicates that an agglutinin with a specificity directed against the hapten PNA is not involved in this reaction. Highly active $H(O)$-substance also failed to show an inhibitory effect. The agglutinating factor could be absorbed from the sera with PNA-erythrocytes, but not with untreated erythrocytes. The activity of this factor was not changed by heating the serum to 56° for 30 min; it was significantly reduced after 30 min heating at 62°, and completely destroyed after 30 min at 65°. All the agglutinating activity could be precipitated by 45 per cent saturation of the serum with ammonium sulphate[4]. The precipitate was dissolved and made up to the original volume in physiological saline. Tested against PNA-erythrocytes, it gave the same titre as the serum from which it was prepared. By paper electrophoresis, it was shown to contain 70–80 per cent β- and γ-globulins, small amounts of α_2- and traces of α_1-globulin.

Table 1

Serum donors	No. of sera tested	No. of sera having a titre of: <1	1	2	4	8	16	32	64	128	>128
Human (healthy)	50	1	4	10	14	11	7	2	1	0	0
Human (pregnant)	78	0	2	9	21	24	13	6	2	1	0
Human (anæmic*)	33	1	5	2	7	11	4	1	1	1	0
Human (leukæmic†)	16	4	2	4	2	0	1	1	2	0	0
Human (hepatitic)	21	0	0	3	1	5	5	1	4	1	1
Human (various other diseases‡)	37	0	2	7	13	6	6	3	0	0	0
Guinea-pig (healthy)	10	0	0	1	6	2	1	0	0	0	0
Rabbit (healthy)	10	0	1	5	2	2	0	0	0	0	0

* Hæmolytic, pernicious or iron deficiency anæmia.

† Lymphocytic and myelocytic leukæmia, lymphogranulomatosis.

‡ Including polyarthritis, rheumatic fever, nephritis, nephrosis, liver cirrhosis and jaundice.

Rabbit and guinea-pig sera and erythrocytes, obtained by carotic bleeding, were also tested. The animal sera were always carefully absorbed with an untreated sample of the cells which were afterwards used for coupling with PNA. After absorption, these sera did not agglutinate the untreated cell control, but did agglutinate human PNA-erythrocytes. The titres were somewhat lower than for human sera (cf. Table 1).

All tested human red blood cells could be rendered panagglutinable without respect to their blood group. When erythrocytes from various donors were tested against the same serum, the titres were identical. The same titre was also obtained when the serum donor's own red cells were used. The PNA-treated erythrocytes are brown in colour and show a high tendency to hæmolyse. When other diazotized amines were used for coupling, as, for example, p-sulphanilic acid or p-aminobenzoic acid, panagglutination titres were significantly lower or absent. However, in these cases, the treated cells did not show such an increased tendency to hæmolysis. Rabbit and guinea-pig PNA-erythrocytes usually gave negative results when tested against human, rabbit and guinea-pig sera; only in a few cases were low titres obtained. All tested human and animal PNA-erythrocytes gave a high titre with a specific rabbit anti-PNA serum.

Since no serological relation to the coupling agent, to $H(O)$-substance, or to blood group agglutinogens could be demonstrated, the agglutination of PNA-erythrocytes appears to be a typical panagglutination. As such, it would be comparable with the agglutination of erythrocytes treated with the enzymes neuraminidase[5] or trypsin[6], which is thought to be due to an uncovering of pre-existing receptors on the red cell membrane, or with the agglutination of erythrocytes treated with the oxidizing agent periodate[7].

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Appearance of Slow α_2-Globulin during the Inflammatory Response of the Rat

Slow α_2-globulin is a protein of large molecular weight which becomes demonstrable by vertical starch-gel electrophoresis in the serum of the rat under a variety of conditions, mostly related to growth. It cannot be demonstrated by this means in healthy, non-pregnant adult animals but has been demonstrated in connexion with normal growth[1–3], neoplastic growth[1,2,4,5], regenerative or compensatory growth[6,7], pregnancy[1,2], tight abdominal bandaging[4], Caesarean section with retention of a single placenta[1], implantation of homologous minced kidney tissue[7], unilateral kidney ligation[7] and in certain states resembling mild infectious diseases[1,6]. The appearance of slow α_2-globulin in the last few of these conditions, together with the observation that it becomes sporadically demonstrable after limb amputation or sham surgery[7], especially in the presence of obvious wound infection, led to the hypothesis that, in addition to growth, the presence of an infection and/or an inflammatory condition can elicit the appearance of slow α_2-globulin in the serum of the rat.

In order to test this hypothesis, rats were subjected to various treatments designed to produce a localized or generalized inflammatory state. Sera were obtained as previously described[2] from Sprague–Dawley rats, of either sex, raised in our laboratory or purchased from the Holtzman Co., Madison, Wisconsin. Little difference in the responsiveness of the two sexes was noted. Vertical starch-gel electrophoresis was performed by the method of Smithies[8], with minor modifications[2]. Lipopolysaccharides (endotoxins) of *Salmonella abortus equi*, *S. typhosa* and *Escherichia coli*, produced by the Westphal method[9] and obtained from Difco Laboratories, Detroit, Michigan, were administered intraperitoneally in the doses indicated in Table 1 as a 1 per cent (w/v) suspension in sterile 0·9 per cent saline. Complete and incomplete Freund's adjuvants (Difco Laboratories) were administered intraperitoneally without dilution in the doses shown in Table 1. For the evocation of a localized inflammation, croton oil (Frank W. Kerr Chemical Co., Detroit, Michigan) was administered intradermally into one or two abdominal sites as a 75 per cent (v/v) solution in peanut oil[10]. Controls

Table 1. APPEARANCE OF SLOW α_2-GLOBULIN AFTER ADMINISTRATION OF VARIOUS SUBSTANCES

Substance administered	Vehicle	Route*	Dose	Presence†	No. of animals in each category at indicated No. of days after administration		
					0	2	4
Endotoxin of *S. arbortus equi*	0·9% NaCl	i.p.	2·5–4 mg/rat	+		18	15
				−		0	0
Endotoxin of *S. typhosa*	0·9% NaCl	i.p.	2·5–3·5 mg/rat	+		6	6
				−		0	0
Endotoxin of *E. coli*	0·9% NaCl	i.p.	3·5–4·5 mg/rat	+		10	6
				−		0	0
0·9% NaCl	None	i.p.	2·0 ml./rat	+		0	0
				−		6	6
Complete Freund's adjuvant	None	i.p.	2·0 ml./rat	+		6	6
				−		0	0
Complete Freund's adjuvant	None	i.p.	0·35 ml./rat	+		6	6
				−		0	0
Incomplete Freund's adjuvant	None	i.p.	2·0 ml./rat	+		7	6
				−		0	0
Incomplete Freund's adjuvant	None	i.p.	0·35 ml./rat	+		3‡	3‡
				−		15	15
Mineral oil	None	i.p.	2·0 ml./rat	+		0	0
				−		6	10
Mineral oil	None	i.p.	0·834 ml./kg	+		0	0
				−		8	5
Croton oil	Peanut oil	i.d.	0·3 ml./rat	+		8	6
				−		0	0
Peanut oil	None	i.d.	0·4 ml./rat	+		0	0
				−		8	6
None (untreated controls)	None	—	—	+	0		
				−	62		

* i.p., intraperitoneal; i.d., intradermal.

† +, slow α_2-globulin demonstrable. −, slow α_2-globulin not demonstrable.

‡, very weak slow α_2-globulin band.

consisted of untreated rats, rats injected intraperitoneally with either sterile 0·9 per cent saline or mineral oil or intradermally with peanut oil.

The results obtained are shown in Table 1. Slow α_2-globulin was demonstrable in every case two and four days after the administration of any of the three endotoxins tested. When the endotoxin of *S. abortus equi* was used, slow α_2-globulin first became demonstrable about 12 h after injection, but was no longer demonstrable in the majority of rats nine days after administration. In none of the control animals injected with sterile saline could slow α_2-globulin be demonstrated. The results obtained here with respect to the endotoxin of *E. coli* are in general agreement with those of Lawford and White[11] which came to our attention during the course of the work.

Since complete Freund's adjuvant contains killed and dried *Mycobacterium butyricum* and is said to modify the cellular reaction about its site of deposition, we investigated the possibility that its administration might provoke demonstrable levels of slow α_2-globulin. Such was the case at both dose-levels tested. However, incomplete Freund's adjuvant, lacking the *M. butyricum* when injected at the rate of 2 ml. per rat, also provokes this response. At the lower dose-level of 0·35 ml. per rat, the incomplete adjuvant, contrary to the action of the complete adjuvant, stimulates only an occasional and weak response. Control injections of mineral oil at either levels proved uniformly negative. The generally superior ability of complete adjuvant in enhancing the immune response as compared to incomplete adjuvant may perhaps be related to the difference in the ability of these two mixtures to provoke demonstrable levels of slow α_2-globulin.

Since the foregoing seems to show that generalized inflammatory responses lead to demonstrable levels of slow α_2-globulin, the question arose as to whether or not a localized inflammation would also do so. The administration of croton oil on to or into the skin provokes a localized inflammatory reaction[10,12]. As shown in Table 1, the intradermal injection into one or two sites of a total of 0·3 ml. of croton oil as a 75 per cent solution in peanut oil evokes demonstrable levels of slow α_2-globulin in all cases tested. Control rats injected with corresponding quantities of peanut oil remained uniformly negative.

The administration of some or all of the foregoing substances may provoke some slight tissue synthesis. There are, then, at least two proximate mechanisms here which might lead to the production of demonstrable quantities of slow α_2-globulin: small amounts of tissue synthesis or a component of the inflammatory response. The former is the less likely as generally no demonstrable slow α_2-globulin level appears during the healing processes following such procedures as limb amputation[7] and laparotomy[6,7] in the absence of wound infection. The alternative conclusion, that the appearance of demonstrable concentrations of slow α_2-globulin is, in these experiments, related to a component of the inflammatory response, is strengthened by the appearance of slow α_2-globulin in conditions of frank tissue degeneration as after the intraperitoneal implantation of minced homologous kidney tissue or after ligation of the blood vessels of one kidney[7].

It may be concluded, then, that slow α_2-globulin becomes demonstrable, by starch-gel electrophoresis, in the serum of the rat, during a localized or generalized inflammatory response, as well as during tissue synthesis.

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PATHOLOGY

Effect on Leukæmic Cells of Ribonucleic Acid extracted from Calf's Spleen

IT has been demonstrated that modifications may be produced in the metabolism of certain experimental tumours by cultivating them with RNA.

Cells of ascites tumour of mice have been incubated with RNA from a normal liver. An impressive reduction was noted in the capacity of these cells to reproduce the original tumour when grafted on to the tissue of other animals. This loss of malignity was due not to the death or diminished vitality of the cells, but to an actual transformation induced by the RNA with which they had been incubated. This was clearly demonstrated by the fact that the cells had acquired characteristics typical of hepatic cells, such, for example, as the ability to produce albumin, tryptophan-pyrrolase and glucose-6-phosphatase[1,2].

This phenomenon was observed not only in the case of Nelson's ascites tumour, but also in other cases of neoplasia (Ehrlich's ascites tumour, Novikoff's rat's hepatoma, etc.). That the transformation induced by RNA is permanent is seen by the fact that not even after a considerable lapse of time do the treated cells regain their original characteristics of neoplastic structures. That the RNA with which they were cultivated provides the essential factor in the transformation of the tumorous cells is proved by the fact that after treating with RNase the effect of RNA from the liver on the tumorous cells ceases completely[2].

On the basis of these observations, we have been, for some time past, carrying out a series of experiments to determine the effect of RNA extracted from various organs on human neoplasias cultivated *in vitro*. The first obstacle encountered in this work was due to the fact that human tumorous tissue is little adapted to cultivation *in vitro*, being constituted of cells presenting, in the majority of cases, phenomena of initial necrosis. Secondly, in the case of solid tumours it is exceedingly difficult to produce a culture of cells similar to each other from a morphological point of view; in consequence, the evaluation of eventual structural or metabolic changes becomes problematic.

Because of these facts I had recourse for the purpose of these experiments to the bone marrow of subjects affected with acute leukæmia or histiocytic medullary reticulosis. In these cases it often happens that the cells of bone marrow are for the most part of the identical, reticular type, which fact notably facilitates the evaluation of eventual morphological or metabolic changes.

Cultivation of leukæmic cells of bone marrow with RNA extracted from the spleen of calf embryo led to the formation of specific structures. At the start of the culture phase, the electron-microscopic investigations strongly suggested the scarcity of endoplasmic reticulum and the abundance of unattached granules lying free in the cytoplasm. In the case of cells in the culture containing RNA, a change in their morphological characteristics was noted in a fairly good percentage (40–48 per cent) after the lapse of 10–72 h (increase of ergastroplasm; parallel orientation of the membrane system; small dense particles studded on the outer surface of the membrane)[3].

The results recorded here refer to the metabolic changes observed in the cells obtained from the bone marrow of 5 subjects affected with acute leukæmia.

The medullary tissue, obtained by means of biopsy, was immediately mixed with sterile Tyrode's solution containing minute quantities of heparin. After having been placed in a refrigerator at a temperature of +2° C and left for 30 min, the particles of medullary matter adhering to the surface of the liquid were removed and immersed in a matrass containing the incubation mixture[4] to be used for the culture. By means of gentle agitation the medullary matter was dissolved in the liquid. The number of cells present in every 1 mm^3 having been counted, a quantity of the incubation mixture sufficient to raise the cellular concentration to 6,500 elements/mm^3 was added. One aliquot of this cellular suspension was immediately used as the control for starting respiratory activity[5]. One aliquot was transferred to a metabolic shaker (control). To the other part was added RNA (2 mg/ml.) extracted from the spleen of calf embryo by a technique already described by other research workers[6]; then the suspension was transferred to a metabolic shaker. Incubation was carried out under sterile conditions for 24 h at 37° C. Then the respiratory activity of cells was examined.

In the case of the cells in the culture containing RNA, a change in their metabolic characteristics was noted; in fact the oxygen consumption of RNA-treated cells is significantly increased (Table 1). The cells in the control culture, where no RNA was present, however, retained the same metabolic characteristics as at the beginning of the experiment.

These results demonstrate that cells of acute leukæmia are affected by the presence in the incubation mixture of RNA extracted from the spleen of calf embryo, an organ well known for its high proportion of cells having hæmopoietic properties. It is important to remember that the oxygen consumption of leukæmic cells is considerably less than that of the normal homologous cells. The increase of oxygen consumption in leukæmic cells indicates that information transfer within hæmopoietic cells can be effected, possibly by incorporating an RNA template containing 'normal' information which competes with pre-existing 'pathological' information systems.

Table 1. OXYGEN CONSUMPTION* OF BONE MARROW CELLS OF 5 SUBJECTS AFFECTED WITH ACUTE LEUKÆMIA BEFORE AND AFTER INCUBATION WITH RNA

No.	Initial value	RNA-treated cells	Control
1	0·16	0·41	0·15
2	0·14	0·36	0·12
3	0·14	0·44	0·11
4	0·21	0·49	0·19
5	0·17	0·46	0·16

* μM/h/10^7 cells.

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Relationship of Adrenocortical Insufficiency to Spontaneous Regression in Mammary Tumours induced in Rats by Oral Administration of Dimethylbenzanthracene

IT has been shown that many of the tumours induced in rats by the oral administration of 9,10-dimethyl-1,2-benzanthracene (DMBA) stop growing and some undergo spontaneous regression[1,2]. It has also been shown that DMBA produces massive adrenocortical necrosis when given in doses which would ultimately give rise to tumours[3]. It appeared possible that spontaneous regression might be due to adrenocortical insufficiency which permitted the development of an immunological mechanism. This communication reports experiments designed to test this hypothesis.

Two hundred and forty rats, descended from stock imported from Sprague–Dawley of Madison, Wisconsin, and commercially available in Britain, were used. They were obtained at 40 days of age and were divided into two groups by a formal randomization process. 50 mg of DMBA dissolved in 2 ml. of corn oil were given to all rats on the 50th day of life by intragastric tube. Starting on the day the DMBA was given, Group 2 was given 3 mg of cortisone acetate daily for 1 week, 2 mg of cortisone acetate daily for a second week and 1 mg of cortisone acetate daily for a third week, after which cortisone acetate treatment was terminated. This dose of cortisone acetate is believed to be adequate to replace the output of the damaged adrenal cortex during the 3-week period of its regeneration[4].

Table 1

Days after DMBA		Rats with tumours	No. of tumours Growing	Static	Regressing	Total	P
100	DMBA	28/34	11	23	4	38	n.s.
	DMBA + cortisone	18/26	10	16	3	29	
150	DMBA	30/34	13	35	10	58	n.s.
	DMBA + cortisone	20/26	14	31	7	52	
200	DMBA	31/34	17	45	13	75	n.s.
	DMBA + cortisone	20/26	15	31	10	56	

Table 2

	Mean induction period (days)	P
DMBA	71·186 ± 2·760	n.s.
DMBA + cortisone	79·696 ± 4·596	

Twenty-three rats in each group were killed 3 days after giving DMBA. The adrenals were examined histologically for evidence of massive necrosis. Rats were examined twice weekly for tumour production from the 4th week onwards and when tumours had been detected these were measured with calipers and their mean diameter recorded.

Depending on their ensuing behaviour, tumours were allocated to one of the three groups, growing, static or spontaneously regressing[1].

Massive adrenal necrosis was found in 21 out of 23 rats examined in Group 1 and in 23 out of 23 rats examined in Group 2. The numbers of growing, static and spontaneously regressing tumours 100, 150 and 200 days after DMBA are given in Table 1. The differences between the incidence of the three tumour types are not significant at these times. It is possible that the induction period of tumours might be altered by the effect of adrenocortical insufficiency and we have given the induction period of all tumours in Table 2. No significant difference exists, however, between these two groups.

It is clear that the replacement dose of cortisone acetate has not in any way influenced either the induction period of tumours or the occurrence of tumours which ultimately fail to grow or regress.

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Renal Immunofluorescence in *NZB/NZW* Mice

INVESTIGATIONS carried out by Helyer and Howie on a hybrid strain of mice derived by crossing the *NZB/BL* and *NZW* strains show that these animals died of renal failure within 8–10 months[1]. The renal lesions in these animals closely resembled those seen in human lupus nephritis. Using conventional microscopical techniques marked glomerular changes, including fibrinoid necrosis, thickening of the basement membrane and wire-loop formation were demonstrated.

The development of the renal lesions in these animals has been examined by immunofluorescent techniques. Groups of mice of both sexes were killed at monthly intervals and similar groups of *CBA/T6* mice were used as normal controls.

A concentrated globulin fraction of a rabbit anti-mouse serum was obtained by three precipitations with a half-saturated ammonium sulphate solution[2]. An electrophoretic pattern of this fraction revealed it to comprise of mostly γ-globulin and some β-globulin. The antiserum was conjugated with fluorescein isothiocyanate[3,4] and purified by dialysis and extraction with charcoal[5]. Non-specific staining materials were absorbed by mouse liver powder (Baltimore Biological Laboratory) prior to use[2,6].

Unfixed kidney tissues, which were obtained as soon as possible after killing the mice, were rapidly quenched in liquid air and 4–5μ sections were cut in a cryostat. The sections were transferred to a glass slide, washed in phosphate-buffered saline and covered with the F.I.T.C. conjugate together with a bovine albumin–rhodamine counter stain (Baltimore Biological Laboratory)[7]. Staining was allowed to proceed for 30 min and the sections mounted in buffered glycerol. Tests proving the specificity of the conjugated serum have been carried out[4,8].

Up to the age of about 6 months the glomeruli appear to be normal and are uniform in size. Although many fluoresce the intensity is notably very pale, at times ghostly, and is markedly patchy generally only involving portions of the tuft. Sharply localized thin areas of brighter fluorescence are evident in a few glomeruli. The tubular basement membranes show as fine structures, with diffuse and moderately bright fluorescence. A similar form and degree of fluorescence has been noted in the control animals examined up to the age of 12 months; this may be explained by the investigations of Tan and Kaplan[9], who showed that in the normal mouse the basement membranes of the renal parenchyma and other organs fluoresced and that the component associated with this staining was related to the β-globulin serum fraction.

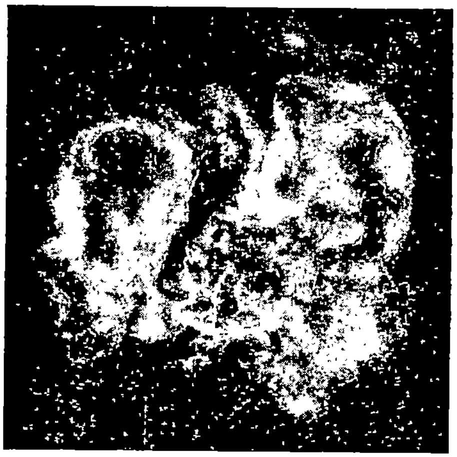

Fig. 1. Glomerulus showing thickened basement membrane and wire-loops. Marked granularity of fluorescence is evident (×420)

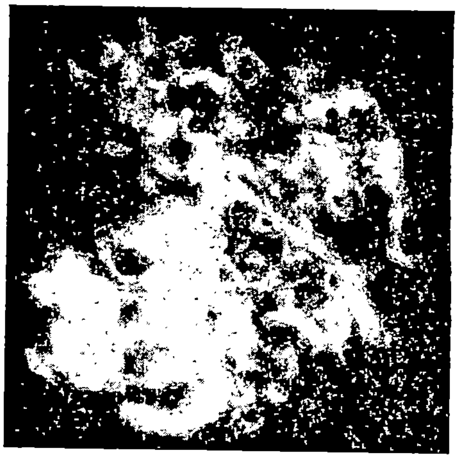

Fig. 2. Large focal lesion with dense and granular fluorescence in a glomerulus (×420)

Beyond the age of 6 months marked fluorescent changes become evident. The glomeruli are generally larger than those seen in the control animals and all fluoresce brilliantly. In all cases there is a progressive increase in thickness of the basement membrane as judged by the areas of fluorescence and, particularly in the older animals, this becomes greatly increased, often producing the appearance of wire-loops (Fig. 1). The fluorescence is irregular, patchy and, in many cases, strikingly granular. In addition occasional glomeruli present focal areas of dense, bright fluorescence involving the whole or part of a lobule (Fig. 2). An associated feature seen in some of the older animals is an increase in lobulation of the tufts. All these changes become evident sooner and more marked in the female animals.

In the tubules no cellular or basement membrane variations have been noted, but in some older animals the tubules are dilated and many are seen to contain faintly fluorescing granular material. There has been little, if any, alteration in the arteries and arterioles in mice examined up to the age of 12 months and no fluorescence has been seen, as yet, in the lymphoid aggregations that form such a striking feature in conventionally stained sections.

The close similarities seen in the renal lesions of human lupus nephritis and the *NZB/NZW* mice have already been indicated. Comparison of the fluorescent patterns seen in these cases as well as in other forms of human nephritis is possible. It is believed that such investigations would be of value in elucidating the nature of the lesions.

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IMMUNOLOGY

Dissociation of Bacterial 0 Antigen from Rabbit Erythrocytes

IT has been shown[1] that rabbit erythrocytes modified *in vitro* with 0 antigens (endotoxins) of *Salmonella typhi* or *Escherichia coli* are rapidly destroyed when injected into rabbits previously immunized with the corresponding bacterial antigen. In contrast, modified erythrocytes administered to non-immune rabbits have a normal life span despite the fact that specific antibody appears within six days after injection of the modified red blood cells. Thus, it becomes important to ascertain whether bacterial antigens attached to erythrocytes *in vitro* remain bound to the red blood cells for their entire period of survival after they are re-introduced into the animal's circulation.

Erythrocyte survival was measured, using autologous red blood cells tagged with radioactive sodium chromate (chromium-51); the half-life of chromium-51 for normal autologous rabbit erythrocytes ranged from 10·5 to 17 days[1]. Autologous rabbit erythrocytes modified with sodium hydroxide treated typhoid 0 antigen (6,000 μg/ml. of 50 per cent red cell suspension) had a half-life of chromium-51 of 10–13 days[1].

An experiment was designed to detect the presence of antigen-modified erythrocytes in the rabbit's circulation by means of direct differential agglutination[2]. With this technique erythrocytes modified with typhoid 0 antigen were readily detected when the proportion of modified to normal erythrocytes was as low as one part in 20 (5 per cent). Approximately 50–70 ml. blood was obtained from each of three rabbits the sera of which contained no detectable antibody against typhoid 0 modified erythrocytes. The red blood cells were modified with the typhoid 0 antigen[1] and were administered intravenously to the original donor animal. Blood samples were obtained from the marginal ear vein 15 min after the injection of modified cells and twice daily thereafter. The presence of modified erythrocytes in each sample was demonstrated by direct differential agglutination using potent typhoid 0 antiserum.

The results of these experiments (Table 1) show that agglutinable red blood cells were not detected 4–6 days after administration of modified erythrocytes. These observations suggest that the bacterial antigen is removed from the erythrocytes *in vivo*. Alternatively, it is conceivable that the agglutination of the modified erythrocytes *in vitro* was 'blocked' by the newly formed antibodies which appear as early as three days after administration of the modified red blood cells.

Table 1. Disappearance of agglutinable erythrocytes from the circulation of rabbits after injection of autologous erythrocytes modified with typhoid 0 antigen

Rabbit No.	Percentage of agglutinable erythrocytes			
	Day 0	Day 2	Day 4	Day 6
98	50	35	25	< 5
95	35	20	< 5	< 5
99	50	30	< 5	< 5

To resolve this problem, experiments utilizing endotoxin labelled with radioactive chromium were undertaken. Sodium-hydroxide-treated *E. coli* 0111 : *B*4 lipopolysaccharide (Difco Lot No. 132562) was labelled with chromic chloride (chromium-51) according to the method of Braude *et al.*[3]. Preliminary work with three rabbits disclosed that the life-spans of autologous erythrocytes modified with non-radioactive antigen (2,000 μg/ml. 50 per cent erythrocyte suspension) were within the normal range (half-life of chromium-51, 12, 14 and 15 days). Approximately 50 ml. blood was obtained from each of three rabbits the sera of which contained no detectable antibody against *E. coli* 0111 : *B*4. The erythrocytes were coated with the chromium-51-labelled antigen; modification of the red cells was confirmed serologically and by demonstrating uptake of radioactivity by erythrocytes. 96 per cent or more of the radioactivity in the final cell suspension was bound to erythrocytes. Autologous red cells were administered intravenously to each rabbit and blood samples were obtained 1/4, 1/2, 1, 2, 24 and 48 h after the injection. The samples were counted in a well-type scintillation detector for a period of time sufficient to ensure a counting error of less than 10 per cent. The results shown in Table 2 support the conclusion that bacterial antigen is removed from modified erythrocytes after they are re-introduced into the animal's circulation.

Table 2. Disappearance of radioactivity from circulation of rabbits after injection of autologous erythrocytes modified with chromium-51-labelled *E. coli* 0 antigen

Rabbit No.	Half-life of ^{51}Cr (min)	Percentage radioactivity remaining after 24 h
168	90	25
171	45	10
179	90	17

In view of these results it seemed advisable to re-examine *in vitro* the avidity of the binding of bacterial antigen to red blood cells[4,5]. Rabbit erythrocytes were modified with the chromium-51-labelled *E. coli* 0111 : *B*4 antigen; the concentration of radioactivity per ml. of packed red blood cells was measured before and after multiple washings with isotonic sodium chloride solution. The results shown in Table 3 demonstrate that radioactivity and presumably antigen is removed from erythrocytes by repeated washing. The observation that a 40 per cent loss of endotoxin from the erythrocytes does not alter agglutinability is not surprising because Luderitz *et al.*[6] demonstrated that one erythrocyte was able to bind approximately 100,000 molecules of an *E. coli* 0 antigen and yet only 5,000 molecules per erythrocyte were necessary for positive hæmagglutination.

Table 3. Effect of repeated washing on rabbit erythrocytes modified with chromium-51-labelled *E. coli* 0 antigen

No. of eight-vol. washings	Hæm-agglutination titre	Radioactivity c.p.m./ml. of packed erythrocytes	Cumulative loss of radioactivity (%)
0	1/800	8,576	0
6	1/800	7,705	12
12	1/800	6,333	28
18	1/800	5,195	40

In order to ascertain whether the loss of radioactivity was due to a disruption of the bond between erythrocyte and antigen, or to a loss of chromium-51 from the antigen itself, two further experiments were carried out. Chromium-51-labelled antigen was dialysed for a period of 6 h with a loss of only 2 per cent of the radioactivity into the dialysis bath. A 50 per cent suspension of erythrocytes modified with chromium-51-labelled *E. coli* antigen was dialysed for 6 h in a bath of isotonic sodium chloride solution. Despite the fact that many of the red cells were hæmolysed, less than 2 per cent of the radioactivity was lost in the dialysis bath. Therefore, it appears that the bond between the chromium-51 and the antigen is stable even after antigen is attached to erythrocytes.

In conclusion, these observations suggest that 0 antigen, or a portion of it, is removed from modified erythrocytes by repeated washing and by re-introduction of the modified red blood cells into the rabbit's circulation.

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Specific Inhibition of Anti-*D* by Human Urinary Mucoprotein

Sialic acid and polymers containing sialic acid[1-3] have been shown to specifically inhibit agglutination of *Rho*(*D*) red blood cells by specific antiserum. Urinary mucoprotein, which consists of 9·1 per cent sialic acid[4] in terms of total mucoprotein, was therefore considered as a possible inhibitor of anti-Rh. The other sugars which make up the prosthetic group of this molecule have been shown not to block specifically Rh agglutination[5].

Two separate preparations of urinary mucoprotein[6], isolated from pooled human urine and designated as *A* and *B*, respectively, were tested for their ability to inhibit the agglutination of trypsinized *Rho*(*D*) red cells by specific antiserum. The results are given in Table 1.

Table 1. Inhibition of Anti-Rh by Urinary Mucoprotein

Urinary mucoprotein preparation	Anti-Rh control	μg of mucoprotein required for inhibition
A	3+	400
B	3+	450

Optimal inhibition was obtained when red cells were added immediately following the mixing of mucoprotein and anti-Rh. Anti-C, anti-E and anti-c were not inhibited by the mucoprotein. Also, a maximal agglutinating dose of anti-D(4+) was not inhibited by the concentrations of the mucoproteins used (400 μg for *A*, 2,000 μg for *B*). 200 μg of preparation *A* inhibited anti-D after previous treatment with a 1 per cent solution of trypsin, a phenomenon somewhat analogous to that of the 'exposure' of Rh receptors on red cells treated with trypsin.

This report supports previous work suggesting an antigenic relationship between sialic acid and the D antigen.

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BIOLOGY

Probable Fertile Region of *Asteroxylon mackiei* K. and L.

Asteroxylon mackiei was first described from the Rhynie Chert by Kidston and Lang[1], but the nature of its reproductive region has remained uncertain, since the peculiar leafless axes and small pear-shaped sporangia, which they provisionally assigned to the plant, have never been found connected to its leafy stems.

During a survey of loose block material containing abundant remains of *Asteroxylon mackiei* and the other organs referred to above, a few small shoots have been discovered bearing sporangia of a quite different type interspersed with veinless 'leaves'. It is the purpose of this communication to give a preliminary description of these and to suggest that they, rather than the naked sporangiophoric axes, may represent the true fertile region of the plant.

The rather large sporangia (up to 7 mm in width when empty) are tangentially flattened and are borne laterally and independently in the shoots between the leaves. Reconstructions based on serial peel sections have shown them to be somewhat reniform. Although most of the sporangia are empty, a few have been found containing spores which are about 50μ in diameter, and it is evident that these were shed through an extended marginal slit.

Each sporangium is borne on a short vasculated pedicel which expands distally into a pad of tissue over which the spore-sac extends. In median radial section no clear distinction can be made between these two regions and the length of the pedicel thus tends to be exaggerated.

Fig. 1 shows the appearance of one of the shoots in longitudinal section bearing on the right two empty sporangia, the lower of which is cut in the median plane. Parts of at least three other sporangia are present on the left side of the section, but the distal regions of the upper

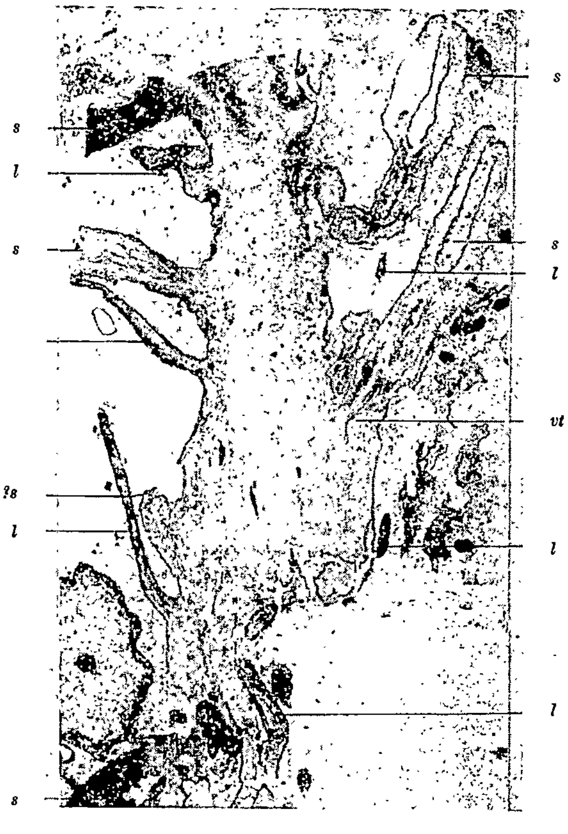

Fig. 1. Longitudinal section through leafy fertile shoot (×*c*. 8). *s*, sporangium; *l*, leaf; *vt*, vascular supply to sporangium

two have been severed and displaced by a fault in the block and lie outside the field of view.

A fortunate series of vertical sections through a shoot tip has indicated that the sporangia reached maturity only a short distance behind the apex. In this region the vascular tissue seems to consist largely of sporangial traces. Older shoot fragments bearing empty or withered sporangia have weakly developed actinosteles the xylem of which is composed of angular, fusiform tracheids with narrow wall thickenings, closely resembling those of *A. mackiei*. No clear evidence of leaf traces has been found, although this may be due to the very indifferent preservation of the material.

The provisional assignation of the leafless axes and small sporangia to *A. mackiei* by Kidston and Lang was based on evidence of close and constant association in the apparent absence of any alternative reproductive organs. Some support for this was later seen in the structure of the more completely preserved German species, *Asteroxylon elberfeldense*, Kräusel and Weyland[2], the leafy stems of which were clearly shown to terminate in leafless sporangium-bearing branches. It is difficult, however, to know how much weight should be attached to this indirect evidence which assumes a close natural relationship between the two plants. Although there are several unquestionable points of resemblance, there are likewise a number of well-marked differences the importance of which may require re-evaluation. Thus, while both leafy and leafless shoots of *A. elberfeldense* are circinately vernate, there is no suggestion of this in the case of *A. mackiei*. Furthermore, the tracheids of *A. elberfeldense* are scalariform or roundly pitted, while those of *A. mackiei* have narrow spiral thickenings; and whereas the stele of the larger stems of *A. elberfeldense* is medullated, no pith is present in *A. mackiei*. So far as the Rhynie species is concerned there are histological difficulties in the way of matching-up the leafy and leafless shoots. In addition to marked differences in tracheid size, those in the leafless axes have never been found to exhibit any traces of wall thickening or pitting although very many examples have been carefully examined (cf. Kidston and Lang). The tracheids of the leafy stems of *A. mackiei*, on the other hand, frequently show clearly recognizable wall thickenings even when preservation, in other respects, is extremely poor. While these differences do not preclude the possibility that the shoots are closely related parts of the same plant, they certainly lend no direct support to such an interpretation of their nature. Assuming, as seems likely, that either one or the other of the two kinds of fertile axis belongs to *Asteroxylon mackiei*, available evidence tends to favour rather strongly the leafy rather than the leafless type. Indeed, it can be said that, had it not been for the sporangia, the new fossils would have been passed over without hesitation as young shoots of that plant. While certainty regarding their nature is clearly impossible owing to the fragmentary condition of the remains, the probability that they represent fertile branches of *Asteroxylon mackiei* seems sufficiently high to justify provisionally assigning them to the plant in place of the leafless sporangiophoric axes. Consequent on this, the latter must be re-interpreted as reproductive portions of an incompletely known, but quite distinct, type of plant for which the name *Nothia aphylla* gen. et sp. nov is proposed.

In its habit and anatomy *A. mackiei* has been compared with *Lycopodium*, and Scott[3] ventured to suggest that "were it not for the peculiar axes associated with it, [it] might be regarded as an early representative, or at least a precursor of the lycopod series". While the new fertile shoots (if correctly assigned) do not establish the plant as a lycopod, they appreciably and perhaps significantly increase its resemblance to one.

A comparison can also be made, however, between the new sporangia and those of the leafless *Zosterophyllum* and it is possible to see in the leafy, fertile shoots a degree of structural complexity which is, in certain respects, intermediate between that of a bractless spike of reniform sporangia (as, for example, in *Zosterophyllum*) and a typical lycopod strobilus of sporophylls. But in the present state of our knowledge it would be facile to suppose that these represent a series of evolutionarily related forms.

Although the sporangia of *Zosterophyllum* are rather lycopod-like in their shape and manner of dehiscence (as Lang and Cookson[4] and Bower[5] have pointed out), any connexion between the Zosterophyllaceae and the Lycopsida, if it exists at all, may well be remote and indirect. Furthermore, while *A. mackiei* exhibits a number of morphological features which it would not be altogether surprising to find in a lycopod precursor—particularly if conceived in terms of the enation theory—the historical record of the group seriously conflicts with such an interpretation of the plant. It must, therefore, be rejected unless the view is taken that at least some of the more simply constructed early Devonian plants may have been relict types.

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Thermophilic Fungi associated with Nigerian Oil Palm Produce

During the course of investigations into factors affecting the quality of palm kernels (that is, the kernels of the oil palm, *Elaeis guineensis* Jacq.) in Nigeria, it was observed that temperatures of the order of 60° C frequently developed within the stacks of undecorticated kernels that accumulated at oil mills[1]. It was further noted that fungal mycelia appeared to be growing profusely within these stacks.

Routine isolations were made from the interiors of a number of such stacks, using a palm oil/peptone/agar medium, incubated at 30° C—a temperature near the normal Nigerian ambient. These yielded predominantly *Rhizopus nigricans* Ehrenberg and occasionally other lipolytic fungi commonly associated with oleaginous material in Nigeria[2].

Thermophilic fungi are frequently found in self-heating plant materials[3,4]. In view of this, further isolations were made, using the same medium, but incubating the isolation plates at 50° C. Under these conditions, an entirely different spectrum of fungal species developed. From thirty-six samples examined, originating from oil mills in various parts of Eastern Nigeria, one or more of three fungi were consistently isolated. A *Thermomyces* species (I.M.I. 96473) was found to be present in every case, *Chaetomium thermophile* La Touche (I.M.I. 96472) was present in twenty-seven of the thirty-six samples, and an ascosporic *Penicillium* sp. (I.M.I. 96474), similar to *P. duponti* Griffon et Maublanc emend. Emerson, in only five: no other fungi developed from any sample, at this temperature.

C. thermophile was originally reported[5] in the United Kingdom, as occurring on straw compost, and as having an optimal growth temperature range of 40°–55° C. This species has also been isolated from coastal grassland soils in the United Kingdom[6]. *P. duponti sensu stricto* has previously been reported from retting guayule in California, when it had an optimal growth temperature range of 47°–50° C, and from fresh manure and wet hay in France[7]. The third organism appears to be a new *Thermomyces* species, distinct from any previously re-

Table 1

		Temperature (°C)	
	Optimum	Maximum	Minimum
Thermomyces sp.	42–47	60–61	31–35
C. thermophile	47–52	60–61	26–30
Penicillium sp.	43–52	58–59	31–35

ported thermophilic *Thermomyces* species. A full description of this species will be published elsewhere.

The temperature/growth relationships found for the Nigerian isolates are as given in Table 1.

All three organisms were found, by the use of an oil/nile blue sulphate agar medium[8], to be actively lipolytic, and were also found capable of utilizing a variety of lipids as carbon sources. When tested on a cellulose-containing medium[9], *C. thermophile* was found to be highly cellulolytic, while the other species were not.

These observations indicate that the three organisms are well adapted to growth in the highly specialized environment from which they have been isolated. In view of their lipolytic nature, these thermophilic fungi may play a significant part in the biodeterioration of oil palm produce during processing and storage[10], which is a problem of considerable importance in Nigeria and other producer countries.

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Influence of Nitrogen Nutrition on Bud and Rhizome Development in *Agropyron repens* L. Beauv.

A PRELIMINARY investigation of the effect of the nitrogen supply on the growth of *Agropyron repens* L. Beauv. showed that reducing the nitrogen level caused an increase in the number of rhizomes produced. This increase in rhizome production was associated with a marked suppression of tillering and seemed to have resulted from a tendency for buds to develop as rhizomes rather than as tillers at the lower nitrogen-levels. A further experiment was therefore designed to investigate this effect more critically.

Seeds were germinated on moist filter paper at an alternating temperature of 15°–25° C and an 8-h photoperiod. The seedlings were selected for uniformity when the coleoptile was approximately 1 cm in length and were planted in 5-in. plastic pots containing 16-mesh quartz sand previously washed with distilled water. The sand was irrigated with Hoagland's solution[1] modified to provide a low nitrogen-level of 2·1 p.p.m. and a high level of 315 p.p.m., these concentrations being obtained by equimolar substitution of K_2SO_4 and $CaCl_2$ for KNO_3 and $Ca(NO_3)_2\ 4H_2O$ respectively.

Twenty plants were grown at each of these nitrogen-levels throughout the experiment. A third set of plants was changed from the low to the high nitrogen-level when the bud in the axil of leaf 1 had just emerged. Previous observations had established that this bud may develop either as a rhizome or as a tiller, being rather variable in this respect. In the present experiment the bud emerged from the base of the leaf sheath in 18 of the 20 plants and was clearly developing as a rhizome. These plants received the increased nitrogen supply when the young rhizomes were still only 1–3 mm in length. In the two remaining plants of this treatment the bud developed as a tiller and the high nitrogen solution was provided when the tiller emerged from the top of the leaf sheath.

The appropriate nutrient solutions were applied in excess at 2-day intervals, evaporation losses being replaced with demineralized water. The experiment was conducted in the greenhouse during the autumn of 1963. A bank of sixteen 8-ft. ('Gro-lux') fluorescent tubes extended the photoperiod to 18 h and provided an additional light intensity of 1,500 ft.-candles. The temperature ranged from 13° to 40° C during the day and from 10° to 21° C at night.

Final observations on bud development were recorded when the sixth leaf of the primary shoot had fully emerged. It is clear from the data in Table 1 that the mode of development of the bud in the axil of leaf 1 was largely determined by the nitrogen supply. At the low nitrogen-level, 90 per cent of these buds developed as rhizomes and 5 per cent as tillers; whereas at the high level of nutrition these proportions were approximately reversed, only 20 per cent developing as rhizomes and 70 per cent as tillers.

Table 1. EFFECT OF THE NITROGEN-LEVEL ON BUD AND RHIZOME DEVELOPMENT IN *Agropyron repens* L. BEAUV.

The data refer only to the bud in the axil of leaf 1

	% buds* developing as:		
Nitrogen-level	Rhizomes	Rhizomes with terminal shoot	Tillers
Low	90 (18)	5 (1)	5 (1)
High	20 (4)	10 (2)	70 (14)
Low to high†	42 (8)	47 (9)	11 (2)

* Number of buds shown in parenthesis.

† Percentages based on 19 plants, one plant having died.

Where a change from low to high nitrogen was made at the bud emergence stage 47 per cent of the young rhizomes, after reaching a length of 1–3 cm, were transformed into shoots. The upward curvature of the rhizome which preceded the transition from rhizome to shoot development was first apparent 5–8 days after the nitrogen supply had been increased.

Data on the behaviour of the bud in the axil of leaf 2 showed a similar effect of the nitrogen supply. At the high nitrogen-level all these buds developed as tillers, while 45 per cent developed as rhizomes at the lower level.

Subsequent experiments, in which similar nitrogen-levels were used, have given essentially the same results in spite of large differences in other environmental factors such as temperature and light intensity. It is therefore apparent that the nitrogen supply is a morphogenetic factor of considerable significance in bud development and seems likely to play an important part in controlling the balance between rhizome and shoot production.

Rhizome behaviour in this species has also been examined by Palmer[2], who showed that the formation of a terminal shoot can be experimentally induced by orientating the rhizome so that the apex points vertically upwards. He also showed that isolated rhizomes, which normally give rise to a terminal shoot, can be prevented from doing so and will continue to grow as rhizomes when rotated on a klinostat. This gravitational influence, when considered in relation to the well-defined nutritional effect revealed by the present experiment, raises the question as to whether these two effects are in some way related or whether the mechanisms involved are physiologically distinct.

It will also be of interest to determine the extent to which the results of the present investigation are applicable to other rhizomatous species.

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A Semi-quantitative Method of Counting Nematode-trapping Fungi in Soil

The usual method of observing nematode-trapping fungi in soil, as outlined by Duddington[1], consists of sprinkling soil in a Petri dish, dispersing in cool but still liquid agar medium, and then examining the whole dish for the trapping structures of these fungi after several days of incubation. Because of the uneven size and distribution of soil particles, and the large area to be examined, this method is difficult and unreliable as a means of estimating numbers of trapping fungi present. However, as outlined here, an adaptation of this method can be used to obtain semi-quantitative estimates.

As a first step in this method, each soil sample is reduced to a slurry or mud of even consistency by mixing in a Waring blender with an approximately equal volume of water. A 0·5-ml. aliquot of each slurry is then pipetted into an empty Petri dish and dispersed in 30 ml. cooled but still liquid 3 per cent water agar. Long narrow strips (3 mm × 60 mm are convenient) of the hardened soil—agar mixtures are cut from these dishes by means of two scalpels tied together with blades parallel. These strips are transferred by spatula to the centres of empty Petri dishes, and the plates are then flooded with 30 ml. of 'Difco' corn meal agar adjusted to 10 per cent of the specified concentration of nutrients. Finally, 1-ml. volumes of a water suspension of saprophytic nematodes are pipetted over the soil—agar strips. The dishes are then set aside to incubate.

Several species of saprophytic nematodes from soil can be grown in Petri dishes in an agar medium supporting good growth of common soil bacteria on which the nematodes feed[2]. The nematodes can later be extracted from this medium by a modified Baermann funnel[3] and standardized to approximately 1,000 nematodes per ml. of water.

After several days of incubation, the soil—agar dishes are examined for the presence of trapping structures in hyphæ which have grown out from the soil—agar strips. Provided these examinations are made in areas close by and parallel to the soil—agar strips, and before extensive branching of hyphæ has occurred, it is generally possible to distinguish between hyphæ originating from separate loci within the strips. Such hyphæ bearing one or more trapping structures are each given a count of one. The low nutrient content of the corn meal agar is a distinct aid in these examinations, both in reducing the extensiveness of branching in hyphæ of trapping fungi, and in reducing growth of contaminating fungi.

By standardizing the procedure described here, counts per dish can be converted to counts per gram of soil. They are then directly comparable to other microbial counts obtained by conventional soil-dilution plating techniques.

We have obtained estimates of nematode-trapping fungi in soil from a replicated field-plot experiment in which soil-treatment variables consisting of plant trash incorporation and nematocide fumigation both significantly affected populations of these fungi.

We thank the Director of this Institute for permission to publish these results.

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Induction of Phototaxis in *Chlamydomonas snowiae* by Indolyl-3-acetic Acid and Ethylenediamine Tetraacetic Acid

In a previous report it was shown that acetic acid and certain other substances could prevent a phototactic response of cells of *Chlamydomonas* without impairing their motility[1]. It was of interest to see whether the reverse action could also be obtained, that is, to induce phototaxis in motile cells which do not respond to light. It will be shown that indolyl-3-acetic acid (IAA) and ethylenediamine tetraacetic acid (EDTA) induce such responses.

The cultures of *Chlamydomonas snowiae* were grown and handled, and phototaxis was measured as previously described[1–3]. The medium containing the algae was adjusted to the required *p*H with hydrochloric acid or potassium hydroxide. In the controls phototaxis was then assayed while to the experiments a suitable amount of the reagent was added and the *p*H checked again.

The effect of IAA on the phototactic response of a fully motile culture of *Chlamydomonas snowiae* is shown in Table 1. The response to IAA is seen to be dependent on concentration and *p*H. Clearly IAA is effective in the non-ionized form, presumably because it is this form which penetrates into the cell. IAA at concentrations between 10^{-3} and 10^{-4} M was able to induce phototaxis at *p*H 6·0 in about 45 out of 50 replicates of the experiments. In a few cases, about 5–10 per cent of the replicates, it failed to induce phototaxis in motile culture, apparently because lack of phototactic response was due to other causes. Analogues of IAA, indole butyric acid and indole propionic acid as well as naphthalene acetic acid and 2,4 dichlorophenoxyacetic acids were also effective, but higher concentrations were required and the induction of response was less consistent than with IAA.

Table 1. Induction of Phototactic Response by IAA at Various Concentrations and Various *p*H

*p*H IAA conc. M	5·4	5·6	5·8	6·1	6·3
0 (control)	−	−	−	−	−
10^{-4}	+ + +	+ + +	+ + +	+ + +	+ + +
$2 \cdot 10^{-5}$	+ +	+ + +	+ + +	+	−
10^{-5}	+ + +	+ +	+ +	−	−
10^{-6}	±	±	−	−	−

−, no response. +, positive response. + + +, very strong response.

Not only did IAA induce phototaxis, but it was also able to reverse to a considerable extent the inhibitory action of acetic acid on the phototactic response. The phototactic response of a culture responding well to light was inhibited by the addition of acetic acid at *p*H values between 6·0 and 5·2. Addition of 10^{-4} M IAA did not reverse the inhibitory action. However, when the IAA concentration was raised to 10^{-3} M, then phototaxis was fully restored between *p*H 5·8 and 5·4 and almost fully at *p*H 6·0 and *p*H 5·2. IAA did not reverse the action of detergents such as 'Tween 80' on phototaxis. Reversal of inhibitory effects could also not be obtained consistently by the IAA analogues.

Other compounds were tested for their ability to induce phototactic response in motile, non-responding cultures of

Chlamydomonas; EDTA at a concentration of 5×10^{-5} M and 10^{-5} M had the same effect as IAA. At higher concentrations, 10^{-4} M and above, EDTA inhibits motility of the organism. The action of EDTA was not so sharply *p*H dependent as that of IAA, being equally effective at *p*H 6·0 and 5·6 at a concentration of 5×10^{-5} M.

It is at present difficult to conjecture about the possible mode of action of IAA and EDTA in inducing phototactic responses and in reversing the inhibitory action of acetic acid. Probably IAA does not act here as a conventional hormone. Although both IAA and EDTA can act as chelates, for example, of calcium it seems unlikely that they do so here, in view of the *p*H range in which they are active and the very weak chelating capacity of IAA. However, it must be noted that IAA and EDTA may be regarded as derivatives of acetic acid. This, together with the interaction of IAA with the latter, makes it possible that they compete for some specific site within the flagella or cell. This site would determine the correct translation of the impulses sent from the light receptor into the flagella. Correct translation results in flagellar beat such as to orient the organism toward the light; prevention of translation also prevents oriented movement.

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Actions and Dosage of Chloralose

Many books recommend chloralose as an anæsthetic for laboratory animals and give a dosage rate of 40–80 mg/kg body-wt.; the drug is considered particularly useful for animals in which autonomic reflexes are to be preserved. I have recently carried out a series of experiments in the dog and rabbit, and in no case did surgical anæsthesia result from intravenous injections of 70 mg/kg or less. If chloralose was used after induction with ether slight improvement was seen, but surgery was still not possible. The heart rate was slowed by 30 per cent in these experiments and excessive secretions caused respiratory embarrassment.

The action of chloralose has not been well understood in the past: "The action is a remarkable one, for the animal does not appear to pass beyond a stage of narcosis, and paddling movements of the legs or nodding of the head may appear throughout. . . . It is suggested that the condition is possibly one of hypnosis superimposed on narcosis, rather than anæsthesia"[1]. Electrocorticographic recordings now show that it makes the Betz cells susceptible to sensory stimuli[2], and that the convulsive response to slight stimulus which readily occurs under chloralose is a cortical reaction, for "chloralose, like the convulsants, facilitates transmission in a reflex arc including the cortex"[3]. Grieg[4] described these convulsions as "strychnine-like". Shukla *et al.*[5], studying its effect on autonomic reflexes, found that it slowed the heart rate by 50 per cent, while Kochmann[6] found that "it reinforces some actions of epinephrine and acts like atropine in reducing vagal activity".

In view of the fact that chloralose does not affect the electrical activity of the brain in the same way as other drugs known to be general anæsthetics, and bearing in mind that it does not leave autonomic reflexes unaltered, it would seem that some substitute should be sought for this drug; a great variety of new substances acting on the central nervous system have been developed since the Second World War, and traditional procedures may not be justified in the light of present knowledge. The fact that chloralose is not used in human medicine casts further doubt on the validity of its use as an anæsthetic for laboratory animals.

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Some Negative Results in the Search for a Lethal Effect of Magnetic Fields on Biological Materials

During recent years there has been an increasing number of reports in the literature of the ability of magnetic fields to affect biological systems. Gerencser and Barnothy[1] noted differences from control of cultures exposed to 15,000 gauss, while Butler and Dean[2] found growth inhibition in tissue culture of *KB* cells exposed to 4,000-gauss magnetic fields. Maclean[3], using intense and 'mild' magnetic fields of unspecified strengths, reported various effects on tumour-bearing mice, but since only 12 animals were used for the entire investigation and these were divided into 3 groups, the results can have no statistical significance. Negative reports have appeared too, and in the most recent of these Halpern and Greene[4] reported that the growth rate of HeLa cells cultured in a 1,200-gauss magnetic field was not significantly different from controls. This communication extends this negative finding to much higher magnetic field strengths. Three separate experiments were performed, using HeLa cells cultured *in vitro*.

The cells were cultured by the methods described elsewhere[5]. In each experiment 200 cells were inoculated into either 50 mm 'Falcon' plastic Petri dishes or into 'Falcon' plastic culture flasks. Two containers were reserved for controls and two were subjected to a magnetic field for various lengths of time as detailed here. The cells were afterwards incubated for 10 days at 37° C in an incubator continuously flushed with a mixture of 5 per cent carbon dioxide and 95 per cent air. At the end of this period the cells were fixed with 10 per cent formalin in normal saline, stained with crystal violet, and the number of macroscopic colonies on each dish or flask counted. The following treatment conditions were used.

(*a*) A steady field of 5,000-gauss was obtained with a permanent magnet having 2-in. diameter pole pieces which were just large enough to cover the 50 mm plastic Petri dishes. The magnet was mounted inside the incubator and the treated plates were subjected to the magnetic field throughout the 10-day period of incubation. The control plates were kept in the same incubator at approximately 12 in. from the pole pieces. Approximately 150 colonies were counted in each dish, but there was no significant difference between the number of colonies counted on the treated against the control dishes (Table 1). There was no obvious difference in the size of the colonies, or their morphology.

(*b*, *c*) To obtain stronger magnetic fields, it was necessary to use electromagnets. Consequently it was not possible to mount the magnets inside the incubator and, for this and other reasons, treatment times were much shorter.

In one experiment an electromagnet was used which had tapered pole pieces, 4-in.–2-in. diameter, with a gap between the poles of 15 mm. Two culture flasks were exposed to a magnetic field of 27 kilogauss for a period of

Table 1

Exp.	Field strength (gauss)	Time of exposure	No. of colonies on control dish or flask	No. of colonies on treated dish or flask
(*a*)	5,000	10 days	150	149
			145	158
(*b*)	27,000	1 h	168	175
			180	165
(*c*)	77,000	15 min	143	161
			151	148

1 h. In another experiment, two culture flasks were placed inside a solenoid, 2-in. diameter, and exposed to a magnetic field of 77 kilogauss for a period of 15 min. In both cases control cultures were handled in a similar manner except that the magnets were not energized. In both experiments, approximately 150 colonies were counted on both treated and control flasks, there being no significant difference between them, either in cell number or morphology (Table 1).

It would seem, therefore, that gross lethal effects on mammalian cells are not produced either by powerful magnetic fields applied for short periods, or by lower field strengths extending over many cell generation times, The work recorded here is subject to the same fundamental limitation as previous reports in the literature—namely, the necessity of using permanent magnets for long-term experiments, with a consequent limit to the field strength of 5–15 kilogauss. For fields higher than this, it is necessary to use electromagnets which consume such vast quantities of electrical power that long-term experiments are out of the question. An obvious alternative would be to use super-conducting magnets, which can provide fields of the order of 50 kilogauss for extended periods at a nominal cost.

We thank Dr. F. Ellis for his advice; Prof. B. Bleaney for the use of the facilities in the Clarendon Laboratory; Dr. N. Kurti and Mr. M. F. Wood for the use of the high-power solenoid; and Mr. J. Good for assistance in the high-field experiment.

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A Persistent Diurnal Rhythm in Photosynthetic Capacity

IN 1939, Meyer[1] discovered a daily rhythm in the rate of photosynthesis in *Ceratophyllum*, a fresh-water angiosperm. In 1957, similar rhythms were found in natural populations of marine phytoplankton[2,3]. Four years later, Hastings *et al.*[4], working on the photosynthetic-capacity rhythm in the marine dinoflagellate *Gonyaulax*, demonstrated that these rhythms would persist for a few days in the laboratory in the absence of day–night environmental cycles, that is, in continuous dim illumination and constant temperature. Negative evidence was presented by Blinks and Givan[5], who performed experiments which suggested the absence of photosynthetic rhythms in 13 species of marine littoral algae. In view of the different results, we have undertaken comparative investigations in an attempt to establish the distribution of persistent rhythms in marine flora.

Pure cultures of the marine diatom, *Phaeodactylum tricornutum*, were used in the work reported here. Cultures of this algae were grown in Guillard's medium *f* (ref. 6) and maintained in a light régime of 12 h of illumination (600 ft.-candles from 'cool white' fluorescent tubes) alternating with 12 h of darkness. When the cultures approached the stationary phase of growth, periodic measurements of photosynthesis were commenced. At the midpoints of each light and dark period, 3 or 4 10-ml. samples were withdrawn from a parent culture and incubated with 1 μc. $Na_2C^{14}O_3$ for 15 min at 600 ft.-candles. Usually, one of these samples served as a 'dark bottle' control, that is, it was incubated in the dark and used to correct for any adsorption of carbon-14 on the cells, etc. At the end of the incubation period the reaction was stopped by adding 2-ml. 1 N hydrochloric acid to the sample; the cells were then collected on a 'Millipore' filter, washed with 5-ml. 0·1 N hydrochloric acid, dried in a desiccator, and the uptake of carbon-14 measured. Concurrently, aliquots of the culture were extracted with methanol and the chlorophyll *a* content determined spectrophotometrically. Cell counts were also made at each sampling time.

This method measured the capacity, or the rate, at which the algae could maintain photosynthesis under a given set of conditions; and, in the experimental design described here, compared the rate at which photosynthesis could proceed at night (when, of course, the photosynthetic machinery is normally inactive) with the midday rate. Fig. 1 shows the results of these measurements; photosynthesis was maximal during the light period and minimal during the dark period, the difference being more than three-fold. (Using identical methods, we found a similar rhythm in the dinoflagellate, *Gymnodinium nelsoni*.) It is clear that some physiological change must have taken place in the cells, which resulted in an altered capacity of the cells to maintain photosynthesis. This change could have been induced in the cells by the different light conditions to which they were subjected (either 6 h of light or darkness) immediately prior to the time at which the rate-measurements were made; or the fluctuations could be controlled by a biological clock, the oscillations being relatively independent of light–dark cycles. By following photosynthesis in cells maintained in continuous illumination—as was done in the next experiment—this problem was resolved.

Cultures were now first grown in alternating cycles of light and darkness for 10 days and then placed in either continuous darkness or in various intensities of continuous light. On the 2 days prior to placing the cultures in static conditions, periodic photosynthetic-rate measurements were made to make sure a rhythm was present; this done, the cultures were then placed in continuous light or darkness at a constant temperature (15° C). As before, and

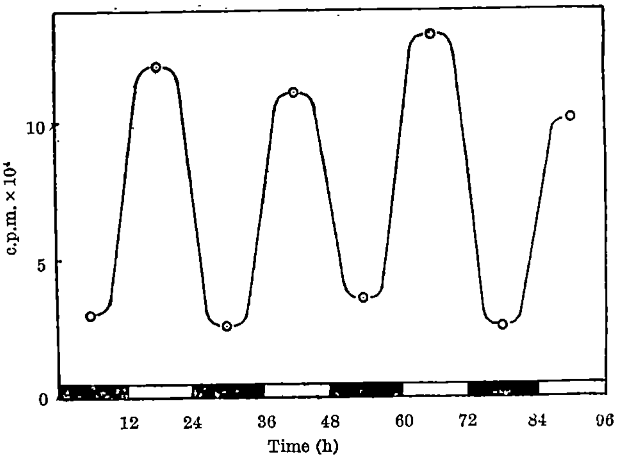

Fig. 1. Photosynthetic-capacity rhythm in *Phaeodactylum tricornutum*. Shaded blocks along abscissa represent times of darkness; open blocks represent periods of light (600 ft.-candles). Ordinate, amount of radioactive carbon assimilated

Table 1. Chlorophyll *a* Content of 10-ml. Samples of *Phaeodactylum tricornutum* Cultures (μg chlorophyll/ml.)

Light treatment	Light period $\bar{x}$	Light period *S.D.*	Dark period $\bar{x}$	Dark period *S.D.*
Constant darkness	1·16	0·05	1·12	0·10
20 ft.-candles	1·37	0·18	1·54	0·23
40 ft.-candles	1·52	0·26	1·53	0·29
80 ft.-candles	1·44	0·21	1·60	0·21

$\bar{x}$, mean of 4–5 chlorophyll determinations. *S.D.*, standard deviation.

in exactly the same manner, photosynthetic rates were determined at 12-h intervals. The results are seen in Fig. 2. In constant darkness the rhythm is not expressed. At 20 ft.-candles, one oscillation—about halved in amplitude—occurs and then all indication of the rhythm disappears. At 40 and 80 ft.-candles, the rhythm will persist for at least three cycles and is characterized by a gradual damping out. As is universally the case of biological rhythm, continuous bright light (600 ft.-candles in this case) inhibits the expression of the rhythm.

The photosynthetic rhythm could well be an indirect result of an underlying rhythm in photosynthetic-pigment content. Mitrakos *et al.*[7] have demonstrated the presence of just this sort of rhythm in the angiosperm *Perilla ocymoides*. They found that the carotenoid and the chlorophyll *a* and *b* content of this plant fluctuated in a diurnal pattern; and that these rhythmic fluctuations would persist in constant light and temperature. Yentsch and Ryther[3] also found diurnal variations in chlorophyll content and in the rate of chlorophyll synthesis, the maxima of which corresponded to the maxima in the photosynthetic rhythm they were investigating. On the other hand, Hastings *et al.*[4], working on the photosynthetic rhythm in *Gonyaulax*, found no change in either the peridinin (the main carotenoid of this dinoflagellate) or the chlorophyll content, which corresponded to the rhythm in photosynthesis. Doty and Oguri[2] report similar findings. Table 1 depicts the differences that we found in chlorophyll *a* content in the various points of the cycles reported here. It is obvious that these differences are not responsible for the photosynthetic rhythm in *Phaeodactylum*; in fact, the day-time chlorophyll values were slightly lower than the night-time ones; but, as can be seen from the size of the standard deviations, the values are not significantly different from one another.

The data also suggest a rhythm in cell division, for virtually all increases in cell number took place during the last half of the dark period and the first half of the light period. Population increases also continued to take place in the same manner in constant conditions. Sweeney and Hastings[8] have reported a similar rhythm in *Gonyaulax*.

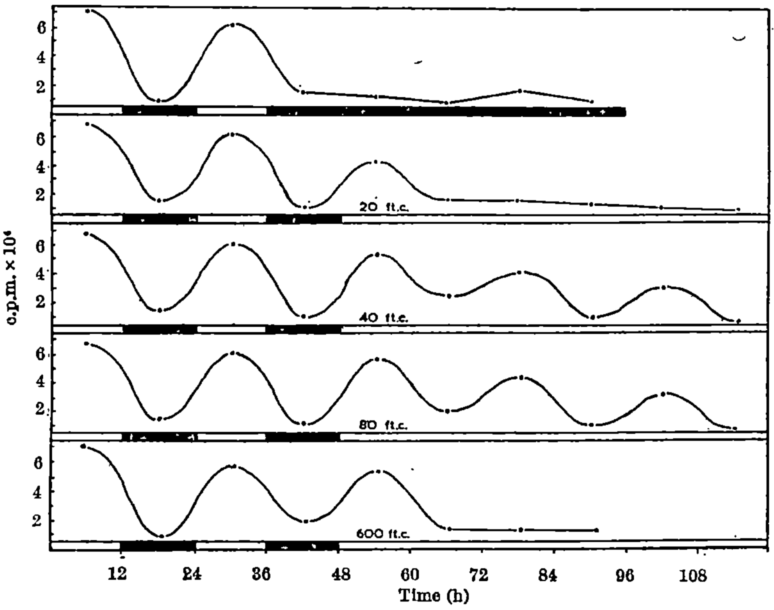

Fig. 2. Persistent nature of photosynthetic-capacity rhythm in various light intensities. Shaded areas are times of darkness; open bars represent periods of illumination (light intensities indicated on figure). Ordinate, amount of radioactive carbon assimilated

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VETERINARY SCIENCE

Enzootic Abortion of Ewes

Enzootic abortion of ewes (EAE) was originally described in Scotland by Stamp *et al.* in 1950[1], and since then the disease has been recognized in several other countries including the United States. The disease has recently been observed in California[2]. A somewhat analogous disease of cattle—epizootic bovine abortion (EBA)—has been recognized in this State for many years. Both diseases are caused by members of the psittacosis lymphogranuloma-venereum (PLGV) group of agents. The placenta is the primary site of predilection for the multiplication of the EAE agent, and the resulting placentitis is presumed to lead to abortion. In EBA, placentitis, although occasionally present, is not so obvious as the changes in the fœtus itself which include a generalized vasculitis with widely distributed petechiæ and focal, generalized hyperplasia of the reticuloendothelial system[3]. The birth of premature, weak young, many of which fail to survive, is a feature of both diseases.

During investigations primarily concerned with the cattle disease, pregnant ewes were inoculated with the agent of EBA. The resulting syndrome in these ewes differed in no substantial way from that of EAE. We did, however, recognize histological changes in the aborted ovine fœtuses. Afterwards, EAE was recognized as a naturally occurring disease in local sheep and the EAE agent was recovered. A group of pregnant ewes was inoculated with this agent, and when these ewes later aborted tissues from their fœtuses were also examined. It is our purpose here to describe briefly the histological changes which have been observed in ovine fœtuses aborted as a result of infection with either EBA or EAE agents.

No gross lesions were observed in the aborted fœtuses although some, which had

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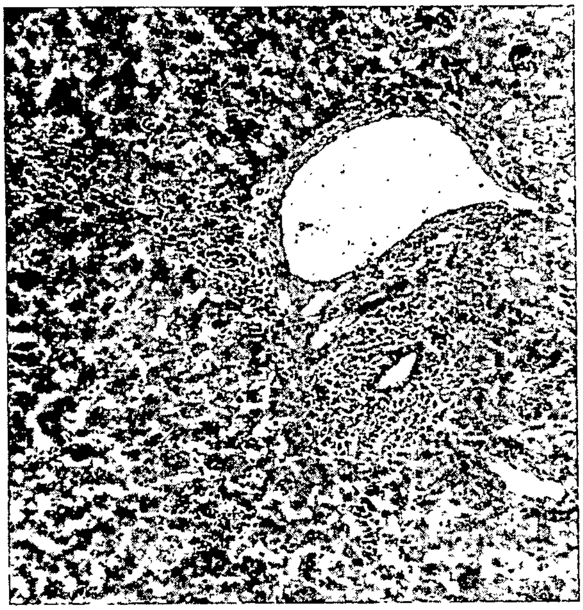

Fig. 1. Perivascular accumulation of reticuloendothelial cells in the liver of a lamb fœtus infected with epizootic bovine abortion agent. The discrete, darkly staining cells are hæmopoietic elements

died and autolysed *in utero*, had blood-stained fluid in the serous cavities. Microscopically, focal accumulations of reticuloendothelial cells were present in the organs and tissues of most aborted fœtuses. In some foci, particularly where necrosis was present, moderate numbers of neutrophils were also present. The foci were always oriented around blood vessels, and in the liver these reactive cells formed thick cuffs around the vessels of the portal triad. Any tissue of the body could be involved in any one fœtus, but the distribution, size, and incidence of the changes were not consistent. The liver was the organ most regularly involved. The proliferated reticuloendothelial elements were often present with the more darkly staining hæmopoietic cells (Fig. 1). Differentiation was usually possible on the basis of distribution and tinctorial quality. Among the other organs in which these reactive foci were observed were : lung, kidney, spleen, lymph node, heart muscle, and skeletal muscle. Occasionally, aborted fœtuses did not have demonstrable changes in their internal organs even though typical elementary bodies were observed in placental smears.

In parallel with the histological examinations, isolation of the agent from various fœtal tissues in the yolk sacs of embryonating hen eggs confirmed the presence of the agent in those tissues in which the pathological changes were occurring. Complement fixation tests on the sera of the dams, bacteriological examination of fœtal tissues and inoculation of pregnant ewes with normal yolk sac material further confirmed that the changes present in the aborted fœtuses were in fact the result of invasion by either the EBA or the EAE agent.

It should be emphasized that placentitis is the major pathological change seen when pregnant ewes are infected with either EAE or EBA agents, but the presence of lesions in the internal organs of lambs born to infected ewes may be important in the neonatal mortality seen in EAE. It is unlikely, however, that these changes are a specific response. Indeed, similar changes have been described in lamb fœtuses aborted as the result of toxoplasmosis[4]. In general a period of at least 60 days intervened between experimental inoculation of EAE or EBA agents and expulsion of the fœtus. It is felt that there is an immunological basis for the lesions related to the engulfment of antigen by reticuloendothelial cells. Because the antigen persists and increases within the cell it is not in fact effectively removed by the macrophages. The generalized distribution of the changes in the fœtus indicates that invasion of the fœtal blood stream occurs. These observations again direct attention to the very close relationships that exist among various members of the PLGV group of agents—particularly those that have been implicated in various diseases in ruminant animals.

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Inhibitory Effect of Lactation on the Breeding Activity of the Ewe

In the course of investigations on the natural breeding activity of a flock of 'Clun' sheep on the College Farm, 37 cases have been recorded, over several seasons in which entire rams have run continuously with the flock, of ewes lambing in one season and conceiving again in the same season. Some of these ewes have lambed in the autumn and conceived again in the winter, and others have lambed in the late winter and conceived again in the spring.

From results available it is possible to compare the inhibitory effects of lactation on renewed breeding activity according to time of lambing. This is shown in Fig. 1.

Of 21 ewes considered to be autumn lambers, all were successfully mated again during the winter with a mean number of days from lambing to next conception of 41·86 ± 2·29 days. Moreover, with the exception of one ewe which lost its single lamb at birth, all these ewes were in full lactation at the time of next conception. The mean autumn lambing date was November 2 ± 6·72 days.

Of 16 ewes considered to be late winter lambers, which were successfully mated again in the spring, the mean number of days from lambing to next conception was 137·6 ± 4·78 days. All the ewes lambed by January 30, the mean lambing date being January 13 ± 2·25 days, and the majority, 13 out of 16, were weaned completely by April 16. In the case of one ewe, partial weaning occurred on April 16 by the removal of a larger lamb

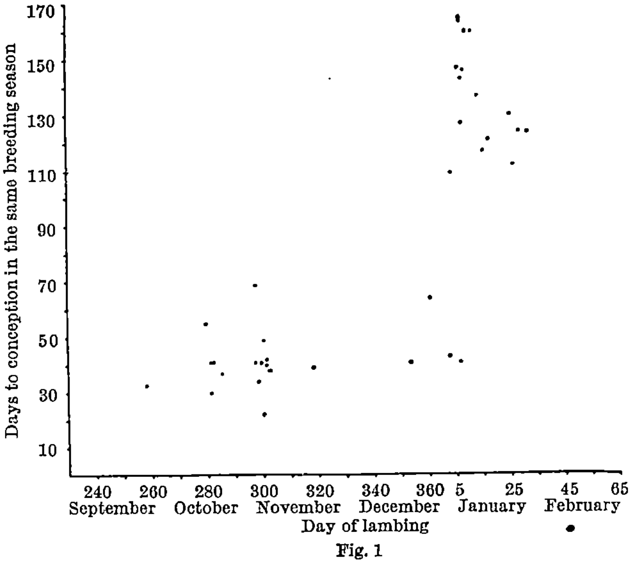

Fig. 1

from a set of twins, allowing a considerably smaller lamb to remain for a much longer period extending beyond the next conception. In the case of the remaining two ewes, which were not weaned until mid-summer, conception took place while they were still suckling. The mean number of days to weaning of 13 completely weaned ewes was 76 ± 3·56 days and from weaning to next conception 61·8 ± 3·96 days.

Twenty-eight flock contemporaries also lambed in January and were weaned by April 16; but they failed to breed again in the same season. 75 others either lambed later than January 30 or were weaned later than April 16, or both. None of these bred again in the same season.

The difference between the mean 'conception periods' (the number of days from lambing to fertile mating) for the autumn and late-winter lambing groups is very highly significant ($P < 0{\cdot}001$) and the change appears to be a very abrupt one occurring at about the turn of the year.

It has not been possible to discriminate between accidental failure and physiological inability to breed. The measurement of renewed breeding activity in terms of actual conception, that is, of effective mating, may thus include a component of error in respect of returns to service due to any accidental failure to conceive (or to early embryonic loss). On the other hand, simply to accept mounting by the ram as an indication of renewed ability to breed could be wholly misleading. Some of these ewes have been pursued or mounted by the ram within 24 h of parturition—others within a few days. In fact, one new-born lamb was lost due to the persistent interference of a ram. But it is very unlikely that mating at such an early stage would be successful and it could not be concluded from mating behaviour by itself that the inhibitory effect of lactation on further breeding ability had ceased. Nevertheless, it is interesting to speculate that, in certain cases among the 21 autumn lambers, for example, the longer 'conception periods' might be attributable to accidental failure to conceive at an earlier œstrus, since, in the four cases where the time to successful mating was 49 days or more, subtraction of average cycle lengths would reduce their recorded 'conception periods' to approximately 38, 36, 33 and 31 days, bringing them very close to a revised mean of approximately 37 days.

The effect of 'lactation anœstrum' thus appears to be markedly different between the two halves of the breeding season. During the period of shortening day-length the increasing intensity of breeding activity may be sufficient partially to offset the effects of 'lactation anœstrum', which then reasserts its influence as the days begin to lengthen, and breeding intensity declines.

It is concluded that the pattern of the breeding behaviour of 'Clun' ewes is not wholly inflexible but can to some extent be manipulated by management practices, particularly early lambing coupled with early weaning, which in this case has resulted in a high proportion (16/44 or 36·4 per cent) of the ewes so treated conceiving again in the same breeding season.

The genetic aspects of this matter and the possibility of selecting for individuals more likely to conceive again after lambing have yet to be examined further.

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ENTOMOLOGY

Secretory Activity in the Corpora Allata of Worker Honey-bees (*Apis mellifera* L.)

The corpora allata are among the endocrine organs in insects controlling larval development[1]. These organs are also active in the adult stage, exerting effects on metabolism and in some insects on the reproductive organs[2].

Volumetric measurements of the corpora allata in adult bees have shown them to be almost twice as large in worker as in queen bees, but no conclusive evidence of secretory activity has been noted[3-6]. In recent work on the salivary glands in worker bees such secretory activity of the corpora allata was observed, and is reported here.

Table 1. RESULTS OF THE EXAMINATION OF THE CORPORA ALLATA OF WORKER BEES AFTER VARIOUS FIXATIVES

Fixatives	No. of bees examined	No. of bees with secretion granules in the cells of the corpora allata
Bouin's (alcoholic)	84	3
Formalin (neutral)	30	2
Zenker's (acetic acid)	52	4
Total	166	9

Adult worker bees from a normal honey-bee colony were fixed at daily intervals in alcoholic Bouin's, neutral formalin and Zenker's fixatives. The age of these insects ranged from 1 to 25 days after emergence. After embedding in methacrylate the specimens were sectioned at 8 microns by the use of the tape method[7]. The histological material was stained by the standard hæmatoxylin and eosin method or Gomori's chromium hæmatoxylin–phloxin stain[8]. From a total of 166 bees examined, 9 animals (5·4 per cent) showed phloxinophilic granules within the cytoplasm of the cells of the corpora allata. Such granules were seen with all fixatives and stains, as reported in Table 1, and illustrated in Fig. 1. The granules within the cells of the corpora allata were noted principally in bees between the ages of 18 and 25 days; in two instances, however, the granules appeared in worker bees at 2 and 5 days following emergence. The phloxinophilic granules were observed near the nuclei of the cells. The structure of such nuclei was quite different from that of nuclei in cells lacking secretory granules. The chromatin was concentrated at the marginal portion, leaving the central area either 'empty' or occupied by one large or several small phloxinophilic inclusions (Fig. 1). These inclusions may be nucleoli.

The secretion granules appeared to be a product of the corpora allata; in no instance was it observed that the granules originated from some other source (that is, the brain or the corpora cardiaca).

It is possible that the granules are released from the corpora allata into the blood. The physiological impor-

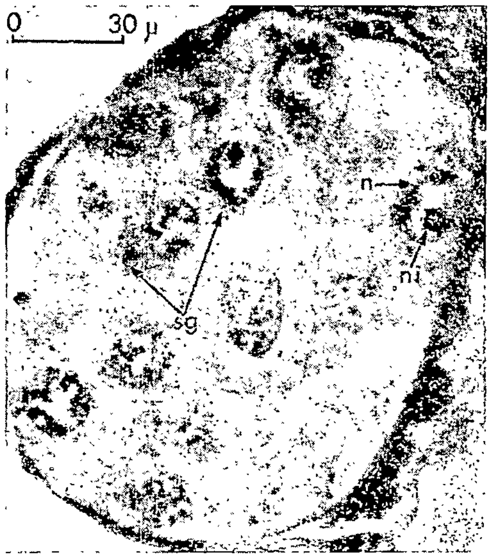

Fig. 1. Photomicrograph of a corpus allatum of an adult worker bee showing secretion granules (*sg*), nucleus (*n*) and nuclear inclusion (*ni*). Thickness of section, 8μ; fixative, alcoholic Bouin's; stain, Gomori's chromium hæmatoxylin

tance of this secretion is unknown, and since the secretion granules were seen in only a few worker bees, it is difficult to interpret their significance. However, this communication supports the claims of other investigators that the corpora allata are secretory organs in adult worker bees.

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MICROBIOLOGY

Antibiotic Sensitivity and Bacterial Numbers

IT is known that the size of inoculum affects results in tests of bacterial sensitivity to antibiotics[1,2]. This is due to individual differences in the bacterial population; the greater the number exposed to a certain concentration of antibiotic, the greater the likelihood that one organism may grow out.

Experiments were carried out in order to establish a quantitative relationship between size of inoculum and inhibiting concentration of antibiotic. Two antibiotics, penicillin and streptomycin, and seven strains of *Staphylococci* (*epidermidis*, *aureus*, 52*A*, 79, 80, 81 and *MG*, a clinical isolate), were investigated. The dilution method was used. Each test-tube contained 10 ml. 'Trypticase Soy Broth' (Baltimore Biological Laboratory). So as to compensate for the dilution caused by the addition of antibiotic, contained in 1 ml. water, and by the addition of the inoculum, also contained in 1 ml. fluid, the powder was dissolved in 80 per cent of the prescribed amount of water. Sixteen to eighteen-hour-old cultures served as inoculum. Bacterial numbers were estimated from optical densities, obtained at 640 mμ[3,4], and appropriate dilutions were prepared. The inocula, per 10 ml., were of the order of: 10^1. 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7. Absence of growth was recorded after incubation for 18 h at 37° C. Preliminary experiments with varying concentrations of antibiotic and varying numbers of bacteria were carried out in order to find the range in which inhibition occurs. Then 20–22 different concentrations of antibiotic, closely spaced, were inoculated with each of the seven inoculum sizes. Each experiment was carried out at least three times.

The mean inhibitive dose, per ml., of penicillin and streptomycin, for two inoculum sizes, namely 10^1 and 10^7, of strains 79, 80, 81 and *MG*, are shown in Table 1. As *Staphylococcus epidermidis*, *aureus*, 52*A* and 79 gave similar results, 79 was chosen to represent the group.

From Table 1 it can be seen that the range of inhibitive doses for penicillin-resistant strains is extremely wide. The penicillin dose needed to suppress growth with an inoculum of 10^7 is 1,000–4,000 times greater than that needed for suppressing growth with an inoculum of 10^1. On the other hand, the range for penicillin-sensitive strains (79) is rather small; the dose is only doubled for inocula ranging from 10^1 to 10^7.

As to streptomycin, inoculum size does not play such a decisive part, even in resistant strains.

Table 1. MEAN INHIBITING DOSES OF PENICILLIN AND STREPTOMYCIN PER ML. FOR INOCULA SIZES OF ORDER OF 10^1 AND 10^7

Penicillin in units, streptomycin in μg

Strain	Inhibiting dose per ml. of:	Inoculum per 10 ml. 10^1	10^7
79	Penicillin	0·033	0·07
	Streptomycin	1·00	6·00
80	Penicillin	0·60	800
	Streptomycin	40	2,000
81	Penicillin	0·60	650
	Streptomycin	1·75	17
MG	Penicillin	0·1	400
	Streptomycin	100	1,000

Table 2. COEFFICIENTS OF CORRELATION BETWEEN LOGARITHMS OF BACTERIAL NUMBERS AND LOGARITHMS OF INHIBITING DOSES OF PENICILLIN AND STREPTOMYCIN

Strain	Coefficient of correlation Penicillin	Streptomycin
79	0·792	0·938
80	0·928	0·894
81	0·877	0·929
MG	0·868	0·957

Table 3. REGRESSION LINES FOR LOGARITHMS OF BACTERIAL NUMBERS (x) AND LOGARITHMS OF INHIBITING DOSES (y)

Strain	Penicillin	Streptomycin
79	$y = 0{\cdot}07745\ x - 1{\cdot}67159$	$y = 0{\cdot}15663\ x - 0{\cdot}20302$
80	$y = 0{\cdot}50300\ x - 1{\cdot}18508$	$y = 0{\cdot}22467\ x + 1{\cdot}33165$
81	$y = 0{\cdot}46924\ x - 1{\cdot}34580$	$y = 0{\cdot}19199\ x - 0{\cdot}17431$
MG	$y = 0{\cdot}47270\ x - 1{\cdot}45345$	$y = 0{\cdot}17550\ x + 1{\cdot}67669$

When plotting the logarithms of bacterial numbers contained in the inocula versus the logarithms of the inhibiting doses of antibiotic, it became apparent that a correlation exists. The coefficients of correlation are shown in Table 2.

There exists a significant correlation between the logarithms of bacterial numbers and the logarithms of inhibiting doses.

When connecting two neighbouring points by a straight line, it was noticed that the resulting curves differed in slope. Regression lines, therefore, were calculated for all strains. Table 3 shows the regression lines.

The regression lines were tested for linearity. The variance ratios indicated that the curves did not differ significantly from straight lines; P values ranged from 0·003 to $< 0{\cdot}001$.

From the coefficients of regression it can be seen that in strains resistant to penicillin the slopes are similar, corresponding to angles of 25°–27°. The slopes for penicillin-sensitive strains correspond to angles of 4°–5°. For streptomycin-resistant strains the slopes correspond to angles of 10°–13°, and for streptomycin-sensitive strains, the angles vary from 8° to 9°.

Differences in slopes were tested for significance. It was found that slopes of penicillin-resistant and penicillin-sensitive strains differ significantly ($P < 0{\cdot}001$). As to streptomycin, there were no significant differences among slopes, irrespective of sensitivity.

In conclusion it may be said that the size of inoculum is of particular importance when testing strains resistant to penicillin. Depending on inoculum size (10^1–10^7), the penicillin dose needed to suppress growth may vary from 0·1 to 400 units per ml. This phenomenon may not be restricted to penicillin. It has been stated: "If 10^{-3} dilution is used for sulphonamide, no activity can be demonstrated", and 10^{-11} dilution is recommended[5].

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Protective Effect of β-Carotene against Lethal Photosensitization by Hæmatoporphyrin

THE carotenoid pigments of certain bacteria can protect the micro-organisms against lethal photosensitization by visible light in the presence of the dye, toluidine blue[1,2]. Since mice can also be lethally sensitized to visible light by the administration of hæmatoporphyrin intraperitoneally, it seemed likely that injection of the carotenoid pigment, β-carotene, would protect against the lethal photosensitization.

An acetic acid–sulphuric acid derivative of hæmatoporphyrin–HCl was prepared by the method of Lipson and Baldes[3]. Suspensions of β-carotene in 'Tween 80' and saline were prepared according to the method of Forssberg *et al.*[4]. Male albino mice of the Charles River Laboratories strain, weighing 18–24 g, were anæsthetized with ether and given 0·2 ml. of suspensions containing 3 mg of β-carotene intraperitoneally. The β-carotene was given 18–24 h prior to exposure to light, in a volume of 0·2 ml. Hæmatoporphyrin (1 mg in 0·2 ml.) was injected in a similar manner just prior to exposure to light. The mice were exposed unshaven to the light from two General Electric blacklight bulbs (maximal emission between 3,400 and 4,400 Å), at a distance of 3·5 in. for 4 h. The mice were held in 0·5-in. wire mesh holders placed in parallel on a board.

Table 1. PROTECTIVE EFFECT OF β-CAROTENE ON PHOTOSENSITIZATION OF MICE BY HÆMATOPORPHYRIN AND VISIBLE LIGHT

Group	Light exposure	No. animals	No. dead	No. alive
Hæmatoporphyrin	Yes	27	21	6
Hæmatoporphyrin and carotene	Yes	27	9	18
Hæmatoporphyrin and 'Tween 80' saline	Yes	8	7	1
Hæmatoporphyrin	No	8	0	8
Carotene	No	8	0	8

Table 1 shows that β-carotene, injected intraperitoneally, protects against lethal photosensitization by the hæmatoporphyrin. The appropriate controls indicate that 'Tween'–saline suspensions are non-toxic, and afford no protection against photosensitization, and that hæmatoporphyrin is non-toxic to animals kept in the dark.

Forssberg *et al.*[4] showed that lycopene, another carotenoid pigment, protected mice against X-irradiation and suggested that the protective action of lycopene might be due to its particulate nature, as well as to some inherent chemical characteristic of the molecule. They were also able to obtain protection against the X-irradiation syndrome by the injection of zymosan, a polysaccharide prepared from yeast cell walls. Experiments were, therefore, performed to see if zymosan could afford any protection against lethal photosensitization of the mice by hæmatoporphyrin and light. Zymosan was injected in saline to give 3 mg/mouse in a volume of 0·2 ml. This amount was found by Forssberg *et al.*[4] to protect against X-irradiation. However, when the zymosan was injected intraperitoneally into mice 18–24 h before injection with hæmatoporphyrin followed by exposure to light, zymosan offered little or no protection against lethal photosensitization (Table 2).

Table 2. RELATIVE LACK OF PROTECTIVE EFFECT OF ZYMOSAN ON PHOTOSENSITIZATION OF MICE BY HÆMATOPORPHYRIN AND VISIBLE LIGHT

Group	Light exposure	No. animals	No. dead	No. alive
Hæmatoporphyrin	Yes	28	20	8
Hæmatoporphyrin and zymosan	Yes	28	17	11
Zymosan	No	8	0	8
Hæmatoporphyrin	No	8	0	8

Recent work has shown that the carotenoid pigments of bacteria are capable of protecting the cells against lethal photosensitization both by endogenous photosensitizers (hæms, chlorophyll) and exogenous photosensitizers (dyes)[1,2,5,6]. Carotenoid pigments are invariably found in bacteria that are exposed to light and air[6]. In green plants, carotenoids are closely associated with chlorophyll, which is a powerful photosensitizer; it is thought that these pigments protect the chlorophyll and other plant porphyrins from photodestruction, and prevent the chlorophyll and other porphyrins from causing cell damage[7]. Carotenoid pigments are also found in skin[8], the cells of which contain potential photosensitizers such as porphyrins and cytochromes. In view of these facts, and of the experimental findings presented here, it is suggested that carotenoid pigments play a part in protecting plant and animal cells against injury from exposure to visible light. This exposure to visible light is presumably injurious because of the presence of potential photosensitizers (such as hæms, porphyrins, cytochromes) within the cell.

The present experiments also suggest that carotenoid pigments might also be administered to patients suffering from skin diseases attributable to visible light. Carotenoids even in large doses seem to be harmless[9]; one successful attempt at carotenoid therapy for light-sensitivity has been reported[10].

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Relation of Ribonucleic Acid Composition to Growth Rate and Dormancy in *Bacillus subtilis*

RECENTLY, Kjeldgaard and Kurland[1] have shown with *Salmonella typhimurium* that the ratio of soluble RNA to DNA is constant at all growth rates, and that the increase in cell RNA content with increasing growth rates results from an increase in the ratio of ribosomal RNA to DNA. Protein synthesis was also directly related to the amount of ribosomal RNA present. The results of these investigations indicate that the ratio of soluble RNA to ribosomal RNA increased at lower growth rates. If this relationship between the two major RNA fractions of bacterial cells is consistent, one might expect a relatively high soluble RNA to ribosomal RNA ratio in spores which are a non-growing stage in certain bacterial species. An analysis of vegetative cells and spores of *Bacillus subtilis* was undertaken therefore to determine if this relationship between RNA composition and growth rate was maintained.

*Bacillus subtilis W*23 cells were grown at different rates in Difco 'Penassay' broth and in synthetic media (0·055 M NH_4Cl; 0·170 M NaCl; 0·054 M KCl; 0·006 M $MgSO_4$; 0·0025 M $PO_4^{\equiv}$; 0·0006 M $FeCl_3$; 0·050 M *tris* buffer, *p*H 7·2; 0·0001 M $MnCl_2$; and 0·0002 M $CaCl_2$), which contained 0·011 M glucose and 0·2 per cent casein hydrolysate (*SCM*-1), 0·011 M glucose (*SCM*-2) or 0·8 per cent sodium glutamate (*SCM*-3) as the energy source. Good sporulation was obtained only in the Difco 'Penassay' broth and the *SCM*-1 medium. The cells were grown at 37° C in a water-bath shaker and their growth followed spectrophotometrically at 660 mμ. Table 1 lists the growth rates for the cells in each medium.

All vegetative cells were collected in the log phase of growth. Spores were grown and purified as described previously[2]. The RNA from the cells and spores was purified by the phenol method[3]. To determine the ratio of soluble RNA to ribosomal RNA, the bulk RNA from individual cultures was fractionated by a methylated albumin column[4]. The RNA was eluted from the column with a linear sodium chloride gradient from 0·3 to 1·2 M. A clear separation of the two types of RNA was obtained as illustrated in Fig. 1. The total amount of soluble and ribosomal RNA from each culture was obtained by summing the optical density readings at 260 mμ of the soluble RNA peak (labelled 4*s*) and the ribosomal RNA peaks (labelled 16*s* and 23*s*). The percentage soluble RNA was determined by dividing the total optical density of the soluble RNA peak by the total optical density of both the soluble and ribosomal RNA peaks.

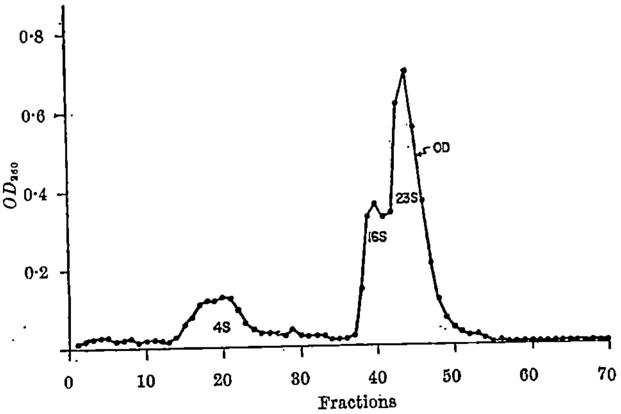

Fig. 1. Methylated albumin column fractionation of bulk *B. subtilis* RNA. The 4*s* peak is soluble RNA; the 16*s* and 23*s* peaks represent ribosomal RNA

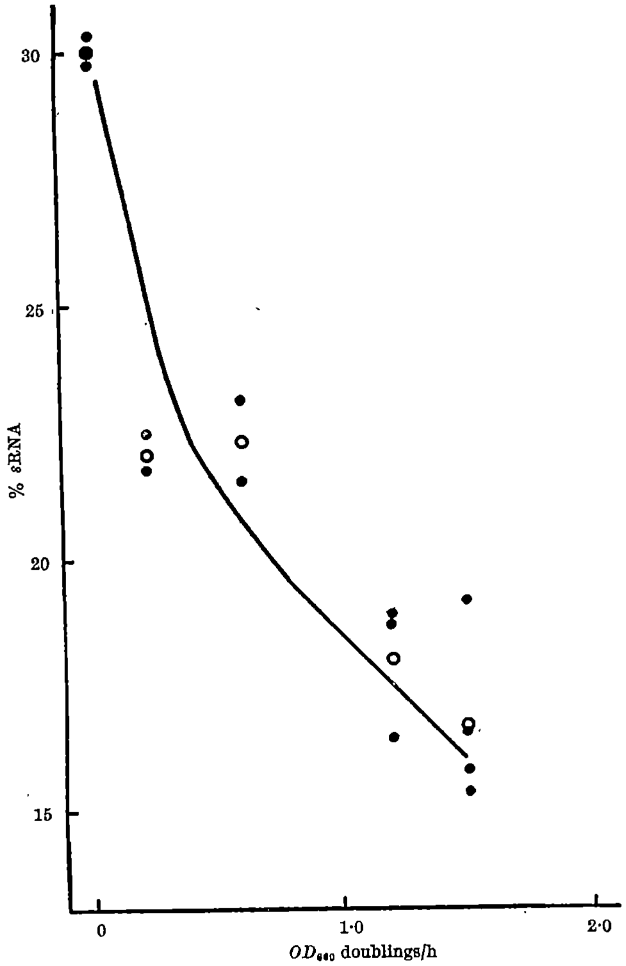

Fig. 2. The ratio of soluble RNA to total RNA at various growth rates. The closed circles represent individual experiments. The open circles represent the average values of the various experiments at a given growth rate

Table 1. GROWTH RATE OF *Bacillus subtilis* IN VARIOUS MEDIA

Medium	Growth rate (OD_{660} doublings/h)
'Penassay' broth	1·50
SCM-1*	1·20
SCM-2*	0·60
SCM-3*	0·22

* See text for description of media.

Fig. 2 indicates the relationship found between growth rate and the ratio of soluble RNA to ribosomal RNA at each of the growth rates listed in Table 1. Each point in the figure represents an independent experiment in which the RNA from cells grown at a specific growth rate was fractionated by column chromatography into the soluble and ribosomal RNA fractions. The results indicate that the ratio of soluble RNA to ribosomal RNA increases with decreasing growth rates. The spore which has a growth rate of 0 has the highest ratio of soluble RNA to ribosomal RNA; the ratio of soluble RNA to ribosomal RNA in spores is twice that found at the highest growth rate examined. When the cells in the stationary phase preceding sporangia formation were analysed, it was found that they contained 26·4 per cent soluble RNA. Since it is difficult to measure growth during the stationary phase, these results were omitted from Fig. 2; however, if the average growth rate is very near to 0, then the ratio between soluble RNA and ribosomal RNA follows the relationship illustrated in Fig. 2.

The transition of the ratio of the two RNA fractions at different growth phases is indicated in Table 2. Cells grown in 'Penassay' broth were examined during the log and stationary phases and as spores. The cells in the stationary phase were obtained 3 h before the appearance of sporangia. The increase in the relative amount of soluble RNA is clearly discernible.

Table 2. RIBONUCLEIC ACID COMPOSITION DURING DIFFERENT GROWTH PHASES OF *Bacillus subtilis*

Growth phase	% soluble RNA	% ribosomal RNA
Log phase	16·6 ± 1·2	83·4 ± 1·2
Stationary phase	26·5 ± 0·5	73·5 ± 0·5
Spore	30·0 ± 0·3	70·0 ± 0·3

These results support the finding of Kjeldgaard and Kurland[1] in terms of the relationship of RNA composition and growth rate. Although they found that soluble RNA could comprise 60 per cent of the total RNA of *Salmonella typhimurium* at low growth rates (0·2 doublings/h), we found a maximum of about 25 per cent at 0·22 doublings/h; it therefore appears that interspecies differences do occur. However, the results do support the expectation that bacterial spores contain the highest soluble RNA to ribosomal RNA ratio.

Furthermore, the results show that there is an increase in the soluble RNA to ribosomal RNA during the stationary phase accompanied by decreased synthetic activity. The ultimate level of soluble RNA is reached then in spores which are completely dormant. Although very little is known about sporangial metabolic activity, it would appear from the conclusions of Kjeldgaard and Kurland[1] and our results that protein synthesis within the sporangium is occurring at a very low rate. Therefore, differentiation into spores may be triggered by events which lead to the over-all decrease in rate of protein synthesis.

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GENETICS

Transferrin Variation in Kangaroos

Inherited variation in the electrophoretic mobility of the serum protein transferrin (siderophilin) has been established for a number of mammalian species[1–12] and for doves[13]. In other mammals[14,15], several colubrid snakes and one amphibian species, variants exist[16] and, although family data are not yet available, it is probable that they are also genetically determined. This communication reports the discovery of inherited variation in the transferrin of an Australian marsupial, *Megaleia rufa* (=*Macropus rufus*), commonly known as the red kangaroo.

Samples of serum or, in a few cases, heparinized plasma were subjected to electrophoresis in starch-gels under essentially the same conditions as those described by Smithies[17], with the exception that platinum electrodes were used at both terminals in a solution of 0·75 M boric acid and 0·125 sodium hydroxide. To several runs, 2–5 μc. iron-59 as ferric citrate were added to the serum. An autoradiograph was made by placing one-half of the gel in a thin sealed polyvinyl chloride bag and leaving it in contact with a 'Kodirex No-Screen' X-ray plate at – 30° C for 24–36 h. The other half was stained with amido black 10*B* (Gurr). Two transferrin patterns were observed. The first type possesses three iron-binding bands, the second four; in both types the fastest and slowest bands are very faint.

The family data are shown in Table 1. Some of them are in the form of mother–offspring combinations. The certainty with which one can ascribe the parentage of a young marsupial to the female in whose pouch it is found should be further exploited in the genetical investigation of this and other marsupial species. It is proposed that the difference between the two types is controlled by a single gene pair, Tf^1 and Tf^2. The first pattern is that of the homozygote $\mathrm{Tf}^1\ \mathrm{Tf}^1$, the second that of the heterozygote $\mathrm{Tf}^1\ \mathrm{Tf}^2$. Each allele determines three bands in a starch gel. The heterozygote on this basis possesses six kinds of transferrin, two pairs of which have identical mobilities in the electrophoretic system employed here. The expected phenotype of the postulated $\mathrm{Tf}^2\ \mathrm{Tf}^2$ homozygote is shown in Fig. 1. Attempts are being made to produce an animal of this genotype by mating captive heterozygous animals. The failure to discover it so far is attributable to its low frequency in the two series examined.

One sample, of 22 individuals, was from a very restricted area on Mount Murchison station near Wilcannia, New South Wales, and had a frequency of $\mathrm{Tf}^2 = 0{\cdot}04$ (95 per cent confidence limits = 0·01 – 0·18).

Table 1. Family Data on Transferrin Variation in the Red Kangaroo

Parents		Offspring	
Female	Male	$\mathrm{Tf}^1\ \mathrm{Tf}^1$	$\mathrm{Tf}^1\ \mathrm{Tf}^2$
$\mathrm{Tf}^1\ \mathrm{Tf}^1$	?	7	0
$\mathrm{Tf}^1\ \mathrm{Tf}^2$	?	2	1
$\mathrm{Tf}^1\ \mathrm{Tf}^2$	$\mathrm{Tf}^1\ \mathrm{Tf}^1$	0	1
$\mathrm{Tf}^1\ \mathrm{Tf}^1$	$\mathrm{Tf}^1\ \mathrm{Tf}^2$	1	1
$\mathrm{Tf}^1\ \mathrm{Tf}^1$	$\mathrm{Tf}^1\ \mathrm{Tf}^1$	9	0

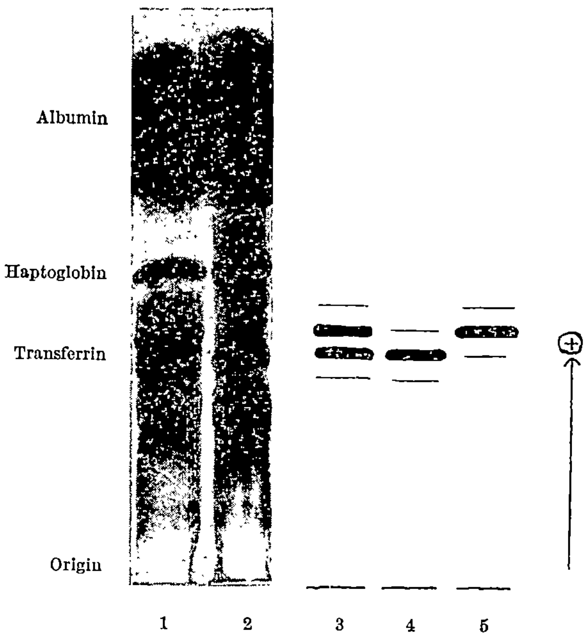

Fig. 1. 1, 2, Starch gel stained with amido black showing transferrin variants in the red kangaroo. 3, 4, Diagram of autoradiograph showing positions of iron-binding bands in 1 and 2 respectively. 5, Expected pattern of the iron-binding bands in the postulated $\mathrm{Tf}^2\ \mathrm{Tf}^2$ homozygote

A series of 34 individuals kept in captivity at the C.S.I.R.O. Division of Wildlife Research, Canberra, also had a frequency of $\mathrm{Tf}^2 = 0{\cdot}04$ (95 per cent confidence limits = 0·004 – 0·11). These came from various widely separated areas of New South Wales and South Australia; in some cases, the point of capture was unknown. Data from the offspring of these animals are included in Table 1.

In the calculation of gene frequencies and confidence limits Cotterman's method of weighting[18] has been used to allow for the inclusion of related individuals. The 95 per cent confidence limits have been obtained using Steven's "Table for the Limits of the Expectation for Binomial and Poisson Distributions"[19].

We thank Dr. H. J. Frith, chief of the C.S.I.R.O. Division of Wildlife Research, for the collection of the Wilcannia samples and Mr. R. Goodwins of the University of Adelaide for preparation of Fig. 1.

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ABO Blood Groups in Sarcoidosis

The distribution of the ABO blood groups is interpreted by Vogel (1961) as the outcome of a dynamic equilibrium between the selective action of epidemic diseases of the world-populations and selection through materno-fœtal incompatibility.

The methods were described at the first and especially at the second International Congress of Human Genetics. The results were reported of investigations being conducted on the association of the ABO blood groups with the varial diseases. Our own investigations regarding the ABO blood groups in sarcoidosis are based on a sample of 518 patients suffering from these diseases.

Table 1. Relative Incidence of ABO Blood Groups in Sarcoidosis and Tuberculosis

	No. of sample control	No.		Comparison	Relative incidence Type A	χ^2 ($m=1$)	P Probability	P Heterogeneity
		Patients	Controls					
Sarcoidosis	1	518	81,985	A : O	1·142	15·22	0·0001	—
Tuberculosis	10	4,505	19,883	A : O	1·1360	12·19	0·0005	0·06

The complete material for ABO blood groups is presented in Table 1. The distribution of ABO blood groups in patients affected with sarcoidosis is compared with the distribution of ABO blood groups in the Federal Republic of Germany. These statistically significant results demonstrate that the relative incidence of type A is 1·142 as against 1 in type O. It means that persons with the blood group A had a 14·2 per cent more frequent probability to acquire a sarcoidosis than persons with the blood group O.

In this connexion it is very interesting that in basal tuberculosis, too, a small preponderance of the blood group A exists. In a second investigation we were able to evaluate the data in 10 samples of former scientific publications. But we could not find a relation of sarcoidosis with the rhesus blood groups and the serum haptoglobins.

So it seems that the gene of the blood group A in the multifactorial genetic systems of sarcoidosis is of some importance.

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Mutant Expression and Canalization

Experiments in which mutant expression was altered by selection have shown that changes in expression are more easily brought about over some parts of the range than others[1-4].

Explanation of this phenomenon, which was interpreted as a consequence of the canalization of normal development, implied that the developmental system governing the expression of the character has at least two components.

One component is sometimes represented as a morphogenetic substance[4,5]. Change of concentration of this hypothetical substance (by environmental factors or selection on expression) would cause a change in mutant expression. The amount of this substance is supposed to vary in different members of a population in a regular and continuous manner. The differences in sensitivity to change (canalization) of expression at different levels of expression, which sometimes result in irregular frequency distributions of the phenotypes[1,4], are a consequence of a second component.

This second component relates the amount of the morphogenetic substance in an individual to the phenotypic expression of the mutant in that individual. The genetic basis of this component must be—at least in part—independent of the genetic basis of the first component[4]. This second component would cause characteristic response curves relating factors which change expression with the magnitude of change in expression. It has been supposed that the mutants involved in the investigations quoted here cause only a decrease in the amount of morphogenetic substance and do not affect the response curve.

I have found[4] that in the mutant *cubitus interruptus* dominant (ci^D) of *Drosophila* genetic differences, differences in environment (temperature) and also differences operating within flies, all act according to the same characteristic response curve. If ci^D affects only the level of the morphogenetic substance, then the response curve for ci^D expression would represent the response curve of the developmental pathway to a normal longitudinal wing vein (canalization cross-section[6]). But neither these experiments nor similar experiments with other mutants could prove that the mutant had no effect on the response curve[4,7].

If the response curve of ci^D represents the canalization cross-section of fourth vein development in the normal fly, one should expect that similar curves can be obtained with certain other mutants which cause a terminal interruption of the fourth vein.

Therefore, two other mutants were tested in temperature experiments, namely, ci^{D-G} (*cubitus interruptus* dominant of Gloor, 4–0·00) and H (Hairless, 3–69·5; in a stock selected for complete penetrance of fourth vein expression).

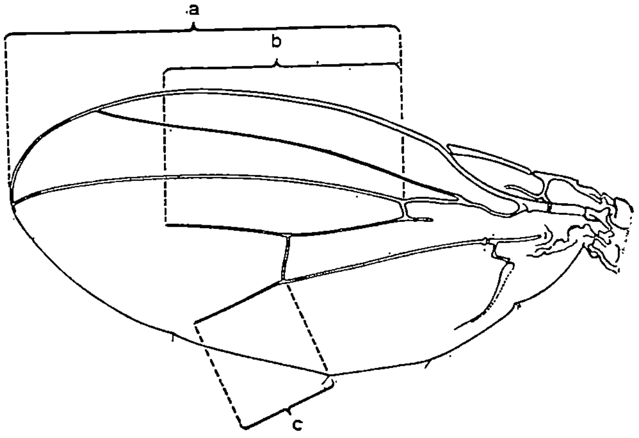

Fig. 1. Measurement of expression on a ci^D-wing. Expression of the 4th vein interruption was measured as the percentage ratio of the length of the 4th vein (*b*) to the length of the 3rd vein (*a*). Expression of the 5th vein interruption as the ratio of the 5th (*c*) and the 3rd vein (*a*)

The expression was measured as indicated in Fig. 1. At each temperature four vial cultures were reared each stocked with 70 larvæ. From each culture 20 females were measured.

The resulting response curves are given in Fig. 2 along with curves obtained earlier for two ci^D stocks with different expressions[4].

The response curves both of ci^{D-G} and H differ basically from the ci^D curves. In ci^{D-G} response is almost linear over the whole expression range. There is no indication of a region of non-linearity around the 70 value which is characteristic for ci^D (ref. 4). Furthermore, there is no indication that on approaching wild-type in expression, further response is becoming more difficult.

In the case of H, change of expression is even facilitated as wild-type is approached.

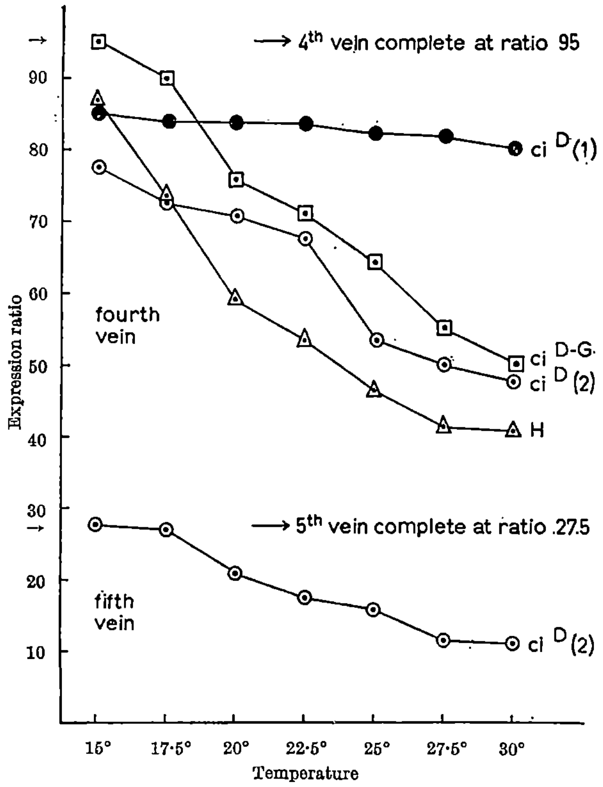

Fig. 2. Relation between temperature and expression (measured on the 4th longitudinal wing vein) of the mutants ci^{D} (2 stocks with different expression ratios), ci$^{D\text{-}G}$, and H. From one ci^{D} stock also expression on the 5th vein is given

Obviously, then, the shape of the response curve of this character (terminal gap in the fourth vein) is dependent on the mutant used in the experiments.

One could reason that nevertheless, in certain cases, as, for example, in ci^{D}, the response curve coincides with the canalization cross-section and that in other mutants, as, for example, in H, the response curve alone is altered, instead of the amount of morphogenetic substance. In the latter case an occasional fourth vein interruption occurring when Hairless is introduced in wild genotypes could be explained by weakening of canalization. Reduced efficiency of canalization would then allow for a certain amount of phenotypic expression of the normal variation in morphogenetic substance, while the same variation in wild-type cannot reach a threshold value set by normal canalization.

The characteristic bimodal frequency distribution of the expression of the mutant deformed recessive of Lüers with modes at either side of wild-type[8], and other cases of 'polar variation'[9] of mutant expression in *Drosophila*, can perhaps be explained in this way.

However, the realization of a morphological character involves a sequence of different processes or, more precisely, a variety of interconnected sequences. It then seems too simple a view to expect that every process in such a sequence, not to mention processes in different sequences, would show the same response curve for sensitivity to environmental and genetic factors. It must be expected that the canalization cross-section of a character could be different at different stages in the realization of the character. The response curves of the three mutants tested in these experiments might therefore represent the canalization cross-section of the fourth vein formation at different times in development.

This suggestion finds support in the fact that the temperature sensitive periods (T.S.P.) for the three mutants are different. While the T.S.P. for ci^{D} starts later than 4 h after pupation, the same period in H begins before pupation and ends before the beginning of the ci^{D} period. The T.S.P. for ci$^{D\text{-}G}$ overlaps with both the period of H and ci^{D} (refs. 10 and 11).

Evidence suggesting that the response curves of the mutants represent the response curve of a process in normal development comes from selection experiments with H and ci^{D}.

Long-term selection for extreme expression of ci^{D} (40–50 generations) did not accumulate a system of modifiers which had an effect on the fourth vein development independent of the presence of the ci^{D} mutant[4,12]. On the other hand, selection on Hairless fourth vein expression (in populations segregating for H and wild-type individuals) showed a rather surprising result. After a few generations of selection, some of the non-Hairless segregants already showed a fourth vein interruption.

It is tempting to relate this easy 'assimilation' of the Hairless fourth vein interruption with the shape of the temperature response curve of this mutant. This curve indicates that change of expression in the neighbourhood of the normal phenotype is relatively easy, while in the case of ci^{D} the opposite is true. Preliminary evidence is available which indicates that also in Hairless genetic factors act according to the same response curve as temperature.

As mentioned before, long-term selection on ci^{D} fourth vein expression did not lead to a break of the fourth vein in individuals without the mutant. But in one line the gap on the fifth vein (which is a regular pleiotropic effect in standard ci^{D}) was increased by selection for increased fourth vein. In this line then, the fifth vein gap appeared independently of the presence of ci^{D} and was shown to be determined polygenically[12].

Now the response curve of the fifth vein break in ci^{D} does not show any indication of canalization (Fig. 2). This, too, points to a relation between the shape of the response curve of a mutant character and the ease of assimilation. The sensitivity to assimilation, of course, is an indication of sensitivity of the pertinent developmental process in normal development.

In this train of thought an essential element is the assumption that at least part of the modifying genes are specifically acting on the sensitive point in the development of the mutant that is revealed by its temperature sensitive period[13]. If this assumption is correct, modifying genes acting on the expression of a given character (for example, fourth vein gap) in different mutants (for example, ci^{D} and H) should be at least partly different.

That such a specificity of modifiers does indeed exist is indicated in the case of ci^{D} and H, where preliminary evidence is available that the modifying genes of ci^{D} have only little effect on the expression of H, and vice versa.

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PSYCHOLOGY

Dynamic Distortions of Perceived Form

It is becoming increasingly clear that the visual perception of form involves the interaction of a number of parallel computing or 'image-processing' operations. Stimuli which preferentially excite one or other of the neural computing networks concerned can give rise to visual anomalies indicative of the way in which the optical information is normally broken down.

I have recently observed a striking anomaly in this class. When two regular patterns such as Figs. 1*a* and *b* are superimposed, they form the 'Moiré pattern' of Fig. 2. If now the second is moved relative to the first, say along the vertical axis of the figure, the lobes of Fig. 2 undergo a continual displacement in the general direction of the arrows, and so long as motion persists the form seen presents an asymmetrical appearance. As soon as motion ceases, however, symmetry returns, the lobes appearing to 'snap back' in a fraction of a second to their positions in Fig. 2. The transition from dynamic to static form is sufficiently striking to evoke incredulity in some subjects. Since the static form is seldom exactly symmetrical, it is necessary (and proves convincing) to control for residual asymmetry by arranging that the figure returns to exactly the same rest-position from opposite directions alternately.

The problem here is that identical figures of excitation on the same retinal area lead successively to different perceptions of form. In general terms the explanation is obvious, that the form seen under dynamic conditions is some kind of running average over the moving figures presented; but since this 'average' is as sharp as the static form, and since it is asymmetrical, it must be computed from a logically dissected representation of the stimulus, in which the velocity-components of the retinal image can apparently contribute to the computation of contour position. With translation of a rigid figure, this 'abstract averaging' need give rise to no anomalies of form perception, though it may be responsible for some illusions of displacement[1]. When, however, the figure is a Moiré pattern the components of which have a different 'phase velocity' from the velocity of the group as a whole, the contributions from contour velocity can grossly bias the perceptual estimate.

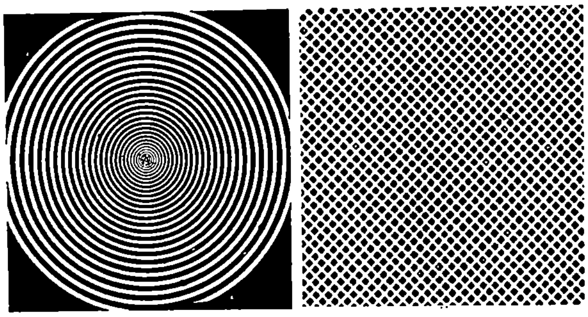
a *b*

Fig. 1

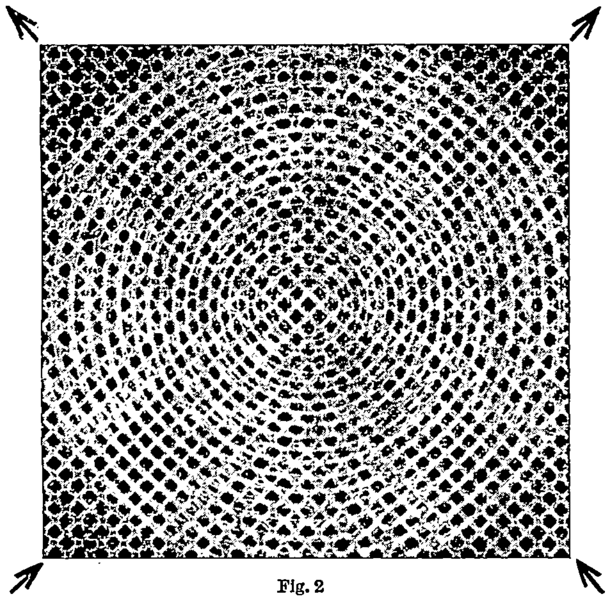
Fig. 2

The phenomenon seems to be in line with other recent evidence[2–4] suggesting that different neural networks are concerned with the signalling of contour direction, contour velocity and contour location.

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STATISTICS

Some Properties of Random Variables

In text-books on probability the following (perhaps curious) properties of random variables do not appear to have been noticed so far.

Consider a bi-variate distribution (Z, U) where both Z and U are positive finite random variables. There will be occasion to refer to Z as the numerator variate. Then one property of positive random variables is that:

$$\mathrm{Cov}\left(U, \frac{Z}{U}\right) \leqq V(\sqrt{Z}) \qquad (1)$$

To prove this inequality we have, by definition:

$$\mathrm{Cov}\left(U, \frac{Z}{U}\right) = E(Z) - E(U)\, E\left(\frac{Z}{U}\right) \qquad (2)$$

Applying Cauchy's inequality, we find:

$$E(U)\, E\left(\frac{Z}{U}\right) \geqq \left[E\left(\sqrt{U}\sqrt{\frac{Z}{U}}\right)\right]^2 \qquad (3)$$

or

$$E(U)\, E\left(\frac{Z}{U}\right) \geqq [E(\sqrt{Z})]^2 \qquad (3{\cdot}1)$$

In equation (3), equality is attained if, and only if, $\sqrt{\frac{Z}{U}}/\sqrt{U}$ is equal to a constant, say $\sqrt{c}$, for all pairs (Z, U), leading to the condition

$$Z = cU^2 \qquad (4)$$

On multiplying each side of (3·1) by -1 and reversing the sign of inequality, and then adding $E(Z)$ to each side, the result given by equation (1) will be obtained. When equation (4) holds, $\mathrm{Cov}\,(U, cU) = V(\sqrt{c}\,U) = cV(U)$, an identity which is to be expected.

Another property of positive random variables is that the correlation with their respective reciprocals, that is, $\rho\left[U, \frac{1}{U}\right] \leqq 0$. The proof of this proposition follows from equations (2) and (3·1) by putting $Z = 1$. Condition equation (4) shows that this correlation is zero only when all the U's are equal.

An interesting question arises as to what is the analogue of the result given by equation (1), when the signs of all the numerator variates are reversed, that is, they now assume finite negative values while their corresponding U's remain positive as before. The probabilities for each pair remain unchanged. In this situation let $(-1)Z = z$, where Z is positive. Thus the variate value of $\sqrt{z} = \sqrt{-1}\sqrt{Z}$ will be a complex number without a real part, and since this circumstance rules out the use of Cauchy's inequality, we adopt the following stratagem to establish the analogous proposition. If the sign of each z is reversed, then obviously equation (1) can be applied. Now multiplying each side of this inequality by -1 we find:

$$-\mathrm{Cov}\left[U, \frac{Z}{U}\right] \geqq (\sqrt{-1})^2 V(\sqrt{Z}) \qquad (5)$$

In equation (5), as it stands, by factoring the coefficient -1 into the covariance function as a coefficient of Z, and $(\sqrt{-1})^2$ into the variance function as a coefficient of $\sqrt{Z}$, we obtain:

$$\mathrm{Cov}\left[U, \frac{z}{U}\right] \geqq V(\sqrt{z}) \qquad (6)$$

in view of the relation $z = -Z$. It is interesting to note that equation (1) has served as a scaffolding for the derivation of equation (6), where equality is attained if, and only if, $-z = cU^2$.

A statistically meaningful interpretation of the inequality equation (6) hinges on the interpretation of the lower bound of its covariance function, namely:

$$V(\sqrt{z}) = V(\sqrt{-1}\sqrt{Z}) = -V(\sqrt{Z}) \qquad (7)$$

That is, the variance of the complex variates $\sqrt{z}$, or let us say the abstract variance of the $\sqrt{z}$'s, is equal to the variance of the square root of their corresponding real variates multiplied by -1.

The last property of random variables as manifested by equation (6) may throw some light on the nature of the underlying variables in linear models used in the analysis of variance in circumstances when their estimates of variance are negative. It is hoped to investigate this question.

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A Probabilistic Similarity Index

Williams *et al.*[1] have recently proposed that, for similarity indices used for numerical taxonomy, each attribute should be weighted according to the sum of the χ^2 values obtained from 2 × 2 tables testing the association of this attribute with all others. This relates the measure of similarity to the distance separating the two individuals in a multi-dimensional space where each axis representing an attribute has a scale depending on the importance of that attribute. Though based on χ^2 calculations, the index is not considered to be probabilistic, though the authors adumbrate the possibility of developing probabilistic methods of similarity analysis.

A similarity index based directly on probability theory is in use in this laboratory, which takes full account of ordered and quantitative attributes as well as of those which in their nature are binary. For each pair of individuals in a sample or population, the exact probability is computed for each attribute in turn that a random sample of two will resemble one another not less closely than the two under test. In computing this probability, the null hypothesis is that the two individuals in question are part of the same population as the rest, while the alternative hypothesis is that these two are a sample from another population.

The phrase "resemble one another not less closely" clearly calls for definition; the appropriate definition varies with the type of attribute.

For purely qualitative attributes, those concordances supporting the alternative hypothesis against the null hypothesis which are less probable under the latter are taken to be evidence of closer resemblance (or better evidence of resemblance). Thus, if an attribute can take three values x_1, x_2, and x_3, and the probabilities that, for any particular individual, the attribute will take these values are respectively 0·1, 0·3 and 0·6, agreement in having the first value would be considered better evidence of resemblance than agreement in having the second or third.

For ordered (but non-metrical) attributes, the degree of resemblance is taken as a function of the proportion of individuals in the same classes as the two under test, or in any intermediate class. Thus, if five ordered classes contain the following proportions of the population: 0·1, 0·2, 0·1, 0·4, 0·2, two individuals in the first and third classes are taken as being more similar than two in the fourth and fifth. For metrical attributes, the range is taken as a criterion of similarity, so that two individuals differing by less in the numerical values of this attribute are regarded as more similar than another pair in which the numerical values are more widely separated. A logarithmic scale might, of course, be used where appropriate.

The probability of the observed, or any closer, degree of resemblance having been computed for each attribute, the problem then remains of combining these probabilities, on the assumption of their independence. Where the probabilities arise from a continuous distribution, the solution has been given by Fisher[2]. He computed:

$$-2\sum_{i=1}^{n} \ln p_i$$

and showed that this quantity was a χ^2 variate with $2n$ degrees of freedom. For most types of attributes, this provides an acceptable approximation to the true multinomial distribution. An exception is formed by binary attributes—the most common type—for which the probability of the observed degree of resemblance between the two individuals is limited to three values. Here, Fisher's continuous solution may lead to a large positive bias, and the method adopted instead has been to combine these probabilities in small groups by the exact multinomial method (to apply it to any large number of attributes would be computationally impracticable), and then combine these group probabilities, now quasi-continuous, by Fisher's method.

When this has been done, the resulting combined probability P_t provides a satisfactory index of dissimilarity, and its complement $(1 - P_t)$ is an index of similarity ranging (like those commonly used) from zero when the two individuals are completely dissimilar to near unity when they are closely similar. Unity can be attained only when the population on which the probability estimates are based is infinite.

The whole procedure outlined in this paper has been programmed in *Fortran II* for the IBM 1620_3 computer, and copies of the programme are available to enquirers. A full account of the technique is being prepared for publication.

I thank Mr. D. W. G. Moore, officer in charge of the Computing Centre of the University of Western Australia, for the facilities used in this work.

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[1] Williams, W. T., Dale, M. B., and Macnaughton-Smith, P., *Nature*, **201**, 426 (1964).

[2] Fisher, R. A., *Statistical Methods for Research Workers*, thirteenth ed. (Oliver and Boyd, Edinburgh and London, 1963).

FORTHCOMING EVENTS

Friday, September 11

F. and M. Scientific Europa N.V. (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 7 p.m.—Dr. Charles C. Sweeley (University of Pittsburgh): "Advances in Gas Chromatography Techniques Applied to the Analyses of Carbohydrates and Related Polyhydroxy Compounds".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Demonstrator (with B.Sc. honours degree in geography and a special interest in cartography and surveying) in the Department of Geography—The Registrar, The University, Nottingham (September 10).

Lecturer in Mathematics; a Lecturer in Statistics; a Senior Lecturer in Biochemistry; a Senior Lecturer in Civil Engineering; and an Assistant Lecturer (Grade B) in Mechanical Engineering—The Principal, Nottingham and District Technical College, Burton Street, Nottingham (September 10).

Lecturer in Organic Chemistry; a Lecturer in Inorganic and Physical Chemistry; an Assistant Lecturer (Grade "B") in Inorganic and Physical Chemistry; and an Assistant Lecturer (Grade "A") in General Chemistry at Birkenhead Technical College—The Director of Education, Birkenhead Education Committee, Education Offices, 63 Hamilton Square, Birkenhead (September 14).

Lecturer (preferably with a special interest in medical statistics) in the Department of Statistics—The Secretary, The University, Aberdeen (September 14).

Lecturer or Assistant Lecturer (preferably with experience or special interest in one or more of the following fields: production engineering, nuclear engineering, thermodynamics and mechanics of fluids, and preferably an interest in design) in Mechanical Engineering—The Registrar, The University, Leeds, 2 (September 14).

Senior Lecturer (well qualified academically and considerable experience in teaching and/or industry and/or research) in Physics or Applied Physics—The Secretary, Welsh College of Advanced Technology, Cathay's Park, Cardiff (September 14).

Lecturer (preferably with a good honours degree in geography) in the Department of Geography, University of Ghana, to teach biogeography or climatology as well as the regional geography of North America, Europe or Asia—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1 (September 15).

Research Fellow (specializing in water chemistry and bacteriology) to undertake research work on the waters of the developing Volta Lake (University of Ghana, Volta Basin Research Project)—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1 (September 15).

Science Librarian (professionally qualified graduate or Fellow of the Library Association, and experience in a special library or in the science section of a university library) in the Balme Library, University of Ghana—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1 (September 15).

Assistant Lecturers (2) (candidates for the one post should have special interests in the evolutionary field studies, and for the other in physiology) in Zoology—The Registrar, The University, Manchester, 13 (September 18).

Research Associate or Senior Research Associate (with an honours degree in engineering and some experience in research in the field of structures, or alternatively experience in the analysis and design of structures) in Engineering (Civil), for work on the application of computer methods to structural analysis and design—The Registrar, The University, Manchester, 13 (September 18).

College Lecturer (with an honours degree in agricultural chemistry, chemistry or biochemistry, with postgraduate experience in soil chemistry, plant biochemistry or other related field) in the Soils Division, Department of Agricultural Chemistry and Soil Science—The Professor of Agricultural Chemistry, University College, Glasnevin, Dublin, Republic of Ireland (September 19).

Newton Drew Chair of Fuel Technology and Chemical Engineering—The Registrar, The University, Sheffield, 10 (September 19).

Lecturer (with experience either in reaction mechanisms or in the application of infra-red spectroscopy) in Organic Chemistry; an Assistant Lecturer (with experience in instrumental and general analysis) in Analytical Chemistry; and a Research Fellow for work involving investigations into polymer technology—The Assistant Registrar (Science), The University of Birmingham, Birmingham, 15 (September 21).

Graduate (with a strong background in physical chemistry) in the Department of Chemistry, to engage on research on nuclear magnetic resonance of simple nitrogen-15 compounds of biological importance—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (September 25).

Lecturer in the Department of Psychiatry—The Registrar, The University, Leeds, 2 (September 26).

Reader in Microbial Genetics—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne (September 26).

Assistant Lecturer (Demonstrator) in the Department of Anatomy (Prof. J. Z. Young), to undertake teaching and research—The Secretary, University College, Gower Street, London, W.C.1 (September 30).

Chair of Applied Mathematics—The Registrar, The University, Manchester, 13 (September 30).

Chair of Electrical Engineering at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

Senior Lecturer or Lecturer in Cytogenetics and Taxonomy (including the development of the Herbarium) in the Department of Botany, University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 30).

Senior Lecturer, Lecturer or Assistant Lecturer in Botany, Mathematics, Physics (2 posts), the Department of Education, and the Institute of Education, University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 30).

Plant Physiologist (with a good degree or equivalent in biology, botany or horticulture and an interest in physics, and preferably some research experience), for work on the use of CO_2, water and energy by glasshouse crops—The Secretary, N.I.A.E., Wrest Park, Silsoe, Bedford (October 1).

Research Assistant in the Department of Chemistry to work on the isolation of the glucose transport system from red blood cells—The Registrar, The University, Manchester, 13 (October 1).

Lecturer/Senior Lecturer in Biometry; and a Lecturer/Senior Lecturer (with special interests in either the physical aspects of geography and, in particular, specific qualifications in climatology or in economic geography and quantitative methods) in Geography at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 5).

Research Fellow (biochemist or physical chemist) in the Department of Microbiology, John Curtin School of Medical Research, Institute of Advanced Studies, Australian National University, to work on the nucleic acids of animal cells and animal viruses—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 12).

Chair of Mechanical Engineering in the Faculty of Engineering, University of Malaya, Kuala Lumpur—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, October 15).

Lecturer in Geophysics at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, October 15).

Research Fellow (graduate with experience in immunology) in the Hugh Adam Department of Cancer Research, University of Otago—The Registrar, University of Otago, P.O. Box 56, Dunedin, New Zealand (October 15).

Lecturer in the Department of Mechanical Engineering, University of Cape Town—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (London and Cape Town, October 19).

Chair of Veterinary Surgery at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 23).

Lecturer or Assistant Lecturer (with a degree in electrical engineering, and an interest or postgraduate experience in the field of electronics, preferably microwaves or semi-conductors) in the Department of Electrical Engineering, University of Melbourne, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 24).

Professor of Organic Chemistry—The Registrar (Room 22, O.R.B.), The University, Reading (October 24).

Chair of Pathology at the University of Tasmania, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 30).

Director (with an honours degree in forestry or its equivalent, considerable experience of research in several branches of forestry, administrative experience, a sound knowledge of two or more modern languages, preferably including German, ability to write good concise English, and preferably editorial experience) of the Commonwealth Forestry Bureau, Oxford—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (October 31).

Post-doctoral Fellows (2) (physical chemists or physicists) to carry out research on atomic reactions—The Registrar, University of Canterbury, Private Bag, Christchurch, New Zealand (November 30).

Biochemist (interested in studying for a higher degree, and preferably with some enzymological experience) for work on isoenzyme purification and properties—Prof. D. N. Baron, Department of Chemical Pathology, Royal Free Hospital, Gray's Inn Road, London, W.C.1.

Demonstrator (Nigerian, with a good first degree in physics), for duties which will include the preparation and testing of undergraduate experiments, laboratory supervision, and organization of tutorials—The Registrar, University of Ife, Ibadan Branch, Ibadan, Nigeria.

Entomologist (between 23–44 years, with a degree in botany, zoology or entomology, and relevant postgraduate experience in applied entomological research) in Northern Rhodesia, to carry out research into the life history and control of pests of agricultural and horticultural importance—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/132/03.

Entomologist (national of the United Kingdom or the Republic of Ireland, with a degree in biology and at least two years' postgraduate research) with the East African Common Services Organization, to study ways of improving methods of controlling or eradicating tsetse flies by insecticides and chemosterilants including both laboratory and field studies—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/214/07.

Head of the Electronics Shop in the New York Medical College, to be responsible for the design and maintenance of research and teaching equipment—Dr. V. B. Brooks, Professor of Physiology, New York Medical College, Fifth Avenue at 106th Street, New York, N.Y., U.S.A.

Lecturer in the Department of Organic Chemistry—The Registrar, The University, Liverpool, quoting Rev. CV/266/N.

Lecturer or Assistant Lecturer in Dairy and Food Technology with particular reference to quality control—The Registrar (Room 22, O.R.B.), The University, Reading.

Leukæmia Research Fellow (non-medical graduate with experience in cytogenetics) to study the cytogenetics of acute leukæmia in children—The Secretary, Institute of Child Health, Great Ormond Street, London, W.C.1.

Physicist (with a good honours degree in physics and preferably some experience in medical physics) to work jointly in the University Department of Medical Physics and the Hospital Department of Nuclear Medicine on problems associated with applications of radio-isotopes to clinical medicine—The Secretary to the Board, The General Infirmary, Leeds, 1.

Professor of Anatomy and Head of the Department—The Dean, Faculty of Medicine, University of Manitoba, Cor. Bannatyne and Emily Streets, Winnipeg, Manitoba, Canada.

Research Fellow or Senior Research Fellow (with a good honours degree in psychology and experience of research in experimental psychology or ergonomics) in Experimental Psychology/Ergonomics, to assist a team working on the effect of variations in job content and design on productivity—The Registrar, Bradford Institute of Technology, Bradford, 7.

Senior Research Assistant in Physics to work on electron spin resonance and relaxation in compounds which exhibit co-operative magnetism—The Registrar, The University, Sheffield.

Senior Technician (preferably with a special interest in microbiology) in the Applied Biology Department—The Registrar, Brighton Technical College, Richmond Terrace, Brighton, Sussex.

TECHNICIAN (with a knowledge of electronics) IN THE DEPARTMENT OF CLINICAL MEASUREMENT—The Secretary, Department of Clinical Measurement, Westminster Children's Hospital, Vincent Square, London, S.W.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Office of Health Economics. New Frontiers in Health: a Review of the Incidence, Impact and Implications of Minor and Unrecognised Illness in the Community. Pp. 32. (London: Office of Health Economics, 1964.) 2*s*. [147

The Radiochemical Centre, Amersham. Technical Bulletins. No. 64/5: Labelled Thymidine. Pp. 2. No. 64/6: Labelled Carbohydrates. Pp. 2. No. 64/7: Tritium Labelled Compounds. Pp. 4. No. 64/8: High Specific Activity Tritium Compounds. Pp. 2. 64/10: Labelled "Nucleics". Pp. 4. No. 64/11: Labelled Steroids and Steroid Esters. Pp. 2. No. 64/12: Labelled Fatty Acids, Alcohols and Derivatives. Pp. 2. No. 64/13: Labelled Amino-Acids. Pp. 4. (Amersham: The Radiochemical Centre, 1964.) [147

The British Steel Castings Research Association. 11th Annual Report, 1964. Pp. ii + 41. (Sheffield: The British Steel Castings Research Association, 1964.) [147

The International Nickel Company (Mond), Ltd. Nickel Plating: Technique and Applications. Pp. 48. (London: The International Nickel Company (Mond), Ltd., 1964.) [147

University Grants Committee. University Appointments Boards: a Report by The Rt. Hon. the Lord Heyworth. Pp. xii + 127. (London: H.M. Stationery Office, 1964.) 12*s*. 6*d*. net. [237

British Baking Industries Research Association. Report No. 72: Some General Principles of Bakery Planning. By D. E. Chapman and E. M. Laing. Pp. ii + 82. (Chorleywood, Rickmansworth: British Baking Industries Research Association, 1964.) 10*s*. [237

The Radiochemical Centre, Amersham. Radioactive Isotope Dilution Analysis. Pp. 12. (Amersham: The Radiochemical Centre, 1964.) [237

United Kingdom Atomic Energy Authority. Tenth Annual Report for the period 1st April, 1963—31st March, 1964. Pp. ix + 89 + 8 plates. 6*s*. 6*d*. net. Atom 1954/64: an Illustrated Summary of the Tenth Annual Report of the United Kingdom Atomic Energy Authority from April 1st, 1963, to March 31st, 1964. (10th Anniversary Issue.) Pp. 48. 2*s*. 6*d*. net. (London: H.M. Stationery Office, 1964.) [237

Building Research Station Digest. No. 90 (First Series). Part 1: Colourless Waterproofing Treatments for Damp Walls. Part 2: Analysis of Water Used or Encountered in Construction. Pp. 5. Revised edition. (London: H.M. Stationery Office, 1964.) 4*d*. [297

Department of Scientific and Industrial Research. The Geological Museum: Museum of Practical Geology. A Short Guide to the Exhibits. Fourth edition. Pp. 36. (London: H.M. Stationery Office, 1964.) 2*s*. 6*d*. [297

National Union of Teachers. The Financing of Education: a Discussion Document. Pp. i + 38. (London: National Union of Teachers, 1964.) 2*s*. [297

Department of Scientific and Industrial Research. Report of the National Chemical Laboratory 1963: Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1963. Pp. viii + 75 + 4 plates. (London: H.M. Stationery Office, 1964.) 5*s*. 6*d*. net. [297

Department of Scientific and Industrial Research. Road Research 1963: The Report of the Road Research Board with the Report of the Director of Road Research. Pp. vi + 168 + 12 plates. (London: H.M. Stationery Office, 1964.) 11*s*. 6*d*. net. [297

Department of Scientific and Industrial Research: National Physical Laboratory. Units and Standards of Measurement Employed at the National Physical Laboratory. II: Light—Photometry, Colorimetry and Radiometry. Second edition. Pp. iii + 10. (London: H.M. Stationery Office, 1964.) 1*s*. 6*d*. net. [297

Building Research Station Digest. No. 88. (First Series): Sound Insulation of Dwellings—1. Revised edition. Pp. 6. (London: H.M. Stationery Office, 1964.) 4*d*. [297

The British Non-Ferrous Metals Research Association. Annual Report, 1963. Pp. 47. (London: The British Non-Ferrous Metals Research Association, 1964.) [297

Robinson Brothers. Chemicals for Industry. Pp. 23. (West Bromwich: Robinson Brothers, 1964.) [297

St. Dunstan's. Special Aids for the War-Blinded. Pp. 14. (London: St. Dunstan's, 1964.) [297

The World Land Use Survey. Occasional Papers, No. 5: The North Sea. By L. E. J. Brouwer. The Law of the Continental Shelf. By The Rt. Hon. Lord Shawcross. (Two Discourses to the 20th International Geographical Congress, London, 1964.) Pp. ix + 42. (Bude: Geographical Publications, Ltd., 1964.) 10*s*. [297

The Pergamon Moon Globe. (Oxford: Pergamon Press, 1964.) 200*s*. [68

Leeds: a Developing University—Descriptive Brochure. Pp. 24. Astbury Department of Biophysics—Descriptive Brochure. Pp. 16. (Leeds: The University, 1964.) [148

The Edinburgh School of Agriculture. Calendar 1964–65. Pp. 64. (Edinburgh: The Edinburgh School of Agriculture, 1964.) [148

Library Association Special Subject List No. 44: Market Research. By David C. Fanning. Pp. 22. (London: The Library Association, 1964.) 5*s*.; (L.A. members, 3*s*. 9*d*.) [148

Department of Technical Co-operation. Research Assistance for the Developing Countries. (Cmnd. 2433.) Pp. 28. (London: H.M. Stationery Office, 1964.) 2*s*. net. [148

Report of the Astronomer Royal for Scotland for year ending 31st March, 1964. Pp. 18. (Edinburgh: The Royal Observatory, 1964.) [148

B.B.C. Engineering Monograph No. 53: Aerial Distribution Systems for Receiving Stations in the L.F., M.F., and H.F. Bands. By Dr. J. B. Izatt. Pp. 20. (London: British Broadcasting Corporation, 1964.) 5*s*. [148

Annual Report 1963/64 of the British Scientific Instrument Research Association. Pp. 42. (Chislehurst: British Scientific Instrument Research Association, 1964.) [148

A Catalogue of the Parasites and Predators of Insect Pests. Section 3: Predator Host Catalogue. Prepared under the direction of W. R. Thompson and F. J. Simmonds. (Commonwealth Institute of Biological Control.) Pp. 204. (Farnham Royal: Commonwealth Agricultural Bureaux, 1964.) 60*s*.; 9 dollars. [148

The Natural Rubber Producers' Research Association. Twenty-sixth Annual Report. Pp. 34. (London: The Natural Rubber Producers' Research Association, 1964.) [148

Change and Tradition in University Extramural Work: Report of the Universities Council for Adult Education 1962–1963. Pp. 24. (Liverpool: Universities Council for Adult Education, The University, 1964.) [148

Other Countries

United States Department of the Interior: Geological Survey. Professional Paper 302-C: Gubik Formation of Quaternary Age in Northern Alaska. By Robert F. Black. (Exploration of Naval Petroleum Reserve No. 4 and Adjacent Areas, Northern Alaska, 1944–53, Part 2: Regional Studies). Pp. iii + 59–91. Professional Paper 303-F: Geology of the Killik–Etivluk Rivers Region, Alaska. By Robert M. Chapman, Robert L. Detterman and M. D. Mangus. (Exploration of Naval Petroleum Reserve No. 4 and Adjacent Areas, Northern Alaska, 1944–53. Part 3: Areal Geology.) Pp. iv + 325–407 + plates 43–49. Professional Paper 341-D: Geology and Ore Deposits of the Belo Horizonte, Ibirité, and Macacos Quadrangles, Minas Gerais, Brazil. By Joel B. Pomerene. Pp. v + 84 + plates 1–13. Professional Paper 349-C: Geology of the Central Great Smoky Mountains, Tennessee. By Philip B. King. Pp. vii + 148 + plates 1–13. Professional Paper 360: Geology and Quicksilver Deposits of the New Almaden District, Santa Clara County, California. By Edgar H. Bailey and Donald L. Everhart. Pp. vii + 206 + plates 1–18. Professional Paper 374-G: Foraminifera from the Northern Olympic Peninsula, Washington. By Weldon W. Rau. Pp. iii + 33 + plates 1–7. Professional Paper 403-G: Fossil and Recent Calcareous Algae from Guam. By J. Harlan Johnson. Pp. iii + 40 + plates 1–15. Professional Paper 420-B: Geology and Oil Resources of the Eastern Puente Hills Area, Southern California. By D. L. Durham and R. F. Yerkes. Pp. iv + 62 + plates 1–4. (Washington, D.C.: Government Printing Office, 1964.) [147

United States Department of the Interior: Geological Survey. Professional Paper 422-E: Channel Geometry of Piedmont Streams as Related to Frequency of Floods. By F. A. Kilpatrick and H. H. Barnes, jun. Pp. iv + 10. Professional Paper 443: Quaternary Geology of the Kenai Lowland and Glacial History of the Cook Inlet Region, Alaska. By Thor N. V. Karlstrom. Pp. iv + 69 + plates 1–7. Professional Paper 454-F: Smaller Foraminifera from the Late Tertiary of Southern Okinawa. By L. W. LeRoy. Pp. iii + 58 + plates 1–16. Professional Paper 454-K: Upper Palæzoic Floral Zones and Floral Provinces of the United States. By Charles B. Read and S. H. Mamay. With a Glossary of Stratigraphic Terms by Grace C. Keroher. Pp. iv + 35 + plates 1–19. Professional Paper 462-E: An Analysis of Some Storm-Period Variables Affecting Stream Sediment Transport. By H. P. Guy. Pp. iv + 46. Professional Paper 427-B: Influences of Strip Mining on the Hydrologic Environment of Parts of Beaver Creek Basin, Kentucky. By Charles R. Collier and others. Pp. x + 85 + plates 1 and 2. Professional Paper 505: Philmont Country: The Rocks and Landscape of a Famous New Mexico Ranch. By G. D. Robinson, A. A. Wanek, W. H. Hays and M. E. McCallum. Pp. viii + 152 + plates 1–6. (Washington, D.C.: Government Printing Office, 1964.) [147

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Map 5–1964: Structure Contours of Base of Favel Formation, Southwestern Manitoba. Map 34–1964: Geological Survey of Canada Field Parties, 1964—Total 111. Index Sheet 75: District of Mackenzie. Index Sheet 85: District of Mackenzie. Index Sheet 95: Yukon Territory–District of Mackenzie. Index Sheet 106, 116: Yukon Territory–District of Mackenzie. (Ottawa: Director, Geological Survey of Canada, 1964.) [147

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Astra Annual Report, 1963. Pp. 25. (Södertälje, Sweden: Aktiebolaget Astra, Apotekarnes Kemiska Fabriker, 1964.) [237

National Museum of Canada. Bulletin No. 199: Contributions to Zoology, 1963. Pp. vi + 110. 1.50 dollars. Bulletin No. 200: The Cultural Affinities of the Newfoundland Dorset Eskimo. By Elmer Harp, Jr. Pp. vii + 183 (34 plates). 3 dollars. Natural History Papers. No. 24, (April 1, 1964): The Northern Prairie Skunk in Manitoba—a Possible Relic Population. By Francis R. Cook. Pp. 11. (Ottawa: Queen's Printer, 1964.) [237

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UTILIZATION OF SCIENTIFIC AND ENGINEERING MAN-POWER

THE Committee on Utilization of Scientific and Engineering Manpower, of which Mr. J. R. Killian, jun., is chairman, was appointed in 1962 by the National Academy of Sciences and financed by a grant from the Ford Foundation. It has thus something of the status of the Committee on Scientific Man-power of the Advisory Council on Scientific Policy in Britain, and its report, *Towards Better Utilization of Scientific and Engineering Talent: a Program for Action**, has its place in a series of reports dealing with aspects of scientific and engineering man-power which have come from the President's Science Advisory Committee during the past two years. It does not deal with the problem of dissemination of information, which was considered in 1963 in the report from that Committee, *Science, Government and Information* (*Nature*, 202, 735; 1964), nor with the support of basic research in the universities, which was covered by a report from the National Academy of Sciences, *Federal Support of Basic Research in Institutions of Higher Learning*. Similarly, graduate training in engineering, mathematics and physical sciences is left to the report under that title from the President's Science Advisory Committee, and the questions of technicians will be dealt with in an impending report from the same Committee.

Nevertheless, as the title of the present report indicates, it is more concerned with action than the reports that have so far come from the Committee on Scientific Man-power, or even, on this subject, from the Advisory Council on Scientific Policy. It includes a brief and clearly presented survey of needs and resources, which is summarized on p. 1125 of this issue of *Nature*, along with the nine study papers which form Part 2 of the report. The bulk of the first part of the report is contained in three chapters, which discuss successively the functions and responsibilities, in respect of the utilization of scientific and engineering man-power, of the Federal Government, industry and the universities. It is with these, and the brief chapter on research, that we are here concerned; the conclusions and recommendations set forth are relevant to the position in Britain, both from the point of view of the expansion of higher education and from that of the reorganization of civil research.

First, as regards Federal Government responsibilities, it should be noted that although only 11 per cent of scientists and engineers engaged in research and development are employed by the Federal Government, 60 per cent of the expenditure on research and development comes from the Government, and 80 per cent of this is dispersed by the Department of Defence and the National Aeronautics and Space Administration. It is scarcely surprising, therefore, that the Committee should recommend that before the Government makes a decision to undertake a great technological programme, such as the lunar landing or the supersonic transport projects, it should make a careful assessment of the impact of the decision on the deployment and utilization of scientists and engineers. How this is to be done depends on the administrative structure generally, but it is worth emphasizing that, in Britain, this appears to have been done reasonably well by the Advisory Council for Scientific Policy. Any changes in the organization of civil science should not be allowed to impair the Council's effectiveness in this field.

In the United States, the Committee on Utilization of Scientific and Engineering Manpower recommends that a unit within the Executive Office of the President should be made responsible for stimulating and co-ordinating planning by Federal Departments and agencies with respect to scientific and engineering man-power. Its responsibilities would also include the promotion of research, inside and outside Government, likely to facilitate the solution of man-power problems, and the initiation of an integrated programme for continuing collection and analysis of information on scientific and engineering man-power. The Committee suggests that such a unit might be placed in the Office of Science and Technology. Besides this, however, it recommends firmly that each department with major scientific or engineering activities should make one of its highest officials responsible for improving the utilization of civilian scientists and engineers, whether employed directly, or by those whose work it finances. Specifically, it recommends that an official in the Department of Defence be given such responsibilities to improve the utilization of civilian scientists and engineers working on defence programmes within the Department and elsewhere.

Such an official should: (1) participate in Government-wide scientific and engineering man-power planning activities and bring to the attention of his colleagues the implications, in terms of man-power, of proposed new programmes; (2) assess the impact on man-power of cancellation, curtailment or alteration of major programmes; (3) analyse the influence of man-power practices and policies on the effectiveness with which scientific and engineering man-power is utilized; (4) provide for the collection and analysis of the necessary information. While it would seem possible that the new organization of the Ministry of Defence could go some way towards securing these objectives in Britain, how far they are consistent with the control at present exercised by the Treasury generally is another matter. Particularly in view of comments by the Estimates Committee on the staffing of the new Diplomatic Services, recommended by the Plowden Committee in its report on *Representational Services Overseas* (*Nature*, 203, 439, 797; 1964), the exercise of Treasury control might well be worth re-examining in the light of the comments in this report. Without such an enquiry, the appointment to major projects of outstanding managers with technical and administrative ability is unlikely to contribute greatly to the improvement of such management, nor are the managers likely to receive the necessary authority, responsibility and resources.

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* *Towards Better Utilization of Scientific and Engineering Talent: a Program for Action*. Report of the Committee on Utilization of Scientific and Engineering Manpower. Publication No. 1191, National Academy of Sciences. (Printing and Publishing Offices, National Academy of Sciences, Washington, D.C., 1964.)

Here the wisdom of assessing these recommendations in the British context seems further emphasized by recent unhappy experience over the fixed-price contract system for guided weapons and the disclosures of the first report of the Inquiry into the Pricing of Ministry of Aviation Contracts, and the next few recommendations underline the same point. Government agencies responsible for developing programmes should continue to place emphasis on accurate estimates of their cost and feasibility, and on the use of multi-phase contracts. In development contracts the use of fixed-price and incentive contracts instead of cost-plus-fixed contracts is recommended. Moreover, Government agencies should take great care to establish meaningful and realistic criteria of performance. The Committee commends the increasing ability of Federal contracting agencies in the fields of defence and space to cancel, curtail or materially alter at an early stage major programmes that do not appear to be worth their cost. However, Federal departments and agencies should work with industry to develop plans and programmes for minimizing any dislocation or wastage of scientists and engineers as a result of such cancellation or redirection. They would also maintain Federal support of contractor-initiated technical programmes at a substantial level and develop incentives for encouraging corporate managements to emphasize quality and continuity, and to orient work towards long-term objectives.

The three last-mentioned recommendations relating to the Federal Government are concerned with its role as employer, and the Committee first recommends greater emphasis on assuring a high level of professional competence in the Federal scientific establishments. This is directed particularly at Congress, which should enact promptly the proposals of the administration for higher salaries at the upper levels in the Government Service. The Committee also recommends that the U.S. Civil Service Commission should take the initiative in improving the working environment of scientists and engineers employed by the Federal Government, and help to foster improved forecasting of their future requirements for scientific and engineering personnel. This recommendation is in line with the ideas of the Fabian Society's Committee regarding the functions of the Civil Service Commission as expressed in a recent report, *The Administrator: The Reform of the Civil Service* (*Nature*, 203, 555, 797; 1964). Finally, the Committee recommends periodic reviews by the Department of Defence, the Atomic Energy Commission, the National Aeronautics and Space Administration and other Government departments of the missions and the programmes of the research laboratories they finance, whether directly or under contract. This would ensure that their resources served national objectives of high priority, that arrangements for their management and location provide the maximum opportunity to be strong and creative, and that their programmes and administrative arrangements are compatible with the objectives of institutions with which they may be linked. In this, it is suggested, the resources of the President's Science Advisory Committee might be used.

Eight recommendations relate to the utilization of man-power by industry, and are concerned primarily with engineers and scientists engaged in research and development, and especially in the aircraft and missiles industry and in electrical equipment and communications. Together, these last-mentioned industries received 5,400 million out of the 6,700 million dollars which the Government spent in 1962 for research and development performed by private industry and accounted for 58 per cent of private and Federal investment in industrial research and development. The recommendations deal first with improving the management of research and with the key role of management. Corporate managers should identify their most promising scientists and engineers and take action to enable them fully to develop and apply their competence, and they should seek to provide a climate for creativity and productivity in keeping with the great potential of highly qualified scientists and engineers.

These have been recurring themes in discussions on the management of research in Britain since the Barber Report on scientific staff and earlier, and the second and third recommendations of the present report reiterate the vital importance of effective management. However, no guidance is offered concerning the selection of technical and administrative talent or on how best such a central core should be given the necessary authority. The emphasis placed on the responsibility of industry, Government and the universities in training and developing more managers and project engineers, who combine thorough understanding of the technology they manage with mastery of the art of leadership, finds some reflexion in the report, and recognition of that responsibility lies to some extent behind the present move towards the establishment of business schools. Beyond this, the report recommends that companies should actively seek to assist their most talented man-power to increase and replenish its professional competence. Furthermore, it advocates more systematic study of the art and science of research management, with support from both industry and Government. Intensive study of the experience of modern corporations that are heavily committed to research and innovation is particularly recommended; this finds support in the evidence which Sir Ronald Holroyd gave to the Sub-Committee of the Estimates Committee, which recently reported on the Treasury Control of Establishments. Finally, the Committee considers that wastage of scientific and engineering talent would best be minimized by improved management on the lines suggested and that no fundamental overhauling of Government procurement methods or imposition of extreme controls over contractors is required. However, it believes that co-ordinated action by industry and Government could stimulate more research and development in areas of the economy where the rate of innovation has been relatively slow.

Precisely how this 'action' could be stimulated is not indicated in the report and it would not appear that the United States has yet any real solution to offer to one of the most acute problems besetting the research associations in Britain, or the utilization of science in backward industries generally. The seven recommendations relating to the universities and other institutions of higher education are likewise of particular interest in the light of the recommendations of the Robbins Committee and emphasize many of the same principles—though without offering much further guidance. While the Committee stresses the responsibility of the universities in maintaining a proper balance between teaching, research and public service—with the systematic co-operation of the Federal Government—and admits that better ways are needed to recognize and reward the distinguished teacher, it can only remind the universities and professional associations that they have given too little attention to this subject. It suggests they might, with the President's

Science Advisory Committee, indicate some more effective means of recognizing great teachers and creative contributions to teaching.

One of the recommendations calls for development of new centres of educational excellence in science as well as further support of the efforts of the present centres and, as is now being done in Britain, increased attention to and larger support of programmes for the development and reform of curricula, particularly programmes which involve outstanding scholars in the universities working with school teachers. It is also in line with the Robbins Report that universities with strength in science should accept a responsibility to provide opportunities for study and research for members of the faculty of independent liberal arts colleges, and that such colleges should strengthen the quality of their science teaching by increased salaries, research facilities and leave for professional development. In view of the present discussion on engineering education in Britain, it should also be noted that the Killian Committee recommends strengthening and expanding graduate study in engineering as well as continued reform of undergraduate education in engineering, reducing its rigidity and increasing its scientific content, and modernization of the laboratory facilities of engineering schools.

The final two recommendations also reflect present-day tendencies in Britain. Universities, it is urged, in close co-operation with Government and industry, should develop a concerted attack on the problem of keeping engineering and scientific man-power up to date by refresher and like courses. Universities should also take the lead in expanding educational research, and the Committee rightly argues that reform of curricula, improvements in engineering schools, expansion of teacher training, and the establishment of new centres of excellence all require a sound foundation in research.

This aspect is further discussed in a separate chapter, "The Need for Research", in which the Committee recommends that the Government, industry and the universities expand or initiate research efforts that will provide the broader perspectives and increased knowledge for dealing more effectively with the issues involved in the utilization of scientific and engineering man-power. As examples, it instances the economics and dynamics of scientific and engineering man-power, the unused potential in human resources, technological aids, the organization in its operating environment, the scientist or engineer in his working environment, and scientific and engineering man-power and public policy. Some of these subjects are already being investigated in Britain under programmes for educational research financed by the Ministry for Education and Science. Others fall within the scope of the Committee on Scientific Man-power of the Advisory Council for Scientific Policy, and there should be no reason to fear that such efforts will be interrupted by the changes which are being made in the organization of Civil science following the recommendation of the Trend Report (*Nature*, 203, 574; 1964).

The significance for Britain of this report from Dr. Killian and his colleagues does not, in fact, lie so much in the novelty of its recommendations as in the clarity and firmness with which they are related to national needs, whether in the United States or in Britain. No Government can now mistake the massive influence which it exerts on the deployment and utilization of scientists and engineers or the concomitant extent of its responsibility to avoid waste. The balanced allocation of such talent, based on adequate and reliable data, is primarily a Government responsibility and, if management also has a key part to play, that in itself indicates the Government's duty to satisfy itself that adequate means for education and training in management are available. Above all, there must be forethought and adaptability to change, and in this report standards are set and challenges offered which must be accepted at least as much in Britain as in the United States.

A LOOK AT LIFE

Atlas of Electron Microscopy

Biological Applications. Compiled by F. Scanga. Pp. xxvi + 331. (Amsterdam, London, New York: Elsevier Publishing Company, 1964.) 210*s*.

THE electron microscope is now firmly established as a tool inseparably bound to future progress in biology, and no biological laboratory can in the future be considered complete without this now essential tool. Most biology students at universities, indeed, already have direct if somewhat glancing acquaintance with the instrument and none can have escaped its influence. So much indeed has electron microscopy become part of the corpus of common biological tools, and so fundamental has been its impact on biological thought, that most sixth formers already know a good deal about the instrument and its achievements, and electron microscopy at a suitable level is about to take its place in the formal curriculum at schools. This is perhaps the first time in which current biological research, however exciting, has found an immediate outlet both in text-books and in school curricula. Whatever satisfaction one may find in this rapid spread of modern knowledge is tempered by a concern that, if the principle is to be maintained that no student should be taught matters which he cannot be called on himself to experience, however remotely, something must be done to bring practical electron microscopy within the ambit of all universities and of all schools dealing with advanced level biology. This has been foreshadowed by recommendations made in several quarters that schools should arm themselves with a sheaf of relevant, informative electron micrographs produced by recognized authorities. Steps are already being taken to publish sets of electron micrographs collected together specifically for this purpose. But steps take time, and time may be something the schools are short of. It was therefore with the keenest anticipation that I received this beautifully presented volume by Prof. Scanga. It constitutes a second edition, in English, of an earlier volume in Italian by the same author. The first edition was chiefly devoted to bacteriology and virology, but this second version "has been supplemented by a large number of micrographs of cytological interest. In all, about 25 per cent of the photographic material is new to the present edition".

The book consists of 482 electron micrographs each briefly annotated together with a "brief but useful survey" of the fundamental techniques and principal biological applications running over little more than eleven pages of text. Some of the micrographs are of historic importance and some are excellent—chance alone would ensure this—but it is disheartening on looking through these pages to see so many which, "notable achievements of workers throughout the world" though they may be, are neither historically important, excellent, recent nor produced by a recognized authority. Most of the micrographs dealing with bacteriology and virology, and some other micrographs are Prof. Scanga's own and it is understandable and welcome that an author should draw on his own richness. 148 micrographs of bacteria in a total of 482 illustrations seems, however, to be going a little far particularly since many of them show exactly the same (few) features. The book would have been greatly improved if most of these had been deleted or, preferably, replaced by more recent plates on most of the topics which are covered in this book and many which are not. Scarcely any of Prof. Scanga's photographs, moreover, come from a named published work so that the viewer has no possibility of following up any point of interest. Of the fewer than 240 items which refer to published papers, only two are more recent than 1960 and more than half were published during the 10 years preceding 1956. One can scarcely believe, therefore, that "the present day scope and utility of electron microscopy" has been adequately documented. One of the potentially good features of the book, the large format "chosen so that the original electron micrographs may be seen to their best advantage", has unfortunately in many cases, though happily not all, gone sadly astray: it has resulted in magnification which many of these earlier photographs could not stand.

This having been said, there are still many photographs in this book which are excellent and well worth recording in this way. It would be invidious to name names, and interested biologists are recommended to leaf through the book to look for these highlights. The total effect, nevertheless, leaves a doubt in my mind about the public for which this book has been prepared. It can scarcely appeal to the expert and, taking into account the amount of information conveyed, can scarcely appeal to schools at this price even though at the same time the price seems moderate for a magnificently produced volume of this kind. Electron microscopy is at this time advancing more rapidly than almost any other facet of scientific endeavour and a collection of photographs is inevitably out of date to-day even if culled from yesterday's efforts. It is sad that this book appears to have been out of date even before it was conceived, for the idea was sound and Prof. Scanga appears to have missed a unique opportunity.

R. D. PRESTON

COPEPODA AND BRANCHIURA

Parasitic Copepoda and Branchiura of Fishes

By Satyu Yamaguti. Pp. vii + 1104 (333 plates). (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1963.) 563*s*.

THE number of known species of Copepoda and Branchiura, mainly parasitic on fishes, has now passed 2,500 and keeps rising from month to month. It includes a diversity of forms which is as bewildering as it is fascinating. Many of these forms have become enmeshed in the tangle of systematic controversy, many have never been properly studied or even sufficiently described. A synoptic look at the entire group has been long overdue.

This book is an attempt to produce a comprehensive account of the copepods and branchiurans infesting fishes. Its author is a noted helminthologist, well known for his magnum opus, *Systema Helminthum*. By his own admission (p. 1), he treats Copepoda as a second string to his bow. Closer acquaintance with his book bears him out entirely.

I began my appraisal of Yamaguti's book by a close investigation of its bibliography. Although the author himself does not consider it complete, the bibliography is surprising by its lack of selectivity. Some important publications are missing. On the other hand, some of the included ones are irrelevant to the subject discussed. Papers by Blake and Bonnier (p. 346) do not deal with the parasites of fishes. The list could have been better edited. Some references are quoted twice under different dates (Brian, 1898, 1908) or under different authors (Nunes-Ruivo, 1952; Pearse, 1952). There is even one reference to a paper never published (p. 362, Kirtisinghe, 1934, in *Spolia Zeylanica*). These, and many more, create an aura of untidiness which does little to inspire confidence. The aura is further augmented by fairly numerous typographical errors. One is rather taken aback at seeing a proposal to amend *Lernaeopodina* to . . . *Lernaeopodina*, particularly when there is really no need for amendment. *Phrixocephalus arboreus* is quoted in the index twice, the second time as *alboreus*. (On the other hand, *Lernaeocera* is missing from the index altogether.)

The book is intended as a complete record of all known species but it falls short of its aim. Many species have been missed out. Even some genera are absent. *Anaclavella* and *Euclavellisa* are not mentioned, though the paper in which they were first described (Heegaard, 1940) is recorded in the bibliography.

A disagreeable feature of the book is its taxonomic confusion with its inevitable consequences. Many species are recorded twice under different names (for example, *Cybicola armatus*, p. 135, and *Pseudocycnoides armatus*, p. 172; *Chondracanthus horridus*, p. 273, and *Disphaerocephalus horridus*, p. 286; *Clavella hippoglossi*, p. 243, and *Hatschekia hippoglossi*, p. 135). The last example is particularly glaring: *C. hippoglossi* was validly transferred to the genus *Hatschekia* in 1902. Similarly, the confusion between *Lernaeocera* and *Lernaea* was resolved in 1915, but it still haunts Yamaguti's pages. The confusion is further increased by the author's revival of long-defunct names. *Salmincola*, a well-established generic name, is replaced by Lamarck's *Entomoda*, on the ground that *Entomoda* (later *Salmincola*) *salmonea* was mentioned first in the account of the genus. Contrary to Yagamuti's view, the International Committee for Zoological Nomenclature decided some time ago that in Lamarck's genera no species will be accepted as type by implication. Equally unfounded, in my opinion, is the substitution of *Chondracanthoides* Oakley, 1930, for *Chondracanthodes* Wilson, 1932.

One of the penalties of nomenclatural inaccuracies is the placing on record of non-existing species such as *Dissonus intermedius* Kurtz, 1932. Kurtz published no paper in that year. She did, however, describe *Pholidopus* (*Achtheinus*) *intermedius* in 1924, establishing in the same paper *Dissonus glaber*. Yamaguti lists also, on p. 114, *Achtheinus intermedius* as synonymous with *Dissonus intermedius*. The two genera are not even in the same family.

It is not surprising, in view of the foregoing, that one tends to approach the author's systematics with caution. Some of it, such as the erection of the new order Andreinidea (consisting of only one species), seems to me to be of questionable value. The chondracanthids are a diverse lot, biologically as well as morphologically, and the isolation of *Andreina* in a separate order seems scarcely justifiable. Why not, for example, *Juanettia*, with its peculiar mandible? In contrast to this innovation is the retention of Chondracanthidae and Lernaeopodidae in the same order, though it is now known that the two families are not related.

The phylogeny of the parasitic copepods is still very controversial and one cannot expect to find agreement with everybody. One must not, however, confuse the sides in the dispute. This is what the author does by mentioning in the same breath, as it were, Lang and Heegaard, on p. 2.

At the level of the lower taxa, thirteen new families are introduced; it would be a bold man who would give a snap judgement on their validity. The author disregards, however, Gurney's (1933) Lernaeoceridae. He decides to retain Lernaeocerinae and Lernaeinae in the same family, in spite of strong evidence, produced during the past two decades, that close affinity exists between *Lernaea* and *Lamproglena* (placed by Yamaguti in Eudactylinidae), and in spite of many indications that a careful revision in that sector might not come amiss. At the generic level, the author retains Leigh-Sharpe's spurious genus *Alella*.

His new combination of *Isobranchia* is an interesting effort to clarify the uncertain status of the *Brachiella* group, but it does require further investigation. Yamaguti's support for Leigh-Sharpe's 'splitting' of *Clavella*, abundantly rejected many years ago, is not acceptable to me.

On the credit side of the book is most of all its existence. The task it set out to fulfil is an onerous one and its author is to be admired for his courage and diligence. The gathering together, in 333 plates, of illustrations, usually dispersed to the point of inaccessibility, is in itself a feat and a tremendous help for anybody working in this field. In spite of its many and obvious shortcomings, in spite of its uncomfortably high price, the book will become essential. Those who use it, however, and beginners in particular, would do well to double-check every piece of information it provides.

Z. KABATA

IMMUNOLOGICAL REVIEWS

Progress in Allergy

Vol. 7. Edited by Paul Kallós and Byron H. Waksman. Pp. xii+334. (Basle and New York: S. Karger, 1963.) 60 Sw. francs.

THE seventh volume of *Progress in Allergy* fully maintains the high standard of its predecessors. This series of volumes consists of reviews of immunology that are usually comprehensive and therefore provide one of the standard sources of reference to the immunological literature. This volume is no exception and contains five important review articles.

In the first article, H. Fudenberg presents a concise review of the "Hereditary Gamma Globulin (Gm) Groups". This field involves the investigation of genetically determined differences in the antigenic properties of human γ-globulin, which can be revealed only by the use of naturally occurring antibodies in certain human sera. It is well known that sera from the majority of patients with active rheumatoid disease contain antibodies to human γ-globulin; it now appears that these naturally occurring antibodies can detect antigenic differences between the γ-globulins of different human individuals. Fudenberg points out that the antigens (allotypes) are readily identified by agglutination-inhibition reactions in a way analogous to the use of inhibition-reactions in investigating the genetically determined antigens of human erythrocytes. He describes the incidence of the allelic γ-globulin determinants (Gm phenotypes) in various ethnological groups; then follows a description of the physicochemical and serological properties of the components of the Gm test system itself.

The author describes his own attempts to simplify the cumbersome test system and discusses the implications of recent work relating Gm specificities to the structure of γ-globulin. It is evident from his review that there is already a need for standardization of nomenclature and test systems in this new field.

Anaphylactic shock has long been considered to involve a reaction of the body to pharmacologically active substances, released from cells or tissues, by the interaction of antigens and antibodies in the immediate vicinity of body tissues. However, little is known of the mechanisms underlying these tissue reactions, and increasing attention is now being directed to the biological effects of preformed antigen-antibody complexes. K. Ishizaka is a leading worker in this field; the second chapter of this volume consists of his review entitled "Gamma Globulin and Molecular Mechanisms in Hypersensitivity Reactions".

In the first part of his article, Ishizaka brings together the biological activity of immune complexes and their immunochemical properties. Complexes formed in moderate antigen excess have the greatest biological activity; they produce acute anaphylaxis in the guinea pig or mouse, and cause contraction of smooth muscle as well as passive cutaneous anaphylaxis. The author discusses the discrepancy between the vascular lesions seen in serum sickness, or in the Arthus phenomenon, and the lesions that result from the injection of preformed immune complexes; he suggests that greater fixation (and hence greater damage) to blood vessels occurs when the immune complexes are actually formed *in vivo*. Both skin reactivity and complement-fixing potency depend on the species of origin of the antibody as well as the ratio of antibody to antigen; it appears that two or more antibody molecules attached to one antigen are required for skin reactivity.

Ishizaka then suggests that important structural changes in antibody molecules may occur when they are brought together by antigen and that certain molecular shapes arise (toxic configurations of the γ-globulin) that acquire the capacity to liberate pharmacologically active substances from skin and other tissues. Ishazaka's findings relate to present thinking on the co-operative properties of large molecules that go beyond the properties of their sub-units; the author's experimental work is discussed in some detail and there is a valuable bibliography.

"Organ specificity with special reference to the lens" is the title of a well-documented review by S. P. Halbert and W. Manski. They examine the concepts of organ-specific and species-specific antigens using their extensive data on ocular lens antigens as a starting point. Further illustrations are drawn from some of the literature on: serum proteins and blood cells; heart and skeletal muscles; spermatozoa and brain; cell nuclei, DNA, enzymes and hormones. It is proposed that organ specificity and species specificity represent extremes of a spectrum of differentiation and that the vertebrate lens provides a classical example of the organ-specific antigens. Antisera to bovine and human lens were made in rabbits and ducks; there were many precipitin cross-reactions with higher vertebrate lenses, but only a few with the lower vertebrate lens extracts, while invertebrate lens did not react at all.

Serum proteins were once thought to reflect the individuality of their species of origin; however, as Halbert and Manski point out, considerable antigenic sharing is now evident in serum proteins from a wide range of species. It is implied that organ- and species-specificity are not mutually exclusive properties of tissue antigens; though few will disagree with this, there will be some differences of opinion concerning the nomenclature used in this review.

The literature on auto-antibodies to lens is reviewed together with selected examples of other tissue auto-antibodies and a critical discussion is given of the status of auto-allergic lens disease. The authors' opinion is that further work will be needed before it is clear that auto-antibodies can damage the lens *in vivo*, or cause damage to cultured lens epithelium *in vitro*.

It is concluded that advances in techniques of immunodiffusion will now permit a greater degree of accuracy in the application of tissue immunochemistry to problems of differentiation and taxonomy.

In a chapter entitled "Induced Synthesis of Histamine, Microcirculatory Regulation and the Mechanism of Action of the Adrenal Glucocortoid Hormones", R. W. Schayer cites evidence implicating histamine in the physiological regulation of the microcirculatory system. It is explained that this view "is in sharp contrast to the usual concept of histamine as a nuisance or toxin". In a closely packed presentation the author enumerates the evidence that regulation of the microcirculation occurs *via* the production of newly synthesized histamine molecules. This is made possible by an increase in histidine decarboxylase activity that occurs after various stimuli; this enzyme activity is shown to depend on the severity of the stimulus and to correlate with the degree of capillary response.* The author closely evaluates other permeability factors (plasma globulins, leucotaxines and plasma kinins) and considers that they are not involved in microcirculatory regulation.

Schayer then discusses the modes of action of glucocorticoids as vasoconstrictors of the smooth muscle of the microcirculation and possible potentiators of catecholamines; thus these two actions of the glucocorticoids would combine to inhibit the effects of induced histamine on the microcirculation. The author tabulates the response of the microcirculation to various circumstances, interpreting the observed changes in terms of a balance between induced histamine synthesis on one hand and glucocorticoid status on the other.

Finally, S. B. Salvin contributes an article entitled "Immunological Aspects of the Mycoses". He reviews the fungal antigens, antibodies and serological tests in eleven mycotic diseases, while he further discusses delayed hypersensitivity, pathogenesis, resistance and immunization in these disorders. The mycoses reviewed are as follows: Actinomycosis, nocardiosis, aspergillosis, candidiasis, cryptococcosis, North American blastomycosis, coccidioidomycosis, histoplasmosis, chromoblastomycosis, sporotrichosis, and dermatomycosis. This is a monumental review, containing nearly 800 references to the literature, though clinical mycologists may notice the absence of data concerning *Mucor*, *Rhizopus*, and *Absidia*. The alarming increase in deep-seated mycotic disease that has occurred during the past ten years makes Salvin's review most timely: its value is thus two-fold, for there is a growing increase in the immunology of Fungi in general.

As can be seen from the contents, this volume richly deserves its place in the reference literature of Immunology. In an introduction, Waksman summarizes recent developments in immunology and makes a plea for the recognition of Immunopathology as a special discipline, and for the introduction of a journal devoted to this speciality. Those who eagerly await the appearance of each, now annual, addition to the *Progress in Allergy* series will agree that these reviews make a great contribution in themselves.

D. C. DUMONDE

ANALYSIS OF PLANT DISEASE EPIDEMICS

Plant Diseases

Epidemics and Control. By J. E. van der Plank. Pp. xvi+349. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 80*s*.

THOSE who know Dr. van der Plank or are aware of his substantial contributions to the epidemiology of plant diseases will not be led astray by the title of this book, but others may be. It is not, as one might anticipate, a description of plant disease epidemics and how to control them, but a dissertation on new methods of epidemiological analysis based on the rate at which the population of the pathogen increases (infection rate) and on the relation between the amount of inoculum and the amount of disease the inoculum produces. It also demonstrates how this kind of analysis can be applied in the control of diseases, whether by the use of resistant varieties, by sanitation or by chemicals, all of which help to reduce the amount of inoculum, or the infection rate, or both.

Plant Diseases: Epidemics and Control is intended primarily for plant pathologists and for plant breeders interested in breeding for resistance to diseases, and some of it is likely to be heavy going for those without an adequate knowledge of mathematics or who lack interest or experience in the application of mathematics to biology. The author, convinced that training in epidemiology will soon become as essential for a plant pathologist as training in mycology, virology or genetics, is clearly aware of this difficulty for he goes out of his way to help his readers. The first of the twenty-three chapters explains simply and clearly what the book is about and there is a continuous explanatory thread, in part taking the form of an introduction to each chapter, which runs through the text, encouraging and maintaining interest even through the more difficult parts. Exercises in epidemiological analysis are included at the end of each of the chapters concerned with methods, and relevant mathematical tables are provided in an appendix. The other early chapters deal with analogies drawn between rates of interest on money and disease infection rates; with methods for plotting the progress of an epidemic; and with the breakdown and analysis of infection rates to reveal how they may be influenced by various factors, such as the time newly infected tissues take to become infectious (latent period) and the time needed for symptoms to develop (incubation period), or by the effects of new host growth, gradual sterility of infected tissues and so on.

The rest of the book is mainly devoted to the illustration of how the practice of sanitation or the use of resistant varieties and fungicides may be related to the analysis of control. The effect of sanitation, very loosely defined as the process of reducing the inoculum from which an epidemic starts, is dealt with in three chapters, with special reference to potato blight, black stem rust of wheat, bunt of wheat and *Fusarium* wilt of tomato. Then follow seven chapters on types of disease resistance, again with potato blight and black stem rust as the cardinal examples. A clear distinction is made between vertical resistance, which is directed against some races of a pathogen but not others, and horizontal resistance (in potatoes at present called field resistance) which operates against all races. The need for and intent of this new jargon is less apparent. Vertical resistance, the usual choice of plant breeders, is preferred by the author only when there is a record of variety stability or when the disease multiplies slowly. Horizontal resistance is preferred when varietal change is frequently imposed by rapid changes in the races of the pathogen or when it is desired to improve the performance of fungicides. In biological warfare with black stem rust, for example, vertical resistance would, it is thought, leave the wheat crop wide open to destruction, whereas horizontal resistance is believed to provide an adequate defence. In chemical control of diseases, regarded here at times as a form of sanitation, the fungicidal action of the chemical is only part of the story. Successful control of an epidemic depends almost equally on weather or other environmental conditions and could be greatly promoted by better horizontal resistance in the host plants.

The text ends with two chapters on the spread, as distinct from the increase, of disease. In the second of these, the author is highly critical of many field experiments intended to provide information that can be applied to farming. In his view, experimental plots frequently do not represent accurately the farmer's fields or orchards they are meant to, because of 'representational' and 'cryptic' errors. Such errors arise, for example, when guard rows in conventional layouts are not efficient against fast-moving pathogens in reducing or preventing plot interference, and the extent of the errors may depend on other factors, including plot size and shape. Special layouts for avoiding interference and reducing representational errors are suggested. More than 200 literature references are listed, most of them to work published in the past decade.

This extremely well-written book can be of absorbing interest, even to one who finds much of the mathematics beyond him. It breaks new ground with new ideas and will without doubt lead to much discussion and argument among plant pathologists and plant breeders as well as in a wider field, for much of it will surely find a place in future training courses.

W. C. MOORE

A COMPREHENSIVE GUIDE TO TEMPERATURE

Temperature: Its Measurement and Control in Science and Industry

Vol. 3. Editor-in-Chief: Charles M. Herzfeld. Part 1: Basic Concepts, Standards and Methods. Edited by F. G. Brickwedde. Pp. xvi + 848. 220*s.* net. Part 2: Applied Methods and Instruments. Edited by A. I. Dahl. Pp. xiv + 1094. 236*s.* net. Part 3: Biology and Medicine. Edited by James D. Hardy. Pp. xii + 683. 180*s.* net. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1962–63.)

THE American Institute of Physics, associated with the National Bureau of Standards and other organizations, has sponsored a series of symposia on temperature measurement. So far there have been four symposia, in 1919, 1939, 1954 and 1961, and the proceedings of the last three have been published as volumes 1–3 of *Temperature: Its Measurement and Control in Science and Industry*. The first volume, of 1362 pages, was so comprehensive and authoritative that it immediately established itself as essential in any laboratory concerned with the measurement of temperature. The second volume did not, perhaps, fully justify the title. It ran to 467 pages, and industrial applications were only very lightly touched on.

The third volume has now appeared and is so large that it has had to be published in three parts, totalling 2625 pages, and one can say at once that it is going to be fully the equal of the famous Volume 1 as an essential reference work. Volume 1, however, has lost none of its usefulness, for little of its contents has become obsolete, but the new volume introduces much new matter. It is clear that many of the recent developments arise from space research, which has posed many problems, notably in the measurement of gas and flame temperatures and in the physiological questions involved in space travel.

The new volume contains 242 papers, ranging from accounts of particular instruments or investigations to general survey papers. In the field of optical pyrometry, for example, we have papers by R. D. Lee of the National Bureau of Standards in Washington and by J. Middlehurst and T. P. Jones of the National Standards Laboratory of Australia, each describing the photoelectric instruments developed in those laboratories, while from Canada D. R. Lovejoy contributes a notable survey paper under the title "Recent Advances in Optical Pyrometry". In this we have an account of work carried out by the author, who also considers the work of many other investigators. References to fifty-three related papers are given. The large number of references is a feature of the book as a whole: there are, on the average, about twenty-five to each paper, and they add greatly to the value of the book as a work of reference.

Part 1, *Basic Concepts, Standards and Methods*, is notable for the contributions from national laboratories in the United States, the United Kingdom, the Soviet Union, Germany, the Netherlands, Canada and Australia, dealing with the relationship between the thermodynamic and practical scales of temperature. One of the main problems in this field at the present time is the method to be used for extending the International Practical Scale of Temperature below 90° K (the boiling point of oxygen) and a group of five papers is devoted to it. Another group of eight papers deals with the establishment of the thermodynamic scale by both traditional and novel methods. "A Review of Recent Determinations of Thermodynamic Temperatures of Fixed Points above 419° C" is another important survey paper. It is contributed by Dr. H. Moser, of the Physikalisch-Technische Bundesanstalt, and summarizes the results obtained by gas thermometry in Germany, Japan, the United States and the U.S.S.R., and discusses possible sources of error.

Notable among a group of papers on platinum resistance thermometry is "Potentiometric Methods of Resistance Measurement", by T. M. Dauphinee, of the National Research Council, Ottawa. Like Lovejoy's optical pyrometer paper from the same laboratory, it is a valuable survey and incorporates some of the author's recent work, notably the design of a resistance comparator utilizing a circuit in which the terms of the quadratic law of a platinum thermometer can be set so that the bridge reads directly in temperature with an accuracy of 0·001° C from − 50° to 700° C with a thermometer having a fundamental interval of approximately 10 ohms.

Part 1 also includes several papers on the measurement of plasma temperatures by spectroscopic and other methods, on temperature measurement in astrophysics and in geophysics.

Nearly all the papers on thermoelectric methods are to be found in Part 2, Applied Methods and Instruments. The contribution "Thermocouple Materials", by F. R. Caldwell, of the National Bureau of Standards, is very comprehensive, and includes most of the physical properties of the metals and alloys which are ever used in thermocouples. This paper gives 124 references. In all, there are 24 papers in the sections on thermoelectric thermometry, dealing with such matters as couples for very high temperatures, e.m.f. stability and performance when exposed to nuclear reaction. There are, however, further papers in other sections on the use of thermocouples for special purposes, such as the measurement of surface temperatures, gas temperatures, transient temperatures, etc. There is also a paper by S. D. Raezer and H. L. Olsen on "The Intermittent Thermometer: A New Technique for the Measurement of Extreme Temperature", which discusses the application of a chromel-alumel thermocouple to the measurement of temperatures well above its melting point by a method of intermittent exposure. Temperature measurements of a plasma jet up to nearly 4,000° K by this method are reported, and the work is being continued in the hope of further extending the range of the method.

Clarence R. Droms reports on an extensive series of tests on the stability of thermistors, while other papers concerned with semi-conductor devices range from the use of thermistors and carbon resistance thermometers at low temperatures to the possible use of ceramic elements for temperatures above 2,300° R (1,000° C).

Radiation thermometry is also well covered in Part 2. There are four papers devoted to both monochromatic and radiation ratio pyrometers, and to their uses in the measurement of gas and plasma temperatures.

Part 3 is concerned with biology and medicine, but many of the experimental techniques will find use in more extended fields. There are, for example, papers on microcalorimetry by T. H. Benzinger and Charlotte Kitzinger and by Robert L. Berger, and a study of the conditions required for accurate surface temperature measurement with thermocouples by George W. Molnar and Joseph C. Rosenbaum, jun. The paper by T. H. Benzinger and G. W. Taylor on "Cranial Measurements of Internal Temperature in Man" gives an ingenious brush design for a thermocouple to maintain light contact on the eardrum which could find other uses. The importance of the measurement of cranial temperatures is clear from the paper on "The Human Thermostat", by T. H. Benzinger, C. Kitzinger and A. W. Pratt. This paper is a very thorough investigation of the mechanism of temperature control in the human body. Other sections of Part 3 are devoted to thermal sensation, hypothermia and the physiological responses to heat and cold.

The three parts of Volume III of *Temperature: Its Measurement and Control in Science and Industry* should all find a place in any laboratory in which the problems of measuring temperature are encountered. The sponsors, publishers, editors and contributors are to be congratulated on a valuable piece of work. J. A. HALL

THE MYSTERY OF THE COSMIC HELIUM ABUNDANCE

By PROF. F. HOYLE, F.R.S., and DR. R. J. TAYLER
University of Cambridge

IT is usually supposed that the original material of the Galaxy was pristine material. Even solar material is usually regarded as 'uncooked', apart from the small concentrations of heavy elements amounting to about 2 per cent by mass which are believed on good grounds to have been produced by nuclear reactions in stars. However, the presence of helium, in a ratio by mass to hydrogen of about 1 : 2, shows that this is not strictly the case. Granted this, it is still often assumed in astrophysics that the 'cooking' has been of a mild degree, involving temperatures of less than 10^8 K, such as occurs inside main-sequence stars. However, if present observations of a uniformly high helium content in our Galaxy and its neighbours are correct, it is difficult to suppose that all the helium has been produced in ordinary stars.

It is the purpose of this article to suggest that mild 'cooking' is not enough and that most, if not all, of the material of our everyday world, of the Sun, of the stars in our Galaxy and probably of the whole local group of galaxies, if not the whole Universe, has been 'cooked' to a temperature in excess of 10^{10} K. The conclusion is reached that: (i) the Universe had a singular origin or is oscillatory, or (ii) the occurrence of massive objects has been more frequent than has hitherto been supposed.

The values of the helium to hydrogen number ratio determined for various objects are shown in Table 1.

Table 1

	He/H
Orion nebula (ref. 1)	0·091
NGC 604 in *M* 33 (ref. 2)	0·102
Small magellanic cloud (ref. 3)	0·11
B stars (ref. 4)	0·16
Planetary nebulæ (ref. 5)	0·09–0·19
Solar cosmic rays (refs. 6 and 7)	0·091
Solar evolution (ref. 8)	0·09

The first five determinations were made by spectroscopic methods. It has been found that the ratios of carbon, oxygen, magnesium and silicon in solar cosmic rays are in good agreement with well-determined spectroscopic values, and it is therefore argued that the composition of the cosmic rays reflects the true solar composition, and hence that the He/O value in the cosmic rays reflects the true solar helium/oxygen ratio. Given reliable spectroscopic values for all elements other than helium, and given accurate opacities within the Sun, it is possible to determine He/H from the requirement that solar evolution must be such that the present-day luminosity is arrived at after $4{\cdot}5$–5×10^9 years, the known age of the solar system. It is of immediate interest that two such different methods as these should arrive at closely the same result for the solar He/H value, particularly as this result—if accepted—is sufficient to establish that the Universe did not have a singular origin, nor can the Universe be oscillatory.

A high helium abundance in some particular star or nebula need not be taken as proof of a high primeval value, since helium may have been produced locally by nuclear reactions. For this reason low values of He/H are of more interest in relation to the original composition of the Galaxy than high values. However, O'Dell's high value of $0{\cdot}18 \pm 0{\cdot}03$ for the planetary nebula *M* 15 is of special interest because O'Dell also finds a low value for the ratio O/H, suggesting that the material of this nebula may not have been adulterated by the products of nuclear reactions. Adulteration by hydrogen-burning could have occurred; but not adulteration by helium-burning, so the situation remains rather uncertain.

We begin our argument by noticing that helium production in ordinary stars is inadequate to explain the values in Table 1, if they are general throughout the Galaxy, by a factor of about 10. Multiplying the present-day optical emission of the Galaxy, $\sim 4 \times 10^{43}$ ergs sec^{-1}, by the age of the Galaxy, $\sim 3 \times 10^{17}$ sec, and then dividing by the energy production per gram, $\sim 6 \times 10^{18}$ ergs g^{-1}, for the process H → He, gives $\sim 2 \times 10^{42}$ g ($10^9 M_\odot$). This is the mass of hydrogen that must be converted to helium in order to supply the present-day optical output of the Galaxy for the whole of its lifetime. Allowance for emission in the ultra-violet and in the infra-red increases the required hydrogen-burning, but probably not by a factor more than ~ 3. Since the total mass of the Galaxy is $\sim 10^{11} M_\odot$ the value of the He/H to be expected from H → He inside stars is only $\sim 0{\cdot}01$. While it is true that the Galaxy may have been much more luminous in the past than it is now, there is no evidence that this was the case.

Next, we shift the discussion to a 'radiation origin' of the Universe, in which the rest mass energy density is less than the energy density of radiation. The relation between the temperature T_{10}, measured in units of 10^{10} K, and the time t in seconds can be worked out from the equations of relativistic cosmology and is:

$$T_{10} = 1{\cdot}52\, t^{-1/2} \qquad (1)$$

In the theory of Alpher, Bethe and Gamow[9] the density was given by:

$$\rho \approx 10^{-4} T_{10}^{3} \text{ g cm}^{-3} \qquad (2)$$

a relation obtained from the following considerations. The material is taken at $t = 0$ to be entirely neutrons. At $t \simeq 10^3$ sec, $T_{10} \simeq 0{\cdot}05$, approximately half the neutrons have decayed. If the density is too low the resulting protons do not combine with the remaining neutrons, and very little helium is formed. On the other hand, if the density is too high there is a complete combination of neutrons and protons, and with the further combination of the resulting deuterium into helium very little hydrogen remains as t increases. Thus only by a rather precise adjustment of the density, that is, by (2), can the situation be arranged so that hydrogen and helium emerge in approximately equal amounts.

It was pointed out by Hayashi[10] and Alpher, Follin and Herman[11] that the assumption of material initially composed wholly of neutrons is not correct. The radiation field generates electron-positron pairs by:

$$\gamma + \gamma \rightleftarrows e^- + e^+ \qquad (3)$$

and the pairs promote the following reactions:

$$n + e^+ \rightleftarrows p + \bar{\nu} \qquad (4)$$

$$p + e^- \rightleftarrows n + \nu \qquad (5)$$

The situation evidently depends on the rates of these reactions. It turns out that for sufficiently small t the balance of the reactions is thermodynamic. This means that not only are protons generated by (4) and (5), but also that the energy densities of the pairs and of the neutrinos must be included in the cosmological equations. At $T_{10} \simeq 10^2$, even μ-neutrinos are produced and these too should be included. The effect of these new contributions to the energy density is to modify (1) to:

$$T_{10} \simeq 1{\cdot}04\, t^{-1/2} \qquad (6)$$

The values of σv for (4), (5), read from left to right, are:

$$\pi^2\left(\frac{\hbar}{mc}\right)^3\left(\frac{W \pm W_0}{mc^2}\right)^2\left(\frac{\ln 2}{(\mathrm{f}\tau)_{\mathrm{lab}}}\right) \qquad (7)$$

in which the well-known Coulomb factor has been taken as unity, and where the symbols have the following significance: m, electronic mass; W, the energy, including rest mass, of the positron or electron; W_0, energy difference between the neutron and proton ($2{\cdot}54\ mc^2$); ± apply to (4) and (5) respectively; $(\mathrm{f}\tau)_{\mathrm{lab}}$, the f$\tau$-value for free neutron decay (1,175 sec).

To obtain the rates of reactions (4) and (5), read from left to right, multiply (7), using the appropriate sign, by the number of positrons/electrons with energies between W and $W + \mathrm{d}W$ and then integrate the product with respect to W from zero to infinity. The corresponding values for the rates of (4) and (5) read from right to left can then easily be obtained by noticing that in thermodynamic equilibrium:

$$\frac{n}{p} = \exp - \frac{W_0}{kT} \qquad (8)$$

where n, p represent the densities of neutrons and protons.

If, however, one is content with accuracy to within a few per cent it is sufficient simply to write $W = mc^2 + qkT$ in (7) and to multiply by the total number density of pairs. The value then chosen for q is that which makes $mc^2 + qkT$ equal to the average electron energy at temperature T; this is a slowly varying function of T and is given by Chandrasekhar[12]. The pair density is:

$$\frac{1}{\pi^2}\left(\frac{kT}{\hbar c}\right)^3\left(\frac{mc^2}{kT}\right)^2 K_2\left(\frac{mc^2}{kT}\right) \qquad (9)$$

where K_2 is the modified Bessel function of the second kind and second order. For $T_{10} \sim 1$ this expression can be closely approximated by the simple form $30\ a\ T^3/\pi^4 k$. The number density of neutrino-antineutrino pairs (of both kinds) is $15\ a\ T_3/\pi^4 k$.

Adopting this simplified procedure, the reaction rate for $n + e^+ \rightarrow p + \bar{\nu}$ is easily seen to be:

$$0{\cdot}071\ T_{10}^3\ (1 + 0{\cdot}476\ qT_{10})^2 \text{ per neutron per sec} \qquad (10)$$

The effect of $n + \nu \rightarrow p + e^-$ is approximately to double the rate at which neutrons are converted to protons. Using equation (8), the rates of the inverse reactions are obtained by multiplying equation (10) by $\exp - W_0/kT = \exp - 1{\cdot}506/T_{10}$. Hence the following differential equation determines the variation of $n/(n + p)$ with time:

$$\frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{n}{n+p}\right) = 0{\cdot}142T_{10}^3\ (1 + 0{\cdot}476qT_{10})^2 \times \left[\frac{n}{n+p}\left(1 + \exp\frac{-1{\cdot}506}{T_{10}}\right) - \exp\frac{-1{\cdot}506}{T_{10}}\right] \qquad (11)$$

To express this in a form convenient for numerical integration use T_{10} as the independent variable. With the aid of equation (6):

$$\frac{\mathrm{d}}{\mathrm{d}T_{10}}\left(\frac{n}{n+p}\right) = 0{\cdot}308(1 + 0{\cdot}476qT_{10})^2 \times \left[\frac{n}{n+p}\left(1 + \exp\frac{-1{\cdot}506}{T_{10}}\right) - \exp\frac{-1{\cdot}506}{T_{10}}\right] \qquad (12)$$

Equation (12) can be integrated from a sufficiently high temperature, at which the neutrons and protons are almost in thermodynamic balance, down to the temperature at which the pairs disappear and deuterons are formed. The results are insensitive to starting temperature if it is chosen above $T_{10} = 2{\cdot}5$. When the protons and neutrons are in thermodynamic balance the right-hand side of (12) is zero and the initial value of $n/(n + p)$ is chosen to make this right-hand side zero.

An important question evidently arises as to the precise value of T_{10} down to which equation (12) should be integrated. Rather surprisingly, it appears the deuterium combines into helium, through D(D,n)He^3(n,p)T(p,γ)He^4, at a temperature as high as $T_{10} = 0{\cdot}3$, in spite of the small binding energy of deuterium. (The concentration of deuterium used in establishing this conclusion was just that which exists for statistical equilibrium in $n + p \rightleftarrows \mathrm{D} + \gamma$.) Hence, equation (12) must not be integrated to T_{10} below 0·3. We estimate that integration down to $T_{10} = 0{\cdot}5$ probably gives the most reliable result, because (12) overestimates the rate of conversion of neutrons to protons below $T_{10} = 0{\cdot}5$.

Mr. J. Faulkner has solved the equation for several starting temperatures. Provided $T_{10} > 2{\cdot}5$ initially, he finds $n/(n + p) = 0{\cdot}18$ at $T_{10} = 0{\cdot}5$, giving:

$$\frac{\mathrm{He}}{\mathrm{H}} = \frac{n}{2(p - n)} \simeq 0{\cdot}14 \qquad (13)$$

a result in good agreement with the calculations of Alpher, Follin and Herman[11]. Allowing for the approximation in our integration procedure we estimate that this value is not more uncertain than 0·14 ± 0·02. It should be particularly noted that, unlike the result of Alpher, Bethe and Gamow, this value depends only slightly on the assumed material density; essentially this result is obtained provided the density is high enough for deuterons to be formed in a time short compared to the neutron half-life and low enough for the rest mass energy density of the nucleons to be neglected in comparison with the energy density of the radiation field.

Before comparing this result with observation we note that variations of the cosmological conditions which led to equation (6) all seem as if they would have the effect of increasing He/H. If the rest mass energy density were not less than the sum of the energy densities of radiation, pairs and neutrinos, the Universe would have to expand faster at a given temperature in order to overcome the increased gravity, the time-scale would be shorter and the coefficient on the right-hand side of equation (12) would be reduced. Similarly, if there were more than two kinds of neutrino the expansion would have to be faster in order to overcome the gravitational attraction of the extra neutrinos, and the time-scale would again be shorter; and the smaller the coefficient on the right-hand side of equation (12) the larger the ratio He/H turns out to be.

We can now say that if the Universe originated in a singular way the He/H ratio cannot be less than about 0·14. This value is of the same order of magnitude as the observed ratios although it is somewhat larger than most of them. However, if it can be established empirically that the ratio is appreciably less than this in any astronomical object in which diffusive separation is out of the question, we can assert that the Universe did not have a singular origin. The importance of the value 0·09 for the Sun is clear; should this value be confirmed by further investigations the cosmological implication will be profound. (A similar situation arises in the case of an oscillating universe. The maximum temperature, achieved at moments of maximum density, must be high enough for all nuclei to be disrupted, that is, $T_{10} > 1$. Otherwise, after a few oscillations all hydrogen would be converted into heavier nuclei, and this is manifestly not the case.)

It is reasonable, however, to argue in an opposite way. The fact that observed He/H values never differ from 0·14 by more than a factor 2, combined with the fact that the observed values are of necessity subject to some uncertainty, could be interpreted as evidence that the Universe did have a singular origin (or that it is oscillatory). The difficulty of explaining the observed values in terms of hydrogen-burning in ordinary stars supports this point of view. So far as we are aware, there is only one strong counter to this argument, namely, that there is nothing really special to cosmology in the foregoing discussion. A similar result for the He/H ratio will always be obtained if matter is heated above $T_{10} = 1$, and if the time-scale of the process is similar to that given by equation (6). In this connexion it may well be important that the physical conditions inside massive objects or superstars simulate a radiation Universe. Hoyle, Fowler, Burbidge and Burbidge[13] were led, for reasons independent of those of the present article, to consider temperatures exactly in the region $T_{10} \simeq 1$. These authors give the following differential equation between the time t and the density ρ, in such a superstar:

$$\mathrm{d}t = \left(\frac{1}{24\pi G\rho}\right)^{1/2} \frac{\mathrm{d}\rho}{\rho} \qquad (14)$$

and also the following relation for an object of mass M:

$$\rho = 2{\cdot}8 \times 10^{8} \left(\frac{M_{\odot}}{M}\right)^{1/2} T_{10}^{3}\ \mathrm{g\ cm^{-3}} \qquad (15)$$

Eliminating ρ and $\mathrm{d}\rho$ we have:

$$\mathrm{d}t = \left(\frac{M}{2{\cdot}44 \times 10^{4} M_{\odot}}\right)^{1/4} \frac{\mathrm{d}T_{10}}{T_{10}^{2.5}} \qquad (16)$$

whereas the differential form of equation (6) is:

$$\mathrm{d}t = -\ 2{\cdot}08\ \frac{\mathrm{d}T_{10}}{T_{10}^{3}} \qquad (17)$$

The difference of sign arises because equation (16) was given for a contracting object. For re-expansion of an object the sign must be reversed, so that the time-scales are identical if $M \simeq 5 \times 10^{5}\ M_{\odot}$. It may be significant that this is about the largest mass in which the temperature $T_{10} \simeq 1$ can be reached without the object being required to collapse inside the Schwarzschild critical radius. If collapse inside this radius followed by re-emergence be permitted, larger masses can be considered. The time-scale is then increased above equation (17) and this has the effect of giving a smaller He/H ratio than that calculated above. If an object is inside the Schwarzschild radius and neutrinos do not escape from it, the conditions are closely similar to the cosmological case. On the other hand, the calculation must be slightly changed for objects that do not enter the Schwarzschild radius, since neutrinos are certainly not contained within them. Thus if the same time-scale were used, that is, $M \simeq 10^{5} - 10^{6}\ M_{\odot}$, absence of neutrinos would reduce the right-hand side of equation (12) by a factor of 2. A corresponding calculation leads to $n/(n + p) = 0{\cdot}22$, also in reasonable agreement with observation, especially as all material need not have passed through massive objects. However, a more detailed discussion of massive objects will be required to decide whether the required amount of helium cannot only be produced but also ejected from them.

This brings us back to our opening remarks. There has always been difficulty in explaining the high helium content of cosmic material in terms of ordinary stellar processes. The mean luminosities of galaxies come out appreciably too high on such a hypothesis. The arguments presented here make it clear, we believe, that the helium was produced in a far more dramatic way. Either the Universe has had at least one high-temperature, high-density phase, or massive objects must play (or have played) a larger part in astrophysical evolution than has hitherto been supposed. Clearly the approximate calculations of this present article must be repeated more accurately, but we would stress two general points: (1) the weak interaction cross-sections turn out to be just of the right order of magnitude for interesting effects to occur in the time-scale available; (2) for a wide range of physical conditions (for example, nucleon density) roughly the observed amount of helium is produced.

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EXTRACTION OF URANIUM FROM SEA WATER

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THERE is now a world surplus of uranium; the production capacity of mines and ore treatment plants in the United States, Canada and South Africa far exceeds the present demand for all civil and military purposes so that the relatively modest uranium supplies to cover British nuclear power requirements seem to be assured for some years. When the work described here was started, more than ten years ago, this situation was far from evident. It is true that the existence of large reserves in these areas was suspected on geological grounds but there was little reason to expect that production capacity could overtake demand so rapidly. In Britain, we were about to embark on a nuclear power programme which it was thought might lead in course of time to dependence on uranium for much of our national electricity supply, yet no reserves of uranium had been discovered in these Islands which were in any way commensurate with requirements.

The possibility of recovery of uranium from natural waters, including sea water, by ion-exchange resins had been considered by the late Dr. F. H. Burstall and his group at the Chemical Research Laboratory (now the National Chemical Laboratory), Teddington, shortly after the War, but it was decided that the effort should be directed towards exploitation of the then known ores overseas. Later some experiments were made at Harwell[1] on the recovery of uranium from sea water by newly developed techniques of continuous counter-current extraction. The solvent—dibutyl phosphate dissolved in butex (dibutoxy diethyl ether) or kerosene—was contacted with acidified sea water in a rotary annular column; extraction appeared to be quantitative and 60 μg of uranium was obtained from 20 l. It was evident, however, that although such a process would be technically feasible on a large scale the solvent losses and reagent costs would render it economically unattractive. Nevertheless, the comparative ease with which uranium could be quantitatively extracted from such an extremely weak solution containing high concentrations of other salts gave encouragement to the idea that in the long term some economically acceptable method might be found.

The total volume of the oceans has been estimated[2] to be $1{\cdot}37 \times 10^{9}$ cubic kilometres and, assuming a uniform concentration throughout, the dissolved uranium should

Table 1*a*. MOLAR CONCENTRATIONS IN SEA WATER; URANIUM AND ASSOCIATED IONS

U	HCO_3'	H^+	CO_3''
$1{\cdot}43 \times 10^{-8}$	$2{\cdot}3 \times 10^{-3}$	$10^{-7{\cdot}8} - 10^{-8{\cdot}2}$	$2{\cdot}2 \times 10^{-4}$*

Table 1*b*. MOLAR CONCENTRATIONS IN SEA WATER—OTHER MAJOR IONS

Na^+	Mg^{++}	Cl'	SO_4''
0·47	0·054	0·54	0·03

* Calculated from the dissociation constant for bicarbonate ion in sea water ($K_{HCO_3} = 10^{-9{\cdot}02}$).

amount to $4{\cdot}16 \times 10^9$ metric tons. Moreover, the uranium concentration in sea water is probably stationary; a large amount, estimated at 27,000 tonnes annually, contributed by rivers, appears to be balanced by an equal amount carried down with the bottom deposits[3].

The uranium concentration (3·34 µg/l.) (ref. 4) is remarkably constant; only second-order variations occur between samples obtained from the Atlantic and Pacific oceans and from near the surface to depths of at least 400 m.

The molar concentrations of uranium and of closely associated species in sea water are given in Table 1*a*, and concentrations of other major constituents are given in Table 1*b* (ref. 2).

A very interesting feature is that the uranium occurs in true solution as the tricarbonato complex, $UO_2(CO_3)_3^{-4}$. This was early established at Harwell[5] where it was also shown that less than about 0·1 per cent of the uranium is associated with suspended matter. Russian workers[6] came to the same conclusion regarding the chemical form of the uranium independently. Thus the ocean is virtually a limitless reservoir of uranium in solution in a well-defined chemical environment. There are, however, two major obstacles to using this reservoir as an economic source of uranium. First it is in a strongly complexed form at extreme dilution in presence of relatively high concentrations of other ions; and secondly, to bring any extraction agent into contact with the very large volumes of sea water that would be involved is not an easy operation. To be successful, an extractant must function efficiently at the usual *p*H and ionic strength of sea water and must be virtually insoluble.

Possible Organic and Inorganic Absorbents

An obvious step following the successful solvent extraction experiments with dibutyl phosphate was to incorporate functional groups of similar type in solid insoluble organic resin matrices. Extraction could only be expected by reagents which form a complex with the uranyl ion sufficiently strong to compete with the uranyl tricarbonate complex in sea water for which the stability constant is about 2×10^{18}. Calculation showed that the stability constant of the competing uranyl complex would have to exceed 10^{12}. Although a number of ligands were known to form uranyl complexes with stability constants of this order, such as β-diketones and 8-hydroxyquinolines, the effective stability constant when the ligand had been incorporated in a resin matrix could not be predicted, so a fairly wide range of functional groups was chosen for investigation in the first instance. Resins were prepared[7] containing mono- and di-basic phosphoric and phosphonic acids, 8-hydroxyquinoline, kojic acid, salicylic acid, amino-carboxylic acid and amino-phosphoric acid functional groups. These were tested first in a simulated sea water enriched about 1,000-fold in uranium and afterwards in fresh sea water in a small laboratory on the Mole at Portland Harbour. Samples of chelating resins available at the Chemical Research Laboratory, Teddington, were tested there by Daborn and Milward[8] using the enriched artificial sea water, and the most promising of them, a condensation product of formaldehyde and resorcinol arsonic acid, was sent to Harwell for further investigation. Greater quantities of some of the more promising resins were specially prepared for us by the Permutit Co., Ltd. A number of commercially available resins was also tested, including the carboxylic acid resin 'Zeo-Karb 226' and an α-hydroxy phosphonic acid resin supplied by Permutit.

The Portland tests were with fresh sea water which was pumped by a stainless-steel pump through a coarse filter into a head tank whence it flowed through a glass-wool filter into the test system. The connecting tubing and vessels were made of polythene, polyvinyl chloride or glass. The resin was held between two sintered glass disks 9 in. apart in a column of internal diameter 1 in. and subjected to upward-flowing sea water. At first, all the preparations showed only a low uptake, the maximum being 10 µg of uranium/g dry resin. However, the form of the resin proved to be critically important and a standard pretreatment procedure was evolved. The resins, initially in the damp H^+ form, were converted to the Na^+ form by 1·0 M sodium carbonate solution and then washed with redistilled water until fully swollen. Where particular mesh sizes were required the fully swollen resin was wet-sieved through stainless-steel mesh. After sizing it was reconverted to the H^+ form, with 0·5 M hydrochloric acid, washed with distilled water, dried for 48 h and bottled. Quantities, normally 2 g, were converted as required to the Na^+ form with 1·0 M sodium carbonate solution before placing in the column for testing. Although there was considerably greater uptake with several of the resins, only one reached values high enough to be of practical interest, namely, the arsonic acid resin. The early preparations of this material both rapidly lost weight in flowing sea water and were soluble in the sodium carbonate eluant used to remove the uranium. Improvements in the method of preparation which were made by Jones and Packham[9] at Teddington, and by Lane, Holdoway, Robinson and Willans at Harwell[10], increased the uniformity of product and enhanced matrix stability and general properties. The uptake of uranium by some typical organic materials is shown in Table 2.

Table 2. UPTAKE OF URANIUM FROM SEA WATER BY ORGANIC ABSORBENTS

Resin	Functional group	Time of contact (days)	Uptake µg/g of dry H^+ form
'Deacidite *FF*'	$-R_3NCl$	84	0·2
'Zeo-Karb 226'	$-COONa$	36	1·5
Polystyrene methylene phosphonic acid	$-CH_2PO(ONa)_2$	88	25
8-hydroxyquinoline	$-N=NC_9H_5N.ONa$	81	13
Resorcinol arsonic acid resin	$-AsO(ONa)_2$	112	1,010

Although the solubility shown by the original arsonic acid resin in sea water was largely overcome there still remained a steady loss of capacity which was traced to a slow hydrolytic attack on the carbon–arsenic bond[11]. This appeared to be an inherent weakness in any form of arsonic acid resin which seemed to exclude it as an absorbent for the economic extraction of uranium from sea water. Fortunately, alternative possibilities had arisen.

Although the concentration of uranium in the sea is far below the saturation level, deposition appears to be taking place at a rate equal to the rate of inflow from rivers. The mechanism is not properly understood but uptake by inorganic material seems to occur. In agreement with this, deposition of uranium from carbonate-containing waters in the Goodsprings District of Nevada has been attributed to absorption by hydrated ferric oxide and basic zinc carbonate. These considerations, coupled with our observation that ferruginous deposits in the filter on the inlet side of the sea-water plant at Portland contained some uranium, led us to investigate a number of inorganic materials as possible absorbents. They were precipitated on glass wool and packed into a glass column for testing. Sea water flowed upwards through the bed at the rate of 2·5 cm/min. Basic zinc carbonate, hydrated titanium hydroxide and certain lead compounds were the only materials which exhibited an effective uptake (Table 3).

Several other lead compounds showed comparable uptakes (lead naphthalene tetracarboxylate gave 1,074 µg/g in 35 days), but unfortunately all were unstable either in sea water or in the sodium carbonate eluant

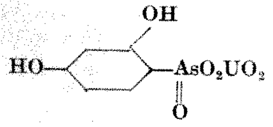
Table 3. UPTAKE OF URANIUM FROM SEA WATER BY INORGANIC ABSORBENTS

Compound	Time of exposure (days)	Uptake (μg/g of metal base)
Basic zinc carbonate	23	512
Titanium hydroxide	21	268
Lead sulphide	21	338
Lead pyrophosphate	21	479

which led to a low uptake when re-used after elution. Moreover, the lead compounds and basic zinc carbonate dissolved too rapidly in the flowing sea water to be economically possible For example, basic zinc carbonate loses 0·5 μg zinc/ml. to sea water flowing at 2·5 cm/min through the bed. With titanium hydroxide, however, the loss under the same conditions was undetectable.

After appropriate contact with sea water, the arsonic acid resin and the inorganic materials listed in Table 3 contain between 0·02 and 0·1 per cent of uranium in terms of dry weight; that is, within the same range as the uranium content of the ores now being worked in the main uranium-producing territories.

The stoichiometry of the reactions between some of these materials and sodium uranyl tricarbonate solutions was examined. Saturation of the arsonic acid resin occurred with an arsenic/uranium atomic ratio of unity. Conductivity determinations on the arsonic acid monomer indicated that the uranyl complex is uncharged, and this is borne out by the behaviour of the resin itself which exhibits no cation exchange capacity when saturated. The structure of the complex is therefore more probably I than II:



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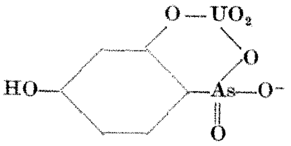

II

Analysis of the wet, basic zinc carbonate precipitate invariably gave a ratio $Zn/CO_3 = 3$, implying the formulation $2Zn(OH)_2ZnCO_3$. This ratio is maintained at all uranium loadings. When absorption takes place from sodium uranyl tricarbonate solution, one mole equivalent of carbonate and two of bicarbonate are released into the solution for each mole equivalent of uranyl absorbed, so that carbonate is not taken up by the absorber. Hydrated titanium oxide behaves in the same way. The absorption mechanism can therefore be represented as follows:

$$M_x(OH)_y + UO_2(CO_3)_3^{-4} \rightarrow M_x(OH)_{y-2}O_2 . UO_2 + CO_3^{-2} + 2HCO_3^{-1}$$

Although this equation may be representative of the conditions when macro concentrations of sodium uranyl tricarbonate are used the absorption reaction in sea water may be different. Tracer experiments with uranium-233 in simulated sea water gave results which, for hydrated titanium oxide, were more indicative of the participation of a partially neutralized species $M_x(OHO^-)_y$.

During the course of this work a report was published describing Russian experiments on the extraction of uranium from natural saline waters containing 60 μg uranium/l. (that is, about 20 times sea water concentration) by absorption or co-precipitation processes[12]. Optimum results were obtained with calcium phosphate, alumino-gel and ferri-gel; titano-gel was also said to be satisfactory, uptakes being in the range 70–170 μg uranium/ml. of absorber. These results, which we have confirmed, indicate a different order of uptake compared with that from solutions with a uranium concentration as low as that in sea water. Those reagents which gave the highest uptakes from Russian lake-waters absorbed relatively insignificant amounts of uranium from sea water.

More recently experiments have been reported by Bayer[13] on the uptake of copper and uranium from sea water by various organic chelating agents containing, for example, the glyoxal *bis*-2-hydroxyanil grouping. The uptake of uranium from 100 l. of sea water from the Gulf of Naples was 50 μg for a bed 2 cm diameter and 30 cm deep, that is, < 50 μg/g absorbent. This is of the same order of uptake as we obtained with some other chelating agents (Table 2).

Properties of Selected Absorbents

Titanium hydroxide is an amorphous material the properties of which depend on the method of preparation. Two forms have been distinguished and designated *ortho* and *meta*: the first, a fully hydrated form, is obtained at room temperatures and the second, a partially hydrated form, is obtained after long standing in air at room temperature or by heating. Although no appreciable difference could be detected in the total uptake of uranium from sea water, the two forms exhibit considerable differences in rate of uptake. Most of the experiments described here were made with titanium hydroxide which was precipitated by means of caustic soda, but it was found, later on, that precipitation with ammonia resulted in a more active product. In some experiments, the absorbent was supported on glass wool, muslin or pumice; in others it was used alone, in the form of granules. The muslin was impregnated by soaking it first in titanium tetrachloride solution (15 per cent titanium tetrachloride in 15 per cent hydrochloric acid) for 5 min, draining off for 30 min and then soaking in 8 N caustic soda solution (A.R.) for 5 min. Before being put into sea water, it was washed with distilled water. Normally the muslin was attached to a frame suspended beneath a vessel in Portland Harbour. The experiments with glass wool were made in glass columns 16 in. × 2 in. with filtered sea water. Glass wool (60 g) was thoroughly wetted with the titanium tetrachloride solution (155 ml.), after which the 8 N caustic soda solution (250 ml.) was stirred into the mixture and stirring continued for 15 min. The treated glass wool plug was positioned and protected in the column by plugs of untreated glass wool at either end, allowed to drain and exposed to flowing sea water without further washing. Granular titanium hydroxide was made by mixing the same reagents in the ratio 155 ml. titanium chloride solution to 250 ml. 8 N sodium hydroxide while stirring vigorously to avoid an appreciable rise in temperature. The precipitate was washed by decantation with distilled water until the *p*H of the washings was less than 8. A slurry of the titanium hydroxide was poured on to trays so as to form a layer 0·5 in. thick and allowed to dry in air for 21 days. When the dried material was placed in distilled water it broke up into granules which could then be separated into different size ranges by sieving.

The uptake curves, shown in Fig. 1, are typical of the results obtained when the methods of preparation described here were used. The precipitate on muslin appeared to be rather finer and more disperse than that on glass wool and the absorption was faster but they both reached the same maximum uptake. Similarly, the smaller granules showed a faster initial rate of uptake than larger granules but, with each, absorption levelled off after a time and the larger granules gave a lower apparent saturation level. If the granules are assumed to be spherical, the ratio of the initial uptake rates proves to be roughly proportional to the ratio of the surface areas. On this model the outer layers of the particles would appear to be progressively saturated until, with the 0·3-mm diameter particles, for example, the thickness of the saturated zone reaches 0·03 mm in 20 days, whereafter the absorption rate falls off rapidly. The irregular shapes of the granules and their porous nature allow such a model to be regarded only as a crude representation; nevertheless it conforms with the idea that the larger the granule the greater is the volume which is inaccessible for uranium absorption.

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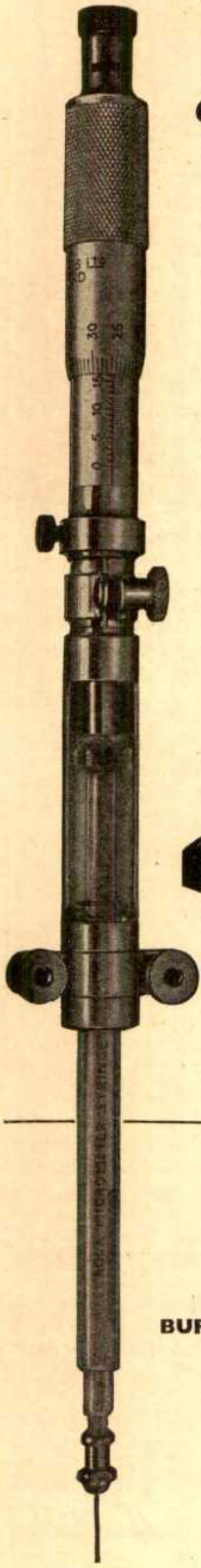

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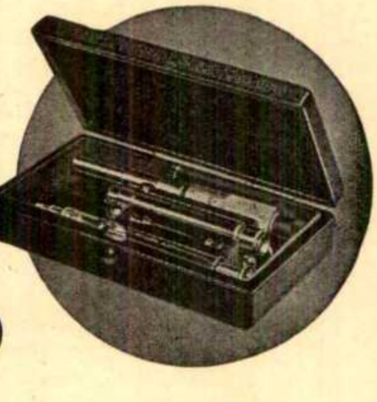

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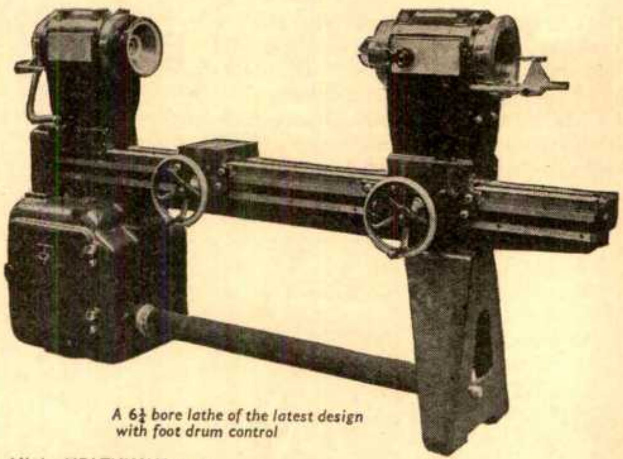

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The model also implies that granules of diameter less than 0·06 mm would be completely accessible.

It has been mentioned that the rate of uptake was higher when the titanium hydroxide had been precipitated with ammonia instead of caustic soda. Granules (14–24 mesh, diameter about 0·9 mm) produced by the ammonia route gave an uptake of 60 μg/g in one day when exposed to flowing sea water. This is more than ten times the initial rate obtained with the same size of granules prepared by the caustic soda method.

Titanium hydroxide is without detectable solubility in flowing sea water, but 0·1 μg/ml. was found in sea water which had been shaken with the precipitate for 72 h. No significant changes in specific uptake occurred after prolonged contact with sea water and after elution with M sodium carbonate solution. In the experimental work, the uranium content was frequently determined after complete dissolution of the sample, but for any large-scale extraction process selective leaching of the uranium would be necessary. Partition coefficients have been determined for a number of alkaline leaching solutions, and, in order to obtain results applicable to conditions of high dilution, trace concentrations of uranium-233 were used (Table 4).

Table 4. PARTITION COEFFICIENTS FOR ELUTION

	Partition coefficients (U per g Zn or Ti/U per ml.)	
Reagent	Basic zinc carbonate	Titanium hydroxide
0·25 M Na_2CO_3	168·0	1,311
0·5 M Na_2CO_3	96·4	602
1·0 M Na_2CO_3	49·8	206
0·25 M ammonium carbonate		45
0·5 M ammonium carbonate		1

Elution of uranium from basic zinc carbonate supported on glass wool is rapid with 1·0 M sodium carbonate, four or five bed volumes giving complete separation. But with titanium hydroxide, elution is slow; for example, less than 60 per cent of the uranium was removed by eight bed volumes of 1 M sodium carbonate eluant. A mixture of sodium bicarbonate (2 vol.) and sodium carbonate (1 vol.) gave 75 per cent elution in eight bed volumes and 1 M ammonium carbonate gave 65 per cent elution in two bed volumes and 93 per cent in eight. Dilute nitric acid is also a possible eluant for titanium hydroxide but ammonium carbonate appears to give the best results. The results in Table 4, obtained by batch equilibration at trace uranium concentration with the uranium initially in the aqueous phase, probably represent true equilibrium conditions since that for 1 M sodium carbonate was confirmed by approaching from the opposite side with titanium hydroxide containing natural uranium absorbed from sea water.

The solubility of titanium hydroxide in these eluants was determined: (*a*) by shaking with the precipitated floc for three days; (*b*) by passing the eluant through a bed of granular material at a flow rate of 1 cm/min.

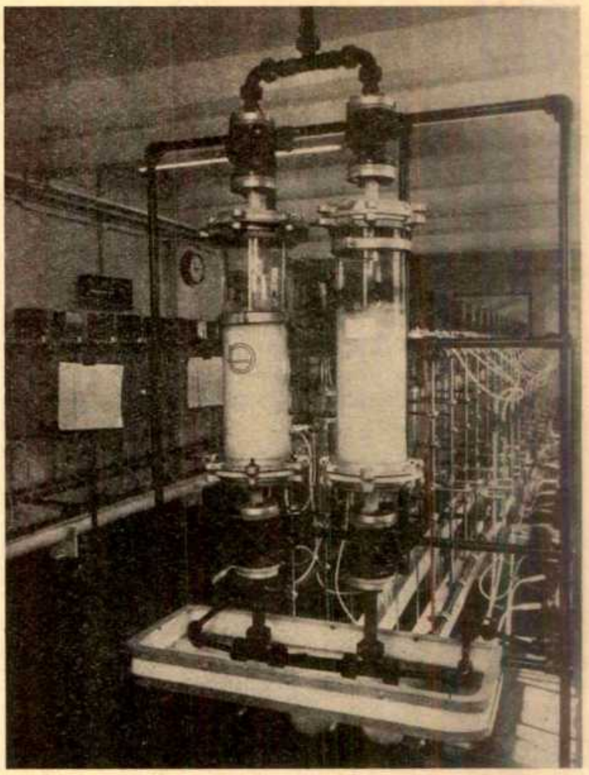

Fig. 2. Sea-water test equipment at Bincleaves Laboratory, Portland Harbour

Table 5. SOLUBILITY OF TITANIUM HYDROXIDE IN ELUANTS

Eluant	Solubility μg Ti/ml.
(*a*) $Ti(OH)_4$ precipitate shaken for 3 days	
1·0 M Na_2CO_3	0·48
0·5 M ammonium carbonate	1·4
1·0 M ammonium carbonate	14·1
(*b*) $Ti(OH)_4$ granules in flowing eluant	
1·0 M Na_2CO_3	0·1
1·0 M ammonium carbonate	11·4

The loss of absorbent in the eluant, although appreciable, would therefore not have a serious effect on the life of the bed.

Engineering Aspects

The engineering problems associated with the large-scale extraction of uranium from sea water are formidable but do not appear to be insoluble. Clearly it is desirable for the extraction plant to be sited so that it can be supplied with fresh uranium-bearing water by natural transport processes. In many coastal areas there is a residual drift superimposed on the purely oscillatory tidal motion which could maintain an adequate supply of uranium provided the rate of extraction was not too great. Certain ocean currents transport very large amounts of uranium. The volume of water transported by the Norwegian Current, which flows past the Orkneys and Shetlands, and along the coast of Norway towards the Arctic Sea, is about 3×10^6 m³/sec, which is equivalent to about 250,000 tons of uranium per annum; much greater quantities, carrying in excess of a million tons of uranium per annum, pass through the Florida Straits and the Japan Strait in comparatively narrow channels.

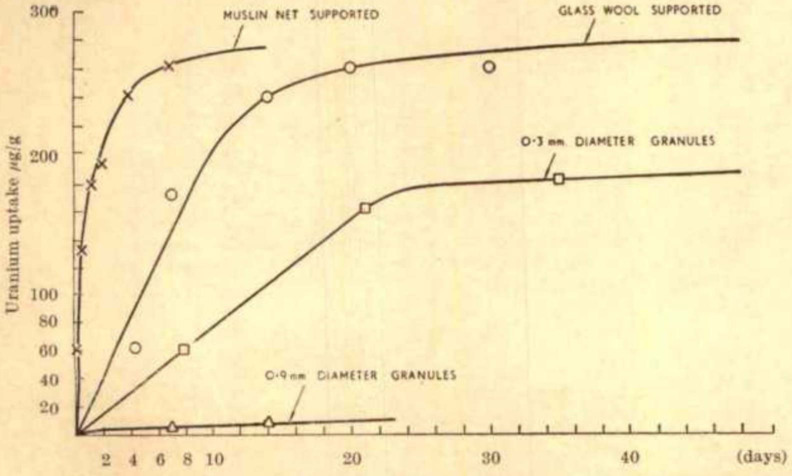

Fig. 1. Uptake of uranium from sea water by titanium hydroxide; typical results

Where there is a plentiful supply of fresh uranium-bearing water it would be possible to consider the advantages of means whereby only a small fraction of the uranium is extracted. Titanium impregnated muslin suspended beneath a vessel in Portland Harbour functioned in this way, giving very rapid uptake. Large-scale application of this technique, though possible in principle, would require a great deal of development to make it workable even before considering cost.

Other methods, in which the flow of sea water has to be channelled or controlled by engineering works, tend to require a high percentage extraction in an absorber system of specific design. Mass transport calculations have been made for different possible geometric arrangements of absorber, making the assumption that uptake was determined only by transport in the aqueous phase and that the diffusion coefficient of uranium species in sea water is about 3×10^{-6} cm²/sec. We obtained a value of $1{\cdot}8 \times 10^{-6}$ cm²/sec in the laboratory using the isotope uranium-233. The calculations showed that absorbers consisting of packs of parallel tubes lined with the absorbent placed across a channel would give a comparatively low yield unless the tubes were excessively long; for optimum absorption a considerable head of water would be required. Experiments were carried out in the southern (partially blocked) entrance to Portland Harbour using a cubic metre steel-framed cell containing asbestos cloth loaded with basic zinc carbonate folded over glass rods so as to give 1/8 in. spacing between layers. Uptake was slow and the percentage extraction was probably small, although this could not be accurately determined; saturation was reached after four weeks, yielding 0·25 g of uranium*.

The requirement for a greater head of water than could be obtained in a simple tidal channel led to an investigation of a fixed bed of absorbent particles operating against a head of sea water. Such a system, based on utilizing tidal energy in a manner similar to tidal power schemes, could be more easily visualized in engineering terms than any of the others considered. Two lagoons would be created, the sea water flowing into one on the rising tide, forming an upper lagoon, and out of the other, the lower, on the falling tide. The plant itself would consist of intermediate lagoons containing the packed beds, situated between the upper and the lower tidal lagoons. Sea water would flow into the plant lagoons from the upper lagoon, down through the packed beds and out along conduits into the lower lagoon. A difference in head of water would exist between the two lagoons which would depend on the level of the tide and the pressure drop through the bed. The beds would thus be operating continuously at all states of the tide, at a rate responding to the variations of head. The plant lagoons would be equipped with gates so that the flow of water could be cut off, enabling the beds to be eluted. The beds themselves would be partitioned so that sections could be eluted in a counter-current manner with a number of bed volumes of eluant, provided from the treatment plant located on the adjacent coast.

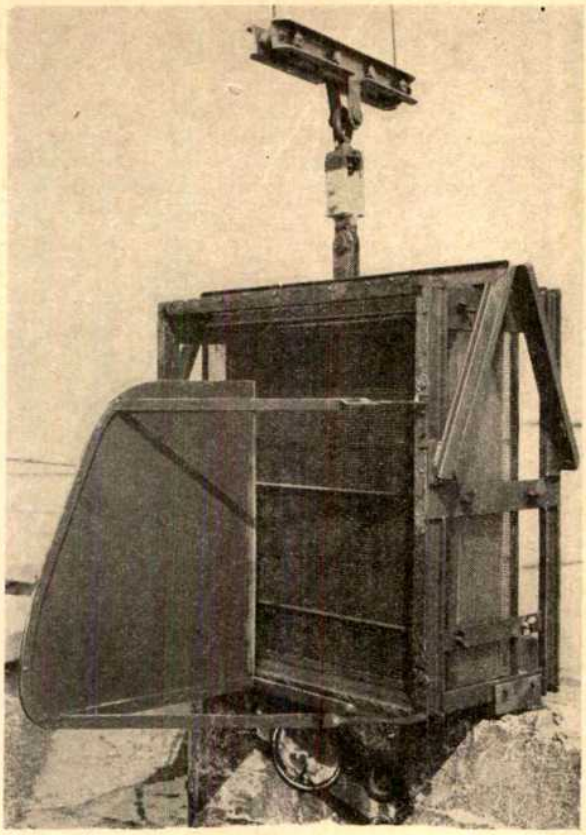

Fig. 3. Cell, 1 sq. metre cross-section, used in south entrance to Portland Harbour, showing frame bearing asbestos cloth impregnated with basic zinc carbonate

In order to obtain some idea of the problems and costs involved, a design investigation was made for a hypothetical plant operating in the Menai Straits. For this it was assumed that there is sufficient north-easterly drift of the water of the Irish Sea past Anglesey to maintain adequate supplies of fresh uranium-bearing water; there appears to be good reason for believing this but experiments were not carried out to establish it. The scheme would involve the construction of dams and sluice gates across the northern entrance between Anglesey and Great Orme's Head, and the division of the enclosed area of Beaumaris Bay into an upper lagoon and a lower lagoon by means of a dam and gates associated with absorber beds running in an approximately southerly direction from the neighbourhood of Puffin Island. A plant providing for the associated chemical operations would be located on shore at the southern end of the beds. Site investigations were not carried out and many assumptions had to be made regarding hydrological and geological factors and further uncertainty was introduced into the cost estimates by the novel engineering problems likely to be encountered. Fortunately we were able to make a comparison with results from the very thoroughly investigated Passamaquody Tidal Power Project through the courtesy of John W. Leslie, chief of the Engineering Division, Army Engineer Division, New England, U.S. Army Corps of Engineers, and this has enabled us to attach considerably greater confidence to our estimates of cost, particularly of the sea dams and gates. It would appear that the Menai site could produce almost 1,000 tonnes of uranium (1,300 short tons of U_3O_8) per annum, assuming adequate absorber performance, at a cost of £10,000–£20,000 per tonne (11–22 dollars per lb. U_3O_8). This is based on a twenty-year amortization; a lower unit cost would be achieved if the amortization period were extended from twenty to forty years. For comparison, the cost of extraction of uranium from shales has been estimated as 40–50 dollars per lb. U_3O_8 (ref. 14) and from granite as 100 dollars[15].

Performance of Titanium Hydroxide Absorber compared with Plant Requirements

The cost and performance of the absorber are, of course, key features. In making the estimate it was assumed that the active absorbent would be supported on low cost inert material in the form of small particles or granules.

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* Mr. N. R. Daly, Atomic Weapons Research Establishment, Aldermaston, has kindly determined the isotopic ratios for this oceanic uranium, namely:

Sample	235/238	236/235	233/235	234/235
A	0·007255 ± 0·000012	0·00005	0·00005	0·0077
B	0·007250 ± 0·000008	0·00007	0·00006	0·0080
Normal for uranium	0·007258			0·0078

Table 6. PERFORMANCE OF BEDS OF SUPPORTED TITANIUM HYDROXIDE IN FLOWING SEA WATER

Support	Bed diam. (cm)	Bed height (cm)	Ti content (g)	Flow rate (cm/sec)	Uptake (mgU)	Time (h)	% Extraction
Muslin	7·6	33	13	0·3	1·215	24	33
Glass wool	5·1	61	12	0·04	0·306	33	93
Glass wool	5·1	61	12	0·04	1·3	166	79
Glass wool	5·1	20	5	0·2	0·821	26·6	62
Glass wool	5·1	20	5	0·75	0·926	7·25	70

A satisfactory bed filling in such a form has not yet been produced but data for beds containing titanium hydroxide supported on muslin and on glass wool are given in Table 6.

Although the glass-wool-supported beds give an adequate performance at flow rates within the range expected in the plant (0·55–0·79 cm/sec) they would not be suitable for large-scale operations both for physical and for economic reasons. It has been shown, however, that titanium hydroxide granules appear to behave as composite particles containing an inaccessible and therefore inert core surrounded by a layer of active absorbent. Beds of those granules should perform similarly to the beds of composite particles specified for the plant. Adequate flow rates were obtained for pressure drops likely to be obtained in the plant using comparatively small granules (mean diam. 0·1 cm), but a larger size would almost certainly be required in practice in order to avoid blocking of the bed.

The results in Table 7 show that uptake rates of the required order can be obtained with titanium hydroxide granules (diam. ~ 0·1 cm) and that a considerable latitude exists for optimizing the absorptive activity of the bed. If these unsupported titanium hydroxide granules were used in the plant instead of the cheaper supported material, the increase in cost would be about £5,000 per tonne (5·50 dollars per lb. U_3O_8). Although preliminary experiments with pumice particles about 1/8 in. in diameter were not very promising, it should be possible to find some other low cost support, such as hydrated silica granules, which is acceptable.

It has not been possible to extend the range of these experiments on account of the closure of the sea water laboratory at Portland, and the present results do no more than indicate that a reasonably favourable basis exists for further development. This would require a substantial effort in bed-material development and manufacture, elution technology and so on. In the Portland experiments blockage and contamination of the absorber beds were avoided by filtration of the sea water through glass wool; the engineering implications of this have not been taken into account and other, unforeseen, difficulties might arise. If we assume that operational difficulties can be overcome there appears to be some hope that the ocean can provide a ready supply of medium cost, if not low cost, uranium.

Table 7. COMPARISON OF EXPERIMENTAL RESULTS USING $Ti(OH)_4$ GRANULES WITH PLANT REQUIREMENTS

Size range of granules, 14–24 mesh (mean diam. ~ 0·1 cm)

Exp.	Bed depth (in.)	Pressure drop (ft.)	Flow rate (ft.³/ft.²/h)	Ti content (kg)	Uptake mgU in 24 h	% Extraction
			Experimental results for columns 1 in. in diameter			
A	25	16	180	0·105	0·071	3
B	1·9	0·25	70	0·008	0·026	3
C	0·5	0·067	70	0·002	0·12	14
			Extrapolated to plant, per sq. ft.			
A	15·6	4·0	70	13	8·3	7·7
B	15·6	2·0	70	13	38	24
C	15·6	2·0	70	13	150	~ 100
			Required in the plant			
		2·0	70–100	2·1	125	80

Notes: (1) The experimental uptake for *A* represents 1/7 of the uptake determined after 7 days.

(2) Absorbents *A* and *B* were made by sodium hydroxide precipitation, but different batches; *C* was by ammonia precipitation.

(3) Results for *B* and *C* are typical of several experiments.

(4) The extrapolations are on a simple linear basis; in *B*, it is assumed that transport in the aqueous phase is not rate determining. This seems to be approximately correct for the range of conditions specified in Table 7 although not for Table 6.

(5) The figures given for plant requirements are typical; satisfactory designs for other performances are not excluded. During actual operation there would be considerable variation depending on the state of the tide.

(6) The quantity of titanium given represents that actually used in the experiments with extrapolations according to note 4. The quantity given for the plant is the minimum needed for a four-day cycle of exposure followed by elution.

This article summarizes work which will ultimately be published in full. The contributions of some of our collaborators have been acknowledged in the text; others who participated at various times and whose work we gratefully acknowledge here are: Mr. F. G. Johnson and Mr. W. G. Berry, Engineering Group, Risley; engineering aspects, Mr B. K. Robinson, Mr. J. W. A. Peckett, Mr. F. A. Burford, Mr. R. J. W. Streeton and Mr. A. C. Fox; A.E.R.E., Harwell, experimental work at Portland and Harwell, and members of the Analytical Chemistry Branch, A.E.R.E., for special uranium analyses. We also thank Prof. P. L. Robinson for his advice.

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A RECONSTRUCTION OF THE CONTINENTS FOR THE UPPER PALÆOZOIC FROM PALÆOMAGNETIC DATA

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AT a recent meeting on continental drift, sponsored by the Royal Society, I reported[1] some new palæomagnetic results from South American rocks and compared these with data from other Gondwanic continents.

It is my purpose here to describe a later stage of this work in which palæomagnetic data from the Gondwanic continents of South America, Africa and Australia are compared with those from the Laurasian continents of North America and Europe.

Since the preliminary results of my South American palæomagnetic work were published[2–4], I have carried out thermal and alternating-field demagnetizing experiments in order to determine more precisely the directions of the fossil magnetization by selectively destroying

Table 1. SUMMARY OF SOUTH AMERICAN PALÆOMAGNETIC DATA

Ref. No.	Rock unit and age	Locality Lat.	Locality Long.	Direction of magnetization *D*	*I*	*α*	Pole position Lat.	Pole position Long.	*α*	Polarity	Treatment
2	Urucum Form. (Silurian)	19° S.	58° W.	37°	+41°	9°	34° N.	16° W.	9°	*R*	Thermal
5	Devonian Red Beds	23° S.	66° W.	56°	+51°	8°	9° N.	22° W.	10°	*R*	Thermal
8	Taiguati Form. (Pennsylvanian)	17° S.	65° W.	294°	−72·5°	5°	28° S.	34° W.	9°	*N* and *R*	Thermal
14	Piaui Form. (Pennsylvanian)	5° S.	43° W.	165°	+44°	9°	65° S.	13° W.	10°	*R*	Thermal
15	Permian Red Beds	30° S.	68° W.	160°	+66°	4°	65° S.	13° W.	6°	*R*	Thermal
26	Serra Geral Form. (Triassic-Jurassic)	22°–32° S.	46°–56° W.	347°	−38°	5°	78° S.	54° E.	4°	*N* and *R*	a.c.

Table 2. SUMMARY OF PALÆOMAGNETIC SOUTH POLE POSITIONS

Period	S. America	Africa	Australia	W. Europe	Russia	N. America
Ordovician						
Silurian	2, 34° N. 16° W.	3, 50° N. 11° W.				1, 18° N. 74° W.
Devonian	5, 9° N. 22° W.		4, 66° S. 10° E.	6, 10° N. 40° W.		
Mississippian						7, 21° N. 73° W.
Pennsylvanian	8, 28° S. 34° W. 14, 65° S. 13° W.	9, 17° S. 23° E.	10, 37° S. 158° E.	11, 37° S. 34° W.	12, 36° S. 35° W.	13, 36° S. 49° W.
Permian	15, 65° S. 13° W.	16, 38° S. 65° E.	17, 46° S. 127° E.	18, 44° S. 17° W.	19, 44° S. 13° W.	20, 37° S. 70° W.
Triassic		21, 68° S. 50° E.	22, 52° S. 154° E.	23, 43° S. 47° W.	24, 50° S. 33° W.	25, 60° S. 74° W.
Jurassic	26, 78° S. 54° E.	27, 67° S. 98° E.	28, 48° S. 151° E.			
Cretaceous		29, 62° S. 79° E.				
Tertiary			30, 67° S. 133° E.	31, 74° S. 47° W.		
Present geographic pole	I	II	III	IV	IV	V

Note. A reference number is given for each palæomagnetic south pole, followed by the latitude and longitude on the present latitude–longitude grid.

secondary magnetizations. Directions of magnetization and palæomagnetic south pole positions, calculated on the hypothesis that the geomagnetic field was, on the average, axial and dipolar during each geological epoch or substantial fraction thereof are listed in Table 1. The poles are plotted (●) in Fig. 1 and a polar wandering curve drawn. The striking feature of this curve is the pronounced shift during the Upper Palæozoic between poles 2 and 14. It is shown below that this feature is also present in the palæomagnetic records of Africa and Australia, the only other Gondwanic continents for which Palæozoic data are available. Furthermore, when the severe and widespread Permian remagnetization of Palæozoic red rocks in Europe and North America, recently proposed by Chamalaun and me[5], is taken into account this pole shift in the Upper Palæozoic is also evident in the palæomagnetic records of these northern continents. This can be clearly seen in Fig. 3, of which a fuller explanation is given later.

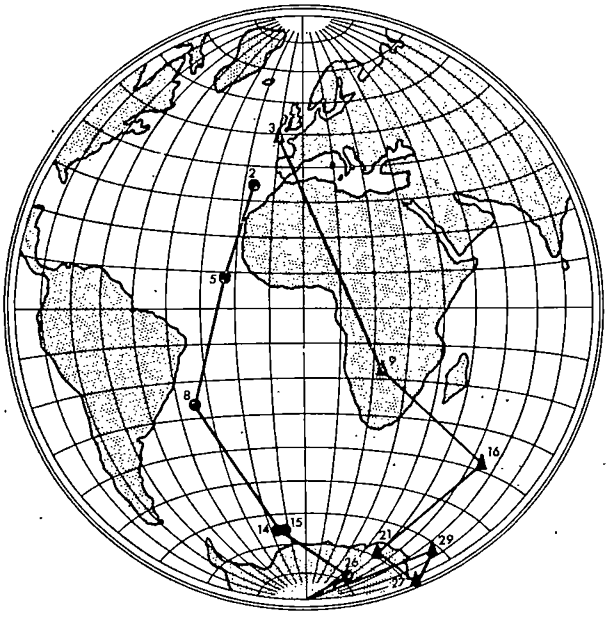


Fig. 1. Palæomagnetic south poles for South America (●) and Africa (▲). See Tables 1 and 2 for description of data. The grid represents the present latitude–longitude system

Table 3. SOURCE OF DATA USED IN THIS ANALYSIS

Number allotted here	Ref.	Notes
2, 5, 8, 14, 15, 26	Table 1 this article	South American poles
1	14	Mean of *C*37 and *C*38
3	15	Calculated from directions given in ref. 15
4	16 and 17	Mean of poles 116 and 120 in list 1 and poles 64, 65 and 66 in list 3
6	5 and 10	Pole of *ORS* lavas
7	14	Pole *C*14
9	14	Mean of *D*52 and *D*54
10	14	Mean of poles *D*50 and *D*51
11	14	Mean of poles *D*2, 5, 8, 10, 11, 12, 16, 20, 31, 33 and 36
12	18	Mean of poles 68–81
13	14	Mean of poles *D*38, 40, 43, 45, 47 and 49
16	19	—
17	20	Mean of poles for lower and upper marine laterites
18	14	Mean of poles *E*4, 6, 8, 10 13, 16, 18, 20, 22, 24 and 26
19	18	Mean of poles 59–67
20	14	Mean of poles *E*53, 55, 59 and 60
21	21	—
22	22	Mean of poles 1 and 2, Table 1
23	8	Keuper marls and sandstones
24	18	Mean of poles 47–58
25	14	Mean of poles *F*39, 79, 91, 93, 96, 98, 100, 102 and 105
27	23	—
28	22	Mean of poles 3, 4, 5 and 6 Table 1
29	24	—
30	16	Pole No. 37
31	8	Eocene lavas

Palæomagnetic south pole positions used in this analysis are listed in Table 2, and notes are given in Table 3 of the sources of these data. An explanation of why certain data are omitted is given later.

First, let us consider the fit of South America and Africa. The shape of the polar wandering curve connecting Upper Palæozoic poles relative to Africa is similar to that for South America[1]. This means that there was no relative movement between these two continents, during this interval of geological time, and by superimposing these parts of the curves the position of these continents relative to one another and to the pole during the Upper Palæozoic may be determined. The polar wandering curves were fitted together by drawing them on a 50-cm globe. One of them was traced on to a transparent fibreglass hemispherical shell. This shell was then moved

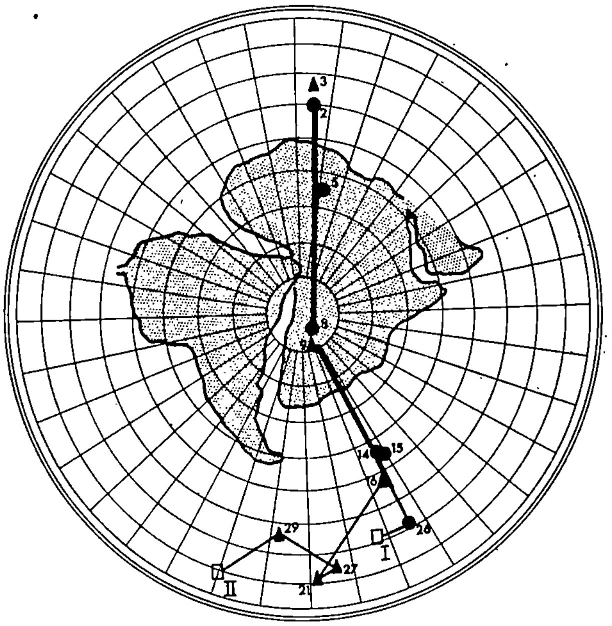


Fig. 2. A palæomagnetic reconstruction of the positions of South America and Africa for the Upper Palæozoic. The continents have been placed so that their polar wandering curves for the Upper Palæozoic coincide. The grid centre has no significance. I ●, South American poles; II ▲, African poles; ☐, present poles

over the surface of the globe until the Palæozoic portion of the curve drawn on it coincided best with the other curve drawn on the globe. The latter curve and also the outline of its continent were then traced on to the shell. Fig. 2 is a projection on a Lambert grid of tracings made on the shell. The polar wandering curves were fitted together by eye because more sophisticated mathematical treatment is not justified yet although it will have an important part to play later. At present we are not so much concerned with detailed fit but with the general overall pattern of distribution of the continents in the Palæozoic and of the movement of the pole across this. In the Upper Permian the two polar wandering curves separate and this indicates that, in the Mesozoic and later, South America and Africa no longer occupied the relative positions shown in Fig. 2 and that they were then drifting apart into their present positions. The fit obtained thus from palæomagnetic data is very close indeed to those which have been proposed by considering the shape of the coastline and from geological reasoning by several workers who have, in the past, advocated the hypothesis of drift and of whom perhaps the most famous are Wegener and du Toit. Recently the goodness of fit of these coastlines has been computed[6] and the best fitting positions so determined are barely distinguishable on a diagram the size of Fig. 2 from those deduced palæomagnetically.

The palæomagnetic data for South America and Africa are now compared with those from Europe and North America. In 1953, I made a palæomagnetic survey of rocks from Great Britain and hence deduced the first polar wandering curve for Britain[7]. Later, in collaboration with Irving and Runcorn, this curve was slightly modified and compared[8] with a similar curve deduced from North American rocks by Runcorn[9]. The shape of these curves is now familiar to most geophysicists and that for Britain is indicated by the broken line in Fig. 3, where north poles are plotted.

Let us now consider the data for the Palæozoic. My original Devonian pole *D* lies very close to the Permian (Nos. 18 and 19) and Carboniferous (Nos. 11 and 12) poles.

Last year Chamalaun and I carried out thermal experiments[5] on Old Red Sandstone rocks from the Anglo-Welsh cuvette and concluded that the natural remanence, although of exceptionally high stability, being unaffected by alternating magnetic fields of amplitude 1,000 œ or more, had most probably been acquired in the Permo-Carboniferous. We deduced that a much weaker magnetization which revealed itself after heating these rocks to 600° C and cooling them in zero magnetic field represented the true Devonian palæomagnetic field. This direction agreed with one previously obtained by Stubbs[10] for Old Red Sandstone lavas from Scotland. We now consider the true Devonian palæomagnetic pole for Britain to be that listed in Table 2 and plotted in Fig. 3 (pole No. 6).

At first we were reluctant to accept this result because it implied that many Russian and North American Devonian rocks the palæomagnetic directions of which agree with my original result for the Devonian from the Anglo-Welsh cuvette had also been remagnetized at the end of the Palæozoic. However, most of these Devonian rocks are red beds. During the Permian very many red beds were also laid down in North America, Western Europe and Russia, so that conditions then must also have been favourable for deposition of new red cement in older rocks which then happened to be near the surface, and thus for their remagnetization. To-day, the palæomagnetic record in tropical countries such as north-east Brazil where laterite is being formed is being slowly masked and even obliterated by magnetization in the direction of the present geomagnetic field of both old and of newly formed iron oxide minerals. During the Permian, the Palæozoic palæomagnetic records of the present northern hemisphere continents were very likely damaged and any directions which are nearly parallel to the Permo-Carboniferous palæomagnetic field from pre-Carboniferous red beds should be treated as suspect and should not be accepted until experiments similar to those described by Chamalaun and me have been carried out on them. The Middle Palæozoic poles for North America listed in Table 2 and used in this analysis have been determined from the magnetization of beds which were not appreciably reddened during the Permian. There is some evidence from the fold test that these magnetizations are post depositional, but even so it is likely that the magnetic age is pre-Carboniferous since the folding was pene-contemporaneous.

For these reasons I now accept that the solid lines in Fig. 3 represent polar movement relative to Western Europe and North America since the Middle Palæozoic. These paths are similar in shape to the path for South

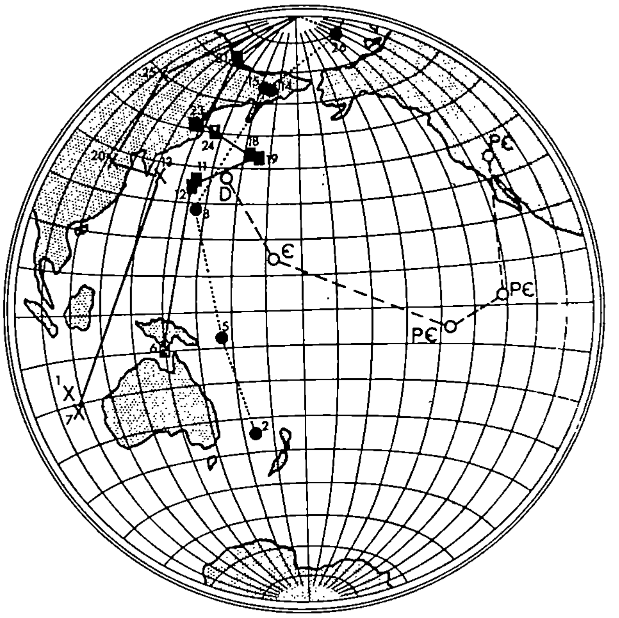


Fig. 3. Palæomagnetic north poles for Europe and Russia (■), North America (×) and South America (●). The new European Devonian pole No. 6 is displaced from the pole (*D*) used in constructing the original polar wandering curve (ref. 7) (– – O –), which connects Pre-Cambrian to Devonian poles. In the Mesozoic the original and new polar wandering curves for Europe are the same

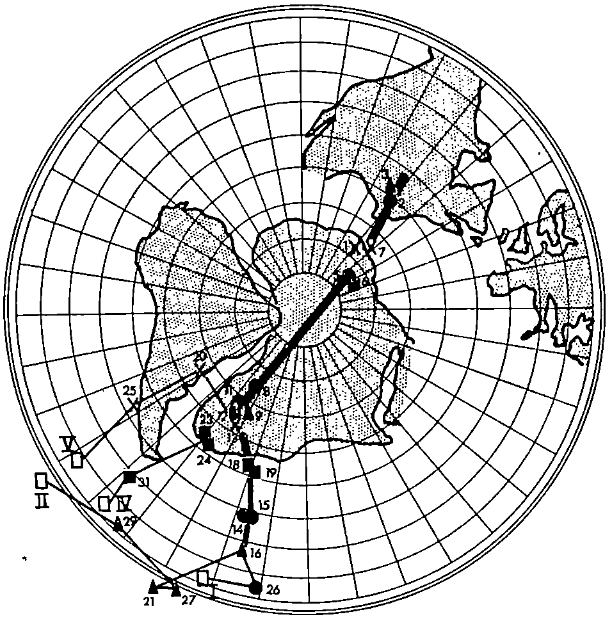


Fig. 4. Palæogeographic reconstruction obtained by fitting the Upper Palæozoic palæomagnetic south poles to the single curve marked by a thick line. The order in which the present continents broke away from the large landmass is indicated by the order in which the individual polar wandering curves separate from the main curve. The numbers are taken from Table 2. The symbols are defined by:

	Present pole	Ancient poles
South America	I	●
Africa	II	▲
Europe and Russia	IV	■
North America	V	×

The grid centre has no significance

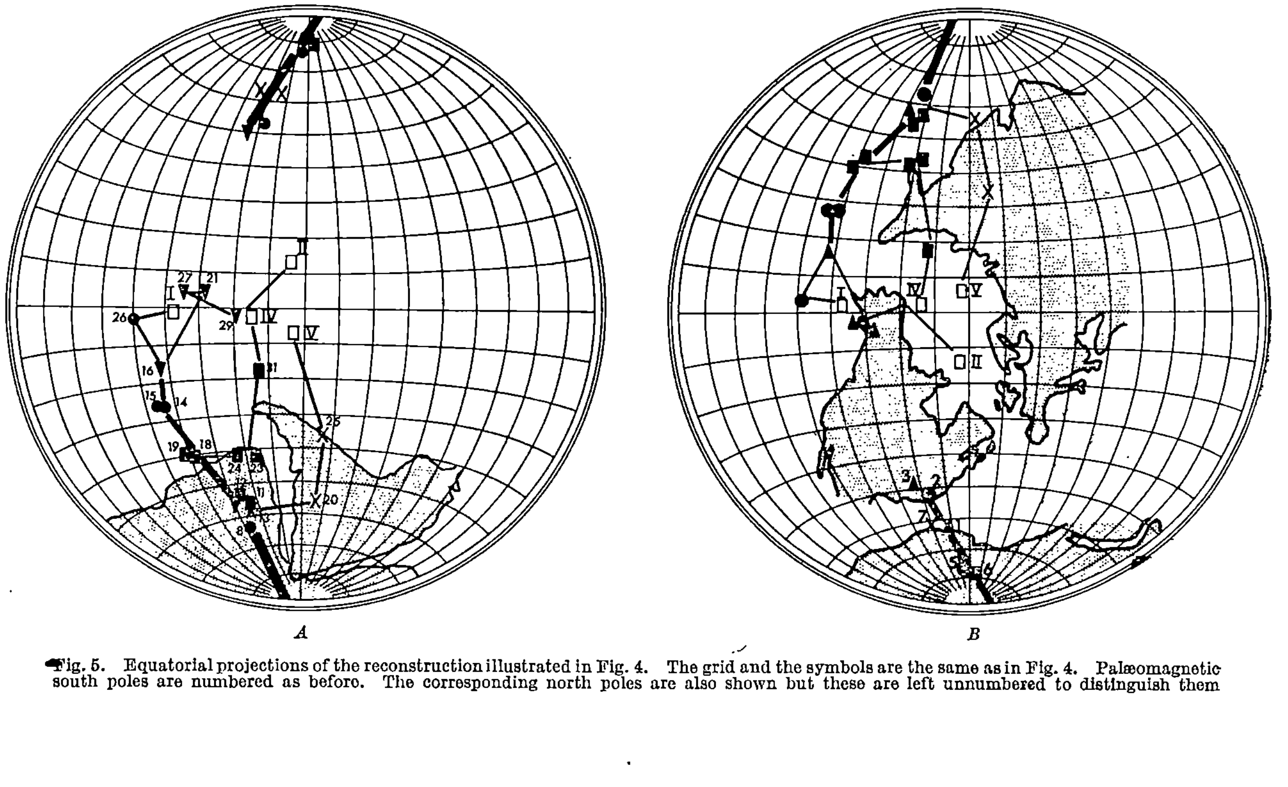
America, of which the north poles are also plotted in Fig. 3 and joined by a dotted line.

The palæomagnetic south poles for Europe and North America were plotted on the 50-cm globe and the Upper Palæozoic portions were fitted together by tracing on to the transparent hemispherical shell on which the curves for South America and Africa had been drawn. The outline of each continent was also traced on to the shell and thus a palæogeographic reconstruction of all these continents for the Upper Palæozoic was made. This and the common polar wandering curve were transferred on to polar and equatorial Lambert grids and the resulting diagrams are shown in Figs. 4 and 5. The polar wandering curves can be fitted remarkably well for the Upper Palæozoic and thus the continents must have then been very close to the positions shown, no relative movement having occurred during this interval of geological time. The fit of Western Europe and North America is similar to that proposed by many geologists, for example, du Toit, the Tethys sea occupying the broad space between Southern Europe and Gondwanaland.

In Figs. 5*A* and *B*, which are equatorial projections, the path of the palæomagnetic north pole is shown as well as that of the south pole. In this figure it has been supposed that Asia and Western Europe still comprised one single continent in the Palæozoic as they do at present. This is assumed tentatively in the absence of reliable Middle Palæozoic data for Siberia. In Figs. 3 and 4, it can be seen that the Russian Triassic, Permian and Carboniferous poles lie very close to the Western European for corresponding epochs. However, only a very small proportion of the Russian data are derived from rocks collected east of longitude 100° E, so there is really no evidence for or against the suggestion that Europe and Asia were then one large landmass.

It thus appears that the palæomagnetic south pole drifted across Gondwanaland from a position to the north-west of Africa, across Africa to a position off the coast of south-east Africa between the Silurian and Permian epochs with the continents reconstructed as shown in Figs. 4 and 5. The corresponding palæomagnetic north pole moved up the east coast of China and Siberia.

The disintegration of the Palæozoic landmass appears to have commenced in the Pennsylvanian at about the time when the Dwyka Tillites were being formed in South Africa. The North Atlantic then started to open up, and this is revealed in the palæomagnetic record by the separation of the North American polar wandering curve (V) from the main curve at poles 9 and 13 (Figs. 4 and 5). The European polar wandering curve (IV) separates from the main curve in the early Permian (poles 18 and 19), and this implies that Europe started to move relative to Gondwanaland narrowing the Tethys Sea. Later in the



A *B*

Fig. 5. Equatorial projections of the reconstruction illustrated in Fig. 4. The grid and the symbols are the same as in Fig. 4. Palæomagnetic south poles are numbered as before. The corresponding north poles are also shown but these are left unnumbered to distinguish them

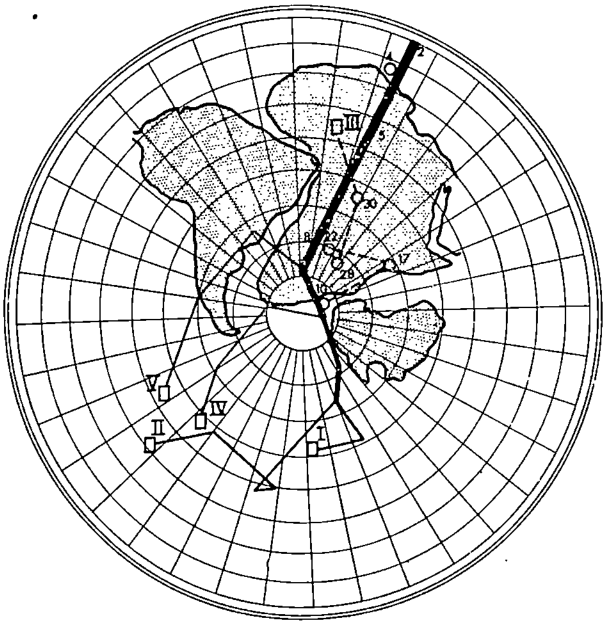


Fig. 6. Australian poles 4 and 10 have been fitted to the main curve and the outline of Australia constrained by these two points has been drawn in. Africa, South America and the polar wandering curves are all in the same positions relative to one another as in Fig. 4, but the grid centre is different from that of Fig. 4. The Australian palæomagnetic curve, for poles younger than 10, is shown by a broken line. Other symbols are as given in the key to Fig. 4

Permian (at pole 16), the South American (I) and African (II) polar wandering curves diverge, implying the South Atlantic then commenced to open out.

An attempt is now made to fit the Australian data to the pattern of events described here. Although much palæomagnetic work has been done on Australian rocks, few data exist for the Palæozoic and these have not been cleaned. The abundant Mesozoic and Tertiary data are obviously of no use in elucidating the arrangement of the continents in the Upper Palæozoic, which happens to be the crucial geological period. The two Upper Palæozoic poles, Nos. 4 and 10, show the large shift illustrated for the other continents (see Figs. 6 and 7) so that it is worth while attempting to fit the Australian data to the main curve. One possibility is to suppose that the Silurian-Devonian pole 4 is reliable and to fit it between the South American Silurian and Devonian poles, Nos. 2 and 5. The transparent shell on which the data of Fig. 4 had been drawn was then moved on the globe so that the pole for the Kuttung Series, No. 10, also lay on the main curve. It was found to lie between the Pennsylvanian and Permian portions (see Fig. 6). The pole path for Australia (III) for post-Kuttung time diverges from the main curve, and this implies that Australia must have started to drift relative to South America and Africa before the South Atlantic opened up between Africa and South America. This is the first important feature of this reconstruction. The second is that Australia is placed adjacent to Africa.

If the position of Australia is adjusted by making pole 10 coincide with the other Pennsylvanian poles at the knee of the main curve, Australia and Africa are found to overlap in regions where the geology is Pre-Cambrian, so such a reconstruction is ruled out and is not illustrated here.

It is stressed that the fit illustrated in Fig. 6 is based on only two not very well dated points and is given here principally to point out that at present the data for Australia are inadequate to reconstruct its position purely from palæomagnetism. Recently another attempt[13] has been made to reconstruct the Upper Palæozoic positions of Africa and Australia omitting to use pole 9 and using the antipole of 2 so that it is not surprising that a different result from that described here was then obtained.

Probably the best solution to the problem is illustrated in Fig. 7, which was made by adding Antarctica and Australia in the positions suggested by du Toit to the drawing on the transparent shell from which Fig. 4 was prepared, and this reconstruction of Gondwanaland is now compared with the Australian palæomagnetic data. The important thing to note is that pole 17 for the Permian Marine Latites now coincides with the South American and African Permian poles. Furthermore, Australia, Africa and South America start to separate and drift into their present positions at about the same time. Gondwanaland thus appears to have started to disintegrate in the Upper Permian. If we suppose that du Toit's reconstruction was the true one it follows that pole 10 is wrong. It is pertinent to note that the pole for the Marine Latites lay close to pole 10 before cleaning[20]. The Devonian-Silurian pole No. 4 lies on the main polar wandering curve, but rather closer to the Pennsylvanian knee than to the other Devonian poles (pole 5 is marked). The explanation for this might either be that these rocks need cleaning or that they are magnetically younger than their quoted age.

(Since this article was written, E. Irving has informed me, during a visit to this Department, that he has recently cleaned his Kuttung collection, and as a result has deduced a new palæomagnetic pole to replace pole 10. This new pole appears to agree with the reconstruction in Fig. 7.)

Finally it is necessary to comment on the accuracy of the palæomagnetic data used in this analysis. The biggest uncertainty is in the magnetic age of the rocks. Sedimentary rocks are frequently magnetized after deposition and their magnetic age may thus be less than their physical age. This is probably true in the case of pole 14 for South America. Fortunately the polar shift revealed by the palæomagnetism of Upper Palæozoic rocks is of considerable magnitude, spanning about 100° of arc. It persisted in roughly the same direction for about 200 million years, the average rate of movement being 0·5° per million years. Hence an inaccuracy in age of an individual pole does not appreciably affect the definition of a polar path. It does, however, mean that there must be some doubt concerning the rate at which the pole moved along this path. It is possible, for example, that a large part of the movement might have occurred much more rapidly

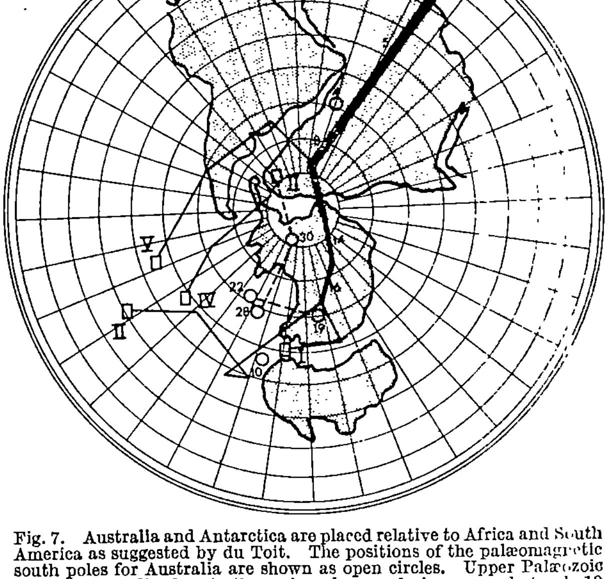


Fig. 7. Australia and Antarctica are placed relative to Africa and South America as suggested by du Toit. The positions of the palæomagnetic south poles for Australia are shown as open circles. Upper Palæozoic poles 4 and 19 lie close to the main polar wandering curve, but pole 10 does not. The pole path between the Mesozoic and the present for Australia is shown by a broken line

than the average rate of 0·5° per million years during a fraction of the total time apparently available. In future, when more well-dated data have been obtained, thus defining the time-scale, it should be possible to determine magnetic ages of Upper Palæozoic rocks from the position of their poles on the master curve.

The standard error of each of the poles listed in Table 2 is less than about 10°. For more details the reader should consult the references noted in Table 3, but he should beware of placing too much reliance on values of circles of confidence given in papers or lists of poles without carefully enquiring how they were calculated, since no single method of doing this has been generally agreed. Frequently the effects of secular variation of the ancient geomagnetic field have not been averaged out because inadequate thicknesses of rock were sampled. Thus the quoted circles of confidence are not realistic because the poles quoted are not true palæomagnetic poles in that they do not coincide with the ancient geographic pole. Sometimes authors have taken no account of systematic errors due to partial remagnetization, and when samples have been collected from too small an area the effects of tectonic movement have not been properly considered.

Lower Palæozoic data have not been considered in this analysis because few are reliable. I am now carrying out more thermal demagnetization experiments on British and South American Cambrian formations and I hope to extend this analysis back in time to the beginning of the Cambrian in the near future.

The prime purpose of this analysis has not been to deduce an exact palæogeographical reconstruction for the Upper Palæozoic, but rather to illustrate that when the reliability of palæomagnetic data is carefully considered, those data thought to be the most reliable fit a rather simple overall pattern which does not conflict unduly with geological evidence. One important result of this analysis is that it illustrates the importance of carrying out much more palæomagnetic work on Palæozoic rocks which have been rather neglected in past studies, and of taking great pains to detect stable secondary magnetization.

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NEWS and VIEWS

Science Policy Research in the U.S. Library of Congress : Dr. Edward Wenk, jun.

DR. EDWARD WENK, JUN., has been appointed chief of a newly created Science Policy Research Division in the Legislative Reference Service at the Library of Congress and will also serve as special adviser to the Librarian in Science and Engineering as from September 28. Dr. Wenk is at present technical assistant to the Director of the Office of Science and Technology in the Executive Office of the President. He has also been serving as Executive Secretary of the Federal Council for Science and Technology. In his new post, Dr. Wenk will serve in the Legislative Reference Service as a Congressional consultant in scientific and technological developments that affect public policy, and he will also serve the Library as a whole, in his capacity as special adviser to the Librarian and as co-ordinator of science information services furnished to the Congress. Dr. Wenk served in the Legislative Reference Service of the Library of Congress from 1959 until 1961 as senior specialist in science and technology. He resigned in 1961 to join the White House staff as assistant to the President's Science Adviser and moved to the President's Office of Science and Technology when it was established in 1962. In addition to serving also as the Federal Council's executive officer, he served as staff specialist in scientific man-power, long-range planning, and oceanography. A graduate of the Johns Hopkins University, where he gained a B.E. degree in civil engineering in 1940, Dr. Wenk holds an M.Sc. degree in applied mechanics from Harvard University (1947), and a D.Eng. degree in civil engineering from Johns Hopkins University (1950). He served in the U.S. Navy during the Second World War and received a Navy Civilian Meritorious Service Award. From 1942 until 1956 he was a naval research scientist and research administrator for the David Taylor Model Basin in Washington, and from 1956 until his first appointment to the Library of Congress in 1959 he was chairman of the Department of Engineering Mechanics at the South-west Research Institute in San Antonio, Texas. Dr. Wenk has been a special lecturer at the University of Maryland, Harvard University, Massachusetts Institute of Technology, Virginia Polytechnic Institute, Purdue University, Yale University, and the University of Texas.

Director of the Water Pollution Research Laboratory, Stevenage : Dr. B. A. Southgate, C.B.E.

DR. B. A. SOUTHGATE, director of the Department of Scientific and Industrial Research Water Pollution Research Laboratory at Stevenage, is to retire at the end of March 1966. Dr. Southgate first joined the Water Pollution Research Organization as a chemist in 1929. Before the Second World War he worked on the development of methods for the treatment of industrial effluents and conducted two major surveys of estuaries. The first, a detailed examination of the effects of pollution on the Tees Estuary, set a pattern for many similar surveys in various parts of the world; the second, on the Mersey Estuary, showed that pollution by untreated sewage was not responsible for siltation of the navigable channels. In 1940 the first central Water Pollution Research Laboratory was set up—though in temporary premises—and in 1943 Dr. Southgate was appointed director. His book on *Treatment and Disposal of Industrial Waste Waters*, published in 1948, was an outstanding contribution to the literature on the subject, and showed clearly his unrivalled knowledge of the field. The activities of the Station expanded steadily after the War and in 1954 it was transferred to a permanent new laboratory (at Stevenage). Under Dr. Southgate's leadership, early empiricism in the research has been replaced by a much more fundamental approach. The benefits are to be seen particularly in the successful outcome of a recently completed survey of the effects of pollution on the Thames Estuary, in which methods have been worked out for predicting the effects on the condition of the water of any given discharge, thus enabling the best way of dealing with the pollution problem to be assessed accurately.

Mr. A. L. Downing

Mr. A. L. Downing is to succeed Dr. Southgate as director of the Water Pollution Research Laboratory. Until Dr. Southgate's retirement in 1966 Mr. Downing will act as deputy director. Mr. Downing, who is thirty-eight, was born at Trentham, Stoke-on-Trent. He was educated at Arnold School, Blackpool, and was awarded a County scholarship to the University of Cambridge where he read natural sciences. Mr. Downing's first appointment within the Department of Scientific and Industrial Research was to Water Pollution Research in October 1946. In October 1947 he was seconded to the Ministry of Agriculture, Fisheries and Food for research at Lowestoft on the effects of radioactive materials on fish. In December 1948 he joined the staff of the Government Chemist in London while studying for an external degree at the University of London, returning to the Water Pollution Research Laboratory in August 1950. Mr. Downing is at present in charge of a large group of sections concerned primarily with biochemical engineering.

Neurophysiological Feedback Research in the National Physical Laboratory : Dr. Thomas B. Mulholland

Dr. Thomas B. Mulholland, jun., of the Veterans Administration Hospital, Bedford, Massachusetts, has been awarded a Carnegie Institution fellowship grant of 12,000 dollars for a year's research on stimulus-brain feedback systems, at the National Physical Laboratory, Teddington. Dr. Mulholland graduated from Tufts College in 1951 and then studied at Clark University, where he received an M.A. in 1952 and a Ph.D. in 1956. He became a post-doctoral trainee at the Veterans Administration Hospital in Bedford in 1956, and is still associated with that hospital. In addition, he is a lecturer in the Division of Continuing Education at Boston University and a lecturer at Clark University. Dr. Mulholland's field is psychology. For the past two years he has been investigating the development of a stimulus-brain feedback system and its application to a variety of psychological and neuropsychological problems.

Mathematics in the University of Strathclyde: Prof. W. D. Collins

The University of Strathclyde has announced the appointment of Dr. W. D. Collins, at present lecturer in applied mathematics in the University of Manchester, to a new professorship in mathematics, from January 1, 1965. Dr. Collins graduated with first-class honours in mathematics at the University of London in 1953 and was awarded the degree of Ph.D. in 1956. He began his career as a scientific officer with the Admiralty Research Laboratory, Teddington, in 1956. In 1957 he was appointed to a lecturership in applied mathematics at King's College, Newcastle upon Tyne, a post which he held until 1961, when he joined the staff of the University of Manchester. For nine months in 1963 he was on leave of absence as a research associate in the Division of Electromagnetic Research at the Courant Institute of Mathematical Sciences, New York University. Dr. Collins's main research interests are in solid mechanics, diffraction theory and methods of applied mathematics, in particular the investigation of integral equations and their application.

Veterinary Microbiology and Parasitology in the Royal Veterinary College, London : Prof. A. O. Betts

Dr. A. O. Betts, at present lecturer in animal pathology in the University of Cambridge, has been appointed to the newly established chair of veterinary microbiology and parasitology in the Department of Pathology at the Royal Veterinary College, London. He himself graduated from the Royal Veterinary College in 1949. Following this he became a research student in the Department of Animal Pathology, Cambridge, where he gained his Ph.D. in 1953. He was appointed demonstrator in that Department in 1952 and became a lecturer in 1956. He was awarded a Commonwealth Fund fellowship in 1955 and studied at the New York State Veterinary School at Cornell, and other centres. His research was at first centred round respiratory diseases of pigs and later his interests spread to include many virus diseases of animals. He has been a pioneer in the study of enteroviruses of domestic animals. He is a member of the World Health Organization's Committee on the Classification of Veterinary Viruses and was co-chairman of a conference on "Comparative Virology" held in New York in 1962 under the auspices of the New York Academy of Sciences. In addition to a number of visits to the United States he has also lectured at several veterinary research establishments on the continent of Europe.

Recruitment of Graduates to School Teaching

The report of the Sub-Committee on Recruitment to School Teaching of the University of Glasgow Appointments Committee notes a disturbing decline in the proportion of men graduates of Glasgow (honours and ordinary) and of women with ordinary degrees in arts and science entering teaching in 1962 (Pp. 16. Glasgow : The University, 1964). The proportion of men graduates with ordinary degrees in science who enter teaching has decreased by 17·5 per cent since 1959, and that of women by 12 per cent since 1960. The Sub-Committee does not consider that salary is now an important deterrent, but the apparent lack of good prospects for promotion in teaching is a serious deterrent and the very considerable change for the better since 1961 is not yet sufficiently widely known. It is also critical of the rigidity of present regulations on certification and recognition, and the Sub-Committee believes that the postgraduate year in a College of Education may also be a deterrent owing to misunderstanding of the nature of the courses, doubts as to necessity or value of the training, and the prolonged dependence on Government grants or parental resources. The limitations of day-school teaching can also deter some, through fear of losing their specialist knowledge, risk of staleness and lack of contact with the adult world. The report makes several suggestions for remedying the defective information position by better descriptive literature and talks to students, but also suggests experiments with trial runs. The defects in the system itself must await Government action, and, while welcoming the Ministry of Education's consultation of the appointments officers of the universities and its commissioning of an enquiry by the Social Survey into what undergraduates know and think about prospects in the teaching profession, and what makes school teaching attractive or unattractive to them, the Sub-Committee recommends immediate attention to three particular defects. First, early consideration should be given to the advisability of pay during training. Secondly, the regulations relating to the certification of teachers should be speedily revised to bring them up to date and make them more flexible. Thirdly, the problem of promotion should be tackled appropriately without delay to create a more uniform and smoothly working system with some real incentive to the able young teacher.

Medical Schools of the World

The third edition of the *World Directory of Medical Schools* will be of great value to everyone who is either concerned with medical education or is planning that kind of education for himself (Pp. 348. Geneva: World Health Organization; London: H.M.S.O., 1963. 20 Sw. fr[illegible] 35*s*.; 6.75 dollars). It provides detailed information about the salient features of undergraduate medical education in 87 countries of the world, the material having been provided by the health authorities of the countries concerned or by the medical schools themselves. To this has been added information about the admission of foreign students to medical schools and about the conditions which govern medical practice by nationals with foreign qualifications or by foreigners who have qualified in the country concerned. There is also information about the laws which govern medical practice in some countries which have no medical schools. Tables show the number of years of medical education required in each country and other conditions that must be fulfilled before a licence to practice is granted. It is interesting to note that, while most countries require a practitioner to complete at least one year of experience in a hospital before he may practise, some countries, such as China and the Sudan, require him to complete two years of such experience; Austria alone requires him to complete three. In some countries one of the years of postgraduate experience is devoted to rural or State service or to specialization and teaching. The *Directory* also supplies data about the population of the countries concerned, the number of medical schools and physicians, and the number of students who enrol and are admitted and who graduate. An interesting appendix compares the numbers of medical, dental and veterinary schools. It may surprise some readers to learn that whereas the United Kingdom has 6 veterinary schools, Japan has 14, the U.S.S.R. 23, the United States 18 and India 16. A study of these pages will enable the reader to obtain what is, in effect, an overall picture of medical education all over the world. It is clear that the total number of years spent, including primary and secondary education, before practice is allowed varies little all over the world. In most countries primary and secondary education followed by pre-medical, medical and hospital experience add up to some 19–20 years of training, while in the Lebanon and the United States the total is 21 years. The time devoted specifically to medical education varies little, being usually 4–6 years.

The Registrar General's Quarterly Return for England and Wales

THE Registrar General's return for the first quarter of 1964 shows that 220,000 live births were registered in that quarter as against 222,000 in the corresponding quarter of last year (*The Registrar General's Quarterly Return for England and Wales: Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, Quarter ended 31st March*, 1964. Pp. 28 (No. 46, 1st Quarter, 1964.) (London: H.M.S.O., 1964.) 2*s*. 6*d*. net). However, Easter fell in the last week of March, and this may have delayed some of the registrations. The preliminary seasonally adjusted figure shows 213,000 births, as against 212,000 for the corresponding quarter of 1963; this is slightly lower than the figure for the last quarter of 1963 (213,300) and below the peak figure for the third quarter of 1963 (215,400). The death rate in the quarter was low at 13·4, reflecting the relatively mild winter. Infant mortality at 22 per 1,000 live births is once again a record low figure for the first quarter. It will be interesting to see whether the figures for the second quarter of 1964 will show a continuation of the decline in the number of births and of the birth rate. The report contains, as is usual in March, a brief analysis of causes of death in 1963. There were only 7 deaths from acute poliomyelitis, the lowest number ever recorded, compared with 18 in 1962, and an annual average of 262 in 1951–53. Deaths from lung cancer increased by 3 per cent for men and 5 per cent for women, compared with 1962, whereas deaths from all malignant neoplasms increased by only 0·8 per cent. Motor vehicle accidents accounted for 6,400 deaths compared with 6,300 in 1962 and 6,600 in 1961.

Radio-isotopes in Hydrology

THE report of the panel on the use of isotopes in hydrology which met in Vienna during December 17–21, 1962, has now been issued (Technical Report Series, No. 23: *Isotope Techniques for Hydrology*. Pp. 36. Vienna: International Atomic Energy Agency; London: H.M.S.O., 1964. 21 schillings; 6*s*.; 1 dollar). More detailed discussions on some of the techniques mentioned by the panel were presented at an International Atomic Energy Agency symposium in Tokyo during March 5–9, 1963, and are published in *Radioisotopes in Hydrology* (I.A.E.A., Vienna, 1963). Ground-water dating techniques, including carbon-14, silicon-32 and tritium dating, are discussed in detail in the report, together with short-term ground-water tracing by techniques involving stable isotopes and activation analysis. It is recommended that the exchange of carbon between the liquid and solid phases in ground-water be investigated by tracing experiments in which tritium in tritiated water and carbon-14 as bicarbonate are used simultaneously. There are many unresolved questions regarding ground-water recharge rates, which depend on climate, season, geography, geochemistry, topography and vegetation, and until more is known the interpretation of ground-water age from tritium concentration data must be treated with caution. One section of the report deals with field experiments, covering investigations in the Snake River Plain, Idaho; an underground connexion in New Mexico; the determination of an aquifer by multiple-well tracing; and reservoir leakage, and another section with specific ground-water problems in Greece, on Kilimanjaro, and in the desert.

The panel concluded that isotope-technique applications to hydrological problems have been somewhat useful in the understanding of the nature of the hydrologic cycle and in the solution of specific engineering problems. However, additional research and development are required on the part of both hydrologists and isotope specialists, preferably working together as teams, to ensure the proper synthesis of scientific advances in their respective fields and the translation of these advances into practical technology. In particular, it considered that the present International Commission on Radiological Protection values, which are used as standards for exposure for large populations and which are based on long-term exposure, are too restrictive for short-term experiments such as are required in hydrological investigations.

Isotope Dilution Analysis

THE monograph *Radioactive Isotope Dilution Analysis* is one of the series of occasional publications of the Radiochemical Centre, Amersham (Pp. 12. Amersham: The Radiochemical Centre, 1964). Classical methods of chemical analysis generally rely either on the quantitative isolation of the component to be determined or on the measurement of some specific chemical or physical property of the component which is not obscured by other substances present. These methods often fail with more complex mixtures, and then isotope dilution analysis may be suitable. The basic principle is that if a radioactive tracer is mixed with the corresponding unlabelled compound the amount of activity per gram of the substance will be reduced, and that if the reduction per gram is measured the amount of diluting material can be calculated. The calculation and method of procedure are explained in the monograph and illustrated by an example. New developments in the field of dilution analysis, particularly derivative dilution where it is necessary to determine one component in the presence of several others, and saturation analysis which involves the use of a binding agent specific for the compound being determined, are explained in some detail. The examples discussed are mainly of organic substances but, as is shown in the final section of the monograph, direct isotope dilution can be applied equally well to inorganic substances. The monograph is not only an excellent introduction to the subject of radioactive isotope dilution analysis but also a valuable laboratory guide for those who wish to make use of the technique. There is a list of 25 references from which additional information can be obtained.

Solid-state Physics

THE *Bulletin de la Société Belge de Physique* is published by the Belgian Physical Society and records the proceedings of meetings on special topics organized by the Society. Regular members of the Society receive the *Bulletin*, but non-members can subscribe at 400 Belgian francs per series of six issues. The number of issues per annum ranges from four to six. The March 1964 issue (Series 4, No. 1) contains the proceedings of the annual meeting on "Solid-state Physics", held in Brussels on October 11, 1963. There were 21 contributions and these, some in abstract or summary form, are printed in English in the issue.

Albright Magazine

THE ingredients of a successful house journal of the type and quality nowadays sponsored by leading industrial concerns in Britain and overseas would appear to be attractive format and presentation, a nice balance between articles of technical and general interest and those concerned with the social activities of employees, high-quality illustration (sensitive blending of black and white and colour), and what may be best described as 'extra-mural' appeal. Circulation of magazines of this type, in many long-established and successful ventures, goes far beyond the patronage of the employees for whom they were primarily instigated, and deservedly so. The merits of these periodicals are that by means of informative articles, lavishly illustrated by photographs and diagrams, and written by administrative and technical experts, often outside the organization concerned, business, technology, general knowledge, and the social humanities are happily blended to the advantage of all concerned. As a tangible means of promoting *esprit de corps* and good staff relationships, such projects can scarcely be bettered. A comparative newcomer to the library of British house journals is the *Albright Magazine*, a bi-monthly periodical published by the well-known Albright and Wilson group of companies with a wide field of chemical and allied interests. The first number appeared in February 1964, to be followed by the April, June and, to date, August issues. A survey of these four issues proclaims at once that a high standard of production was initially aimed at, and certainly achieved, and that it persists. This journal has originality plus all the ingredients mentioned here. As it appears to be the policy of the editorial board not to publish regular editorials, the first issue, likewise the second, was not introduced from the 'chair'; it was as though the *Magazine* just happened, leaving the reader to judge its merits. However, in the June issue, the Editor relents and gives the reader a glimpse of the policy behind this project, particularly information on how the first two numbers had been received. Generally, the reception of this publication has been extremely good, including comments from an independent critic well accustomed to reviewing house journals. Two points in presentation, however, have been raised: the use of the + sign in place of an ampersand connecting Albright and Wilson, and the 'readability' of the sans serif type so far used in the text. The writer of this note frankly considers the former rather ugly and incongruous; the matter of type, on which the Editor asks for comments, is largely a matter of opinion; at first sight it appears rather formal, like some government specifications, and not so easy to the eyes as many house journals. Apart from these queries, it will be surprising if in the next National House Journal Competition (one has already been held for 1964) the *Albright Magazine* does not receive an award for merit, which it undoubtedly deserves.

Mechanizing Library Services

THE June–July issue of *Science Information Notes* (6, No. 3; 1964), issued by the National Science Foundation, records the commencement of an investigation, under an arrangement by the Council on Library Resources, to demonstrate that bibliographic information, suitably punched into a perforated paper-tape record by a tape-producing typewriter, can be reproduced in any form required for the records used in library operations. The purpose is to lay the basis for seeking general agreement on standards in the conversion of such information to machine-readable form. The National Science Foundation is itself supporting the American Institute of Physics in a programme directed towards improving the communication of scientific information. This will enable the Institute to continue its work on creating an adequate reference retrieval system for physicists, its collection of information from authors using the property–object–method scheme of analysis, and its investigation of the relations between cited and citing physics papers, including an investigation of the reasons why articles are cited. An experimental system which can use the data already collected will also be tested. Research is continuing at the Massachusetts Institute of Technology on the development of a realistic test environment for evaluating ideas and systems components capable of making the flow of scientific information more effective. The National Aeronautics and Space Administration has established an unusual search tape system which provides up-to-date comprehensive search capabilities at individual research centres of the Administration's entire collection of reports.

The Sarawak Museum

THE latest issue of the *Sarawak Museum Journal* (11, Nos. 21–22 (New Series); July–December 1963) maintains the essential regional character of this publication. Mrs. Barbara Harrisson records some interesting experiments in educating orphan young orang-utans for normal life in the jungle, and the editor, Mr. Tom Harrisson, has several papers on local natural history. Additional information regarding the animal bones found in Niah Cave are given by Lord Medway and the Earl of Cranbrook. In all, there is a total of 31 interesting and well-illustrated papers.

South Australian Museum

THE report of the South Australian Museum for the year ended June 30, 1963 (Pp. 14 + 1 plate. Adelaide: Government Printer, 1964), is the first issued under the directorship of Dr. W. P. Crowcroft, and records the continued anxiety regarding accommodation. The installation of a mezzanine floor in the Stirling Gallery provided an extra 1,500 ft.^{2} for storage. Several collecting projects were undertaken during the year, with the view of acquiring detailed field observations in support of material collected for research purposes, and the recording of the destruction of native habitats. Trips were thus undertaken to the Gulf of Carpentaria, the Simpson Desert, and Sturt's Stony Desert. Some of the records of this work are indicated in an impressive list of publications by the staff.

Physics Conference, Gothenberg

THE twelfth General Physics Conference arranged by the Swedish National Committee for Physics was held in the Chalmers Institute of Technology, Gothenberg, during June 4–7, 1963. The attendance of some 300 members, and particularly the large number of contributed papers, required the meetings to be held in three parallel sessions in adjacent lecture halls. One general lecture was given each day by an invited speaker. Prof. D. Amati (CERN) spoke on the present situation in the high-energy theory; Dr. J. J. Quinn (Radio Corporation of America) on the properties of a quantum plasma in a uniform magnetic field; Prof. G. Ekspong (Stockholm) on experimental results concerning elementary particles; and Dr. T. Ericson (CERN) on some statistical phenomena in nuclei. The members were entertained to a dinner and dance by the city council on the evening of June 6, and demonstrations were arranged in the physics and electrical engineering departments of the Institute. A brief report of the proceedings of the Conference, together with summaries of the papers presented, is given in *Arkiv for Physik*, 26, 233 (May 22, 1964).

American Society of Plant Physiologists

THE American Society of Plant Physiologists has announced the following awards: *Stephen Hales Prize*, to Prof. F. C. Steward, Cornell University, New York, in recognition of his work on the salt uptake of plants and the nitrogenous constituents of plant tissues, and his investigations of growth substances; *Charles F. Kettering Award*, to Dr. L. N. M. Duysens, University of Leiden, in recognition of his contributions to photosynthetic research; *Charles Reid Barnes Life Membership Award*, to Prof. W. Z. Hassid, University of California at Berkeley, in recognition of his work in the field of sugar biochemistry which has led to discoveries in the metabolism of carbohydrate compounds. Charles A. Shull Life Membership Certificates have been presented to the following members, who were present at the founding of the Society and have retained their interest in its activities: Prof. C. H. Arndt, Clemson College, South Carolina; Prof. W. M. Atwood, Oregon State University; Prof. A. L. Bakke, Iowa State University; Prof. J. P. Bennett, University of California; Dr. W. B. Davis, U.S. Department of Agriculture; Dr. R. B. Dustman, University of West Virginia; Dr. F. M. Eaton, University of California; Prof. G. R. Johnstone, University of South California; Dr. J. P. Jones, U.S. Department of Agriculture; Prof. A. E. Murneek, University of Missouri; Prof. A. C. Saeger, University of Kansas City.

The following were elected officers of the Society: Dr. S. Aronoff, U.S. National Science Foundation (president); Prof. G. G. Laties, University of California (vice-president); Prof. H. Beevers, Purdue University, Indiana (member of the executive committee); Prof. W. D. Bonner, jun., University of Pennsylvania, and Prof. P. J. Kramer, Duke University, North Carolina (members of the editorial board of *Plant Physiology*). These elections will take effect from October 1.

The Night Sky in October

NEW moon occurs on Oct. 5d 16h 20m U.T. and full moon on Oct. 21d 04h 46m. The following conjunctions with the Moon occur: Oct. 1d 00h, Mars 2° S.; Oct. 2d 03h, Venus 5° S.; Oct. 16d 18h, Saturn 3° N.; Oct. 22d 23h, Jupiter 1° N.; Oct. 29d 09h, Mars 3° S.; Oct. 31d 23h, Venus 4° S. Venus is in conjunction with Regulus on Oct. 5d 07h, Venus being 0·4° S. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 2h 00m, 2h 35m and 3h 15m on October 1, 15 and 31, respectively. Its stellar magnitude is −3·6; its distance from the Earth increases during the month from 89 to 108 million miles and the visible portion of the apparent disk from 0·654 to 0·765. Mars rises at 0h 20m, 0h 15m and 0h 05m at the beginning, middle and end of the month, respectively. Its stellar magnitude is +1·5 and its distance from the Earth on October 15 is 165 million miles. Mars is in Cancer until October 14, when it passes into Leo. Jupiter rises at 19h 10m, 18h 10 m and 17h 05m on October 1, 15 and 31, respectively. It is now moving westwards near the boundary between Aries and Taurus, its stellar magnitude is −2·4 and its distance from the Earth on October 15 is 384 million miles. Saturn sets at 2h 15m, 1h 20m and 0h 20m at the beginning, middle and end of the month, respectively. It is in Aquarius, moving westwards among the stars. Its stellar magnitude is +0·8 and its distance from the Earth on October 15 is 850 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Oct. 13d 18h 34·0m, 51 Sgr. (*D*); Oct. 13d 19h 06·3m, 52 Sgr. (*D*); Oct. 19d 01h 41·2m, 30 Psc. (*D*); Oct. 28d 01h 22·6m, γ Cnc. (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The Orionid meteors are active during October 15–25, with maximum on October 21, but conditions for observation are unfavourable.

Announcements

PROF. ALEXANDER WEINSTEIN, of the University of Maryland Institute for Fluid Dynamics and Applied Mathematics, has been elected a foreign member of the Accademia Nazionale dei Lincei, Rome. Prof. Weinstein is well known for his outstanding contributions to applied mathematics, especially to the theory of partial differential equations and the calculation of vibrations.

THE 1964 symposium of the Technicon Instruments Company, Ltd., on "Automation in Analytical Chemistry" will be held in London during October 12–14. Further information can be obtained from Technicon Instruments Co., Ltd., Hanworth Lane, Chertsey, Surrey.

THE twenty-seventh annual meeting of the American Documentation Institute on "The Parameters of Information Science" will be held in Philadelphia during October 4–8. Further information can be obtained from A. W. Speakman, E. I. du Pont de Nemours and Co., Wilmington, Delaware.

AN international symposium on "Laser-Physics and Applications" will be held in the University of Berne during October 12–14. Further information can be obtained from R. Dändliker, Institut für Angewandte Physik, Universität Bern, Sidlerstrasse 5, Bern.

THE annual conference of the British Association for Commercial and Industrial Education on "Policy in Perspective" will be held in the University of Nottingham during September 24–26. Further information can be obtained from the Conference Secretary, British Association for Commercial and Industrial Education, 26A Buckingham Palace Road, S.W.1.

A SYMPOSIUM on "Relation of Measurements of Radioactive Contamination in the Working Environment to Personal Exposure", arranged by the Society for Radiological Protection in association with the Joint Health Physics Committee, will be held in the Manchester College of Science and Technology on October 7. Further information can be obtained from Mr. E. T. Wray, Radiological Protection Division, U.K. Atomic Energy Authority, Harwell, Didcot, Berks.

THE annual meeting of the Conference on Electrical Insulation of the Division of Engineering and Industrial Research, National Academy of Sciences—National Research Council, will be held in Cleveland, Ohio, during October 12–14. Further information can be obtained from D. W. Thornhill, Conference on Electrical Insulation, National Academy of Sciences—National Research Council, 2101 Constitution Avenue, Washington 25, D.C.

THE national conference of the Institute of Personnel Management on "Economic Growth and Human Resources" will be held in Harrogate during October 9–11. In conjunction with the conference, five one-day courses will be held on October 9 on: evaluation and development of the personnel function; organization and staffing; staff assessment and counselling; improving selection techniques; training as an aspect of personnel management. Further information can be obtained from the Institute of Personnel Management, Management House, 80 Fetter Lane, London, E.C.4.

SCIENTIFIC AND ENGINEERING MANPOWER IN THE UNITED STATES

THE report of the Committee on the Utilization of Scientific and Engineering Manpower in the United States*, which is discussed on p. 1101 of this issue of *Nature*, bases its recommendations and conclusions largely on the facts set forth in the second chapter of Part 1 of the report, "Needs and Resources". Between 1940 and 1960, the proportion of scientists and engineers in the working population greatly increased, and now, at 1·7 million, exceeds 2 per cent. Of this total, about 950,000 are engineers, 500,000 scientists and 250,000 teachers of science or mathematics at the secondary school level. By 1970, the total is expected to reach 2·8 per cent of the working population. About 40 per cent are engaged in private industry or commerce, 20 per cent in education, and between 20 and 30 per cent in work affecting national security and its position in the exploration of space. No major division of science includes more than 7·5 per cent of the scientists and no branch of engineering more than 15 per cent of engineers. Of those with doctorates, 29·2 per cent are scientists, 15·1 per cent being in the physical sciences and 7·4 per cent in the biological sciences. Secondary school teachers account for 14·7 per cent and engineers for 56·1 per cent; 6·7 per cent are psychologists or social scientists. Two out of three scientists and two out of five engineers are engaged in research and development. It is estimated that in 1963, of the total graduates in science and engineering, 57 per cent were employed in industry, 11 per cent by the Government, 12 per cent in colleges and universities and 15 per cent as secondary school teachers.

These figures and profiles are supported in Part 2 of the report by nine study papers. Mr. F. H. Harbison discusses measures towards the development of a comprehensive man-power policy; G. Colm and L. A. Lecht outline requirements for scientific and engineering man-power in the 1970's; and A. M. Ross considers how the United States uses its engineers and scientists. Proposals for development of an improved man-power-related information programme are detailed by A. O. Gamble, while P. W. Charington considers the impact of Government policy and action on the utilization of scientific and engineering man-power in the acquisition of weapons and space systems in a paper entitled "Systems-Acquisition and the Utilization of Scientific and Engineering Man-Power (Requirements and Programme-determination, Contracts and Grants)". In a further paper, he submits two case studies of the utilization of scientific and engineering man-power in the major military systems, *Titan II* and Naval Tactical Data Systems: this is summarized and its principal findings only are presented. In the last three papers, A. B. Kinzel presents an industrial view of the Government's impact on civilian research and development man-power, L. M. Hartman describes industrial practice affecting the utilization of scientific and engineering man-power, and R. H. Bolt takes a look ahead at man–machine partnership in intellectual pursuits.

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* *Towards Better Utilization of Scientific and Engineering Talent: a Program for Action.* Report of the Committee on Utilization of Scientific and Engineering Manpower. Publication No. 1191, National Academy of Sciences. (Printing and Publishing Offices, National Academy of Sciences, Washington, D.C., 1964.)

FILM AND TELEVISION IN THE WORK OF THE UNIVERSITIES

A ONE-DAY conference on the film and television in the work of the universities was held at the Royal College of Art, London, on July 21. Mr. Michael Clarke, tutor in the Department of Television and Film at the College, deserves every praise and admiration for arranging the meeting. The interest and enthusiasm shown by his audience were a welcome indication that audio visual media may shortly emerge from their adolescence.

The British Universities Film Council conference at Senate House in March, 1963, set out to survey the field of closed-circuit television within institutions of higher education from the severely practical point of view of application, technique, instrumentation and cost. At the Royal College of Art, Members of Parliament, university vice-chancellors and professors, members of the University Grants Committee Sub-committee on Audio Visual Aids Facilities in Higher Education, directors and senior executives of the independent television companies and of the B.B.C., as well as a catholic selection of experts in the arts and sciences came together to hear and to discuss a wide-ranging and more general search for the present and future role of film and television in the universities.

In opening the morning's session on "Potential Functions of Film and Television in Higher Education", Mr. Clarke pointed out that the meeting was too large to achieve any concrete results and that the main aim should be to gain information from each other. The Television and Film Department within the Royal College of Art would accept up to ten students a year who had completed their art-school training. They would go through a three-year course on film and closed-circuit television to learn how to think in terms of moving pictures. From among these students, it was hoped, would come some of the producers and directors of the future. A one-year course exists for those who are already in industry but wish to experiment or enlarge their knowledge. The nucleus of an academic film unit has been created and has started to make teaching films in collaboration with Imperial College of Science and Technology, London.

Mr. Clarke claimed that film and television should be regarded as modes of communication in their own right. If they were to be fully exploited in this way, both users and producers would have to rethink and learn how to use visual concepts. This should apply not only to the professionals but also to the teachers; all should learn to think in terms of illustration.

Prof. Collin Cherry, Department of Telecommunication, Imperial College, and author of *On Human Communication* (John Wiley and Sons and Science Editions, Inc., 1961), spoke on his experimental use of simple closed-circuit television equipment as an enlarging device during his lectures on engineering, where this device enabled him to use small models to illustrate abstract concepts. He also recorded a twenty-minute summary immediately after each lecture, so that his students could listen to the tape-recording either to supplement their notes or to catch up on a lecture they might have missed.

Prof. Cherry emphasized that the academic lecture should impart a basic understanding and interest in the subject, not detailed information. He expressed the hope that television would cut its traditional cinema apron strings and make greater use of its essentially electronic genes. Future use of television in the lecture theatre might bring outside events and phenomena to the student to enrich his understanding and visual experience. A great expansion could be envisaged as soon as the restricted availability of wave-lengths had been overcome.

As a complete contrast, Mr. Edgar Anstey of British Transport Films and vice-president of the International Scientific Film Association recalled that Percy Smith produced superb classroom films on biological subjects almost fifty years ago. While films had been produced ever since, lamentably little effective use had been made of them. This might possibly be explained by the lack of appreciation of their enormous potential and, particularly, by the ignorance of the film language and its ability to appeal not only to logic but also to the emotions.

Prof. David Daiches, Department of English, University of Sussex, recounted his experiences in mounting twelve experimental television broadcasts on English literature and how he attempted, and evidently most successfully, to impart a visual understanding of concepts which could not be explained by words alone. So, for example, he illustrated the concept of stylization by showing a photograph of a real lion followed by a drawing of a heraldic lion. This demonstration was followed by the reading of poems on Nature, some realistic recounts of what the poet had seen, others giving a stylized account of another poet's experiences. As a further example, Prof. Daiches mentioned the film of Dublin streets which was used as background illustration to the reading of part of Joyce's *Dubliners*.

Prof. Eric Crook, Department of Biochemistry and Chemistry, St. Bartholomew's Hospital Medical College, London, brought the meeting back from open broadcasting to the uses of television and film within the university. He outlined the vocational rather than academic nature of the medical curriculum and how his college was revising the sequence and manner of teaching the preclinical course. He emphasized that an effort should be made to exploit the visual aspects of the material to be learned, in order to help the student who was very largely visually orientated in his method of learning and retention of knowledge.

Mr. Peter Laslett, Trinity College, Cambridge, bridged the transition from the morning to the afternoon session on "Immediate Problems and Needs" with his account of the "Dawn University" experimental broadcasts from Cambridge and the television links between Cambridge and Norwich, and Cambridge and Imperial College, London. Mr. Laslett felt that few students would be able to proceed to a degree examination unless they were given a great deal of other help and guidance, and that the main bulk of the viewers would merely look for a "cultural uplift".

While such broadcasts could well train or retrain mothers who wanted to go back to teaching, a degree course of the air would require a new central national body. Television teaching could not be cheap. At an estimated cost of £2,000 per hour a degree course of 500–600 hours would cost £1,200,000; and yet every university offers not just one but many degree courses. To be effective each hour of television broadcasting would have to be accompanied by something like four hours of radio instruction, eight hours of correspondence course work and time when the students would actually visit the university to which they were attached.

Mr. Laslett made it clear that the subject was far too complex to be discussed satisfactorily in a short space of time. An organization had now been set up to discuss ways and means, and the University of Cambridge was prepared to mount a comparative experiment. Unfortunately, research into education did not appear to attract financial support, and no action could be taken at the present time.

Mr. S. Pitt Corder, Department of English, University of Leeds, gave a stimulating account of the important pioneering work now being translated into reality at his university. This University Television Centre will assist and stimulate the use of closed-circuit television as a visual aid and as a tool in research at department level. The central organization will develop television as a medium of teaching in its own right, and central studio facilities have been created for the distribution of instructional material to departments of the university. The Centre will also institute its own researches into educational television.

The staff is planned to provide a small team to advise on departmental uses and to offer a loan and repair service. Central activities will be carried out by a bigger team equipped with high-quality apparatus. The director, who will have an academic background, will probably be supported by a studio manager-producer, an assistant producer, two technicians, an art director and assistants, clerks, drivers and handymen to form a team of some ten to fourteen people. Naturally the Centre will wish to lean quite heavily on existing university film and photographic facilities. It may not prove easy to find suitably qualified and experienced personnel, and it is interesting to note that school teachers have already asked to be trained, so that they may be enabled to use television in their schools.

Mr. Thorold Dickinson, Slade School of Fine Art, University College, London, joined the discussion at this point to direct attention to the American and Russian trend towards smaller and more portable film cameras for more flexible documentary and dramatic recording. He advanced the argument that this would make filming rather less the preserve of the professional and that the teacher could acquire the necessary expertise within a short period of two to three months, and that this might apply equally to the use of television.

As a partial reply to the problem of selection, storage and availability of films and television recordings (video tapes), raised by Mrs. Eirene White, M.P., Mr. Dickinson pointed to the enormous amount of documentary film material stored by the various television companies. He had been able to obtain some interesting and valuable examples from the United States and from France on condition that these films remained under his supervision. Even with this safeguard of copyright it had not yet proved possible to secure the same facilities from British companies.

Mr. R. Postgate, deputy controller of educational broadcasting, B.B.C., gave a survey of the Corporation's past, present and future policy. He stated that it had always been thought desirable to appoint members of the teaching profession to lead educational broadcasting policy. Apart from the educational activities which were already well known, the Corporation was planning to use its second television channel during the day-time for educational programmes as soon as the channel had achieved wider coverage. Some twenty-four broadcasts were planned to assist those who were working for the social economics diploma of the University of London. On the whole, however, it was the policy of the B.B.C. to think in terms of a national service, and the main emphasis had to be on schools because of the extreme shortage of teachers.

Mrs. Mary Adams of the Television Viewers Council and the Mental Health Film Council made a strong plea for a national education television channel. She defended the point of view that schools and universities should formulate the purpose, nature and contents of educational broadcasting, instead of leaving the initiative to the broadcasting companies. Mrs. Adams proposed that an information centre be created, and that a fellowship might be financed to prepare a survey of present activities in instructional television.

The chairman, Sir John Newsom, chairman of the I.T.A. Education Council, in summing up the discussion, proposed that a further meeting be called early in the new year to concentrate on more specific topics. In the meantime, the conference on the use of film in teaching and research, to be held at the University of Birmingham in September, will serve as a further stimulus.

The conference at the Royal College of Art proved to be a useful forum for the exchange of information and opinion. The next meeting may accept the view expressed by a number of discussion speakers that film and television should not be considered in isolation but in the wider context of all methods of learning and of teaching. Almost invariably the appropriate use of several methods in combination will produce the best results.

While film and television can be expressions of art in their own right, the primary usefulness will probably be as aids in communication. The selection of the most effective methods of communication presupposes a clear understanding of the end product, what the student is supposed to be able to do, and how he is expected to think and react at the end of his course of studies. An appreciation of the teaching situation and the context in which learning is to take place will be just as important. Furthermore the producer of audio visual aids should base his planning on a clear definition of objectives, on existing knowledge of the proposed audience, and on the purpose of the contents. Finally, students should be given an opportunity to learn how to learn from visual information. Visual literacy, a concept cited by Sir Arthur Elton, is essential for the effective construction, use and acceptance of information in visual terms by producers, teachers and students respectively. The literature on the effective construction and use of audio visual teaching devices, both for mass instruction and for individual learning, as well as the literature on methods of testing, is considerable and should be made more widely available. It is, therefore, a pleasure to learn that Lord Robbins will be concerned with a new journal on the technology of education, *New Education*, which is to commence publication in November of this year.

C. E. ENGEL

OXFORD CHROMOSOME CONFERENCE

AN international conference on chromosomes, attended by more than a hundred delegates from twenty countries, was held at Magdalen College and the Botany School, University of Oxford, during July 28–31. The deliberations of the conference were of five kinds: dissertations, demonstrations, business meetings, social functions, and extra-mural informal gatherings.

The proceedings began with an address of welcome by Prof. C. D. Darlington in which he recalled for the conference the content of Weismann's classic paper published in *Nature* (36, 607; 1887). This he saw as still a charter for those who examine chromosomes.

Thirty other papers were delivered in four sessions. The first, on plants, included a report by Prof. A. Müntzing (Sweden) on accessory (*B*) chromosomes in *Poa* and *Secale*. The latter was also the subject of an investigation by Dr. J. Sybenga (Netherlands) on the possible role of interchanges in determining selective pairing in polysomics. Preferential autosyndesis was not observed, indicating that interstitial changes in homology have no detectable effect on pairing which is localized near the ends. The control of pairing was considered also by Dr. R. Riley (Great Britain), who described work on wheat undertaken jointly with Dr. E. R. Sears (U.S.A.). It revealed a genetic condition which determined asynapsis but only under certain temperature conditions. Other polyploids were the subject of papers by C. J. Marchant (Great Britain) and Prof. J. K. Morton (Sierra Leone). A different aspect of chromosome mechanics, diplotene repulsion, was discussed by Dr. K. Pusa (Finland), who concluded that such a force exists. The chairman of this session was Dr. E. R. Sears and it also contained a paper by Mrs. E. R. Sansome (Nigeria), who presented evidence in favour of the diploid condition of the vegetative phase in the Oömycetes.

The second session was concerned with the structure of the nucleus and with chromosome abnormalities induced by mutagens. Prof. Müntzing acted as the chairman of this session, which included papers by Dr. H. Ris (U.S.A.) and L. F. La Cour (Great Britain), who considered chromosome organization as revealed by the electron microscope and nucleolar structure respectively. There were accounts also by Drs. H-G. Keyl (Western Germany) and B. A. Kihlman (Sweden), who discussed various aspects of DNA synthesis. Papers by Dr. R. C. von Borstel (U.S.A.) and Dr. G. R. Lane (Great Britain) reported investigations on X-ray chromosome breakage while Mr. C. J. Grant (France) discussed the delay in mitosis caused by chemical mutagens. This session included a report by Prof. G. Östergren (Sweden) on the effects on chromosome behaviour of induced point mutations, and he gave a demonstration on the same subject. Of special importance were his observations of chromosomes which entered pollen grain mitosis although they had not divided into chromatids.

Insects and mammals were the subject of the third session, which was chaired by Prof. H. Bauer (Western Germany). It began with a beautiful film of meiosis in *Pales* by Dr. R. Dietz (Western Germany), who also exhibited three other films at a supernumerary session held on the last day of the conference. These showed that the spindle could function in a normal way even in the absence of centrioles and they threw considerable light on the mechanical behaviour of the hereditary sex univalents of this genus. Other aspects of chromosome movement and their control were described by Dr. I. Geyer-Duszýnska (Poland), while the effects of α-heterochromatin on crossing-over were considered by Dr. B. E. Wolf (Western Germany). Gene action in *Drosophila* was the subject of two papers, one by Dr. O. Hess (Western Germany) and the other by Dr. H. D. Berendes (The Netherlands). Chromosome polymorphism in black-flies was considered from the taxonomic point of view by Dr. R. W. Dunbar (Great Britain), who also gave a demonstration, while intra-specific chromosome variation of the Robertsonian type was discussed by Dr. C. E. Ford (Great Britain) and Dr. J. Wahrman (Israel).

Prof. E. Battaglia (Italy) was the chairman of the final session which contained papers dealing with mammals and the recent, far-reaching, discoveries in man. Putative Robertsonian-type variation in humans was described by Dr. J. L. Hamerton (Great Britain), but there was a suggestion that the condition was better regarded as a monosomic one following unequal interchange between acrocentric chromosomes. Dr. M. Fraccaro (Italy) considered the occurrence of iso-chromosomes and deficiencies in the long arm of the X-chromosome in the same species. Attempts to identify the chromosomes of man individually by different methods were discussed by Dr. D. T. Hughes (Great Britain) and by Dr. C. H. Ockey (Great Britain), who supported his paper with a demonstration. Miss U. Mittwoch (Great Britain) reviewed the problem of the Barr-bodies in human females. This session also contained a very interesting account by Dr. S. Ohno (U.S.A.), who described a hitherto unique condition in mammals, namely, an *XO* soma as the normal state in females of the creeping vole. It is associated with mechanisms leading to the formation of only *X*-carrying eggs and *Y*- or *O*-containing sperm.

The thirty demonstrations included thirteen by members of the host department. They covered a wide range of organisms from microbes to man and a variety of techniques including experimental breeding, autoradiography and electron microscopy. Giant models of meiosis made at the Botany School were exhibited. The spiral structure of chromosomes was demonstrated on closed-circuit television by Mr. Canio Vosa (Italy), now of the Botany School, Oxford, and visits to the Genetic Garden of this School were conducted by Prof. Darlington and Mr. Kenneth Burras, the superintendent of the Botanic Garden. This garden contains a collection of variegated plants, chimæras and chromosomally interesting plants used principally for teaching purposes.

These activities, the demonstrations and the papers, served the immediate purpose of the conference which was the exchange of information and ideas. Chromosome investigations have had a tremendous impact on all branches of biology, especially in recent years, and they have established connexions with medicine, agriculture, sociology (anthropology) and the physical sciences. But the extension and expansion of this technique have led to the unforeseen dispersion of chromosome workers themselves and reduced communication between them. Enquiries, beginning at Oxford, had suggested that the need for greater contact could best be fulfilled by regular meetings organized by an international body. With this in view, the chairmen of the four sessions were asked to function as a steering committee and they gave their recommendations at the business meetings.

It was decided that they, by co-option, should increase their number to nine with Dr. K. R. Lewis (Great Britain) as secretary and that this body should serve as an international committee responsible for the second conference of this kind which will be held in Oxford in 1966. At that conference the members of the original steering committee should be replaced by members elected by the conference. Such elected members would then be responsible for the organization of later international conferences every two years. The committee would, by rules or example, indicate the form of future conferences and suggest other activities by which chromosome investigations could be advanced.

The spirit of this first conference indicated a vigorous future for this new venture and anyone interested in it is asked to communicate with me (Botany School, Oxford). The full proceedings of the conference will be published as a supplement to *Heredity*.

K. R. LEWIS

SPECTRAL EVIDENCE ON THE ORIGIN OF TYPE I SOLAR RADIO STORMS AND THE CORONAL MAGNETIC FIELD

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THE dynamic spectrum of metre-wave radiation emitted by the Sun during type I storms[1,2] consists of a conglomeration of storm bursts which are scattered in the time-frequency plane over periods of hours or days and within frequency bands of tens or hundreds of megacycles per second. Owing to the complexity and lack of systematic features in the macroscopic disposition of these bursts, spectrographic investigations have yielded no real clues about the physical nature of the storms as a whole. The purpose of this article is to direct attention to a particular class of systematic macroscopic feature which we have found to be repeatedly evident on the spectral records taken at Dapto, New South Wales, during the last solar cycle. The feature in question is the occurrence of bursts grouped together in tens or hundreds to form narrow-band 'chains' with durations of the order of 1 min or several min and band-widths of the order of 10 Mc/s. An isolated example of this phenomenon was briefly reported some years ago[3].

The characteristics of the type I burst-chains are illustrated by the examples given in Figs. 1 and 2 and the left-hand columns of Table 1; many more examples can be found in the records and indeed it is our subjective impression that the majority of type I radiation may be resolved into chains. The chains may apparently occur in any part of the metre-wave spectrum (30–300 Mc/s) and may drift systematically in frequency at a rate given by $\frac{1}{f}\left|\frac{df}{dt}\right| \lesssim 0{\cdot}1$ per min, the average rate being about half that for type II bursts. Although instances have been found in which the drift is positive (that is, from low to high frequency), the predominant drift is negative as in type II bursts. The chains may occur insignificantly among other complex storm activity, but sometimes dominate the record for periods of up to 1 h in which a sequence of chains with negative drift follow one another (Fig. 1*b*). Measurements using the Dapto swept-frequency interferometer have shown that variations in position on the Sun's disk of several minutes of arc may accompany the drift in frequency.

It is common, as in the lower record of Fig. 2, to find a pair of chains running roughly (but not exactly) parallel to one another at different frequencies, providing an example of the split-band phenomenon described by Elgaroy[2]. It is not clear whether this circumstance is always due to two independent sources simultaneously

Table 1. OBSERVED AND DERIVED CHARACTERISTICS OF CHAINS OF TYPE I BURSTS

Date	U.T.	Drift in frequency (Mc/s)	Duration (min)	Movement in height (10^5 km)	Upward velocity (km/sec)	*H* (gauss)
April 27, 1953	0018	210 → 185	1·7	0·9 → 1·15	250	2·5
	0038	230 → 180	4·8	0·7 → 1·2	160	1·6
	0045	178 → 161	2·7	1·2 → 1·4	120	1·0
August 12, 1957	0223	56 → 48	4·2	4·3 → 4·9	240	0·6
	0229	50 → 44	3·4	4·75 → 5·3	260	0·6
	0239	55·5 → 48	4·1	4·4 → 4·9	260	0·7
	0449	63 → 58	1·8	3·9 → 4·2	270	0·8
	0458	58 → 51	2·0	4·2 → 4·7	410	1·1
	0512	48 → 44	5·5	4·9 → 5·3	110	0·25
	0514	61·5 → 60	2·1	4·0 → 4·1	65	0·2
March 29, 1960	0602	47·5 → 45·5	0·8	5·0 → 5·15	380	0·9
		53 → 51·5	0·4	4·5 → 4·65	500	1·3
	0610	58 → 52	1·9	4·2 → 4·6	360	1·0
	2345	48 → 42	2·1	4·9 → 5·5	460	1·0
	2352	56 → 44	2·0	4·3 → 5·3	800	2·0

The heights and velocities are derived assuming the radiation to originate at the plasma levels determined from Newkirk's model of the solar atmosphere above an active region. The column *H* gives the radial component of the magnetic field derived from the assumption that the velocity is equal to the speed of Alfvén waves.

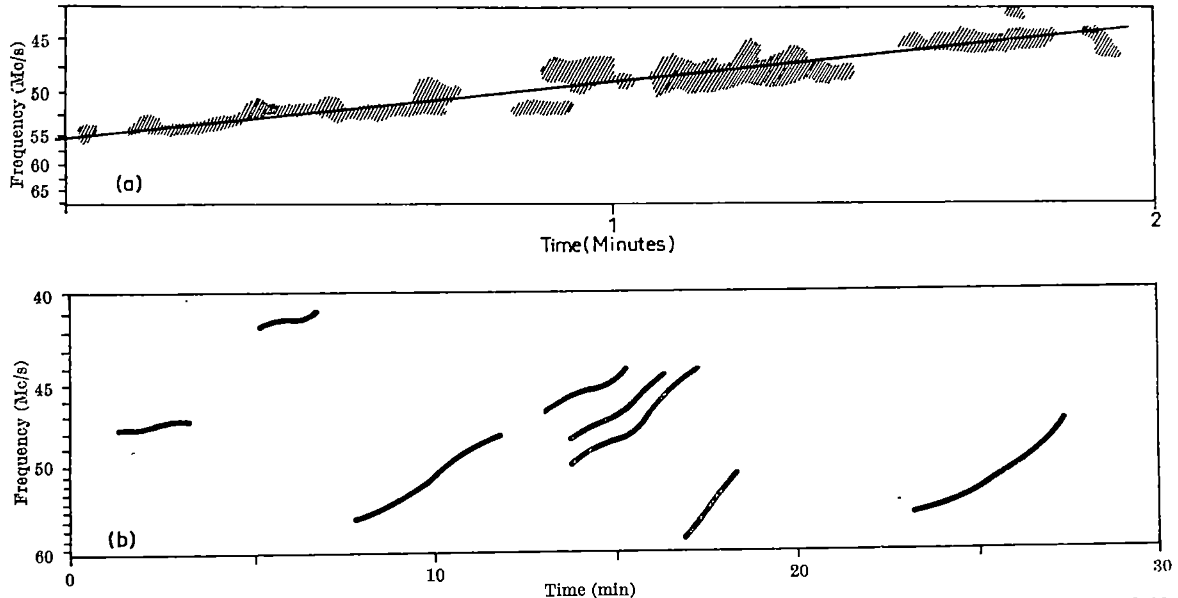

Fig. 1. Tracings of dynamic spectra. (*a*) An individual burst chain, the hatched areas indicating concentrations of type I bursts (March 29, 1960, 2352–4 U.T.); (*b*) several chains occurring in succession (August 12, 1960, 0215–0245 U.T.)

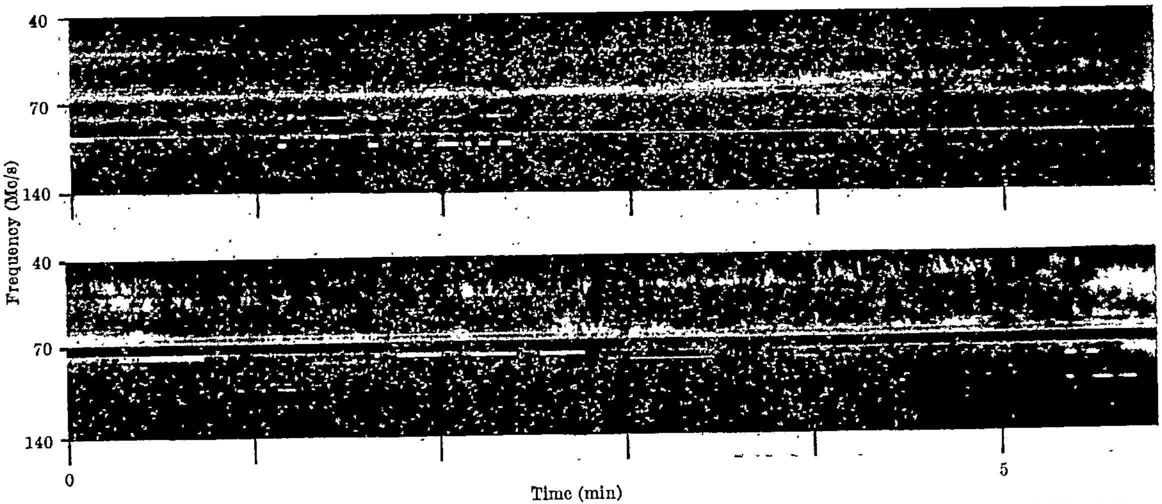

Fig. 2. Dynamic spectra of type I burst chains, showing examples of a simple and a double event (August 12, 1957, 0238–0244 and 0512–0518 U.T.)

present or whether it may sometimes be due to the excitation of two frequencies by the same source : there is often complete lack of detailed correspondence between the two bands though sometimes simultaneous brightness variations repeatedly occur in the two; the latter condition need not necessarily imply a common source but could be due, for example, to two independent sources linked by a fluctuating electron stream or magnetic field. Differences in position between two bands of a pair of up to several minutes of arc have been found.

We suggest that the two most important characteristics of type I chains—their narrow-bandedness and their frequency drift—could be due to either (*a*) effects of propagation limiting and varying the frequency range of emission from a relatively large, broad-band source, or (*b*) the motion of a localized source passing through regions of different electron density or magnetic field. Bearing in mind the preponderance of frequency drift in the one sense (negative) we strongly favour the latter. Other evidence and the majority of present theories support the view that type I bursts are generated by plasma oscillations in which the frequency is determined by the local plasma frequency ; and on this basis, the typical chain originates in a localized source moving upwards through the solar atmosphere. This hypothesis has been used in Table 1 to determine the heights and speeds of the events listed, for which purpose the plasma frequency is assumed to be determined by Newkirk's model of the solar atmosphere above an active region; the gyro-frequency has been assumed negligible in comparison with the plasma frequency. The sources are seen to rise through a height range of up to 10^5 km at speeds of several hundred km/sec, and to be capable of existing at heights of up to some 5×10^5 km.

The deduced velocities are too small to be identified with electron streams, since they are less than the speed of thermal electrons, and they are generally in excess of the field-free velocity of sound in the corona by a factor of between 1 and 5. We suggest that their most likely identity is that of a magnetohydrodynamic wave or shock wave, and that the evidence implies that some features, at least, of type I storms originate in such waves occurring in succession with predominantly ascending motion. The finding of any detailed optical correlation with these features would clearly be valuable, and we suggest that blue-shifted *H*α observations made with the necessarily high spatial and time resolution would provide the most likely chances of success.

It is important to distinguish between the phenomenon reported here and the type II burst : the latter also exhibits slowly drifting features and again is believed to originate in a magnetohydrodynamic shock wave. The distinction between the two phenomena lies in (i) the much greater magnitude and isolation of the type II events (which are associated with outstanding flares), (ii) the strong circular polarization of the type I (absent in the II), (iii) the much greater fluctuation (burstiness) of the type I, and (iv) the generally higher velocity of the type II. There thus appears to be a clear distinction in the generating mechanisms of the two. If our interpretation is correct, one may ask what causes a magnetohydrodynamic wave or shock wave sometimes to generate type I radiation and at other times type II. We suggest two possible distinctions : first the nature of the wave itself, whether it be transverse (Alfvén) or longitudinal (quasi-acoustic) and whether it be a linear disturbance or a shock wave ; and secondly whether or not the kinetic energy of the disturbance exceeds the magnetic energy in the region of the source.

If it is assumed that the disturbances responsible for the type I chains travel at the speed of Alfvén waves, which is given in terms of the magnetic field (H gauss) and plasma frequency (f_p Mc/s) by:

$$v \approx 2 \times 10^4 \, (H/f_p) \text{ km/sec}$$

then one is able to derive a value for the radial component of the coronal magnetic field. Such values are given in the last column of Table 1 and are seen to range from 1–2·5 gauss for heights near 10^5 km and mainly from 0·2–1 gauss for heights near 5×10^5 km. If the disturbances are actual Alfvén waves, these derived velocities are formally appropriate; if they are any other kind of magnetohydrodynamic wave or shock wave (other than a purely acoustic shock wave travelling along the field), the derived values represent upper limits to the magnetic field.

The detailed process of radio-wave generation is not considered here. It is interesting to note, however, that Takakura[4] has proposed a theory of type I bursts in which the radiation is attributed to plasma waves excited by superthermal electrons which are accelerated between two packets of Alfvén waves travelling in opposite directions. In terms of this theory a chain of type I bursts might conceivably be attributed to encounters between a single, outward-moving wave packet and a series of inward-moving packets following one another in quick succession. The detailed process proposed by Takakura is not, however, qualitatively compatible with an application of this hypothesis to the present observations, since Takakura requires the Alfvén waves to travel at about the mean speed of thermal electrons (~7,000 km/sec in the corona) and hence the magnetic fields to be of very much greater intensity than those inferred above.

We thank Mr. K. V. Sheridan and other colleagues who have contributed towards the observational programme of Dapto field station and Mr. S. F. Smerd for helpful discussion. This work was carried out at the Radiophysics Laboratory, Sydney, while one of us (A. T.) was on leave of absence from Ondřejov Observatory during the tenure of a C.S.I.R.O. fellowship.

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ANION ADSORPTION ON PLATINUM BY THE MULTIPULSE POTENTIO-DYNAMIC METHOD

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ADSORPTION of anions on platinum has previously been investigated by a variety of non-voltammetric techniques. These methods, most commonly applied to platinized platinum, include: (1) observation of the effect of anion adsorption on hydrogen deposition[1-3]. (2) measurement of the electrode potential shift resulting on addition of the adsorbable ion to a solution of sulphuric or perchloric acids[4-6]; (3) measurement of the pH or conductivity change of a relatively small volume of liquid, caused by displacement of hydrogen ions from the ionic double layer by anions[7]; (4) direct determination by quantitative analysis of anions lost from solution[8]; (5) direct determination of anion surface concentration by means of radioactive tracers[9-11].

The results reported for any particular anion vary with surface preparation, pH, potential, etc. One fairly clear-cut conclusion is that the halide ions specifically adsorb, with the extent of adsorption increasing as one descends down the series to the higher atomic weights[12]. Perchlorate ion is assumed to adsorb very slightly or not at all.

Recently, Breiter[13] made measurements of the capacity of a smooth platinum wire in perchloric-hydrochloric acid solutions under the conditions of periodically varying potential. This method served to bring the electrode to a more reproducible surface state than was generally accomplished in the non-voltammetric measurements of his predecessors. One disadvantage of the method is the inability to assign quantitative interpretation to the measured capacities.

The purpose of work recorded here was to develop methods for the investigation of ion adsorption along the same lines as previously applied to carbon monoxide[14-16] and oxygen 'adsorption'[17]. A 'multipulse potentiodynamic' approach, for example, has the advantage, when successfully applied, of providing simple initial boundary conditions, and allowing for quantitative interpretation of data. Two anions of different nature were chosen for this investigation. Chloride is highly adsorbed and is an oxidizable ion. Phosphate is relatively slightly adsorbed and is non-oxidizable. The investigation resulted in development of the desired quantitative methods for measurement of anion adsorption. These methods permit, in principle, the measurement of kinetics of adsorption and desorption, and determination of equilibrium surface coverages as a function of potential.

The electronic equipment, glassware and electrodes have been described previously[14]. The working electrode was a length of C.P. platinum wire of 0·064 cm^2 geometric area. The saturation hydrogen surface coverage, Q_{SH}, determined as described previously[15], was 0·272 mcoul./cm^2, suggesting a roughness factor of approximately 1·3. Q_{SH} was not found to vary appreciably during the measurements, suggesting little or no etching of the electrode in the dilute hydrochloric acid solutions used, and under pulsed conditions. Solutions of hydrochloric acid and of phosphoric acid were prepared by addition of the analytical-reagent grade chemicals to a stock solution of 1 N analytical-reagent grade perchloric acid. All measurements were made in a thermostatic bath at 30° C. All potentials are referred to a reversible hydrogen electrode in 1 N perchloric acid.

The multipulse potentiodynamic method can be used to detect extremely small amounts of adsorbed anions if proper attention is directed toward the establishment of a reproducibly clean initial surface state. The criteria previously used for such an initial condition are con-

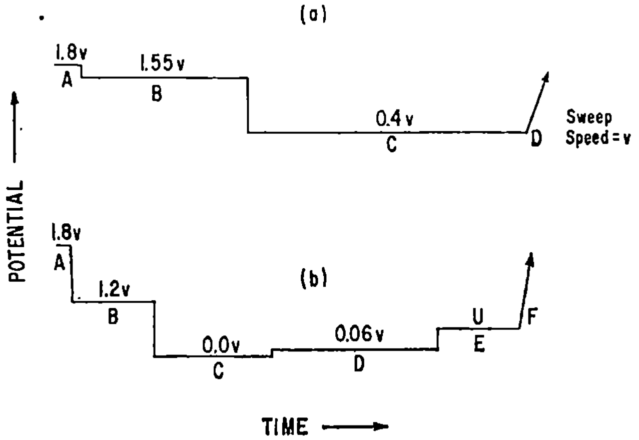

Fig. 1. Signal sequence applied to the working electrode

stancy of: (1) saturation hydrogen coverage, Q_{SH} (refs. 15, 17); (2) 'oxygen adsorption', Q_0 (refs. 15, 17); (3) differential electrode capacitance[17]. Sequence (*a*) of Fig. 1 consists of electrode pre-treatment steps *A* through *C* and measurement pulse *D*. The details and significance of each step have been described previously[15]. Steps *A* and *B* serve to cleanse the surface. During step *C*, the surface is reduced and adsorbable materials in the solution, including impurities, may be adsorbed. The presence of adsorbates on the surface after any period of time T_C at step *C* may be detected by examination of the 'oxygen adsorption' trace obtained on application of linear anodic pulse *D* (refs. 15–17). If a linear cathodic pulse is substituted for *D*, the resulting 'hydrogen adsorption' trace serves as an indication of surface cleanliness[15,17]. Both these criteria reveal a reproducible surface state for 10 msec $< T_C \leqq 10$ sec if a 1 *N* solution of analytical-reagent grade perchloric acid is used as the electrolyte. Longer 'clean times' are possible if the electrolyte is purified[15]. Sequence (*b*) of Fig. 1 is a modification of sequence *a* for use in 1 *N* perchloric acid containing hydrochloric acid. This modification is intended to reduce the extent of possible chlorine evolution and platinum dissolution during steps *A* and *B*. Steps *C* and *D* are chosen so that by the end of step *C* there are no chloride ions adsorbed on the surface and the concentration of molecular hydrogen in the adjacent solution is small. Steps *A* and *B* are of 2- and 15-sec duration, respectively. Step *C*, the purpose of which is to eliminate adsorbed anions, is of 2-min duration. Paddle-stirring of the electrolyte is continued from the beginning of step *A* to the end of the first 0·5 min of step *C*. By the end of step *C* the solution is quiescent, but the concentration of hydrogen near the surface is excessive. This is reduced to a tolerable level by application of step *D*, which is of 10-sec duration. If the value of *U* of step *E* is taken as 0·4 V and the perchloric acid solution contains no added adsorbable anion, the 'oxygen adsorption' trace measured by application of linear anodic sweep *F* is identical with that obtained by means of sequence (*a*), Fig. 1. If, on the other hand, the perchloric acid is made 10^{-3} *N* in hydrochloric acid, *U* is taken as 0·7 V and pulse *F* is set at $U = 1{,}000$ V/sec, the 'oxygen adsorption' trace varies gradually with time as indicated in Fig. 2. Trace 1 of Fig. 2 is the trace obtained in the absence of hydrochloric acid, under which conditions the same trace would be obtained for values of T_E over 10 sec. The difference in area (in mcoul.) between trace 1 and any other trace of Fig. 2 is the decrease in 'oxygen adsorption' (ΔQ_0) for that particular value of the adsorption time, T_E. A plot of ΔQ_0 versus $T_E^{\frac{1}{2}}$ results in excellent linearity. The particular slope obtained is precisely that predicted for the following conditions:

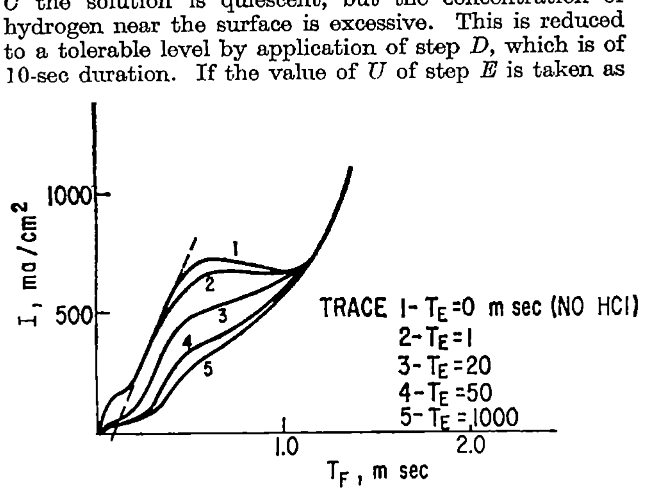

Fig. 2. 'Oxygen adsorption' traces obtained by means of signal sequence (*b*), Fig. 1. Electrolyte composition $= 10^{-3}$ *N* hydrochloric acid, 1 *N* perchloric acid

(1) The adsorption of one equivalent of chloride ions hinders the 'adsorption' of two equivalents of oxygen.

(2) Adsorption of chloride ions is controlled by semi-infinite linear diffusion for the short time required to reach maximum coverage.

(3) No reaction (for example, chlorine evolution, platinum dissolution) occurs at an appreciable rate compared with the rate of adsorption of chloride ions (for this particular concentration, and sweep speed, *v*). Condition 3 is made possible for any concentration of adsorbable anion by choosing *v* so that the duration of step *F* is small compared with the time required for the diffusion of an appreciable amount (compared with a monolayer) of the anion to the surface.

If step *E* is made sufficiently long, and *U* is taken as 0·7 V, the rate of adsorption of chloride ions eventually drops to zero when the amount of chloride is still smaller than a monolayer. This amount is an equilibrium value since it can be obtained by adsorbing chloride ions at 0·8 V, and allowing description to proceed to completion at 0·7 V. Equilibrium surface coverages may be measured for any value of *U* up to 0·8 V. The equilibrium surface coverage is approximately linear between 0·15 and 0·80 V. The equilibrium surface coverage at any fixed potential in this range appears to depend approximately on the log of concentration.

Observations on phosphate adsorption are similar to those for chloride, except that the equilibrium surface coverages are approximately 1/5 as large.

Additional observations suggest that adsorbed chloride is not oxidizable at any appreciable rate at potentials up to 1·6 V. This is probably related to the fact that the surface undergoes a valence change during adsorption (starting at a potential no higher than 0·7 V), resulting in a species having the stoichiometry (but not the structure) of $PtCl_2^-$. Evidence for this last statement is obtained by measuring the anodic current which flows during chloride adsorption at constant potential.

It is likely that the multipulse potentiodynamic method might be applied successfully to the quantitative examination of the adsorption of many different anions on platinum and other metals.

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AN ANTIFOULING AND ANTI-CORROSION SYSTEM

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THE growth of barnacles or other fouling organisms on the hulls of modern ships increases residual friction and leads to decreased travelling speeds. Although the speed losses involved are usually small (less than 2 knots), they can cause large increases in fuel consumption; Redfield and Hutchins[1] indicated that fouling on ships of 1,850–35,000 tons (standard displacement) might increase their fuel consumption by 35–50 per cent after 6 months in temperate waters or after 3 months in tropical waters. The method commonly used to reduce fouling is to apply an antifouling paint, containing cuprous oxide, which has a deterrent effect on the establishment of fouling growths. Unfortunately such paints can also accelerate hull corrosion. As the British Corrosion Committee pointed out more than 20 years ago[2], antifouling paint performance can be completely satisfactory only when the underlying anticorrosive paint prevents any corrosion of the hull surface. For best results the antifouling and anticorrosive systems need to be individually effective and mutually compatible.

The hull protection system proposed here, and now under trial, consists of a cuprous oxide antifouling paint of the soluble matrix type applied over a solventless epoxy resin anticorrosive layer. It is intended for use with a conventional cathodic protection system. Antifouling paints operating on the soluble matrix principle have been available for many years, but their potential has not been utilized fully, probably because of inadequacies in the underlying anticorrosive paints. The use of a solventless epoxy resin as a replacement for conventional anticorrosive paint systems is a new development.

Shipfouling and external underwater corrosion arise from endemic and interacting processes located in four basic strata which are referred to here as the larval, antifouling, anticorrosive and hull layers.

Although 374 shipfouling species have been listed[3], many of these are not important. Redfield and Deevy's tabulation of data[4] for 131 ships docked in northern hemisphere ports[5,6] and Skerman's analyses for 89 ships docked in the southern hemisphere[7] show clearly that the important fouling groups involved are acorn barnacles, bryozoans, tubeworms, hydroids, algae, bivalve molluscs and tunicates. The shipfouling problem is initiated by the larvæ of these organisms (or the equivalent stages in the algae) which have arrived at the hull surface and are attempting to attach to it. Antifouling methods should prevent this larval layer from colonizing the hull successfully and so avoid, from the beginning, the development of bulky adult fouling organisms on the hull.

The antifouling layer usually consists of a paint containing cuprous oxide as the toxic agent. Biological observations show that such paints can be effective against larvæ of four of the seven important fouling groups mentioned earlier. Wisely[8] suggested that a larva approaching an active antifouling paint faces several distinct possibilities: (1) it may be repelled and move away; (2) it may become moribund before it can attach properly; or (3) it may attach, commence metamorphosis into a young adult and become moribund at some stage during this process. The terms 'repellent', 'pre-attachment mortality' and 'post-attachment mortality' have been applied to these respective effects[9,10]. Recently, Crisp and Austin[11] showed that cyprids of the barnacles *Balanus balanoides* (L.) and *Elminius modestus* Darwin were killed during, or often after, metamorphosis when they attempted colonization of a cuprous oxide antifouling paint (post-attachment mortality). Pre-attachment mortality resulting from contact with similar paints has since been reported for larvæ of the bryozoans *Watersipora cucullata* (Busk)[8] and *Bugula neritina* L.[10] and for larvæ of the tubeworms *Spirorbis lamellosa* Lamarck and *S. convexis* Wisely[12]. A repellent effect has now been recorded for larvæ of the bivalve molluscs *Mytilus edulis planulatus* Lamarck and *Lasaea australis* Lamarck[9]. Comparable investigations have yet to be made on hydroid and tunicate larvæ, and on the equivalent stages of larger marine algae. However, representatives of these groups and of other less important groups are common in Sydney Harbour, where cuprous oxide paints have successfully prevented fouling for 1–2 years, so it can be assumed that such paints are also capable of preventing colonization by these remaining groups.

The value of cuprous oxide as an antifouling agent has been established from biological observations and practical experience. It has advantages not possessed by other toxic substances so far tested for this purpose. These include low cost, suitable solubility and stability, and safe-handling characteristics. Organic and other inorganic substances tested have not been as effective or have had other disadvantages. The solubilities of cupric oxide and cupric hydroxide are too low for them to be used in antifouling paints, whereas the solubility of mercuric oxide is excessively high[13]. Organic toxicants more active than cuprous oxide have been found[14,15], but the majority of these were unsatisfactory in paint films[16]; some of these are specific in their action; for example, DDT is toxic to barnacles but not to other groups[17,18]; other organic toxicants, for example, methyl mercuric chloride, are toxic to man as well as fouling organisms[19].

Experimental work has established[20] that cuprous oxide antifouling paints will prevent fouling so long as the copper ions continue to leach out at a rate greater than 10 $\mu g/cm^2/day$. The difficulty lies in maintaining a leaching rate safely, but not wastefully, above this value for long periods. Ferry and Ketchum[21] described the two main types of antifouling paints available in 1952. The continuous contact type has a high cuprous oxide loading in a virtually insoluble matrix, which allows the copper ions to be extracted only down to a depth of about 50µ (0·002 in.)[22]. Consequently, there is no advantage in applying the paint layer thicker than this. In contrast, paints of the soluble matrix type usually have a lower toxic loading, but the leaching rate can be more readily controlled by adjusting this loading and the dissolution rate of the matrix. The outstanding advantage of the soluble matrix paints is that their effective lives can be extended by increasing the thickness of the layers applied. Paints of both types, and more recently developed ones, have resisted fouling for 1–2 years in Sydney Harbour during acceptance testing to Australian Standards

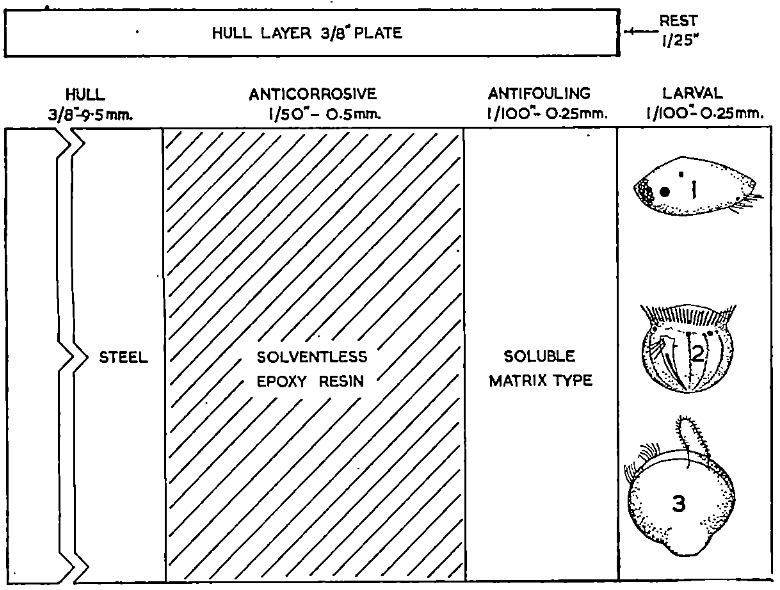

Fig. 1. Above, scale drawing showing the relative widths of the hull and the rest (anticorrosive, antifouling and larval layers); below, diagrammatic enlargement of the latter layers. 1, 2 and 3 represent an approaching barnacle, bryozoan and bivalve mollusc larva respectively

Specification K129. These tests include raft evidence of 12-months' fouling- and corrosion-free performance, followed, after retouching and re-immersion of the test plates, by similar performance for a further 6 months. One of these paints afterwards resisted fouling satisfactorily for two years when applied to a ship moored in reserve[23]. These encouraging results were obtained with thin layers of antifouling paint (0·0015–0·0030 in.); it would be of considerable interest to ascertain how long thick coats (0·005–0·01 in.) of the cuprous oxide, soluble matrix types now available would remain effective.

The function of the anticorrosive layer is to prevent sea-water penetrating through to the hull and causing corrosion. The use of cuprous oxide for antifouling raises an additional problem, since copper compounds can enter into galvanic reactions with the iron of the hull. This is the main disadvantage with cuprous oxide antifouling paints and one of the reasons for seeking alternatives among the toxic organic compounds. Redfield[24] suggested that the logical method of overcoming this kind of problem was to use an anticorrosive layer with impermeable and electrically insulating properties. Such a layer should prevent copper ions from the antifouling layer reaching the hull and should also insulate the hull from sea-water and thus prevent the completion of local circuits through that medium. Wisely[25] tested a solventless epoxy resin as an anticorrosive layer, since those resins were reputed to have the qualities stipulated by Redfield, together with the very desirable feature of strong adhesion to properly prepared metal surfaces. The results of these experiments were promising. The steel beneath the epoxy resin was still bright after 44 months' continuous immersion in the sea. Moreover, the antifouling paint applied over the resin resisted fouling for nearly 32 months, which was longer than could have been expected from previous experience in the use of this paint.

These results suggest that an effective hull protection system can be established by appropriately preparing the hull surface and then applying a solventless epoxy resin to it, followed by a thick layer of cuprous oxide, soluble matrix type, antifouling paint (Fig. 1). The thickness of the antifouling layer shown in this figure is that estimated for a relatively soft and erosion-prone type of paint; this might be expected to last several years. However, if one of the harder plasticized paints was used the layer could be much thinner. Experience in the use of epoxy resin is as yet too limited to indicate the optimum thickness. One of the critical points concerns the ability of the epoxy resin to maintain its characteristic properties and integrity during prolonged immersion on hulls. Ship patch trials would seem to be the most satisfactory method for evaluating these and related issues.

A test area of 250 sq. ft. aimed at assessing the potential of the foregoing system was applied to an Australian ship in September 1963, using conventional dockyard methods and labour. The area was wet sandblasted, using a soluble rust inhibitor additive (trisodium phosphate/potassium bichromate mixture), followed by washing down with fresh water containing a trace of additive. The area was then allowed to dry thoroughly. The epoxy resin applied was 'Araldite Resin 714' (now known as 'Resin MY752') supplied by Ciba Co. Pty., Ltd., Sydney. This was used in the proportions: resin 714, 3·7 kg; hardener 'HY830', 2·3 kg; accelerator 'DY830', 66 g; thixotropic agent (SiO_2), 113 g. Three brushed coats gave an estimated thickness of 0·025–0·035 in., which was thicker than desired. Four coats of 'International Anti Fouling Red 92027', supplied by International Majora Paints Pty., Ltd., Sydney, were then rolled on, giving an estimated thickness of 0·004–0·008 in. This particular paint was used because after 33 months' raft immersion testing there was no evidence of a surface residual matrix layer building up; this suggested it was not a continuous contact type in the strictest sense, and might have had affinities with the plasticized soluble matrix types. It should be noted that the thickness applied was much greater than that recommended normally by the manufacturers. The performance of the complete system, though not identical with that shown in Fig. 1, should permit an estimate of the latter's potential. Application details will be presented later with docking reports.

This work is part of a programme on the biology of fouling organisms carried out in co-operation with the Royal Australian Navy.

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ACTINOMYCIN *D* AND MESSENGER-RNA TURNOVER

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RATES of metabolic turnover of messenger ribonucleic acids (*m*RNA) from various sources have been investigated intensively in recent years. Levinthal *et al.*[1] observed that *m*RMA of *B. subtilis* possessed a rapid turnover with an average half-life of 2·5 min. In sharp contrast, others have reported that *m*RNA of reticulocytes appears to be stable[2,3]. These extreme cases emphasize the necessity of investigations on *m*RNA turnover with particular reference to the regulatory mechanisms of *m*RNA synthesis and decay in different systems.

Original investigations with rat liver gave rise to the speculation that the template for protein synthesis in this tissue may be stable[4]. However, these results were contradicted by subsequent findings that inhibition of RNA synthesis with actinomycin caused a breakdown of polysomes which, according to the authors, resulted from the rapid *m*RNA turnover[5,6]. In recent experiments, Revel and Hiatt[7], utilizing actinomycin *D*, have again suggested that a stable template exists in rat liver. The stability of rat liver *m*RNA is obviously a subject of present-day controversy. In pulse-labelling experiments to be published elsewhere, we have found that, under steady-state conditions, the average half-life of mouse liver polysomal *m*RNA is approximately 2 h. It therefore becomes important to investigate the decay of *m*RNA of mouse-liver polysomes after inhibition of RNA synthesis by actinomycin *D*. In this situation the metabolism of *m*RNA may differ from that existing in a normal steady state and a comparison of turnover rates of *m*RNA under these two conditions may provide information regarding the regulatory mechanism of *m*RNA metabolism. Indeed, using actinomycin *D*, we have found the average half-life of mouse liver *m*RNA to be 8–12 h.

Male adult mice of the *C57Bl/6J* strain purchased from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, were used in our experiments. Actinomycin *D* was a generous gift from Dr. Elmer Alpert of Merck Sharp and Dohme, West Point, Pennsylvania. Orotic-6-^{14}C acid hydrate (specific activity = 3·45 mc./m.mole) and DL-leucine-1-^{14}C (specific activity = 22·3 mc./m.mole) were obtained from the New England Nuclear Corporation, Boston, Mass. Preparation of purified ribosomes (*p*-ribosomes) by centrifugation through 2 layers of sucrose, and their fractionation by zone centrifugation analysis in a linear sucrose gradient have been described previously[8]. RNA was liberated from *p*-ribosomes by treatment with sodium dodecyl sulphate[9] and fractionated on a linear sucrose gradient. Radioactivities of the various RNA fractions were determined by the procedure of Staehelin *et al.*[10] with minor modifications as follows. 1 ml. of 0·6 N perchloric acid was added to each sample after addition of 20 mg of 'Celite' (diatomaceous silica of John S. Manville Co.) and 0·5 ml. of a bovine serum albumin solution (4 mg per ml.) serving as a co-precipitant. Resulting precipitates were collected by suction on filter-paper disks (Whatman No. 540) layered with 20 mg of 'Celite' in a stainless-steel filtration apparatus (*E*–8*B* apparatus, Tracerlab) and washed successively with 6 ml. of 0·5 N perchloric acid, twice with 5 ml. of isopropanol–ethyl ether (1 : 2) and, finally, with 5 ml. of isopropanol–ethyl ether–chloroform (2 : 2 : 1). Dried precipitates were transferred to glass-counting vials and 1 ml. hydroxide of hyamine (methanol solution of *p*-(diisobutylcresoxyethoxy ethyl) dimethyl benzyl ammonium hydroxide, Packard Instrument Co.) solution added. After 1 h at room temperature, 9 ml. of a 0·5 per cent solution of 2,5-diphenyloxazole in toluene were added and radioactivity measured in a Packard 'Tri-Carb' scintillation counter.

A typical experiment demonstrating effects of varying doses of actinomycin *D* on pulse-labelled liver RNA is shown in Fig. 1. The sedimentation pattern observed in the control animal (Fig. 1, I) has been analysed extensively in recent investigations on the sedimentation behaviour of pulse-labelled liver RNA[10–12]. This diagram clearly shows 3 peaks, *a*, *b* and *c*, and an area between *b* and *c* with high radioactivity. The radioactive peaks *a* and *b* coincide with the 29*S* and the 18*S* ribosomal RNA (*r*RNA), respectively. Peak *c*, which corresponds to an

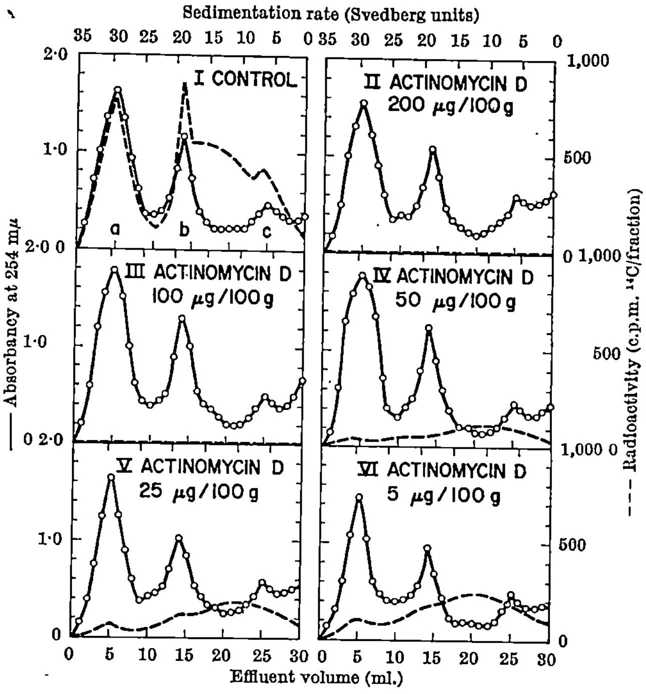

Fig. 1. Sedimentation patterns of pulse-labelled liver RNA from normal (I) and actinomycin *D*-treated (II–VI) mice. Mice weighing 25 g were injected intraperitoneally with the indicated amounts of actinomycin *D*. After 4 h, each mouse received 15 µc. of orotic-6-^{14}C acid hydrate by intraperitoneal injection and was killed 4 h later by decapitation after ether anæsthesia. Liver *p*-ribosomes were prepared and RNA liberated by stirring the ribosomal pellet for 3 min at 37° C in 1 ml. of 0·5 per cent sodium dodecyl sulphate. The mixture was layered over 30 ml. of a cold linear sucrose gradient (0·5–1·0 M) containing 0·005 M *tris*-HCl, *p*H 7·2. After centrifugation for 40 h at 25,000 r.p.m. and 1° C in the *SW* 25 Spinco rotor, 1 ml. fractions were collected from a needle inserted through the bottom of the centrifuge tube and their absorbancies at 254 mµ and radioactivities determined. Sedimentation constants of RNA fractions were determined by calibrating the sucrose linear gradient with the 18*S* and 29*S* ribosomal RNA (ref. 10)

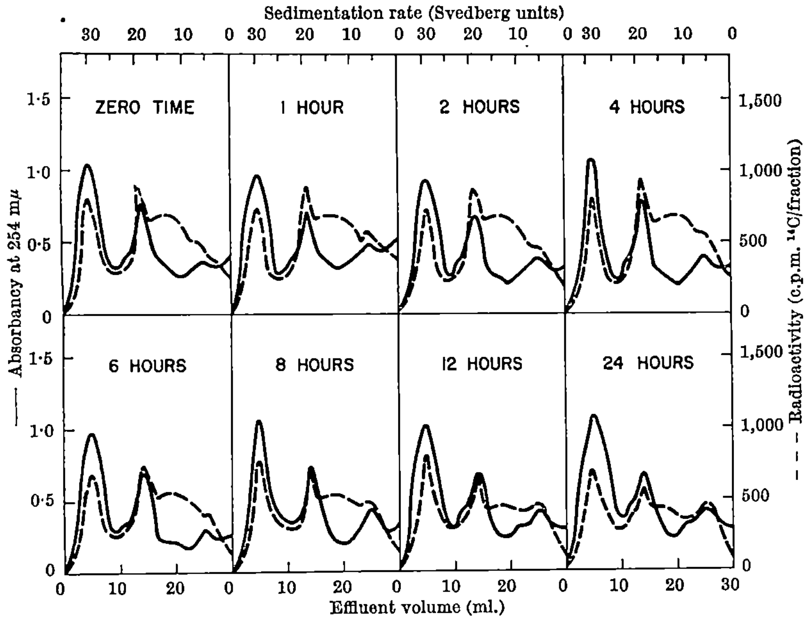

Fig. 2. Sedimentation patterns of pulse-labelled liver RNA at varying times after administration of actinomycin *D*. Mice weighing 25 g were injected intraperitoneally with 15 μc. of orotic-6-^{14}C acid hydrate. Four hours later, each mouse received intraperitoneally 100 μg of actinomycin *D*/100 g body-wt. A mouse killed immediately after injection of actinomycin *D* served as the zero time control. Other animals were killed as indicated at the varying time-intervals after injection of actinomycin *D* and sedimentation patterns of pulse-labelled RNA determined as in Fig. 1

RNA component with an approximate sedimentation constant of 4·5–5*S*, consists of labelled transfer RNA (*s*RNA). The radioactive area *b–c* possesses a high specific activity and is heterogeneous with components possessing sedimentation rates ranging from approximately 4·5*S* to 20*S*. Accumulated evidence supports the view that this area consists of a spectrum of *m*RNA molecules[10–12]. Intraperitoneal injection of 200 μg/100 g body-wt. actinomycin *D* 4 h prior to administration of orotic-6-^{14}C acid hydrate blocked completely the synthesis of *m*RNA and *r*RNA (Fig. 1, II). In a separate experiment, we noted that 9 of 10 mice receiving this dose of actinomycin *D* died within 30 h. Philips *et al.*[13] have reported that the LD_{50} of actinomycin *D* for the mouse is approximately 76 μg/100 g body-wt. RNA synthesis was also blocked completely by administering actinomycin *D* at a dose-level of 100 μg/100 g body-wt. (Fig. 1, III). Doses of 50, 25 and 5 μg of actinomycin *D*/100 g body wt. produced approximately 85 per cent (Fig. 1, IV), 70–75 per cent (Fig. 1, V) and 60 per cent (Fig. 1, VI) inhibition of RNA synthesis, respectively. Percentage inhibition was computed from the total c.p.m. recovered, the control values being taken as 100 per cent, Similar results were obtained when actinomycin *D* was injected only 30 min prior to injection of orotic-6-^{14}C acid hydrate.

The decay time of radioactively labelled *m*RNA was determined in the experiment recorded in Fig. 2. Mice received an intraperitoneal injection of labelled orotic acid followed after 4 h by an intraperitoneal injection of actinomycin *D*. Animals were killed at varying times thereafter and radioactivity of RNA components determined. Since synthesis of RNA is inhibited completely by the dose of actinomycin *D* used, one can with this procedure determine the rate of decay of existing RNA molecules which had been labelled by injection of orotic acid. Decay of *m*RNA became apparent 6 h after injection of actinomycin *D* and continued until the experiment was terminated at 24 h (Fig. 2). Total c.p.m. of *m*RNA were calculated at various times after administration of actinomycin *D* and expressed as percentage of the zero time or control value (broken line curve in Fig. 3).

Capacities of liver ribosomes to incorporate DL-leucine-1-^{14}C at varying times after injection of actinomycin *D* were determined and results plotted in Fig. 3 (unbroken line). The inhibitory effects of actinomycin *D* on DL-leucine-1-^{14}C incorporation paralleled the rate of decay of labelled *m*RNA subsequent to administration of the same dose of actinomycin *D* (Fig. 3). It should be noted that the addition of actinomycin *D* (1–2 μg/ml.) *in vitro* to the amino-acid-incorporating system had no effect on ability of ribosomes to incorporate DL-leucine-1-^{14}C. In other experiments utilizing zone centrifugation analysis of purified ribosomes, we observed that 40–60 per cent of liver polysomes were de-aggregated to 73*S* ribosomes 8 h after injection of 100 μg of actinomycin *D*/100 g of body-wt. These results have been noted previously[5] and are in agreement with the pulse-labelled and incorporation investigations described here.

An average half-life for mouse liver *m*RNA of about 8–12 h was obtained from our investigations reported here (Fig. 3). This value is considerably greater than that of 2 h which we observed under steady-state conditions[12]. This apparent decrease in decay of polysomal *m*RNA after inhibition of RNA synthesis by actinomycin may be attributed to several factors. We have observed in an experiment similar to that described in Fig. 2 that

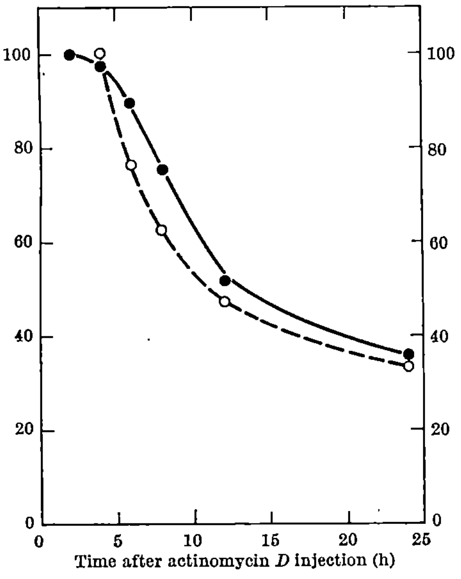

Fig. 3. Effect of injection of 100 μg of actinomycin *D* per 100 g of body-weight on decay of labelled liver *m*RNA and capacity of liver ribosomes to incorporate DL-leucine-1-^{14}C. Broken line represents the decay curve of labelled *m*RNA and was plotted from data presented in Fig. 2. Incorporation investigations were conducted as follows. Mice were injected intraperitoneally with actinomycin *D* and killed at the indicated times. The complete cell-free amino-acid incorporating system contained purified ribosomes (derived from 0·6 g of liver and containing 500 μg of RNA) suspended in 0·4 ml. Hoaglands's salt buffer (ref. 8), 3·2 μmoles $MgCl_2$, 30 μmoles *tris*-HCl (*p*H 7·6), 2·0 μmoles β-mercaptoethanol, 10 μmoles phosphoenolpyruvic acid, 10 μg pyruvate kinase, 1 μmole ATP, 0·4 μmoles GTP, 0·1 μmoles DL-leucine-1-^{14}C and 0·1 ml. of dialysed post-microsomal supernatant derived from a 25 per cent liver homogenate in Hoaglands's medium A (0·005 M *tris*, 0·025 M KCl, 0·005 M $MgCl_2$ and 0·25 M sucrose) after removal of the microsomal fraction (ref. 14) and dialysis at 2° C for 25 h against a 50-fold volume of Hoaglands's salt buffer containing 0·005 M β-mercaptoethanol. Final volume was brought to 1 ml. with Hoaglands's salt buffer. After incubation at 37° C for 30 min, incorporation was stopped by addition of 1 ml. of 1 M NaOH. The mixture was kept at room temperature for 1 h and the protein then precipitated with $HClO_4$, filtered, washed, solubilized with hyamine and radioactivity measured in a Packard 'Tri-Carb' scintillation counter (ref. 5). Controls utilized in each experiment were kept at 0° C and 1 M NaOH added at the beginning of incubation. A mouse killed immediately after injection of actinomycin *D* served as the zero time control. Degree of incorporation of DL-leucine-1-^{14}C (c.p.m. per mg RNA) is expressed as percentage of the zero time control value (3,012 c.p.m. per mg RNA) in the unbroken line

Left: ●, incorporation ability expressed as percentage of the control; right, ○, total c.p.m. of *m*RNA expressed as percentage of the control a labelled nuclear RNA fraction with sedimentation pattern similar to that of polysomal *m*RNA can be found in nuclei as long as 4 h after injection of actinomycin *D*. Gradual release of this labelled nuclear RNA fraction, presumably *m*RNA, into the cytoplasm would obscure the experimental determination of the true decay time of ribosomal *m*RNA. An apparent increase in decay time of labelled *m*RNA would be observed and the true metabolic turnover of polysomal *m*RNA could be determined only after the labelled nuclear *m*RNA fraction had been exhausted. However, we cannot dismiss the possibility that *m*RNA synthesis may be related to *m*RNA breakdown via regulatory mechanism of as yet unknown nature. Inhibition of *m*RNA synthesis by actinomycin *D* would then be expected to influence the rate of *m*RNA breakdown, perhaps, as noted in this article, in the direction of inhibition.

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HEAT OF RESPIRATION AS A POSSIBLE CAUSE OF ERROR IN THE ESTIMATION BY PSYCHROMETRIC METHODS OF WATER POTENTIAL IN PLANT TISSUE

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TWO types of miniature wet and dry junction thermocouple psychrometer have been used for the determination of water potentials in plant tissues[1–5]. Spanner[5] devised a psychrometer in which one junction is wetted by the Peltier cooling effect. The quantity of water involved is very small, and the wet junction so formed lasts only for a short time, being renewed before each reading. Between humidity readings, when the 'wet' junction is dry, the instrument may be used to compare the dry bulb temperature within the chamber with that of the bath. In the second type of psychrometer[6] the wet junction is permanent, being formed by dipping a silver cylinder, which is soldered to the free junction, into water before inserting the couple into the chamber. It is not possible to observe dry-bulb temperatures within the chamber owing to the permanent nature of the wet junction.

This article is a preliminary report of a comparison of results from the two types of psychrometer when used to determine water potentials of leaves of plants.

The couple design referred to here as due to Spanner differs slightly from the original in that chromel-*p* and constantan wires have been substituted for bismuth and bismuth–tin[7]. In other respects the techniques used were essentially as described by Spanner[5], and Richards and Ogata[6], the outputs from both types of couple being measured with a reflecting galvanometer. The chambers were immersed in a controlled temperature water bath and the design of the couples ensured the reference junctions were held at bath temperature soon after immersion.

Early experiments using *Pelargonium zonale* indicated that the Spanner couple was consistently measuring larger water potentials than the Richards couple. A closer examination of the results showed that, between humidity readings, and while the 'wet' junction of the Spanner couple was dry, there was still an appreciable output. This was in the reverse direction to the output when recording humidities with the wet junction present, indicating that the 'wet' junction was warmer than the reference junction. During calibration when the chamber contained filter paper wetted with sodium chloride solution, there was no output from the dry couple, suggesting that the chamber was warmer only when it contained plant material. Further information on this point was obtained using dry Richards couples (that is, silver ring without water). Fig. 1 shows that these also measured temperature rises within the chamber when living tissue was present. The temperature rise with two layers of *Pelargonium* leaf (curve *a*) is clearly greater and maintained for a shorter time than the rise using a single layer (curve *b*). Conversion of these temperature differences to water potentials gives values of approximately 7 and 1·8 bars respectively, though the signs of the thermocouple outputs are, of course, the reverse of those normally obtained when a wet junction is present. It seemed likely that this rise was due to the liberation of heat accompanying the respiration of the tissue, and that the decline to bath temperature occurred when the oxygen-level within the chamber had fallen to some critical level and respiration at normal rates could no longer be maintained.

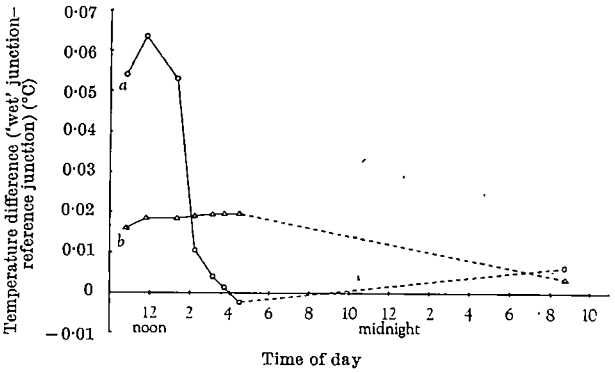

Fig. 1. Effect of two layers (curve *a*), and one layer (curve *b*), of *Pelargonium* leaf tissue on temperature within equilibration chambers, referred to bath temperature. Set up 10 a.m. with dry Richards couple

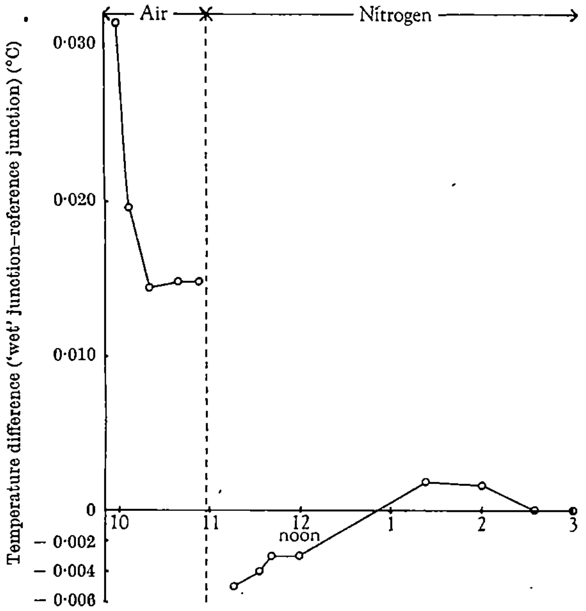

Fig. 2. Effect of replacing air by nitrogen on temperature within chamber referred to bath temperature. Set up 9.50 a.m. with dry Richards couple and one layer of *Pelargonium* leaf

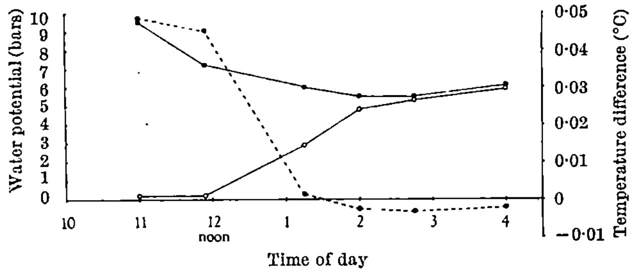

Fig. 3. Water potential (●——●) and chamber temperature referred to bath temperature (●- - -●) using a Spanner couple and water potential (○——○) using a Richards couple

Fig. 2 shows how the rise in temperature within the chamber could, in fact, be abolished by substituting nitrogen for air ; the leaf apparently becoming slightly colder than the reference junction for some time before thermal equilibrium was finally reached. This latter behaviour has frequently been noted although not always. The effect of nitrogen in abolishing the temperature rise suggests that the rise is largely dependent on continued aerobic respiration. Fig. 3 compares water potentials estimated by a Richards and a Spanner couple. The couples were in separate chambers and each chamber contained a double layer of the same *Pelargonium* leaf. Fig. 2 also shows the temperature of one chamber as measured by the Spanner couple when dry. These latter measurements indicate a very considerable reduction in respiration between twelve noon and 1.30 p.m. ; it can be seen that at the same time the reading of the Richards couple began to rise markedly and by 2.45 p.m. approximated closely to the reading of the Spanner couple. Fig. 2 also shows water potential as rising finally. There is evidence, to be described in detail elsewhere, that this is associated with the death of the tissue and liberation of the cell contents. The difference between the observed water potentials before 2.45 p.m. is suggested as being largely due to the fact that the Richards couple only permits measurement of wet-bulb depression against bath temperature (the temperature of the reference junction), whereas the Spanner couple permits a measure of actual chamber dry-bulb temperature immediately before the cooling current is passed and the wet-bulb depression noted. Reference to Fig. 1 curve *b* shows that for one layer of *Pelargonium* leaf the rise in temperature within the chamber could be maintained for more than 4 h and in fact one layer has usually been able to maintain such an elevation for 8 h or longer. Ehlig[4], working with the Richards couple, reports that equilibrium and a steady output are obtained in 2·5–6 h for cotton, pepper and trefoil. This has been found to apply to *Pelargonium*. Hence it is quite possible for a Richards couple to reach equilibrium in a chamber the dry-bulb temperature of which is higher than bath temperature. This may lead to an underestimate of the water potential of the tissue, here presumably by about 1·8 bars.

The Spanner couple, on the other hand, would appear, to a first approximation at least, not to be affected by moderate rises in chamber temperature. This is provided that the reference point for its output as a psychrometer is taken as its output when the 'wet' junction is dry, rather than the mechanical zero of the indicating galvanometer. An example may clarify this; suppose bath temperature to be 25·00° C, chamber temperature to be 25·01° C and wet-bulb temperature to be 24·98° C, then the correct depression will be 25·01–24·98 = 0·03° C, and the Spanner couple will approximate to this provided it has been used to estimate chamber temperature as well as wet-bulb depression. The chamber temperature cannot be measured by the Richards couple, owing to the permanent nature of the wet junction, hence the observed depression will be 25·00–24·98 = 0·02° C, resulting in a 33 per cent error. On this basis it should be possible, given a sufficient quantity of turgid tissue, to so heat the chamber that the wet junction of the Richards couple becomes warmer than the dry junction, reversing the output of the couple compared with the normal direction found during calibration. As Table 1 shows, this has indeed been observed using two layers of *Pelargonium* leaf. The final change in sign of output and rise to a high level would be explicable in terms of a marked decline in respiration.

Table 1

Time	10.45	11.12	12.00	1.18
μV	−0·46	−0·52	−0·33	+3·87

(Set up 9.45 a.m. with dry Richards couple)

Table 2

Plant	Cotton	Pepper	*Pelargonium*
Respiration	10·6, 11·8	10·4, 11·6	31·5, 32·8

(Respiration as μLO_2/15 min/9 cm^2 leaf)

The results presented here are perhaps extreme. Table 2 shows that the respiration rate of the *Pelargonium* was three times as high as that of cotton and pepper. It may be that errors due to temperature rise in the equilibration chamber are less important for many species. This is being investigated together with the possibility of preventing temperature rise by providing a suitable heat sink for the leaf. A more critical evaluation of the performance of the Spanner couple, and in particular the assumption that when dry it approximates leaf temperature, is in progress. A full account of this work will be published shortly.

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TRIGONAL OR TETRAGONAL DISTORTIONS AND THE MAGNETIC PROPERTIES OF TRANSITION METAL IONS WITH TRIPLY ORBITALLY DEGENERATE GROUND TERMS

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THE magnetic properties of transition metal complexes have long been of interest in determining stereochemistry and valency in transition metal ions[1]. Recently, measurements have been used to differentiate between octahedral and tetrahedral stereochemistry in complexes, distinction being based on the absolute value of the magnetic measurements on complexes with these stereochemistries[2]. The temperature variation of the magnetic properties of octahedral and tetrahedral complexes of the transition elements has been interpreted in terms of the splitting of the metal d orbitals by the combined effects of the ligand field and spin-orbit coupling[1,3,4]. In most previous treatments the ligand field has been assumed to be of cubic symmetry. However, the importance of the deviation of the ligand field from cubic symmetry has been recognized in certain cases. Most frequently the low symmetry has been dealt with for systems with A or E ground terms[5-7] because these terms respond to departures from cubic symmetry in a fairly simple manner. Systems with T ground terms, however, present a much more difficult problem, and with the exception of the ${}^2T_{2g}$ term[8,9] they have been the subject of detailed consideration in only few instances[5,8-10].

We have reported[9,11] calculations for the effect of a trigonal or tetragonal distortion on the configurations t_{2g}^1, t_{2g}^5 and $e^4 \cdot t_2^5$, which give rise to a ${}^2T_{2(g)}$ ground term*. These calculations have now been extended to cover configurations which give rise to ${}^3T_{1(g)}$, ${}^4T_{1(g)}$ and ${}^5T_{2(g)}$ ground terms, this covering all the T ground terms possible for the transition elements.

Our results refer only to the average magnetic moments as determined on powdered samples, and as pointed out previously[8] they do not give as accurate values of the parameters which determine the distribution of the energy levels in a T ground state, as do more sophisticated techniques such as electron spin resonance or magnetic anisotropy. However, they do enable a great deal of information to be obtained from the more readily available powder data.

In considering magnetic properties of complexes two different classes of T ground terms must be recognized. They are, on one hand, the $T_{2(g)}$ terms arising from the configuration t_{2g}^1, $e^2 \cdot t_2^2$, t_{2g}^5, $t_{2g}^4 \cdot e_g^2$ and $e^4 \cdot t_2^5$, and on the other hand, the $T_{1(g)}$ terms from the configurations t_{2g}^2, $e^2 \cdot t_2^1$, t_{2g}^4, $t_{2g}^5 \cdot e_g^2$ and $e^4 \cdot t_2^4$. It is convenient to discuss the two classes separately.

T_2 terms. The effect of asymmetric ligand fields on the ${}^2T_{2g}$ ground terms has been considered previously[8,9,11]. A similar treatment using a tetragonal or trigonal distortion has been carried out here for the ${}^5T_{2(g)}$ terms. The asymmetric ligand field component (l.h.s.) and spin-orbit coupling (r.h.s.) split the ${}^5T_{2(g)}$ term as shown in Fig. 1. For an intermediate perturbation by both spin-orbit coupling and asymmetric ligand field components the splitting is given in the centre of the figure. It is this last condition which is the major concern of this discussion.

We define Δ as the splitting of the orbital degeneracy of the ${}^5T_{2(g)}$ term by the asymmetric ligand field component, spin-orbit coupling being neglected, and it is taken to be positive when the orbital singlet is lowest. Fig. 2 shows the behaviour of the magnetic moment for the ${}^5T_{(2)g}$ term as a function of the parameter kT/λ. Curves are given for various values of ν, the ratio of the distortion to the spin-orbit coupling constant: that is, $\nu = \Delta/\lambda$. (Here we use λ to denote the value of the spin-orbit coupling constant *in the complex* and λ_0 for the free-ion value.)

Previous work has indicated that it is also necessary to consider a reduction in the orbital part of the magnetic moment operator. This reduction has been associated with electron delocalization on to the ligand atoms[8,9,12]. It is normally allowed for by introducing a constant, k, into the matrix elements of the orbital angular momentum operator. The magnetic moment and spin-orbit coupling operators for the Z direction then become: $(kL_z + 2S_z)$ and $kL_z S_z$ respectively.

We have allowed k to vary from 1 to 0·5. The calculated curves for two values of k and negative values of the spin-orbit coupling constant are shown in Fig. 2. We have only provided results for negative values of λ because these apply to the configuration $t_{2g}^4 \cdot e_g^2$, as our experimental results for the ${}^5T_{2(g)}$ case are confined to compounds of octahedral bivalent iron.

As expected, and as observed in the corresponding calculations for the ${}^2T_{2(g)}$ ground terms, a decrease in the value of k reduces the magnetic moment, bringing it closer to the 'spin-only' value of 4·90 Bohr magnetons. For negative values of Δ (ν positive) the overall variation of the moment with temperature is reduced as the magnitude of the distortion is increased. On the other hand, for positive values of Δ, a very significant change in the

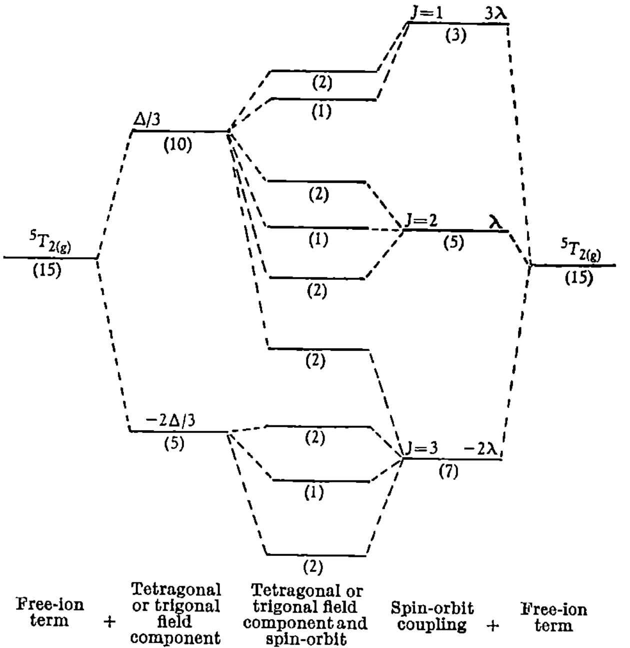

Fig. 1. Splitting pattern for the ${}^5T_{2(g)}$ term under the action of a tetragonal of trigonal ligand field component and spin orbit column

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* The brackets are included in the term symbols, for example, ${}^2T_{2(g)}$, to indicate that the results apply to both octahedral and tetrahedral stereochemistries.

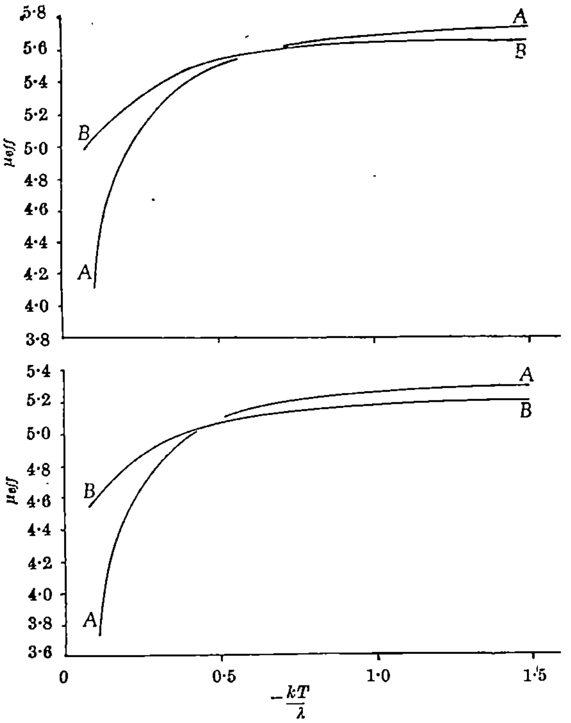


Fig. 2. Above, k, 1·0; below, k, 0·5. A, ν, −3·0; B, ν, +3·0

variation of the magnetic properties with temperature occurs, the dependence of the moment on temperature being much more marked.

In general, ligand field components of low symmetry have comparatively little effect on the magnetic properties of the ${}^5T_{2(g)}$ ground term unless they raise its orbital degeneracy by an amount greater than the spin-orbit coupling constant. It is, therefore, important to consider the effects of asymmetric ligand fields for the compounds of 1st row transition elements rather than for 2nd and 3rd row elements. For values of ν greater than about 10 our treatment is not satisfactory, as the asymmetric component of the ligand field potential is then of the same order as the primary cubic ligand field. Under such conditions the perturbation methods which we have used are no longer applicable.

$T_{1(g)}$ *terms.* Any configuration of a transition element ion that gives rise to an F ground term, in addition, leads to a P term of the same spin multiplicity. This P term is separated from the ground F term by an energy which depends on the inter-electronic repulsion parameters of the system. Under the influence of a cubic ligand field, both these terms give rise to $T_{1(g)}$ terms which interact. The degree of interaction depends on the relative magnitudes of the interelectronic repulsion and the ligand field parameters. It is convenient to express the composition of the ground $T_{1(g)}$ term as a linear combination of the $T_{1(g)}$ terms in the free-ion limit:

$$U_{T1} = (1 + c^2)^{-\frac{1}{2}}[U_{T1(F)} + cU_{T1(P)}]$$

where:

$$c = \frac{6Dq + E}{4Dq}$$

E is the lower root of the equation:

$$E^2 + (6Dq - Z)E - 6Z - 16D^2q^2 = 0$$

Z is the ratio of the $F - P$ term separation in the complex to the cubic ligand field parameter, namely:

$$Z = [E_{(P)} - E_{(F)}]/Dq$$

The admixture coefficient c, which may often be determined with the aid of the optical spectrum of the complex, lies between 0 and −0·5. For the weak field limit ($Z \rightarrow \infty$), $c = 0$, while for a medium or strong field ($Z \rightarrow 0$), $c = -0{\cdot}5$. For more general application it is convenient to employ a constant A, which is a measure of the strength of the ligand field relative to the interelectronic repulsion energy, given by $A = (1{\cdot}5 - c^2)/(1 + c^2)$. The classification of ligand field strengths has been discussed elsewhere[3].

The variation of the magnetic moments of the ${}^3T_{1(g)}$ and ${}^4T_{1(g)}$ terms is given in Fig. 3 as a function of the parameters k, ν and kT/λ. These results are applicable to systems for which λ is negative, for example, $e^4{\cdot}t_2^4$ (tetrahedral Ni^{2+}) and $t_{2g}^5.e_g^2$ (octahedral Co^{2+}). It is obvious from these curves that changes in the parameters make a significant difference to the variation of the magnetic properties of the ions with temperature. As with the ${}^5T_{2(g)}$ term, the variation is much more marked for positive values of Δ.

In considering the data it is important to take into account the relationship between the orbital splitting parameter, Δ, and the magnitude of the low symmetry ligand field component which is responsible for it. A ligand field potential of cubic symmetry with a tetragonal or trigonal component superimposed may be put in the form:

$$V = D(x^4 + y^4 + z^4 - 3r^4/5) + C(z^2 - r^2/3)$$

Conventionally, the matrix elements arising from the first, cubic, term in this potential are expressed as multiples of a number, q, and the splittings of the free-ion terms are then integral multiples of the parameter Dq. Similarly, if we define a parameter Cp for the second, low symmetry, term in the potential, then the splittings of T terms by an asymmetric ligand field component are simple multiples of Cp. In the parameter Cp, p is equivalent to q of cubic symmetry. In order to express Δ as a function of Cp we must consider tetragonal and trigonal symmetry separately. The splitting of the T terms in units of Cp are given in Table 1, as a function of both the magnitude of the cubic ligand field and symmetry. For a given low symmetry ligand field component the splitting of the various terms may be greatly different. In the case of $T_{1(g)}$ ground terms, μ_{eff} is very dependent on the degree of mixing with the higher $T_{1g}(P)$ term.

By using curve fitting procedures we have applied the above theory to magnetic measurements we have performed on a number of complexes of octahedral divalent

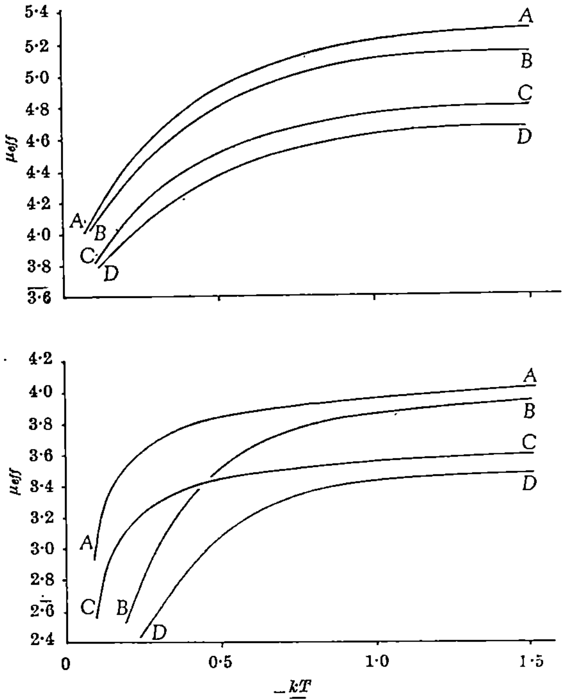


Fig. 3. Above, ${}^4T_{1(g)}$; below, ${}^3T_{1(g)}$
k, 1·0. A, ν, −3·0; B, ν, +3·0; k, 0·7. C, ν, −3·0; D, ν, +3·0

Table 1. The relationship between the potential of a tetragonal or trigonal ligand field component and the magnitude of the orbital splitting of T ground terms. See the text for the definition of Cp. 'Weak' and 'stronger' refer to the cubic ligand field

Term	Tetragonal symmetry	Trigonal symmetry
$E_{(g)}(D)\ d^1$	$-8Cp$	0
$T_{2(g)}(D)d^1$	$6Cp$	$-6Cp$
$T_{1(g)}(F)$: d^2		
Weak field ($A = 1{\cdot}5$)	$-2{\cdot}4Cp$	$0{\cdot}6Cp$
Medium or strong field ($A = 1$)	$-6Cp$	$6Cp$

iron, octahedral bivalent cobalt and tetrahedral bivalent nickel. Figs. 4–6 show the agreement which may be obtained in this way and Table 2 contains results for a representative list of the complexes we have investigated. It is important to appreciate that the value of Δ obtained by this procedure may, in certain instances, be in error by as much as 50 per cent. This uncertainty arises from ambiguity in the curve-fitting process.

In some instances it is possible to compare Δ obtained from our data with its value assessed by other techniques. In the case of Fe(II) comparison may only be made with the low-temperature measurements on the fluorsilicate complex. From data gathered at liquid helium temperatures, Δ has been reported to be 1,200 cm^{-1} (ref. 13). For Co(II) complexes there may be some ambiguity in the interpretation of the experimental results. The data often may be interpreted in terms of either a negative or a positive value for the distortion parameter, although one of the alternatives usually gives a distinctly better fit. The ambiguity arises from the small curvature of the susceptibility–temperature plots.

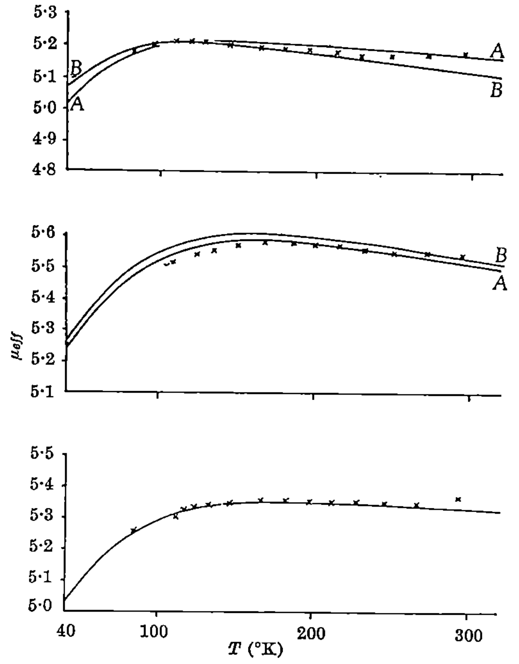
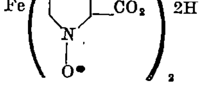


Fig. 4. Top, $Fe(H_2O)_6\ SiF_6$; A, ν, $-2{\cdot}0$; B, ν, $+3{\cdot}0$. Middle,  $2H_2O$; A, ν, $-2{\cdot}0$; B, ν, $+2{\cdot}0$. Bottom, Fe(oxalate), $2H_2O$; ν, $-2{\cdot}8$

×, Experimental results

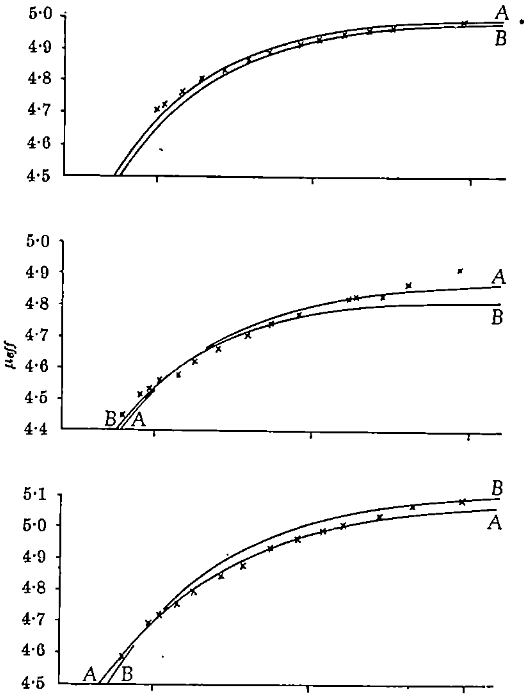


Fig. 5. Top, $Co(NH_4)_2\,(SO_4)_2\ 6H_2O$; A, ν, $-2{\cdot}4$; B, ν, $+4{\cdot}5$; middle, $CoCl_26H_2O$; A, ν, $-2{\cdot}5$; B, ν, $+6{\cdot}0$; bottom, $Co\,py_2Cl_22H_2O$; A, ν, $-2{\cdot}5$; B, ν, $+4{\cdot}5$
×, Experimental results

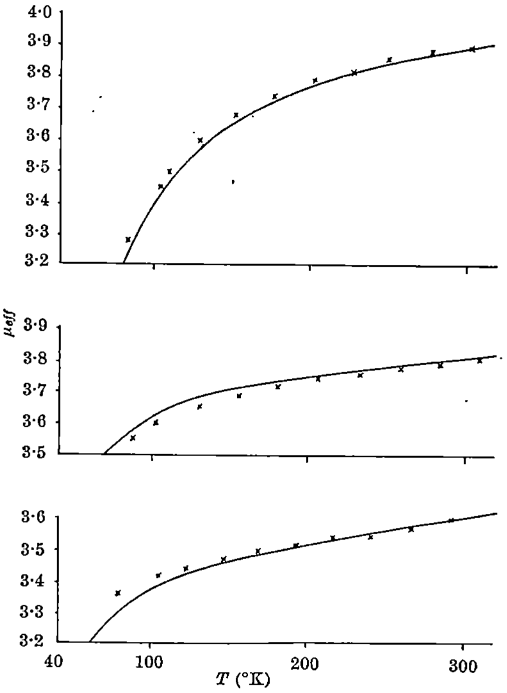
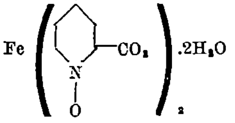


Fig. 6. Top, $[Ph_3AsCH_3]_2\ NiCl_4$; ν, $-2{\cdot}4$; middle, $[Ph_3AsCH_3]_2\ NiBr_4$; ν, $-3{\cdot}2$; bottom, $[(C_2H_5)_4N][(Ph_3P)NiBr_3]$, ν, $-2{\cdot}8$
×, Experimental results

The electron-spin resonance[8,14] and low-temperature magnetic susceptibility investigations[15,16] on the complex $Co(NH_4)_2(SO_4)_26H_2O$ give values of Δ of about −700 to −1,300 and approximately −1,100 cm^{-1} respectively, although it was found that in the latter case[15] Δ had to be varied somewhat with temperature in order to fit the results. These estimates are in general agreement with the value obtained in this work, that is, −775 cm^{-1}. Low-temperature magnetic susceptibility measurements on the complex $[Co(H_2O)_4Cl_2]2H_2O$ have not been interpreted unambiguously, values of Δ/λ of −1·0 or −5·0 being deduced[17], that is, Δ is approximately +200 or +900 cm^{-1}. The value of Δ obtained from the present work, 375 cm^{-1}, is in reasonable agreement with the lower of these estimates. No independent estimation of Δ in the Ni(II) tetrahedral complexes is available.

For tetrahedral Ni^{2+} the ground term is $^3T_1$. Table 2 contains the data for some of the complexes, the tetrahedral stereochemistry of which has been assigned previously on the basis of spectral and room-temperature magnetic susceptibility data[18,19]. It has been suggested that the reduction in the magnetic moment of the complexes on the replacement of halogens by phosphine

Table 2

Ground term	Complex	k	$\nu = \frac{\Delta}{\lambda}$	$\frac{\lambda}{\lambda_0}$	Δ	Δ cm^{-1}	μ_{eff} at 300° K B.M.
$^5T_{2g}$	$FeSiF_6.6H_2O$	0·5	−2·0	0·68	–	+135	5·18
		0·5	+3·0	0·78	–	−225	
	Fe(oxalate)$.2H_2O$	0·6	−2·8	0·86	–	+240	5·36
		0·9	−2·0	1·0	–	+200	5·54
		0·9	+2·0	1·0	–	−200	
$^4T_{1g}$	$CoSO_4(NH_4)_2SO_4 6H_2O$	0·9	−2·4	0·8	1·4	+330	4·99
		1·0	+4·5	1·0	1·4	−775	
	$CoCl_2 6H_2O$	0·8	−2·5	0·87	1·4	+375	4·92
		0·9	+6·0	1·0	1·4	−1,030	
	$Copy_2Cl_2 2H_2O$	1·0	−2·5	1·0	1·3	+430	5·09
		1·0	+4·5	1·0	1·5	−774	
$^3T_1$	$(Ph_3CH_3As)_2NiCl_4$	0·95	−2·4	0·89	1·5	+670	3·89
	$(Ph_3CH_3As)_2NiBr_4$	0·95	−3·2	0·90	1·5	+855	3·81
	$[(C_2H_5)_4N][(Ph_3P)NiBr_3]$	0·80	−2·8	0·75	1·4	+650	3·60
	$Ni(Ph_3P)_2Cl_2$	0·50	−5·0	0·40	1·5	+650	3·23
	$Ni(Ph_3P)_2Br_2$	0·60	−5·1	0·55	1·5	+900	3·27

ligands arises from distortions from regular tetrahedral symmetry. From Table 2 it appears that the value of Δ is more or less constant, and the reduction in the magnetic moment on the replacement is to be associated with reduction in k and λ. These observations emphasize that it is important when discussing magnetic results to consider the effects of distortion, reduction in the spin-orbit coupling and orbital reduction parameters together.

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A SENSITIVE DOUBLE ANTIBODY IMMUNOASSAY FOR HUMAN GROWTH HORMONE IN PLASMA

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DESPITE the successful use of electrophoretic and electrochromatographic methods[1-3] of assaying human growth hormone, many workers have had difficulty with these relatively elaborate procedures. This has prompted us to re-investigate the double antibody method. Non-specific interference from substances in plasma have complicated previous use of the double antibody method, and extraction procedures proved to be rather laborious[4,5]. A double antibody immunoassay for human growth hormone, patterned after a similar immunoassay for insulin[6], using guinea pig anti-human growth hormone serum and rabbit anti-guinea pig γ-globulin serum has proved to be successful. While this method is relatively simple and easy to use, it provides a high degree of sensitivity along with good reproducibility of results.

All dilutions were made with 5 per cent bovine serum albumin (BSA Armour, 30 per cent solution) in a barbital buffer, ionic strength 0·1, *p*H 8·6.

Anti-HGH serum was obtained ten days after the last of three weekly subcutaneous injections of 1 mg human growth hormone (Raben) emulsified in complete Freund's adjuvant.

Rabbits were immunized with 1 mg of guinea pig γ-globulin in complete Freund's adjuvant injected into each foot pad. Subsequent monthly injections were given subcutaneously. Animals were bled by cardiac puncture 10–14 days after the third or subsequent monthly boosters.

Human growth hormone was labelled with iodine-131 by the method of Greenwood, Hunter and Glover[7] as modified by Glick *et al.*[8]. Specific activities of 300–350 mc./mg were obtained.

Pooled normal guinea pig serum was diluted 1 : 200 with 5 per cent BSA barbital buffer.

Duplicate standards of human growth hormone are freshly prepared for each assay with the following concentrations: 0, 0·25, 0·5, 1, 2, 4 and 8 mμg/ml. 0·5 ml. of each standard solution is used in duplicate. Disposable glass tubes, 10 mm × 75 mm, have been found to be most satisfactory for the incubation tubes. 50 μl. of each plasma sample is added to 0·45 ml. 5 per cent BSA barbital buffer for an initial dilution of 1 : 10. 10–100 μl. of plasma in an initial total volume of 0·5 ml. can be used as well. 0·1 ml. of diluted guinea pig antihuman growth hormone serum is then added to each tube. The appropriate dilution of antiserum is that which binds approximately 35–55 per cent iodine-131-labelled human growth hormone added when no competing unlabelled human growth hormone is present in the system. After gentle shaking of the tubes, 0·1 ml. (0·1–0·2 mμg) of iodine-131-human growth hormone is added, the tubes are again shaken and incubated at 4° C for 72 h. At this time, 0·1 ml. of appropriately diluted rabbit anti-guinea pig γ-globulin serum, and finally 0·1 ml. of 1 : 200 normal guinea pig serum are added, the tubes shaken and incubated another 18–24 h at 4° C. The appropriate dilution of rabbit anti-guinea pig γ-globulin serum is one which will completely precipitate all guinea pig γ-globulin in the system. The tubes are then counted in a well autogamma spectrometer. They are then centrifuged at 2,500 r.p.m. for 20 min and the supernatants are aspirated and discarded. The precipitates are washed with cold (4° C) distilled water, recentrifuged at 2,500 r.p.m. for 20 min and the supernatants again aspirated and discarded. The tubes, containing the precipitates, are recounted and the percentage iodine-131-labelled human growth hormone precipitated is calculated.

Table 1. MEAN DIFFERENCE IN PERCENTAGE PRECIPITATION OF HUMAN GROWTH HORMONE BETWEEN DUPLICATE STANDARDS

Human growth hormone standard concentration (mμg/ml.)	Mean difference in % human growth hormone-iodine-131-labelled precipitation between duplicates ± *S.D.*
0	0·8 ± 0·2 (9)*
0·25	1·8 ± 1·2 (9)
0·5	1·7 ± 1·2 (9)
1	1·7 ± 1·5 (9)
2	1·0 ± 0·6 (9)
4	0·9 ± 0·8 (9)
8	0·7 ± 0·3 (9)
Average	1·0 ± 0·7

*No. of duplicate values in brackets.

A standard curve is constructed for each assay (Fig. 1) and the concentration of human growth hormone in each plasma sample is then determined from the percentage

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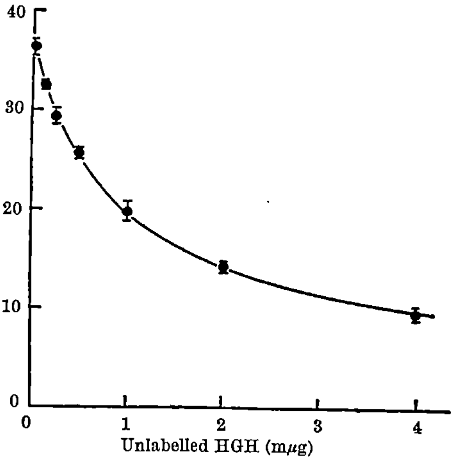

Fig. 1. Human growth hormone-^{131}I (0·1 mμg), guinea pig anti-human growth hormone serum (1 : 120,000), rabbit anti-guinea pig γ-globulin serum (1 : 6), First incubation period, 72 h; second incubation period, 24 h. Each point is the average of 5 determinations. I = ± *S.D.*

iodine-131-labelled human growth hormone precipitated by comparison with the standard curve.

In 9 assays, the mean difference (± *S.D.*) in the percentage precipitation of iodine-131-labelled human growth hormone in duplicate standards was found to be 1·0 ± 0·7, with a predicted variability at each standard level (Table 1).

When the percentage precipitation of iodine-131-labelled human growth hormone is converted into the concentration of human growth hormone in plasma samples by using the standard curve run in each assay, duplicate values in the same assay agree within 20 per cent on the average throughout the whole range of values

Table 2. Plasma Human Growth Hormone Concentrations during Insulin Tolerance Tests

Subject	Plasma human growth hormone concentration (mμg/ml.)					Blood glucose (mg%)				
	0 min.	20 min.	40 min.	60 min.	90 min.	0 min.	20 min.	40 min.	60 min.	90 min.
P. B.	1·2	1·5	16·5	44·0	60·0	105	28	52	50	66
	1·0	1·0	19·5	48·0	62·0					
G. B.	15·5	35·0	56·0	8·0	24·0	86	36	45	73	72
	8·0	32·5	64·0	10·0	20·0					
L. C.	2·5	3·4	3·0	8·0	3·0	113	66	66	94	102
	6·0	3·8	6·0	9·0	3·5					

Plasma human growth hormone concentrations obtained during three insulin tolerance tests are seen in Table 2.

Excellent agreement has been found in low, medium and high ranges between the values of plasma human growth hormone obtained by the double antibody method and by the chromatoelectrophoretic method of Glick *et al.*[8] at present in use in our laboratory. The double antibody method moreover offers two distinct advantages: greater sensitivity can be obtained for a given specific activity of iodine-131-labelled human growth hormone and all manipulations in this procedure can be carried out at room temperature.

We thank Dr. William H. Daughaday for his advice and assistance.

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THREE DEGREES OF GUANYLIC ACID — INOSINIC ACID PYROPHOSPHORYLASE DEFICIENCY IN MOUSE FIBROBLASTS

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LOSS of the enzyme guanylic acid–inosinic acid pyrophosphorylase confers resistance to the analogue 8-azaguanine in bacteria and mammalian cells[1]. In the latter this loss might be expected to be step-wise, reflecting gene dosage, and recently sub-lines of mouse fibroblasts (*L* cells) partially deficient in pyrophosphorylase activity and partially resistant to this analogue have been described[2]. Further investigations now suggest that there are three degrees of enzyme deficiency and resistance to 8-azaguanine in these aneuploid cells.

Cell culture methods were the same as those used previously[2] except that the medium containined 10 per cent fœtal calf serum (Microbiological Associates, Inc.), rather than calf serum. Chromosomes were enumerated by standard methods[3,4] after the cells were exposed for 16 h to colchicine (5 × 10^{-6} M). The pyrophosphorylase activity of cell extracts was determined with hypoxanthine-8-^{14}C as before[2], except that the reaction mixture was incubated at 37° C for 1 h, rather than at room temperature for 2 h. A factor relating these two conditions was determined; and to allow comparison with previous investigations, all results are expressed as c.p.m./μg of cell extract protein per 2 h at room temperature. In certain assays, guanine-8-^{14}C (1 μc./m.mole; 7 × 10^{-5} M) (Schwarz Bioresearch Inc.) was used in the reaction mixture instead of hypoxanthine-8-^{14}C.

The specific activities of guanylic acid–inosinic acid pyrophosphorylase in extracts of 38 sub-lines of *L* cells are shown in Fig. 1. The average activity of wild-type cells was 332 ± *S.E.* 11 c.p.m./μg protein in 9 extracts during a 2-year period. Three clonal derivatives (*A*19, *A*23 and *A*24) were in the same range. At the bottom of Fig. 1 are shown the activities of cloned or uncloned lines highly resistant to 8-azaguanine, which were selected with 1·0–2·0 μg of 8-azaguanine/ml. as described previously[2]. The average enzyme activity in extracts of these lines was 1 per cent of wild-type activity.

Lines of *L* cells moderately resistant to 8-azaguanine were obtained in two ways[2]. In suspension culture 3 uncloned populations (*A*70*M*, *A*100*M* and *A*110*M*) were selected with 0·1 μg of 8-azaguanine per ml. In Petri dishes 2 clonal lines (*A*41 and *A*46) were selected with 0·1–0·3 μg of 8-azaguanine/ml.; afterwards one of these lines was recloned once (*A*46–3) and again (*A*46–3–6 and *A*46–3–7). The average activity of these 8 lines was 113 ± *S.E.* 8 c.p.m./μg protein.

The activities of these moderately resistant lines were not statistically separable from those of two revertant populations (*A*55*R* and *A*63*R*) and 3 clonal derivatives thereof[2] (average activity 116 ± *S.E.* 11 c.p.m./μg of protein). Altogether the activities of these 13 lines averaged 35 per cent of wild-type activity.

When on two occasions wild-type cells in suspension culture were exposed to an even lower concentration of 8-azaguanine (0·01 μg/ml.), cell multiplication was not inhibited. However, after growth for approximately

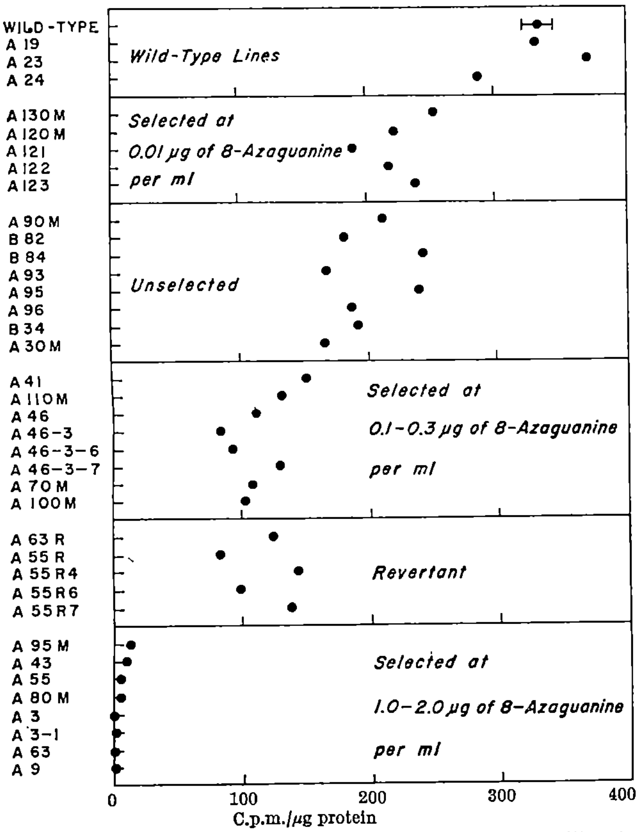

Fig. 1. The guanylic acid–inosinic acid pyrophosphorylase activities of 38 cell lines. As indicated, certain lines were obtained by selection of wild-type cells with 0·01, 0·1–0·3 or 1·0–2·0 µg of 8-azaguanine per ml. All lines were clonal except wild-type, *A55R* and *A63R*, and those lines designated with the suffix *M*. Cell extracts were prepared and assayed as described in the text and previously (ref. 2). The points represent the assay in duplicate of a single extract of a line, or the average activity of several extracts. For wild-type cells nine extracts assayed over a 2-year period averaged 332 ± *S.E.* 11 c.p.m./µg protein

2 months with the analogue, the pyrophosphorylase activity of cell extracts (*A*120*M* and *A*130*M* in Fig. 1) was decreased in comparison with untreated wild-type cells assayed at the same time. It was not possible to select lines of this sort as clones in Petri dishes, presumably because these cells were almost as sensitive to 8-azaguanine as wild-type cells. However, one of these lines was cloned to exclude the possibility that it was a mixture of wild-type and moderately or highly resistant cells; extracts of these clonal derivatives (*A*121, *A*122 and *A*123) were again less active than wild-type extracts. The average activity of these 5 lines was 221 ± *S.E.* 10 c.p.m./µg of protein, or 67 per cent of wild-type activity. Thus these lines were intermediate in regard to resistance and enzyme content between wild-type cells and the 13 moderately resistant lines described above.

Furthermore, after continuous growth for a number of months, untreated wild-type cells were found to contain less enzyme than previously. These cells (*A*30*M*) were discarded, and a new culture begun from a frozen sample of the original common stock which had been maintained previously in more complete medium in another laboratory[5]. The new cells were as active as before, but again after several months their activity had declined (*A*90*M*). In neither case had the generation time appreciably shortened. Several clonal derivatives of both *A*30*M* and *A*90*M* (*A*93, *A*95, *A*96, *B*34, *B*82 and *B*84) also contained less enzyme activity. The average activity of these 8 'unselected' lines was 197 ± *S.E.* 10 c.p.m./µg of protein.

The activities of the 5 lines selected at 0·01 µg of 8-azaguanine/ml., and the 8 'unselected' lines, were not statistically separable, and together averaged 63 per cent of the activity of wild-type cells. This group was statistically distinct (Student's *t* test yields $P < 0{\cdot}001$) both from wild-type cells and from the moderately resistant lines described above.

In mammalian cells it is likely that guanylic acid and inosinic acid are pyrophosphorylated by the same enzyme[1], although Kalle and Gots[6] have presented evidence that this may not always be the case in *Salmonella typhimurium*. We determined the pyrophosphorylase activity of extracts of 12 lines with both guanine-8-^{14}C and hypoxanthine-8-^{14}C. The results, calculated for both radioisotopes as a percentage of the activity of wild-type cells, are shown in Table 1. Although the results with guanine-8-^{14}C are consistently slightly higher than those with hypoxanthine-^{14}C, both radioisotopes gave the same ordering of lines in regard to pyrophosphorylase activity, as if guanylic acid and inosinic acid were substrates for the same enzyme in *L* cells.

Chromosome counts were performed on a number of the cell lines described above. The average of all counts was 53 ± *S.E.* 0·9. More detailed karyotype analyses[3,4] have not been carried out.

It was possible to estimate the frequency of moderately or highly resistant cells in various lines from the day of appearance and generation times of such cells in cultures exposed to 0·1 or 1·0 µg of 8-azaguanine/ml.[2]. The frequency of moderately resistant cells in the wild-type culture was approximately 1·6 and $0{\cdot}4 \times 10^{-3}$ on two occasions. In single experiments the approximate frequency of highly resistant cells was 3×10^{-2} in a moderately resistant culture, 3×10^{-4} in a slightly resistant culture, and 10^{-5} in a wild-type culture[2].

These results indicate that in *L* cells three statistically distinct degrees of loss of guanylic acid–inosinic acid pyrophosphorylase activity can be distinguished, leaving an average of 63, 35 and 1 per cent of wild-type activity. Previous investigations suggested that these cells contained a decreased amount of active enzyme, rather than an unchanged amount of enzyme of altered kinetic properties[2]. The synthesis of this enzyme cannot be 'de-repressed'[2]. Therefore, it might be expected to be present in proportion to gene dosage. While the chromosome number of normal mouse cells is 40, that for several *L* cell lines averaged 53. It may be that in these cells there are usually 3 homologous chromosomes (trisomy) with the pyrophosphorylase locus. The 3 degrees of enzyme deficiency may reflect the inactivation of 1, 2 or 3 such loci, or the loss of 1, 2 or 3 such chromosomes.

The decreasing frequency of highly resistant cells in moderately resistant, slightly resistant and wild-type cultures is in accord with this concept. Moreover, the frequencies in wild-type cultures of highly resistant (10^{-5}) and moderately resistant (10^{-3}) cells suggest that the loss of one pyrophosphorylase locus to produce slight resistance, the frequency of which cannot be determined directly, is an even more common event. There are other examples of changes in drug resistance in *L* cells which are frequent and may involve only one chromosome: the acquisition of high resistance to puromycin[7], reversion in 2 lines highly resistant to 8-azaguanine[2], and the acquisition of partial resistance to 5-bromodeoxyuridine[8]. Similarly frequent changes occurred in an established line of human bone marrow cells[9].

Table 1. PYROPHOSPHORYLASE ACTIVITY DETERMINED WITH HYPOXANTHINE-8-^{14}C AND GUANINE-8-^{14}C

Cell line	Wild-type specific activity (per cent) Hypoxanthine-8-^{14}C	Guanine-8-^{14}C
Wild-type	100 (90–110)	100 (89–108)
*A*24	109	108
*A*90*M*	68	71
*A*120*M*	67	74
*A*41	52	66
*A*110*M*	40	61
*A*55*R*7	38	46
*A*46	33	40
*A*100*M*	31	48
*A*95*M*	4	9
*A*55	1	3
*A*3–1	0	1

The wild-type activity represents the average activity of four extracts.

Rather than mutation, these changes probably reflect the large number of mitotic abnormalities in aneuploid lines[4,10,11], including uneven distribution of chromosomes to daughter cells as well as intrachromosomal rearrangements. Indeed, Szybalski has been unable to demonstrate mutagenesis in an aneuploid line with a number of agents known to be mutagenic in bacteria, and has suggested that this may be because of the high frequency of mitotic abnormalities[12].

A change in one chromosome with a pyrophosphorylase locus seems to have occurred also in the wild-type population during growth under new conditions (the 'unselected' lines in Fig. 1). This is analogous to the shifts in karyotype observed by Hsu[10]. The new genotype was presumably advantageous for cell survival, perhaps due not so much to the loss of a pyrophosphorylase locus as to the loss of an unknown linked locus. If this genotype occurred frequently, as discussed here, it need have produced only a slight shortening of the generation time, or other advantage, to replace the original wild-type cells during growth for several months.

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BIOSYNTHETIC LABELLING OF MAMMALIAN INSULINS *IN VITRO*

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AND

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INCORPORATION of labelled amino-acids into insulin is now being examined for information it may give on the synthesis of a protein of known structure. Attempts have been made to label insulin *in vivo* by Voelker *et al.*[1] but, because of their greater convenience, most other workers have used methods of labelling *in vitro*, using for this purpose ox or calf pancreas[2–4], rat pancreas[5], or giant islet tissue from teleostean fish[6,7]. In mammalian pancreas, however, a very rapid incorporation of labelled amino-acids also takes place into the proteins of acinar tissue, and the presence of so much other labelled protein makes for great difficulties in isolating insulin in a state of radiochemical purity. In the rat, a further difficulty is in the presence of two insulins, differing in structure and properties[8].

It will be shown here that insulin is labelled by a process which is compatible with synthesis, when either fresh ox pancreas or rat pancreas is incubated with tritium labelled amino-acids.

Incubation of pancreas and purification of insulin. A satisfactory medium for incubation of pancreas with amino-acids appeared to be a bicarbonate buffered saline solution[9], to which glucose had been added to give a concentration of 250 mg/100 ml. After incubation, the isolation of insulins, or peptides derived from labelled insulins, proved to be a major problem and, even after very exhaustive extraction procedures, it proved very difficult to get rid of radioactive contaminants. Similar problems have been reported by Humbel[7], in the purification of labelled toadfish insulins. In all the isolation procedures used, insulins were first extracted by acidified ethanol, and a procedure based on that of Best *et al.*[10]. After removal of salts, the crude insulin was first purified by paper chromatography. This by itself proved inadequate in our hands for isolation of either ox or rat insulins in a state of radiochemical purity. With rat insulins further purification was achieved after paper chromatography, and elution from paper, by repeated isoelectric precipitation in the presence of carrier insulin[11]. Some similar considerations also applied to the purification of *A*- and *B*-chains derived from rat insulins. It was found that purification of these from radioactive contaminants was best achieved by paper electrophoresis at *p*H 2 in 5 M formic acid. These difficulties have been discussed at length elsewhere[11].

One possible way in which the high degree of radioactive contamination of insulin labelled *in vitro* may be avoided is by the use of immunological methods to isolate the insulin. After incubation of pancreas with tritiated leucine, radioactive insulin was precipitated using antibody by methods which have already been outlined, the insulin being regenerated with acid, and then purified by paper chromatography[12]. Such insulin appeared to be homogeneous on electrophoresis (Fig. 1).

Incorporation into insulins. In both rat insulins, leucyl residues are present in both *A*- and *B*-chains, though isoleucine is confined to the *A*-chain of each (Smith, L. F., personal communication). Accordingly, if labelling of the insulin proceeds by protein synthesis, both chains should be labelled when pancreas is incubated with leucine. Rat pancreas was accordingly incubated with tritiated leucine or isoleucine, and after addition of carrier ox insulin, *A*- and *B*-chains were made from it by the method of Sanger[13]. After electrophoresis on paper, only *B*-chain which had been derived from pancreases incubated with leucine was labelled[11]. This suggested that labelling is in accord with the structure of the insulins. In addition, the distribution of label among amino-acids obtained by the hydrolysis of labelled rat insulins suggested that neither leucine nor isoleucine was metabolized by rat pancreas in such a way as to give rise to significant amounts of radioactivity in other amino-acids[11].

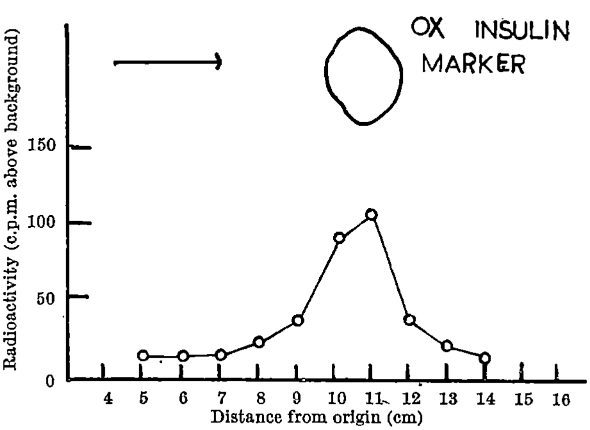

Fig. 1. Distribution of radioactivity on paper after electrophoresis of insulin labelled with tritium and purified by antibody precipitation. Ox insulin was labelled *in vitro*, and after chromatography of an insulin extract precipitated with antibody, an appropriate area of paper was cut out and sewn on to a second strip of paper for electrophoresis at *p*H 2, in formic acid. Radioactivity was determined on strips of paper as shown

Table 1. INCORPORATION OF TRITIATED LEUCINE INTO INSULIN AND OTHER PROTEINS OF OX PANCREAS *in vitro*

	Conc. of glucose in medium 25 mg/100 ml.	250 mg/100 ml.
* Radioactivity in proteins of acid–ethanol extract of pancreas (c.p.m./g wet pancreas × 10^{-4})	2·8 ± 0·67	3·4 ± 0·51
Specific activity of insulin (c.p.m./μg of insulin)		
Exp. 1	0·91	2·10
2	0·62	1·91
3	0·03	0·48
†4	0·14	1·30

* Mean of four results, ± *S.E.M.*

† Insulin purified by both chromatography and electrophoresis.

Pancreas was incubated with tritiated leucine and after extraction with acid ethanol, insulin was precipitated with antibody. Insulin was then regenerated from the precipitate and purified by chromatography. Insulin was assayed by elution of dyestuff. Concentration of radioactivity was different in the two groups of experiments.

Evidence that ox insulin was being labelled has been obtained by differential labelling of the *A*- and *B*-chains. Using a mixture of amino-acids of high specific activity (^{14}C-leucine, 160 mc./m.mol, and tritiated isoleucine, 315 mc./m.mol), it could be shown that the ratio of leucine to isoleucine was obtained for labelled material which was compatible with the known distribution of leucine and isoleucine in the molecule. Moreover, agents such as 2,4-dinitrophenol or puromycin, which are agents known to inhibit protein synthesis, characteristically reduce incorporation into ox insulin *in vitro*[14]. Labelling of both rat and ox insulin appears, therefore, to be by a biosynthetic mechanism.

Effect of glucose concentration on incorporation into insulin. Incorporation of leucine into insulin appears to be greatly enhanced by increasing the glucose concentration from 25 mg/100 ml. to 250 mg/100 ml. (Table 1). By contrast, only a very small effect of varying the glucose concentration on incorporation of leucine into a crude acid alcohol extract of ox pancreas was found, and it was not statistically significant. A similar lack of effect of variations in glucose concentration on incorporation of leucine into toadfish pancreas has also been reported[15]. It is not at present known whether this effect on incorporation is confined to ox insulin or is a widespread phenomenon in the pancreas of other species. If this should be the case, effects of glucose on incorporation into insulin might be of considerable physiological significance, perhaps as a regulatory mechanism for controlling the quantity of insulin stored in pancreas. A direct connexion has been suggested by Haist[16] between the blood glucose-level and the amount of insulin which is extractable from the pancreas of rats.

In summary, it is concluded that segments of mammalian pancreas, incubated *in vitro*, may be used for an investigation of factors which affect incorporation into insulin, though special purification methods have to be used to ensure radiochemical purity. A high concentration of glucose in the incubating medium appears to be important in achieving a rapid rate of incorporation into insulin in these experiments.

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ENERGY TRANSFER IN PROTEINS

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A NUMBER of workers have reported that when proteins or amino-acid mixtures containing tryptophan are excited with ultra-violet light in the range 250–300 mμ, the fluorescence emitted has many of the properties of that from the constituent tryptophan, whereas fluorescence typical of tyrosine arises from those proteins which contain tyrosine but no tryptophan (an extensive review of the original literature can be found in ref. 1). Further, Steele and Szent-Györgyi reported that the wave-length of maximum phosphorescence from tryptophan-containing proteins is the same as that for phosphorescence from tryptophan[2]. On this basis, it has often been concluded that in proteins an excited phenylalanine will transfer its energy to either tyrosine or tryptophan residues nearby and an excited tyrosine will either transfer its excitation to a neighbouring tryptophan or be quenche without emitting[3–5]. Evidence that intra-protein energy transfer is probably efficient and occurs via a so-called resonance transfer mechanism has been reviewed by various authors[1,6]. However, Weber and Teale calculated from the fluorescence excitation spectra of many tryptophan-containing proteins that energy transfer from tyrosine to tryptophan probably occurred with only very low efficiency[7]. Also, since 1960 several workers have reported finding a weak tyrosine fluorescence component in proteins containing both tyrosine and tryptophan[8–10]. Furthermore, there has been a recent abstract reporting phosphorescence characteristic of tyrosine from some proteins which also contain tryptophan[11].

Accordingly, it seems worth while to report here experiments which raise further questions about the efficiency of transfer from tyrosine to tryptophan as well as about the nature of the electronic states of tyrosine residues in proteins. That is, in accord with Longworth's recent report, phosphorescence characteristic of that from tyrosine can be readily detected from tryptophan-containing proteins such as trypsin, bovine plasma albumin, alcohol dehydrogenase, pepsin and pepsinogen. However, 'tyrosine' phosphorescence is not observed from all tyrosine-containing proteins—for example, none could be detected from either chymotrypsin or chymotrypsinogen. Further, only slight evidence of tyrosine fluorescence can be found in any of these proteins. This report is concerned primarily with the investigations of phophorescence.

To prepare the solutions for spectroscopy, 10-mg portions of either a highly purified crystalline sample of tyrosine, or tryptophan, or one of a number of proteins was dissolved in 25 ml. of water. To these solutions was added sufficient glucose (0·5 per cent) or spectral grade ethylene glycol (1 : 1 by volume) to minimize the formation of microcrystals during cooling to liquid nitrogen temperature. The phosphorescence spectra shown in Fig. 1, obtained at 77° K in an Aminco spectrophosphorimeter[12], are representative of the data derived. There it can be seen that, on excitation at 280 mμ for tryptophan and both the proteins, the maximum phosphorescence is observed at 435 mμ and there are obvious shoulders at 410 and 460 mμ. However, the shortest wave-length of phosphorescence observed from both proteins (345 mμ) agrees with that from tyrosine: the short-wave-length portion of the alcohol dehydrogenase phosphorescence spectrum is quite obviously unlike that from tryptophan and seems to mimic that from tyrosine both in shape and the shortest wave-length observed. Further, the decay times measured at 380 mμ agree with those for tyrosine whereas the decay observed at 480 mμ is dominated by a component which decreases at the same rate as tryptophan: at intermediate wave-lengths the decay curves appear to be a mixture of these two components having respective decay times of 1–2 and almost 6 sec. (See discussion in ref. 13 of the effect of intramolecular environments on phosphorescent decay times.)

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Assuming that the shapes of the phosphorescence emission spectra for constituent tyrosine and tryptophan residues are the same as those shown in Fig. 1 for these two amino-acids in a glass, it can be estimated that approximately 0·5 of the phosphorescence from alcohol dehydrogenase and 0·06 of that from trypsin arises from constituent tyrosine when excitation is by 280 mμ light (the maximum absorption of both tyrosine and tryptophan is near 280 mμ). Furthermore, it is estimated that tyrosine accounts for almost 0·7 of the phosphorescence emitted when alcohol dehydrogenase is excited by 240 mμ light (that is, primarily excitation of phenylalanine): the peak at about 400 mμ is almost equivalent in intensity to the usual maximum at 435 mμ.

It seems unlikely that the phosphorescence in the interval 350–390 mμ arises from tyrosine contamination in the protein samples, inasmuch as the crystalline protein preparations which were of the highest purity available commercially were further purified by passage through a 15-cm 'Sephadex' column. Preliminary results with the seven proteins listed also indicate that the intensity of tyrosine phosphorescence relative to that from tryptophan cannot be correlated with the ratio of tyrosine/tryptophan content nor with the absolute number of tyrosine or tryptophan residues in the various proteins.

Unequal distribution of tyrosine and tryptophan residues within a given protein could result in large yields of emission from tyrosine residues which might be sufficiently isolated—either because of distance or mutual orientation—from potential acceptor tryptophans so that resonance transfer of excitation energy is unlikely. However, it would be expected that, if this were an important cause of the observed results, then in addition to the phosphorescence a significant amount of tyrosine fluorescence should be detected also, since a fluorescence/phosphorescence ratio of approximately 1 has been recorded from tyrosine in glasses[13]. Although resolution near the peak of tyrosine fluorescence at 300 mμ is made difficult by Rayleigh scattering of the 280 mμ exciting light from the frozen (cracked) solutions, the amount of fluorescence which would be expected from 'isolated' tyrosines has not been observed. Further, excitation with wave-lengths less than 260 mμ permits adequate separation of fluorescence and scattered light to show that, in fact, only a minimal fraction of the fluorescence emitted from alcohol dehydrogenase is typical of that from tyrosine.

The present results indicate that interactions with neighbouring residues in proteins so distort the electronic properties of tyrosine residues that their normal emissive, and thus energy transfer, properties are drastically altered. In this regard, previous investigations of the wave-length corresponding to maximum phosphorescence, the F/P ratio and the kinetics and lifetimes of phosphorescence decay, indicated that approximately three of the six tyrosines in RNase interact strongly with neighbouring residues[13]. In fact, Weber suggested that the low efficiency of tyrosine fluorescence was a result of quenching arising from interaction of the phenolic OH groups with neighbouring carboxylate moieties[9].

The variation in relative tyrosine emission among the proteins investigated indicated that either: (*a*) all tyrosines are sufficiently perturbed so that practically all fluorescence is quenched, but the interactions of some residues are such that phosphorescence can still occur; or (*b*) most tyrosine residues are relatively unperturbed but the interactions of a few are sufficiently strong so that inter-system crossing from singlet to triplet configurations competes both with tyrosine fluorescence and with resonance transfer to tryptophan.

Precise measurements of quantum yield as a function of exciting wave-length, as well as better resolution of emission lifetimes, are needed to select among these two possibilities. Even so, it seems worth while considering possibility (*b*) further since the great increase of tyrosine phosphorescence relative to that from tryptophan, when phenylalanine is preferentially excited (by 240 mμ light), suggests that, at least in alcohol dehydrogenase, perturbations of tyrosine inter-system crossing may be very important.

Approximately equal fluorescence and phosphorescence are observed when tyrosine is excited at 77° K in an organic glass. Thus, in relatively unperturbed tyrosine residues the rate constant for intersystem crossing must be essentially equivalent to that for fluorescence. Accordingly, in a protein in which all tyrosines are unperturbed, no tyrosine emission would be anticipated, since the relative rate constants for energy transfer should be about two orders of magnitude greater than the rate constant for fluorescence emission. That is, fluores-

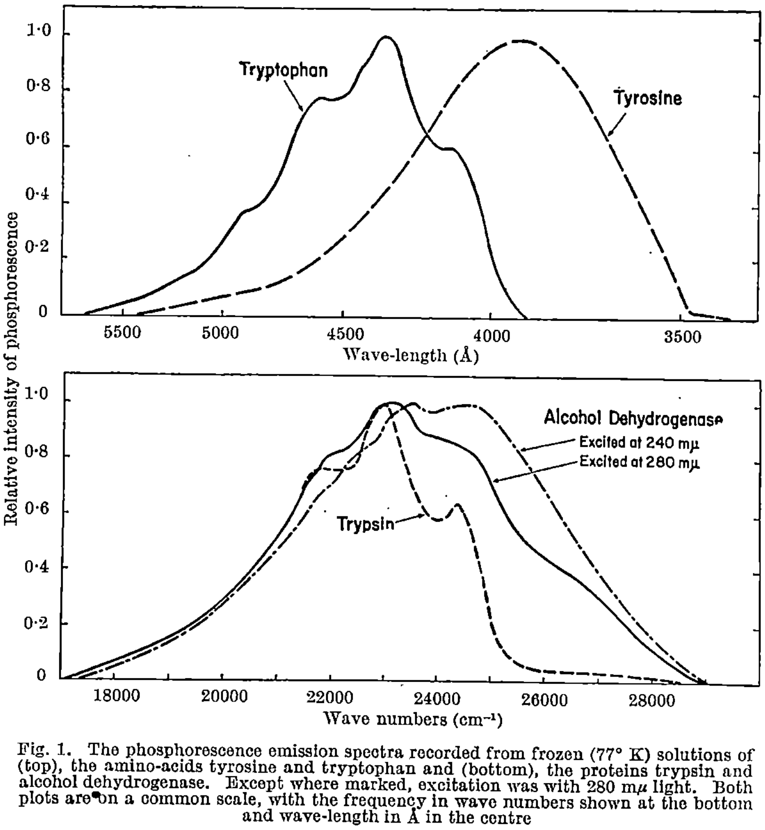

Fig. 1. The phosphorescence emission spectra recorded from frozen (77° K) solutions of (top), the amino-acids tyrosine and tryptophan and (bottom), the proteins trypsin and alcohol dehydrogenase. Except where marked, excitation was with 280 mμ light. Both plots are on a common scale, with the frequency in wave numbers shown at the bottom and wave-length in Å in the centre

çence lifetimes are normally about 10^{-9} sec, whereas the conditions of resonance required for Förster-type energy transfer from tyrosine to tryptophan are presumed to occur in 10^{-12}–10^{-11} sec during vibrational relaxation[6]. Indeed, there are some proteins containing tyrosine in which only tryptophan emission is observed. By contrast, in those proteins where significant levels of tyrosine phosphorescence are observed, it would be concluded that the interactions of tyrosine with neighbouring residues are such that the rate constant for inter-system crossing is increased to a value comparable to that for vibrational relaxation. Such a large perturbation could presumably be the result of heavy atom contamination[14] or perhaps more probably the strong interaction between tyrosine and neighbouring disulphide groups (spin-orbit coupling and/or charge transfer)[15]. In this respect, interactions between aromatic residues and cystine have been postulated to account for the disruption of specific cystine residues during protein inactivation by ultra-violet irradiation[16,17].

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COMBINATION WITH RED CELLS OF IMMUNE RABBIT 7S ANTIBODY AND ITS SUB-UNITS

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WE have shown[1], in the case of immune rabbit anti-blood group *A* antibodies, that 7*S* γ-antibody is a much less efficient agglutinating agent than 19*S* ($\beta_2 M$) antibody: that is to say that at the end point of a hæmagglutination titration with human group A_1 red cells, each cell is carrying 19,500 molecules in the case of 7*S* antibody, and only 25 molecules in the case of 19*S* antibody. We have also shown that the mean distance separating *A*-antigen sites on the red cell is only about half the length of a 7*S* molecule. Even supposing uniform distribution of *A*-sites on the cell there must be approximately 18 sites within a circle radius 240 Å (the length of a 7*S* molecule). In these circumstances, if there were no steric impediment, it would seem most likely that the free combining site of an antibody molecule already bound to a red cell by one of its specific groups would also attach itself to the same red cell. Molecules so attached could not take part in agglutination; conversely if, as we believe, 19*S* antibody is multivalent it is unlikely that all the specific groups on one molecule could be attached to a single red cell.

In order to throw further light on this question we have investigated the reactions with red cells of fragments derived from immune rabbit anti-A 7*S* antibody by means of papain digestion and pepsin digestion followed by reduction with cysteine or 2-mercaptoethylamine with subsequent blocking of the free —SH groups with iodoacetate. This treatment results in the production from each 7*S* antibody molecule of two univalent fragments which retain the antibody specificity of the original molecule[2-4]. We find that treatment with cysteine and iodoacetate is, in the present context, without effect on intact 7*S* antibody.

The binding constant (*K*) of the reaction between an antibody and antigen can be expressed as the reciprocal of the concentration of antigen (red cells in this case) at which half the antibody is combined, if the concentration of antibody is sufficiently low for the combined antigen to be a small fraction of the total antigen. Aliquots of iodine-131-labelled antibody preparations pre-absorbed with O-cells and diluted in bovine albumen have been added to varying quantities of centrifuged A_1 red cells. The red cells have been resuspended and, after allowing 30 min for the reaction with antibody to take place, the supernatant has been removed and the amount of antibody adhering to the red cells has been measured in a scintillation counter[1]. By suitable techniques it has been possible to avoid the necessity of washing the red cells, which would involve the elution of unavid antibody. Curves, of which Fig. 1 is typical, have been constructed from which the concentration of red cells necessary to absorb half the antibody has been measured.

In plotting these curves, the total antibody available has, in the case of intact 7*S* antibody and 5*S* peptic digest (which is bivalent), been taken as that amount absorbed by 16 per cent cells. It is clear from the asymptotic shape of the curves that the error in this assumption is small. It has been assumed that reduction of peptic digest has not destroyed any antibody and that the reduced preparations contain the same proportion of antibody radioactivity as the 5*S* peptic digest from which they were derived. The shape of the curves, which are still rising steeply, lends

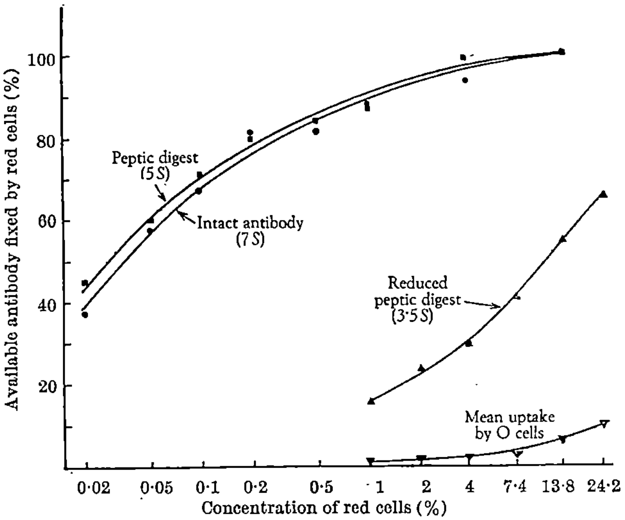

Fig. 1. The uptake by A_1 and *O* red cells of antibody preparations

support to this assumption. For papain digest we have assumed that the active fragments are the same as those produced by reduction of peptic digest[3] and that the proportion of radioactivity absorbable is the same as that in the whole (before dialysis) peptic digest of the $7S$ preparation.

Comparisons have been made of the $7S$ antibody (separated by DEAE-cellulose chromatography) and its derivatives from three rabbits, all the derivatives from one serum being investigated simultaneously. In each case, the curves for $7S$ antibody and peptic digest have been almost identical. The curves for univalent fragments, however derived, have been very close together and parallel. The concentrations of A_1 cells necessary to absorb half the antibody from $7S$ or $5S$ preparations from each of the three rabbits were, respectively, 0·16, 0·03 and 0·02 per cent; the cell concentrations required to absorb half the univalent antibody derived from these preparations were, respectively, 19, 10·5 and 6 per cent. That is to say $\frac{K \text{ bivalent } Ab \text{ } (7S \text{ or } 5S)}{K \text{ univalent } Ab \text{ fragments}}$ was 119, 350 and 300 respectively for the three rabbit sera.

The greatly diminished binding constant of univalent antibody fragments could be explained if the process of splitting the molecule had distorted the antibody specific group; this seems unlikely since, at least for a small hapten, K remains unaltered after splitting the antibody molecule[5]; moreover, if the binding constant of antibody sites were drastically diminished it seems improbable that a comparatively small excess of univalent fragments would inhibit precipitation in the ovalbumin – anti-ovalbumin system[2]. Labelling with iodine does not appear harmful since labelled anti-ovalbumin fragments which we have prepared inhibit precipitation in the same proportion as described by Porter. It is possible that reduction in the size *per se* of the antibody would reduce K by eliminating some non-specific binding of the antibody molecule, but we find no difference in K between the $7S$ and the bivalent $5S$ fragment derived therefrom.

We consider that the great diminution in binding constant which accompanies the reduction of antibody valency from 2 to 1 gives support to our hypothesis that rabbit immune $7S$ anti-A antibody mostly combines with the red cell by both its specific groups. The possibility of this type of antigen–antibody attachment does not appear to have received consideration and is even excluded from his calculations by Goldberg[6], although it is not necessary to postulate that the bivalent attachment of antibody on a red cell is to a single antigen molecule. However, Almeida, Cinader and Howatson[7] have shown electron micrographs which may be interpreted as bivalent attachment of a single antibody molecule to a wart virus particle.

A final resolution of this point may be possible by the examination, which we are about to attempt, of univalent fragments recombined to the bivalent $5S$ form[8], where if our hypothesis is correct, recombined pure antibody should regain its $5S$ binding constant, whereas $5S$ recombinants made up of one antibody and one non-antibody $3·5S$ fragment would have the binding constant of a univalent fragment.

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QUINACILLIN: A COMPARISON WITH OTHER PENICILLINASE-RESISTANT PENICILLINS

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QUINACILLIN, 3-carboxy-2-quinoxalinylpenicillin, a recently described semi-synthetic penicillin, was found[1] to have 'unusual properties', namely, antibacterial activity highest against *Staphylococcus aureus*, enhancement of antibacterial activity and rate of diffusion in agar with decrease in pH of the medium, and poor inducing ability for staphylococcal penicillinase. In view of these findings, we have examined the interactions of quinacillin with various bacteria and their penicillinases, and are able to confirm and extend the description of this penicillin as possessing "unusual properties".

Quinacillin as a substrate for penicillinase. (*a*) *Gram-positive bacteria.* The rates of hydrolysis of quinacillin at various concentrations in 0·025 M sodium phosphate buffer pH 7·4 were measured using the hydroxylamine method[2]. With staphylococcal penicillinase made, as previously described[3], from *Staph. aureus E*3, the Michaelis constant (K_m) for quinacillin was 61 mM (28 mg/ml.) with a $V_{\max}$ 8 per cent that of benzylpenicillin; at an initial concentration of 4·3 mM (2 mg/ml.) quinacillin was not appreciably hydrolysed by this preparation. Richards *et al.*[1], who used the iodometric method of assay at pH 6·5, found that the K_m value for staphylococcal penicillinase (Parker strain) was 5 mg/ml. and the $V_{\max}$ was 5 per cent that of benzylpenicillin. They also found no appreciable hydrolysis of quinacillin at lower concentrations.

Penicillinase preparations[4] from *B. cereus* and *B. licheniformis* were found to hydrolyse quinacillin (initial concentration 4·3 mM) at 11 and 13 per cent, respectively, of the rates at which they hydrolysed benzylpenicillin. Both these preparations had K_m values for quinacillin of about 54 mM.

(*b*) *Gram-negative bacteria.* Cell-free preparations made from *Pr. morgani G* (ref. 5), *Pr. rettgeri* 410, *K. aerogenes* 366 (ref. 6) and *E. coli 214T* (ref. 7) did not appreciably hydrolyse quinacillin (initial concentration 4·3 mM). A cell-free extract of *A. cloacae* 53 (ref. 6) hydrolysed quinacillin (4·3 mM) at about 7 per cent of the rate at which it destroyed benzylpenicillin (5·4 mM) and intact cells of this organism hydrolysed quinacillin at about one-seventh of this rate (Table 1). Disruption of this strain has been shown[6] to increase its hydrolytic activity towards benzylpenicillin 25 times. Thus it seems that quinacillin can pass the substrate accessibility barrier in this strain more readily than benzylpenicillin.

Quinacillin as an antibacterial agent. Minimum inhibitory concentrations of quinacillin were determined, by means of the tube test, for six strains of *Staph. aureus*, *E*3, 13137, 6637, 5974 (all of which produce penicillinase, the last three being resistant to methicillin and cloxacillin[8]), *E3T* and Oxford (benzylpenicillin-sensitive, penicillinase-negative strains). Two inoculum sizes were used, about 10^6 and about 10^2 viable units per tube. The results

Table 1. DESTRUCTION OF 2 PENICILLINS BY *A. cloacae* 53
(rates expressed as μg/min/10^9 cells /ml.)

	Intact cells	Disrupted cells	Ratio (to nearest whole number) of penicillinase activity of disrupted to intact cells (= 'permeability factor')
Quinacillin	3	20	7
Benzylpenicillin	12	303	25

Table 2. ANTIBACTERIAL ACTIVITY OF QUINACILLIN AGAINST SIX STRAINS OF *Staph. aureus*

Minimum inhibitory concentrations (μg/ml.)

	Penicillin	Methicillin		Cloxacillin		Quinacillin	
	Inoculum	10^6	10^3	10^6	10^3	10^6	10^3
Strain of *Staph. aureus*	*E*3	7·5	3·7	5·0	1·0	6·3	3·1
	13137	1,000·0	125·0	500·0	5·0	50·0	12·5
	6637	> 1,000·0	25·0	1,000·0	5·0	400·0	6·3
	5974	500·0	100·0	1,000·0	5·0	25·0	6·3
	E3T	3·2	1·6	0·8	0·4	6·3	6·3
	Oxford	1·6	0·8	0·4	0·2	6·3	1·6

Table 3. MINIMUM INHIBITORY CONCENTRATIONS OF QUINACILLIN, TESTED AGAINST GRAM-NEGATIVE BACTERIA BY TUBE DILUTION METHOD

Inoculum size approximately 10^6 organisms

K. aerogenes 83, 370, 415, 418	> 4,000
K. aerogenes 1, 43, 366, 373, 407, 414	> 8,000
K. ozaenae 61	> 4,000
E. coli 214T	> 2,000
Pr. morgani G	680
185	> 2,000
Pr. rettgeri 410	500
Achromobacter 510	4,000
A. cloacae 53	> 20,000

Table 4. INDUCING ABILITIES OF FOUR COMPOUNDS FOR PENICILLINASE OF *Staph. aureus* E3

	Concentration μg/ml. to give maximal induction	Concentration μg/ml. to give half maximal induction	Ratio of induced to basal penicillinase activity at maximum
Quinacillin	12·5	2·07	17·8
6-Aminopenicillanic acid *	10·0	3·0	16·3
Methicillin *	3·0	0·27	36·0
Cloxacillin †	0·1	0·013	71·3

* Ref. 3. † ref. 15.

(Table 2) show that there is cross-resistance between cloxacillin, methicillin and quinacillin, but it appears that resistant strains are slightly more susceptible to quinacillin than to the other two, at least using large inocula. The 'inoculum size effect' obtained with this series of organisms is probably a reflexion of populations that are not homogeneous, in that they contain small proportions of highly resistant bacteria[9]. In common with other penicillins, quinacillin was extremely poor at inhibiting the growth of *B. cereus*, the minimum inhibitory concentration being 5 mg/ml. for an inoculum of 10^6 organisms. Quinacillin was relatively inactive as an antibacterial agent against Gram-negative bacteria (Table 3). Richards *et al.*[1] also found that Gram-negative bacteria were resistant to quinacillin.

Quinacillin as an inducing agent for penicillinases. The ability of quinacillin to induce penicillinase production in *Staph. aureus E*3 was determined as described previously[3] and the results are shown in Table 4. It can be seen that the concentrations for half maximal induction ('induction constants'[10]) with quinacillin, methicillin and cloxacillin were 2·07, 0·27 and 0·013 μg/ml., respectively. Thus the 'induction constant' for quinacillin is some eight times larger than that for methicillin and 160 times larger than that for cloxacillin. In addition, as judged by the concentrations required for maximal induction and by the amount of enzyme production at these concentrations, quinacillin was a less effective inducing agent than either methicillin or cloxacillin. Using the Parker strain of *Staph. aureus*, Richards *et al.*[1] reported a twenty-five-fold difference between the 'induction constants' for methicillin and quinacillin, so, allowing for strain differences, it seems that quinacillin is a much poorer inducing agent than would be expected from its activity against penicillinase-producing staphylococci.

It appeared that the figures obtained here for quinacillin in induction experiments were very similar to those found for 6-aminopenicillanic acid in an earlier series of experiments[3]. To determine whether quinacillin was broken down to 6-aminopenicillanic acid (which could explain these similarities) during the process of induction, quinacillin was incubated at 37° C with washed cells from an overnight culture of *E*3 in phosphate-buffered saline at *p*H 7·4 for 3 h. The cells were centrifuged down, and enough of the supernatant fraction to contain approximately 50 μg quinacillin was applied to Whatman No. 1 paper and chromatographed in *n*-butanol 4 : acetic acid 1 : water 5, descending overnight; markers of quinacillin, the penicilloic acid derived from it (made by dissolving quinacillin in N soda, and neutralizing with N hydrochloric acid after 30 min at room temperature) and 6-aminopenicillanic acid were also applied to the chromatogram. When the run had been completed, the paper was dipped in 0·2 per cent ninhydrin in acetone[11], heated for 5 min at 100° C and then 'fixed' in alcoholic copper nitrate. Under these conditions, the quinacillin developed a pale-yellow colour, fluorescent in ultra-violet light (this yellow fluorescence could also be observed before ninhydrin treatment). The penicilloic acid developed a red colour which did not fluoresce. The R_F values of these two compounds in the solvent used were 0·82 and 0·65, respectively. No trace of a spot similar to that derived from 6-aminopenicillanic acid (R_F value 0·53—which develops a golden-yellow colour, slightly fluorescent[11]) could be observed in the incubation mixture. Nor was any evidence of a penicillin acylase obtained when the butyl acetate extraction technique[12] was used, with benzylpenicillin as substrate, with this strain of *Staph. aureus*. Hence, it appears that the resemblance between the values of certain induction parameters of quinacillin and 6-aminopenicillanic acid is a mere coincidence.

Quinacillin, benzylpenicillin, methicillin, cloxacillin and 6-aminopenicillanic acid were tested for inducing ability towards the penicillinase of *B. cereus*. Various concentrations of each inducing agent were dissolved in broth, 2 ml. of a 6-h 37° C culture of *B. cereus* 569 added to each 100 ml., and bottles were incubated overnight at 37° C. Portions were centrifuged (3,000*g* for 15 min), after mM *p*-chlormercuribenzoate had been added to prevent further induction, and the turbidity measured at 700 mμ. The supernatant fraction was adjusted to *p*H 7·4 with N sodium hydroxide and the rate of hydrolysis of benzylpenicillin estimated by the hydroxylamine method. Results were expressed in terms of the amount of increase in penicillinase activity as compared with a control culture grown in broth alone; Table 5 shows the results obtained. It is clear that quinacillin, although a better inducing agent than benzylpenicillin (probably due to its greater stability to this penicillinase), is not so efficient at the higher concentration as methicillin, cloxacillin or 6-aminopenicillanic acid.

Quinacillin did not cause any increased enzyme production in five strains of *K. aerogenes*—43, 370, 373, 407 and 414—nor in *K. ozaenae* 61; this is in common with the non-inducibility of these penicillinases by all other compounds tested[6]. When *Pr. morgani* 185 (ref. 5) and *Pr. rettgeri* 410 were grown overnight at 37° C in the presence of half the minimum inhibitory concentration of quinacillin, penicillinase was induced. The activities of these cultures after disruption, expressed in terms of μg benzylpenicillin hydrolysed/min/10^9 cells/ml., were 5·6 and 3·7, respectively. Uninduced preparations of these two organisms hydrolysed less than 0·1 μg benzylpenicillin/min/10^9 cell/ml.

Quinacillin as an inhibitor of benzylpenicillin hydrolysis by penicillinases. Penicillins which are only slowly hydrolysed by penicillinases have been shown to inhibit benzylpenicillin hydrolysis by *B. cereus*[13], *B. licheniformis*[11], *K. aerogenes*[14], *E. coli*[14] and *Pr. morgani*[14] penicillinase preparations. Mixtures of 1·3 mM (0·5 mg/ml.) benzylpenicillin and mM quinacillin were incubated with enzyme

Table 5. INDUCING ABILITIES OF FIVE COMPOUNDS FOR PENICILLINASE OF *B. cereus* 569

	Ratio of concentration of inducing agent to minimum inhibitory concentration for that inducing agent	
	1/10	1/4
Benzylpenicillin (1,250)	9	7
Quinacillin (5,000)	31	39
Cloxacillin (500)	21	54
6-Aminopenicillanic acid (10,000)	66	92
Methicillin (313)	25	140

Figures in brackets are minimum inhibitory concentrations of drugs for *B. cereus* (large inoculum, in μg/ml.).

Figures in body of the Table are enzymatic acitivity after induction, taking basal level = 1.

preparations from *Staph. aureus E*3, *B. cereus* 569 and *B. licheniformis*, which had been adjusted to hydrolyse 2 μg benzylpenicillin/min[11,15]. The rates of hydrolysis in the presence of quinacillin, measured by the hydroxylamine method, were compared with those obtained in the absence of quinacillin but no differences were observed. Hence, quinacillin, in contrast to the other poorly hydrolysed penicillins, does not inhibit any of the penicillinase preparations from Gram-positive bacteria examined.

When cell-free penicillinase preparations of *E. coli* 214*T* and *Pr. morgani* G were tested by the modified spectrophotometric method[11], no inhibition of the hydrolysis of benzylpenicillin (2·7 mM) could be detected in the presence of concentrations of quinacillin as high as 260 μM. However, under similar conditions 50 per cent inhibition of the penicillinase activities of *K. aerogenes* 366 and 373 was observed with 70 μM and 161 μM quinacillin, respectively; thus, quinacillin is about as active an inhibitor of the *Klebsiella* penicillinases as oxacillin and *BRL* 1577 (ref. 14). The fact that quinacillin does not inhibit the *E. coli* and *Proteus* penicillinase preparations is in contrast to the inhibitory action of other penicillins stable to the penicillinases of these bacteria.

Discussion. Richards *et al.*[1] found that quinacillin possessed unusual properties. We have confirmed that this penicillin is a poor inducing agent for staphylococcal penicillinase, and in addition we have shown that it is a relatively inefficient inducer of *B. cereus* penicillinase.

As a substrate for penicillinases derived from various sources, quinacillin shows in general a marked resistance to hydrolysis; its affinity for staphylococcal and Bacillus penicillinases was uniformly low, but it was hydrolysed rather more rapidly by the Bacillus enzymes. Penicillinase preparations from *K. aerogenes*, *E. coli* and *Pr. morgani* did not detectably destroy the compound, although a strain of *A. cloacæ* did hydrolyse quinacillin. An interesting feature of this penicillin is that it does not inhibit penicillinases from *B. cereus* and *B. licheniformis*, which is in contrast to the action of other poorly hydrolysed penicillins. Similarly, penicillinase preparations from *E. coli* and *Pr. morgani* were not inhibited by quinacillin, and it was found to be only a very weak inhibitor of *K. aerogenes* penicillinases.

The discovery that a penicillin which is a poor inducer of staphylococcal penicillinase can also be an effective antistaphylococcal agent discounts the correlation that has been made by some authors[15–18] between inducing ability and antibacterial activity for penicillinase-producing staphylococci. Until now penicillins which are biologically active against penicillinase-producing *Staph. aureus*, besides being resistant to hydrolysis by this enzyme, have also been found to be very good inducing agents and can markedly inhibit various penicillinases. It is interesting that although quinacillin does possess the first two characteristics it is not a particularly good inducing agent nor an efficient inhibitor of penicillinases.

We thank Boots Pure Drug Company, Ltd., for gifts of quinacillin.

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A COMPARISON OF EFFECTS OF SOME CHEMOTHERAPEUTIC AGENTS AND THOSE OF X-RAYS ON THE REPRODUCTIVE CAPACITY OF MAMMALIAN CELLS

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WHILE chemotherapeutic agents have been extensively investigated for ability to 'cure' a wide range of transplanted tumours *in vivo*[1] and have less frequently been investigated qualitatively *in vitro* in cultures of mammalian cells[2], there has been a paucity of quantitative investigation of the effect on cell reproductive capacity of agents known to have clinical usefulness in palliation of human cancer[3,4]. The technique of clonal cell culture described by Puck *et al.*[5] by which macroscopically visible colonies can be grown *in vitro* from single mammalian cells has been extensively applied to the examination of effects of ionizing radiation (see Porter[6] for a representative list of references). It is only recently, however, that this method has been used to investigate the dose-response to a chemotherapeutic agent[7].

The present communication reports investigations of the dose-response relationship for the reproductive capacity of two strains of mammalian cells exposed *in vitro* to several commonly used anticancer drugs and compares these relationships with that for X-irradiation of the same cells.

The drugs used in these experiments were of the following types: (1) *Nitrogen mustard.* (*a*) 'HN3' (tri-(2-chloroethyl)-amine, 'Trillekamin', Crookes). (*b*) Mannitol mustard (Mannomustine dihydrochloride, 'Degranol', Chinon). (2) *Ethylene imine* ('Thio-TEPA', Lederle). (3) *Epoxide* ('Epodyl', I.C.I., 32865). (4) *Antimetabolite* (5-fluorouracil, Roche). All drugs were diluted with sterile normal saline to desired final concentrations immediately prior to addition to the growth medium.

The mammalian cell lines used were HeLa S-$3_{\text{oxf.}}$, an aneuploid derivative of human cervical carcinoma kindly supplied by Dr. C. E. Ford, Medical Research Council, Harwell, and re-cloned in this laboratory, and the mouse strain $L_{\text{oxf.}}$ fibroblast kindly provided by Dr. J. Paul, Department of Biochemistry, Glasgow, and re-cloned in this laboratory by Dr. A. H. W. Nias. They were grown in Medium 199 (Glaxo) supplemented with 5 μg/ml. amphotericin *B* ('Fungizone', Squibb), and for HeLa: 10 or 20 per cent freshly drawn human AB serum; for strain *L*: 2 per cent pooled calf serum (Oxoid) and 0·5 per cent 'Bacto-Peptone' (Difco) for stock growth and 10 per cent pooled calf serum for clonal growth.

The technique of clonal culture was modified only slightly from that of Puck *et al.*[5]; cells were trypsinized ('Bacto-Trypsin', Difco, 0·25 per cent in 0·3 per cent sodium citrate made isotonic with sodium chloride) from a stock bottle containing a nearly confluent sheet of cells. They were re-suspended in the appropriate medium and

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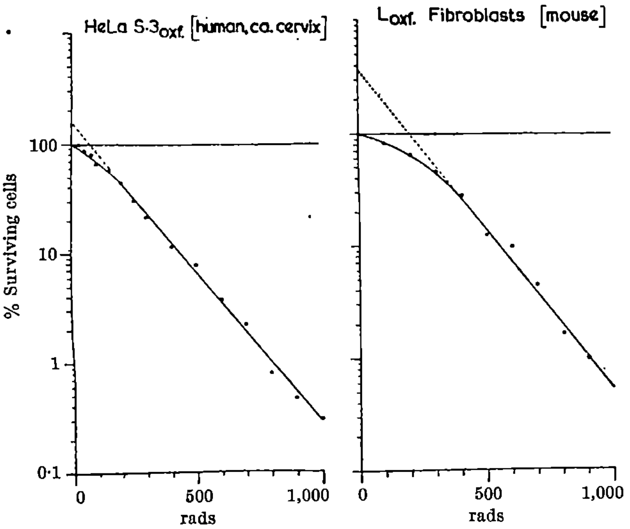

Fig. 1. Dose-response to X-rays, fraction of surviving cells (log scale) versus dose in rads (linear scale)

counted electronically (Coulter model *D*, 140μ orifice, calibrated previously against hæmacytometer count under phase microscopy). Appropriate dilutions were made and the cells were finally suspended in medium to which the drug under test had just previously been added in known concentration and 4 ml. aliquots were plated in 60 mm 'Falcon *TC*' polystyrene Petri dishes. The dishes were incubated in a humidified 5 per cent carbon dioxide–air environment at 37° C for 14 days to allow growth of visible colonies. If the drug given was stable for extended periods of time, it could be removed at any time by aspirating the medium, gently washing the plates with Medium 199 without serum, and then adding 4 ml. of the appropriate medium without the drug to each Petri dish. When cultures were to be irradiated, this was done 12–16 h after plating to allow the cells to attach firmly to the polystyrene dishes. Dishes were individually irradiated from below with 75 kVp X-rays (no additional filtration) at 15 cm FSD at a dose-rate of 140 rads/min; during irradiation 5 per cent carbon dioxide–air was circulated rapidly over each dish.

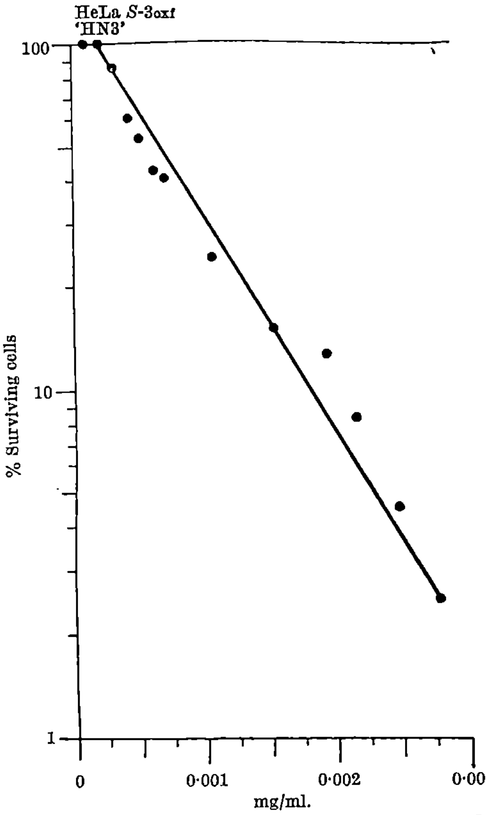

Fig. 2. Dose-response to nitrogen mustard 'HN3', HeLa *S*-3oxf. fraction of surviving cells (log scale) versus dose in mg/ml. (linear scale)

In all experiments, the number of cells plated was calculated so that the number of colonies produced was approximately 200 per dish. After 14 days' incubation, all dishes were washed twice with cold normal saline, fixed and stained with Leishman's stain. Colonies containing more than 50 cells were scored as reproductively intact, and the fraction of surviving cells (*S.F.*) after various drug or radiation treatments was calculated from the expression:

$$(1) \qquad S.F. = \frac{\text{control colonies}}{\text{experimental colonies} \times \text{dilution factor}}$$

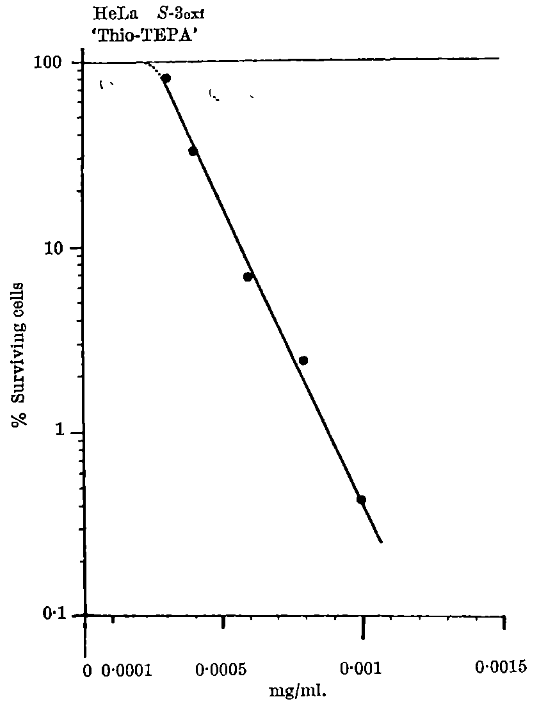

Fig. 3. Dose-response to 'Thio-TEPA', HeLa *S*-3oxf., fraction of surviving cells (log scale) versus dose in mg/ml. (linear scale)

The dose-response curves obtained in these experiments are shown in Figs. 1–6, where *S.F.* is plotted against the dose of the drug used. Each point represents the average of at least four independent determinations of surviving fraction and most points represent several thousand counted clones. Experiments in which control plating efficiency $\left(\frac{\text{No. of colonies reproductively intact}}{\text{No. of cells initially plated}}\right)$ was lower than 30 per cent were rejected, as it was felt that surviving clones from these experiments might represent an inadequate and potentially biased sample of the cell population.

Dustin[8] in 1947 coined the term 'radiomimetic', which has been widely used by many authors, uncritically accepted by many clinicians responsible for the care of cancer patients, and recently advanced again by Elson[9], to describe the action of chemotherapeutic agents which mimicked effects of total-body *X*-irradiation. The present results allow us to examine at the cellular level whether this term is justifiable in any but the most superficial evaluation.

The reproductive survival of mammalian cells of many types both *in vivo* and *in vitro* to X-irradiation has been shown to be of the type illustrated in Fig. 7 (for examples see Porter[6]). When the fraction of cells surviving (on a logarithmic scale) is plotted against dose of radiation (on a linear scale), there is a 'shoulder' region followed by what appears as a simple exponential (straight-line) response. When these same data are re-plotted with a 'logarithmic' dose-scale, the curve has a continuing downward inflexion, as also shown in Fig. 7. This type of dose-response is clearly seen in the present results with X-rays (Fig. 1). Both 'HN3' and 'Thio-TEPA' produce dose-response curves of this 'X-ray type', as did the nitrogen mustard 'HN2' in another cell strain in Levis's recent studies[7]. However, in these curves the 'shoulder' region is much sharper and more closely resembles a true threshold below which no effect of the drug on cell survival is seen and above which survival decreases exponentially with dose (Figs. 2 and 3). The dose-response to 'Epodyl' is of the same general type, but without evidence of a 'shoulder'; the curve falls as a simple exponential with depression of surviving fraction at even the smallest doses (Fig. 4). In investigations with ionizing radiation, the presence or absence of a 'shoulder' has been closely correlated with the ability or failure of cells to recover a certain amount of sub-lethal damage when doses are delivered as multiple fractions separated by intervals in time[10]. Whether this also applies to fractionated doses of chemotherapeutic agents is at present being investigated in this laboratory.

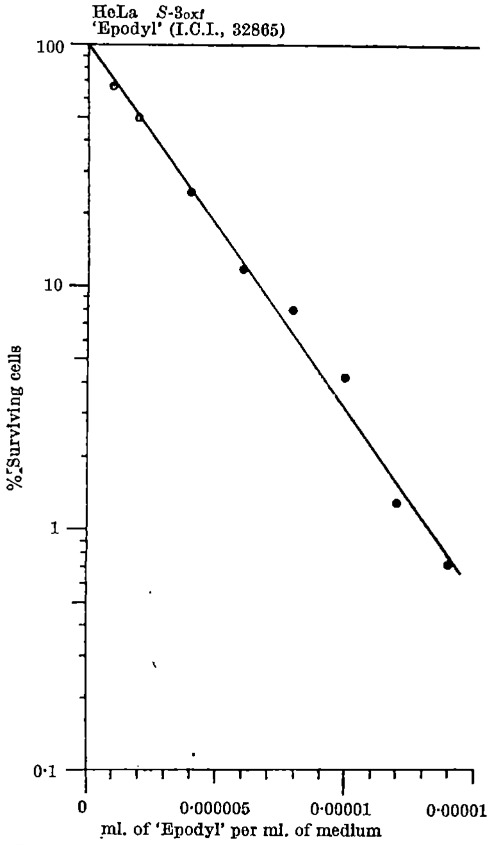

Fig. 4. Dose-response to 'Epodyl', HeLA S-3oxf., fraction of surviving cells (log scale) versus dose in ml. per ml. of growth medium (linear scale)

The dose-response to mannitol mustard ('Degranol') in both cell types is shown in Fig. 5. In both HeLa and the strain *L* fibroblast, the dose-response curve describes a straight line when dose on a logarithmic scale is plotted against percentage survival (log). This is typical of the response to many drugs[11], and its theoretical form is called the 'poison' type of curve in Fig. 8. Thus, with 'Degranol', the same percentage increment in dosage produces the same (log) decrease in survival, whereas with radiation, 'Thio-TEPA' and 'HN3' the same absolute increment in dose produces the same (log) decrease in survival. Clearly, at a cellular level, 'Degranol' is not radiomimetic, although its biologically active moiety is presumably the same as that of the 'radiomimetic' nitrogen mustard 'HN3'. This difference may have clinical significance, for an agent which produces an 'X-ray' type dose-response will be far more liable to catastrophic over-dosage than an agent showing the 'Degranol'-type response where the same percentage error in dose will produce the same percentage change in survival regardless of the absolute dose given, large or small.

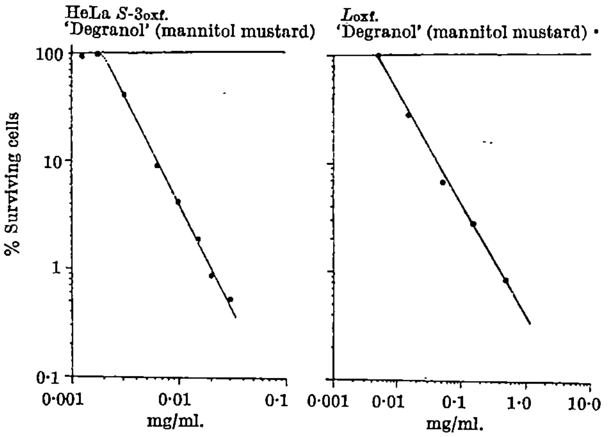

Fig. 5. Dose-response to mannitol nitrogen mustard ('Degranol'). Note that fraction of surviving cells (log scale) is plotted versus dose in mg/ml. on a logarithmic scale

The dose-response to the thymine analogue 5-fluorouracil (Fig. 6) is of different interest. Although the continuous presence of this agent produces a catastrophic dose-response curve with minute increments in dose producing vast decreases in cell survival once a threshold dose is exceeded, the dose-response to 24-h exposure to the drug starts similarly steeply above a threshold but

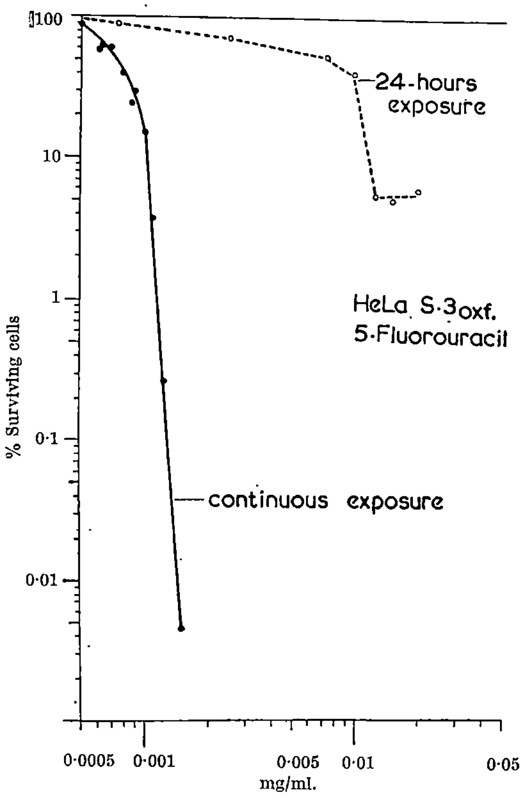

Fig. 6. Dose-response to 5-fluorouracil, HeLa S-3oxf., fraction of surviving cells (log scale) versus dose in mg/ml. on a logarithmic scale. Note that for 24 h exposure to the drug, the percentage of surviving cells is never decreased below 6 per cent (see text for discussion)

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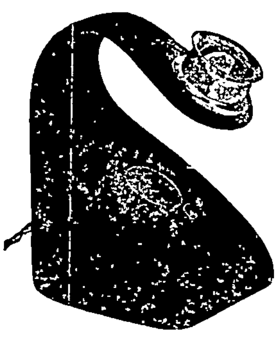

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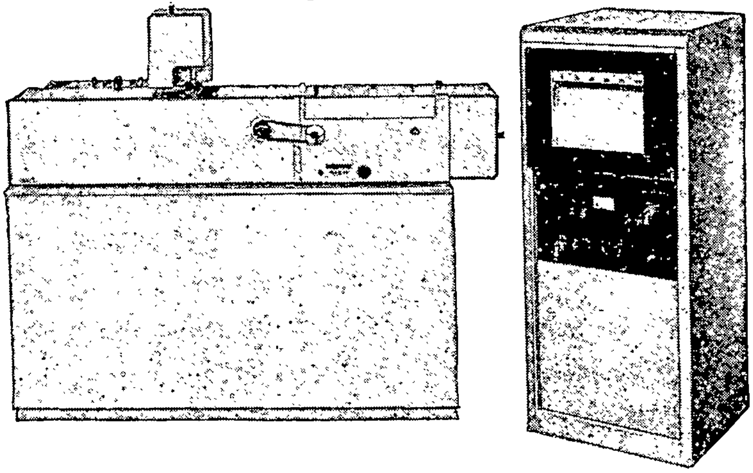

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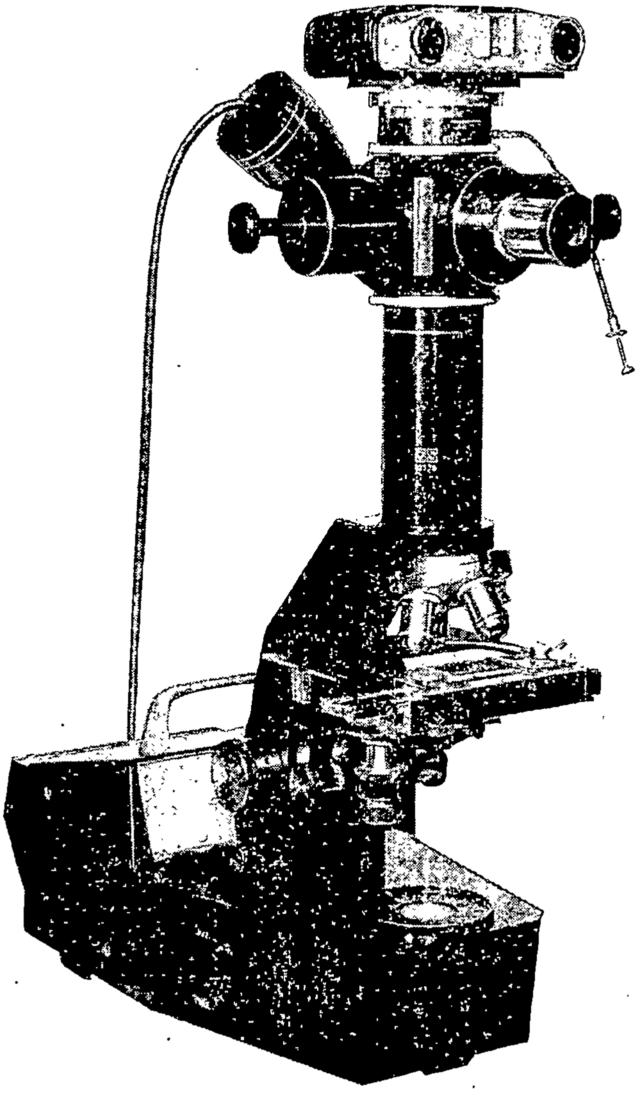

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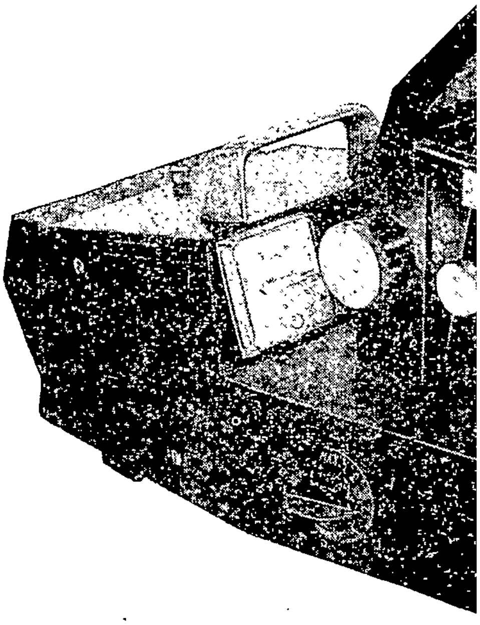

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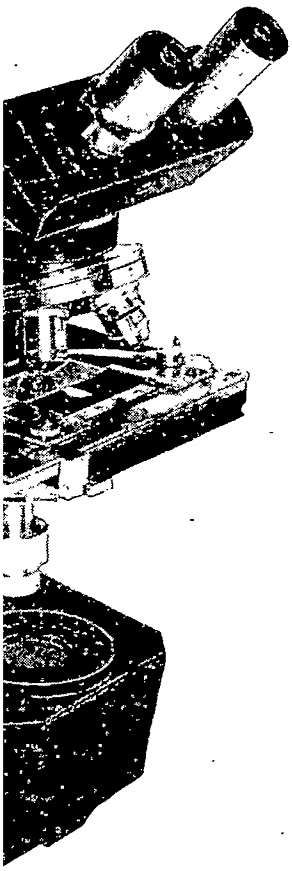

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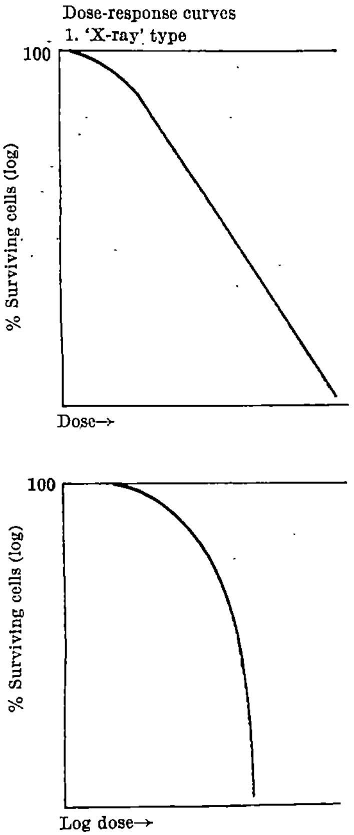

Fig. 7

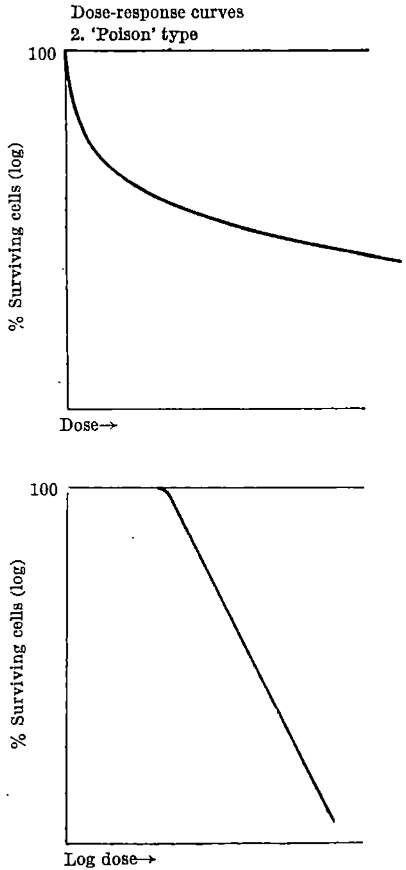

Fig. 8

reaches a point at which the percentage of surviving cells does not change in the face of increased concentrations of the drug. This becomes understandable when it is realized that in the HeLa *S*-3_{oxf}. line, with its average doubling time of 40 h, a certain proportion of cells do not enter DNA synthesis within any 24-h period. If tritiated thymidine is added to the growth medium for 24 h, the cells then fixed, and stained autoradiographic preparations made, it is seen that approximately 6 per cent of the cells fail to take up the radioactive DNA precursor and remain unlabelled[12]. It is this group of cells which survive a 24-h exposure to 5-FU because they are never called on to make DNA in the presence of the analogue, and no reasonable increase in the drug dose when administered for only this 24-h period can be expected to alter the approximate 6 per cent survival (Fig. 6). This type of observation at a cellular level may have great relevance to the schedules by which anti-tumour agents are administered to the cancer patient.

I thank the Damon Runyon Memorial Fund for their continued support of the cell culture laboratory, and Dr. F. Ellis, director, Radiotherapy Department, and Mr. R. Oliver, principal physicist, for helpful discussion. Misses M. A. Luschen, M. Jackson and J. Restall gave invaluable technical assistance. 'Trillekamin' ('HN3') was supplied by the courtesy of Crookes Laboratories, Ltd., and 5-fluorouracil by the courtesy of Roche Products, Ltd. The Regional Blood Transfusion Centre, Churchill Hospital, Oxford, kindly provided the freshly drawn human serum used in these experiments.

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REGULATION OF BACTERIAL ENZYME SYNTHESIS BY INDUCTION AND REPRESSION

By PATRICIA H. CLARKE and W. J. BRAMMAR
Department of Biochemistry, University College, London

ENZYME synthesis in bacteria can be induced or repressed by changes in the external environment. The essential similarity of these two processes was explicitly recognized by Jacob and Monod[1] when they put forward the repressor-operator model for the regulation of enzyme synthesis. In general, enzyme repression has been associated with biosynthetic systems and enzyme induction with degradative enzyme systems. The common feature of these two types of regulation is thought to be the possession of one or more regulator genes, responsible for producing a regulator molecule termed the cytoplasmic repressor. In a typical repressible system the cytoplasmic repressor is assumed to be incomplete, and active in repressing enzyme synthesis only when combined with the low

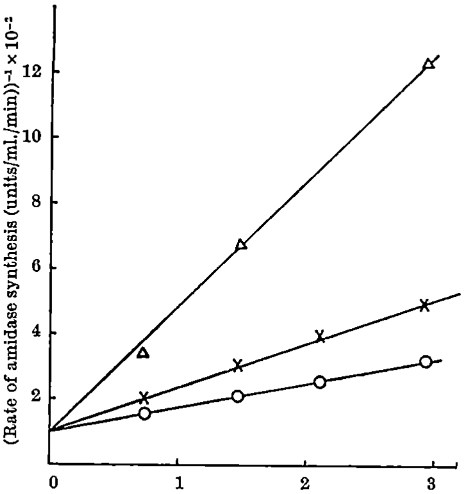

Fig. 1. Effect of cyanoacetamide on the rate of amidase induction by *N*-acetylacetamide. *Pseudomonas aeruginosa* 8602/A was grown overnight at 37° on a mechanical shaker in minimal salt medium with 0·01 M succinate (growth limiting concentration). 2-ml. samples were incubated with appropriate concentrations of *N*-acetylacetamide and cyanoacetamide. (Total volume, 2·5 ml.) 0·25 ml. samples were removed after 10 and 40 min for enzyme assay by the hydroxamate method (ref. 6). △, $1{\cdot}4\times10^{-4}$ M cyanoacetamide; ×, $3{\cdot}4\times10^{-5}$ M cyanoacetamide; ○, No cyanoacetamide

molecular weight co-repressor molecule. In a typical inducible system the cytoplasmic repressor is considered to be fully effective, and able to prevent enzyme synthesis, until it is rendered inactive by combination with the inducer molecule. Inducible enzymes are also subject to regulation by other cell metabolites and since many, though not all, enzymes are repressed by glucose, this was known for a long time as the glucose effect. Magasanik[2] considered that repression by glucose and other carbon compounds was a specific effect due to particular cell metabolites, and suggested the term 'catabolite repression'. McFall and Mandelstam[3] showed that glucose repression of β-galactosidase, serine deaminase and tryptophanase in *Escherichia coli* is due not to glucose directly, but to metabolites of glucose which are also intermediates in the degradative pathway of the substrate inducers of these enzymes. These findings suggest that catabolite repression is not a general property of cell metabolites but can be as specific as end-product repression or enzyme induction. McFall and Mandelstam[3] concluded that catabolite repression operated at a site other than the cytoplasmic repressor. Among the evidence for this view were the findings that strains of *E. coli* which were either i^- or 0^c constitutive for β-galactosidase were still subject to catabolite repression, and that increased inducer concentration did not reverse catabolite repression[3,4].

We have some evidence from studies with *Pseudomonas aeruginosa* to show that catabolite repression can be affected by changes in the inducer concentration. *P. aeruginosa* produces an amidase which hydrolyses a number of aliphatic amides and also transfers the acyl moiety to hydroxylamine to form the corresponding hydroxamate[5,6]. The enzyme can be induced by adding acetamide, one of its substrates, to exponentially growing cultures. A few amides, including *N*-acetylacetamide, are inducers but not substrates. Other substituted amides including cyanoacetamide prevent enzyme induction by both substrate and non-substrate inducers. We have shown that in exponentially growing cells this effect of cyanoacetamide can be reversed by increasing the concentration of either acetamide or *N*-acetylacetamide[6]. The relative concentrations of these amides giving enzyme induction or repression under these conditions suggested that they all acted at the same site in the cell and that the affinities for binding at this site were in the order acetamide > cyanoacetamide > *N*-acetylacetamide. The amidase is also subject to catabolite repression, and we examined the effect of increasing the inducer concentration under conditions similar to those used for the experiments on amide analogue repression. We were able to show that with acetamide as inducer, catabolite repression by propionate could be partially relieved by increasing the concentration of acetamide. There are serious disadvantages in attempting to examine this phenomenon in exponentially growing cells. The concentration of carbon compounds required in the medium to give a rapid growth rate is such that there is likely to be a high concentration of metabolities in the cells, and in particular, the conditions are favourable for producing a high concentration of catabolite repressor molecules. We have therefore re-examined amide analogue and catabolite repression with cells the growth of which has been limited by the supply of carbon compounds in order to deplete the cell carbon pool.

Amidase synthesis is induced by acetamide or *N*-acetylacetamide in carbon-starved cells with little or no lag and continues for at least 1 h. Fig. 1 shows the results of experiments to determine the effect of cyanoacetamide on the rate of induction by *N*-acetylacetamide under these conditions. The induction rates were measured for *N*-acetylacetamide alone, and at two different concentrations of cyanoacetamide. The reciprocal of the rate of enzyme synthesis is plotted against the reciprocal of the inducer concentration. The graph obtained is similar to a Lineweaver–Burke plot for competitive inhibition of enzyme activity by substrate analogues. These results support the conclusions drawn from the experiments with exponentially growing cells that amide analogues compete at the same site in the cell. From the data of Fig. 1 a K_{Inducer} (the concentration of inducer giving half-saturation of the induction system) was calculated. For *N*-acetylacetamide the K_{Inducer} was approximately 10^{-3} M. In the same way, the 'inhibitor constant' for cyanoacetamide repression was calculated to be approximately 5×10^{-5} M.

Fig. 2 gives the results of a similar experiment in which *N*-acetylacetamide is used as the inducer and succinate as the catabolite repressor. The graph shows that succinate repression is also dependent on inducer concentration, but the relationship in this case is not that of a simple competitive inhibition. It seems reasonable to conclude that repression by succinate is different in character from that produced by cyanoacetamide. We have not yet determined whether there is a single specific catabolite repressor for this system. Propionate was also examined under these conditions and gave results similar to those obtained with succinate.

One possible site of action of both amide analogue and catabolite repressors is at the point of entry of amides into

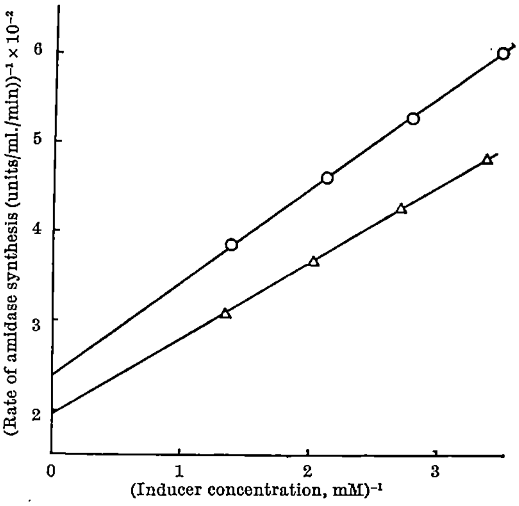

Fig. 2. Effect of succinate on the rate of amidase induction by *N*-acetylacetamide. Experimental conditions as for Fig. 1 with succinate replacing cyanoacetamide. ○, 10^{-2} M succinate; △, No succinate

the cell. This possibility was examined by measuring the uptake of *N*-acetylacetamide labelled with carbon-14 by exponentially growing cells under conditions allowing enzyme induction in the absence of added repressors. Cyanoacetamide at a concentration of 10^{-2} M completely repressed amidase induction by 5×10^{-3} M N-acetylacetamide and had no effect on the uptake of labelled *N*-acetylacetamide. Amidase induction by 10^{-3} M *N*-acetylacetamide was almost entirely repressed by 10^{-2} M succinate. There was no significant effect on uptake of labelled *N*-acetylacetamide. It seems clear that neither cyanoacetamide nor succinate represses the synthesis of this enzyme by preventing the entry of inducers into the cell.

The inducer of an enzyme is thought to react with the cytoplasmic repressor molecule and thereby release the structural genes from repression. For theoretical reasons the cytoplasmic repressor is likely to be a protein and there is some experimental support for this view[7]. The specificity of the cytoplasmic repressor for its inducer would be expected to be similar to the specificity of an enzyme for its substrate. In these terms we have no difficulty in accounting for cyanoacetamide repression of enzyme induction, by both substrate and non-substrate inducers, as direct competition for the inducer site of the cytoplasmic repressor. The site of action of catabolite repression is more difficult to identify, but it could also be the cytoplasmic repressor. One possible interpretation of the kinetic results given in Fig. 2 would be that succinate represses induction by reacting with the cytoplasmic repressor at a site other than the inducer binding site. This would be essentially the same phenomenon as the allosteric interactions between an enzyme and its substrate and feed-back inhibitors[8]. Catabolite repressors would then be expected to combine with the cytoplasmic repressor in such a way that its repressing activity was increased, and the inducer to combine with it in such a way that its repressing activity was decreased. This could happen if combination of the cytoplasmic repressor with the catabolite repressor increased its affinity for the operator and combination with the inducer decreased it. On this basis the mechanisms of action of the cytoplasmic repressors of inducible and repressible enzymes become almost identical. Catabolite repression is widespread among inducible enzymes but in a few cases only has the possibility of reversal by increased inducer concentration been examined[3,4,9]. If conditions had been used in which the concentration of compounds in the metabolic pool had been severely reduced it might have been possible to show some effect of inducer concentration on catabolic repression. In this connexion it is perhaps significant that the concentration of isopropyl-β-D-thiogalactoside required for the maximum rate of induction of β-galactosidase is $1{\cdot}4 \times 10^{-3}$ M in glucose medium and only 5×10^{-4} M when glycerol is used as the carbon source[10]. The antithesis of catabolite repression of inducible enzymes would be metabolite induction of repressible enzymes. An example of this is ornithine transcarbamylase which is repressed by arginine and induced by glutamic acid[11].

The first recognizable event in enzyme induction is the production of a specific messenger RNA, and this has been demonstrated by isolating enzyme specific DNA RNA hybrids from several inducible enzyme systems[12]. Nakada and Magasanik[13] have shown that induction of β-galactosidase and catabolite repression by glucose operate at the stage of synthesis of the specific messenger RNA. However, Loomis and Magasanik[10] rejected the hypothesis of allosteric interactions of inducer and catabolite repressor with the cytoplasmic repressor. They examined catabolite repression by glucose under conditions in which it was reasonable to suppose that the cells did not contain any *i* gene product. Since $i^-_{\text{del}}\ z^+$ mutants were not available they used mutants with a complete Lac region deletion, which were $i^-z^-y^-$, as F^- recipients, and examined the effect of glucose on the constitutive phase of β-galactosidase synthesis which occurs during the first hour after gene transfer[14]. There was marked repression by glucose, but it is by no means certain that *i* gene products were absent. The period of constitutive enzyme synthesis after i^+z^+ transfer represents a very complex situation. The simple explanation is that the *z* gene can express itself immediately but the *i* gene can only do so after some time. Loomis and Magasanik[10] point out that the *i* gene product is probably also made immediately, and the constitutive enzyme synthesis covers the time required for the cytoplasmic repressor to reach an effective concentration. If this were the case, then during the early stages, when the concentration of the cytoplasmic repressor was very low, any factor which increased its repressing activity, such as combination with a catabolite repressor molecule, would repress enzyme synthesis. We suggest that to resolve the problem of catabolic repression far more quantitative data are required on the interactions of inducers and catabolite repressors.

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EVOKED RESPONSES TO NUMERICAL AND NON-NUMERICAL VISUAL STIMULI WHILE PROBLEM SOLVING

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EXPERIMENTS attempting to deal with the relation between evoked responses and 'meaningfulness' may be classified into the following categories: (1) distraction procedures where evoked responses to test stimuli are compared with and without 'distraction stimuli'[1]; (2) attention procedures where the subject is instructed to attend to some stimuli and not to others[2]; (3) habituation and learning procedures[3]. Experiments using these procedures have shared three common difficulties: (1) assurance of the 'meaningfulness' of the stimuli to the subject, (2) separation of effects due to general states of the organism, such as activation-level, (3) adequate controls for the physical stimulus reaching the receptors[4].

The present experiment introduces a new procedure. Subjects were instructed to solve simple problems that required the perception of numerical visual stimuli presented

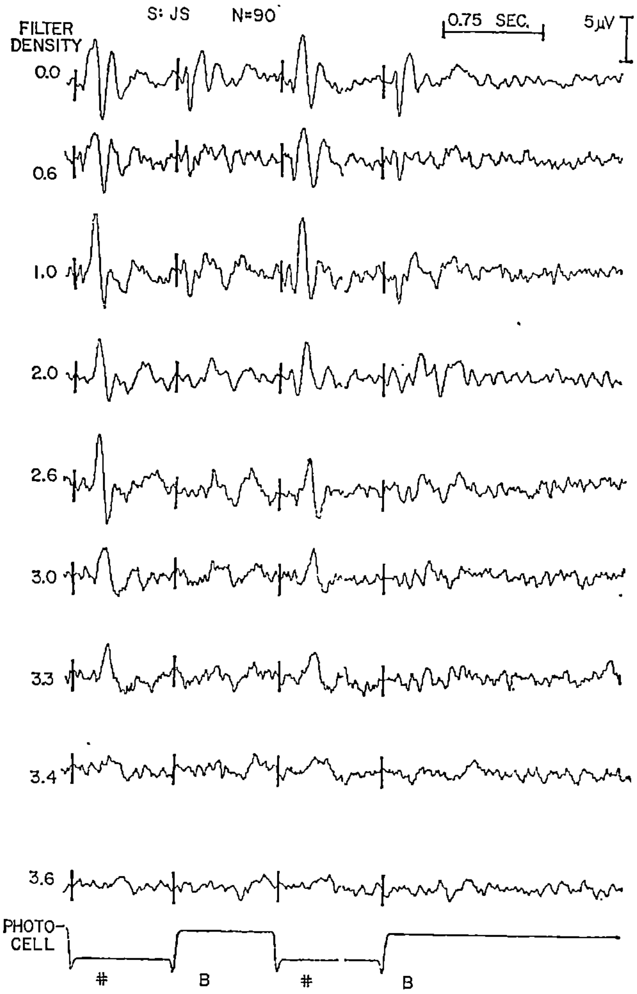

Fig. 1. Evoked responses to numerical (# = number) and non-numerical (*B* = blank) visual stimuli of various luminances while the subject is using the numbers. Positive at the central-parietal electrode is up in the evoked response. The photocell average shows the relative energy of the number and blank stimuli. Relative luminance of runs indicated by neutral filter density; blank at 0·0 filter density was 7·6 ft. lamberts

in sequence. Other visual stimuli, irrelevant to solving the problems, were a regular part of the stimulus sequence. Electrical responses evoked by these stimuli and the subjects' performance were recorded. Differences in evoked responses were found that may not be attributed to the physical energy of the stimuli but are related to their meaningfulness in the sense of their task relevance.

The stimuli consisted of white luminous figures presented every 3/4 sec in sequence, for example, number, blank, number, blank. Bina-View decoder display units (Industrial Electronic Engineers, Inc.) were automatically controlled by a paper tape reader driven by a synchronous motor through a gear train. Sync pulses also were derived from the punched tape. The subject viewed two adjacent windows (36 × 33 mm) at a distance of 160 cm. In the left window appeared the stimulus sequence. In the right window, covered by a green filter, appeared the answer stimuli. The blank occurred when no figure mask interposed between the stimulus light and the window of the display unit and appeared as a bright, blank field. During the time when a number or plus was not being presented, the blank was maintained at both windows. For some of the data (for example, Fig. 2) a different light source made the problem stimuli approximately 1 log unit more intense. The maximum luminance of the blank stimulus was 76·4 ft. lamberts measured by a MacBeth illuminometer. The subject's room was dimly illuminated (< 0·01 ft. lamberts). The luminance was controlled by Wratten neutral density filters placed in front of both windows (for example, Fig. 1). The subject's task was to say which of the two numbers was numerically smaller, 'first' or 'second', or whether they were 'even'. Then the correct answer was presented as a green luminous figure. This sequence constituted a trial lasting 7·5 sec. Each stimulus was a brief darkening (0·04 sec) followed by the new figure which was maintained until the next stimulus occurred (Fig. 1, photocell trace). Ninety trials constituted an uninterrupted run (11¼ min). During each run the numbers (0–9) were randomized and the sequence of numerical and non-numerical stimuli was constant.

The evoked responses were obtained by bipolar recording from EEG silver disk electrodes fixed to the scalp on the midline 2·5 cm below the vertex and 2·5 cm above the inion[5] with the ground electrode attached over the mastoid. Eye movements were monitored by electrodes placed in the external canthis and below one eye. Other kinds of activity were recorded on additional channels at various times. After suitable amplification the evoked responses were revealed by computer averaging of the activity during 90 trials. The electrical activity was amplified by a Grass Model 6 EEG machine and recorded on *FM* magnetic tape. The computer system was designed by W. Kropfl. The sampling rate was 8 msec/sample for each channel. The computer (Packard-Bell 250) was programmed to average 1536 samples over the 90 trials and to graph the average waveforms on an X–Y plotter. The low frequencies were limited only by the EEG machine which was set to be 20 per cent down at 5 c/s. High-frequency muscle activity was scarcely ever obtained from the evoked response sites.

Representative evoked responses from one of the subjects are shown in Fig. 1. Each of the stimuli in the trial sequence evoked an average response. The evoked responses were consistently different to the number and to the blank stimuli. Although the physical intensity of the blanks was approximately 4 times greater, the responses to the numbers were larger. This effect held over a considerable range of luminances (2,000 to 1, Fig. 1).

With all neutral filters from 0·0 down to 3·3 this subject answered 100 per cent of the problems correctly. His accuracy dropped to 58 per cent correct at a density of 3·4 and 50 per cent at 3·6. This was the luminance region for threshold of number recognition. Appropriately this was the luminance region where the evoked responses abruptly reduced in amplitude. In the luminance range where the subject performed with 100 per cent accuracy (0·0–3·3), the size and waveform of the evoked responses were controlled to some extent by the light luminance. Generally as the luminance was decreased the amplitude decreased, latency increased and the waveform simplified. However, the amplitude of the responses to the number stimuli did not decrease appreciably until the recognition threshold was reached. In marked contrast, the amplitude of the responses to the blank stimuli decreased rapidly with lowered luminance. From this comparison it appears that the amplitude of the evoked response is only weakly controlled by the physical luminance of the visual stimulus. The waveforms of the responses to number and blank stimuli are different and the differences may not be attributed to the stimulus luminance. Data from monopolar recording show that the larger components of these evoked responses come from the central-parietal site rather than the occipital site.

The main finding appears to be general. Larger evoked responses were obtained to number stimuli than to blank stimuli in all persons tested (3, Fig. 2; another, Fig. 1).

A variety of control runs have been made, some of which are illustrated in Fig. 2. The effect of a different irrelevant stimulus and different sequences of stimuli are illustrated in the top 3 lines. A plus sign (+) was used because it was similar to the number stimuli in having approximately the same luminance and general meaning by virtue of the subject's language history. When a plus

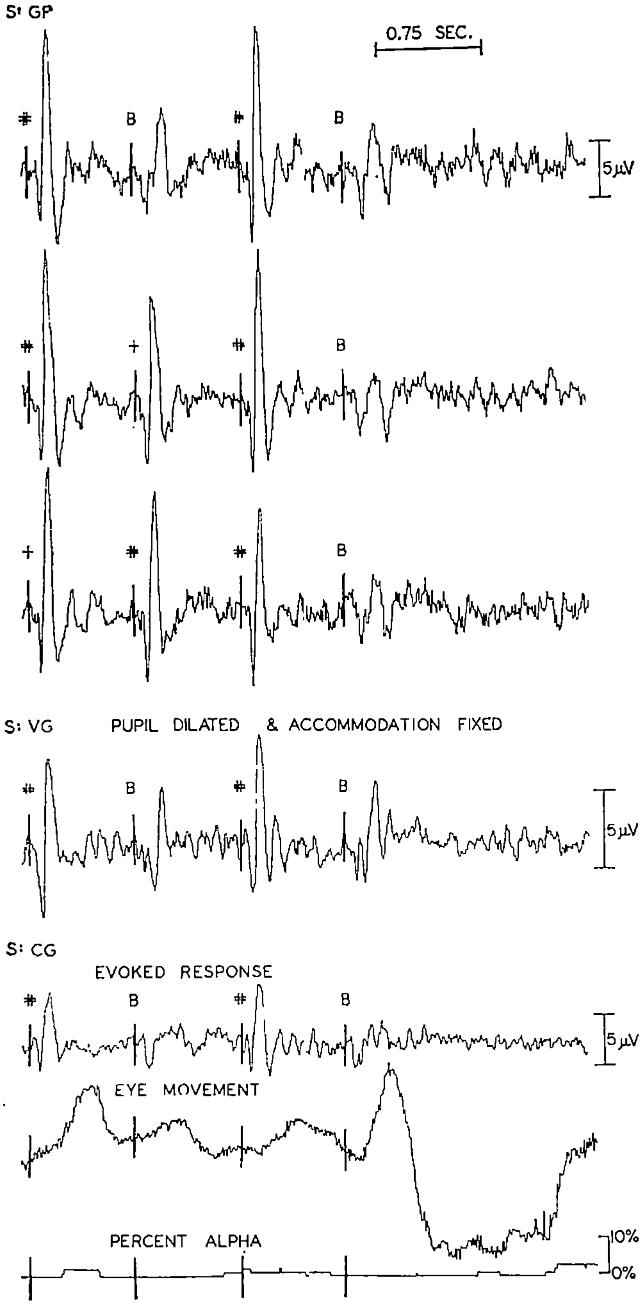

Fig. 2. Evoked responses from three subjects illustrating control runs. Blank luminance for *GF* and *VG* was 76 ft. lamberts; for *CG*, 7·6 ft. lamberts

was used in the second position in place of a blank (line 2), the response tended to be larger and have a waveform more like the number response. Nevertheless, the response to the irrelevant plus was smaller than the response to the relevant number. When a plus was used in the first position (line 3), its response was larger. It is suggested that its task relevance is increased in this position in that it signifies the start of each trial.

The differences in evoked responses may not be attributed to changes in pupil size or accommodation, since the middle section of Fig. 2 was recorded with a cyclopegic drug. These monocular data also show that binocular viewing is not necessary.

The differences in evoked responses may not be attributed to eye movements, which were monitored by averaging the electro-oculogram. Eye movements occurred but were not consistently correlated with the size or waveform of the evoked responses (compare evoked response and eye movement lines at the bottom of Fig. 2). Eye movements were reduced during the stimulus sequence by training the subjects to fixate the centre of the window and to delay their verbal answer after the end of the stimulus sequence.

The differences in evoked responses may not be attributed to activation-level as indicated by amount of alpha rhythm in the EEG. The electrical activity which was averaged to obtain evoked responses was also delivered to an electronic scorer of alpha rhythm[6]. Each alpha burst above 15 μV was indicated by a voltage step which was averaged to indicate the course of alpha activity across the trial. Fig. 2 shows a case where the alpha activity was more frequent during the number stimuli than during the blank stimuli. Other subjects have shown the opposite time pattern of alpha activity and still others have had negligible alpha activity. Thus, the pattern of alpha activity varied considerably whereas the differences between evoked responses to number and blank stimuli were quite consistent. The experimental design also makes it unlikely that the evoked response differences are due to changes in general activation-level, since the relevant and irrelevant stimuli change every 3/4 sec within each stimulus sequence.

During some control runs the electroretinogram (ERG) was recorded with a contact lens electrode[7]. In contrast to the evoked responses, the ERGs were not smaller to the bright blank than to the number stimuli. These data indicate that the differences in central-evoked responses between number and blank stimuli are not due to peripheral mechanisms.

These findings may be explained by a variety of hypotheses.

Several features of this experiment bear emphasizing. Control stimuli (blank and plus) were used in every averaging run by making them a regular part of each stimulus sequence. Since they occurred in temporal proximity with the number stimuli, they rule out the possible role of any longer term changes, they save experimental time, and they avoid the problem of what the subject should do during control runs. In this procedure, the subject is busy with the numbers and presumably does not have time for extraneous activity during the control stimuli. The subject is required to do something with the stimuli and his performance is readily scored. Obtaining evoked responses from visual form stimuli such as numbers leads to many interesting experiments dealing with cognitive processes, perception, learning, etc. The use of meaningful stimuli and subject performance might also be a useful technique to reduce variability in experiments purporting to deal with sensory processes.

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LETTERS TO THE EDITOR

PHYSICS

The Temperature Scale

THE proposal made by Prof. J. C. Georgian[1] is in my view unsatisfactory in so far as it involves not only a change of unit but a change in the definition of the quantity, temperature. Temperature is a macrophysical quantity and confusion arises if it be given a pseudo-microscopic redefinition.

It would, however, be possible and unobjectionable were there to be defined a new unit of temperature, say 'the Georgian' with the unit symbol Geo, such that the molar gas constant became 1 J/kmole Geo. This would achieve many of the aims of Prof. Georgian without involving a redefinition of the quantity.

One must, however, note that a system involving a new unit of temperature, say, the Boltzmann with a unit symbol Bol, such that the molar gas constant became 1 J/molecule Bol, might prove to be even more attractive.

In my view, however, a more radical reform should be made. It would be appropriate to make entropy a primary quantity and temperature a derived quantity. I would introduce a unit of entropy, say the carnot with a unit symbol Car, such that the molar gas constant became 1 Car/kmole. The unit of temperature would then be the J/Car.

This change would lead to relations between temperature and entropy which were symmetric relative to the relations between pressure and volume or force and distance. When considering pressure and volume it has long proved convenient to regard thermodynamic pressure as defined as minus the derivative of internal energy with respect to volume at constant entropy. According to my scheme, one would define (thermodynamic) temperature as the derivative of internal energy with respect to entropy at constant volume. This is indeed used in many presentations of thermodynamics, but the traditional scheme of units in which temperature is regarded as primary and entropy as derived militates against a clear understanding of this approach.

Whatever may eventually be done about changes in this field it cannot in my opinion be too strongly stressed that these macroscopic quantities should retain definitions which do not confuse them with related but non-identical microscopic quantities. It is from such a confusion that the controversy about certain electromagnetic quantities stemmed.

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[1] *Nature*, 201, 695 (1964).

I BELIEVE Mr. Le Fevre's objections are due to the long-term habits of considering temperature measured by a degree (centigrade, Celsius, Kelvin, Fahrenheit, Rankine, etc.); hence, he feels it is necessary that the temperature should have some type of unit name. There is, of course, nothing wrong with this since, for example, we call the unit of energy, newton-metre, a joule, and power a newton-metre/second or joule/second, a watt. I am only proposing that the temperature be called by what it actually represents, that is a joule/kilomole, in the same manner that pressure is a newton/square metre.

While I appreciate Mr. Le Fevre proposing calling the new unit in my temperature scale a Georgian, I think that, if he feels it necessary to name this new unit, I suggest calling it a degree Joule, as it most closely names the temperature dimension of joule/kilomole. I maintain, however, that the molar gas constant can be dispensed with, as the temperature unit in the ideal gas law is determined from the fundamental M.K.S. units.

I suspect Mr. Le Fevre is also objecting to the idea that temperature has the dimensions of L^2/T^2 and feels that temperature needs a separate dimension. The ideal gas law shows from a macroscopic or phenomenological point of view that temperature is a measure of energy and on a specific energy basis has the units of L^2/T^2. On the other hand, from the microscopic or kinetic theory of gases it is shown that:

$$\overline{c^2} = \frac{3\theta}{M} \qquad (1)$$

where $\overline{c^2}$ is the mean square velocity of the molecules; M is the molecular mass number (molecular weight) of the gas; θ is the absolute temperature.

Equation (1) indicates that the temperature is directly proportional to the mean square velocity of the molecules in an ideal gas. Consequently, either from the phenomenological or statistical theory of gases we find that temperature is indeed an energy unit with the dimensions of L^2/T^2.

Only by understanding clearly that temperature is a measure of energy do we see that entropy from its definition is nothing but a dimensionless quantity which, however, is of great convenience and utility in thermodynamics. In addition, the probability theory of entropy shows clearly that entropy is a dimensionless quantity. Entropy is given by the expression:

$$s = k \log_e W + s_0 \qquad (2)$$

where now Boltzmann's constant, k, equals $1\cdot6604 \times 10^{-27}$ kilomole in the M.K.S. system with $\bar{R} = 1\cdot0$, and W is the probability. Therefore, Mr. Le Fevre's idea of introducing entropy as a primary quantity is extremely objectionable as he is attempting to force an essentially dimensionless quantity into some type of unit.

I believe that the real confusion has arisen from the fact that temperature has not been clearly defined as the energy unit it is, with the consequent proliferation of large numbers of extraneous units. Finally, the original proposal was to place temperature in the M.K.S. system of units and, hence, relieve scientific and engineering calculations of the unnecessary burdens of conversion factors.

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Stress Waves in a Liquid Column

EXPERIMENTS have been carried out in which stress waves are propagated upwards through a vertical liquid column. For this purpose the apparatus previously described by me[1] was used except that a piston of shorter length (3 cm) was used.

Briefly, the liquid under test was contained in a vertical steel cylindrical tube fitted with a steel piston at its lower end and the upper end open. A positive pressure wave was generated in the liquid by firing a lead bullet of 0·22-in. calibre so as to strike the lower end of the piston normally at its centre. When this pressure wave reached the upper free surface it was reflected as a wave of tension. The pressure changes in the liquid were recorded by means of a horizontal steel pressure bar inserted into a 'porthole' below the liquid surface.

Records using ordinary tap water showed that tensions of the order of 7 atm. could be generated in the liquid. It was then decided to introduce gas nuclei into the water in order to examine their effect on the wave propagation. This was first done by using fresh 'soda water' in the tube;

the results were the same as those using tap water, that is, a tension wave could be transmitted. Secondly, a number of experiments were performed by arranging for a steady stream of small nitrogen bubbles to rise in ordinary tap water past the inner end of the pressure bar. The rate of production of these bubbles could be varied from about one bubble per sec to a fast turbulent rate. With a slow rate of bubble production the results were like those for ordinary water. With a fast bubble rate, however, the records showed that no stress wave, either of compression or of tension, was propagated.

In another type of experiment the effects accompanying wave propagation across a plane interface between two liquids which do not mix were investigated. For this purpose water occupied the tube to a level of 5 cm above the pressure bar and about 50 cm^3 of lubricating oil were then added. In this way records of events in the water at 5 cm below the water–oil interface were obtained. One would expect the upward wave of compression to be transmitted across this interface, but the behaviour of the downward wave of tension is not as predictable. The results showed, however, that a tension wave was propagated downwards.

It is hoped that further experiments on these lines will shed more light on the 'cavitation threshold' of liquids, that is, on the maximum tension that can be built up before cavitation sets in.

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GEOPHYSICS

Collision Frequency in the Ionosphere and Solar Activity

IN a recent publication, Belrose and Hewitt[1] have produced evidence for a variation in collision frequency with solar activity in the lowest ionosphere (at about 60 km height). In this communication we direct attention to similar results which we have obtained for a somewhat higher altitude (about 110 km).

In 1960–62 we carried out a series of vertical incidence absorption measurements using pulse signals on frequencies in the range 1·9–4·0 Mc/s. The measurements were made near local noon and at this time the absorption on 1·9 Mc/s was always predominantly 'non-deviative' (*D*-region). Simultaneously with those on 1·9 Mc/s, absorption measurements were also made on 2 frequencies just below, and 2 frequencies just above, the normal region *E* critical frequency (*foE*). At these latter frequencies the observed absorption was predominantly of the 'deviative' type associated with considerable group retardation. Expanded scale ionograms were available at the times of these measurements and from these the appropriate *E*-layer electron density/height profiles were calculated. These $N(h)$ profiles and the experimental measurements of absorption on the 5 frequencies were then used to calculate a best-fit collision frequency/height profile. Reliable results were obtained on 47 days during the whole period of the measurements, and it was found that theory and experiment could only be satisfactorily reconciled if the collisional frequency at *E*-layer heights varied by quite a large factor from day to day.

Fig. 1 shows the daily values of ν at a true height of 110 km, deduced from this work, plotted against the 10-cm solar radio wave flux. It will be seen that there is a fairly clear positive correlation between the two parameters. The slope of the best line to fit in Fig. 1 represents a change of about 1 per cent in ν at 110 km per unit change in the flux φ. We consider that part of this apparent change in ν with solar activity may be due to a change in *D*-region ionization with solar activity and the true change is probably smaller than the figure quoted.

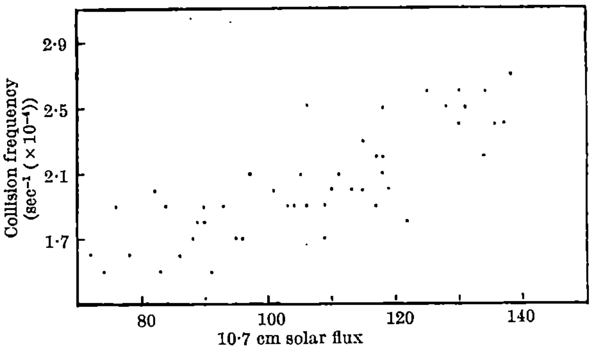

Fig. 1. Variation of collision frequency at 110 km with the 10·7 cm solar flux

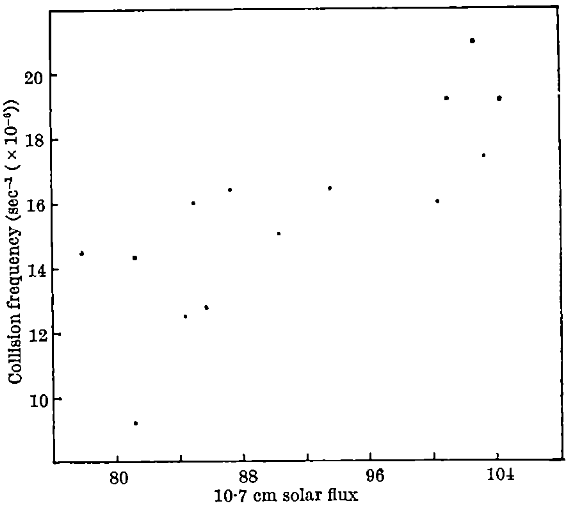

Fig. 2. Variation of collision frequency at 59·5 km with the 10·7 cm solar flux (ref. 1)

In Fig. 2 the values of ν given by Belrose and Hewitt for a height of 59·5 km are also plotted against 10 cm solar flux. The rate of increase of ν at this height is about 2 per cent per unit change in φ. The first published evidence for a dependence of ν on solar activity was given by Schlapp[2], and his results indicate that at a height of about 110 km ν changes by rather less than 1 per cent per unit change in φ.

Some data on collision-frequency measurements made in India have been published by Ramana and Rao[3] and provide supporting evidence for this dependence of ν on solar activity. These workers give values of ν at the peak of the *E* layer for each month in the period March 1958–November 1959. We have made some first-order corrections to these results to give estimates of ν at a fixed height, and find that for an altitude of about 105 km there is again evidence for an increase in collision frequency with solar activity of the same order of magnitude as that deduced here.

There are thus three independent sets of data showing a significant positive dependence of the *E*-layer electron collision frequency on solar activity. Various authors have suggested modifications of the usual Appleton–Hartree magneto-ionic theory to take account of the dependence of the collisional cross-section on the electron energy. Consideration of the collision frequencies deduced from the present work indicates that at 110 km the electron temperature at noon may exceed the neutral gas temperature by a factor of about two.

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Disappointing Interim Palæomagnetic Results from the Seychelles

RECENT seismic refraction experiments made in connexion with the International Indian Ocean Expedition have confirmed that the shallow Seychelles Bank has crustal structure of a continental type and represents an isolated continental fragment in the middle of the north-west Indian Ocean[1,2]. The late Precambrian granites exposed on the central islands of the Seychelles group have been shown to be cut by two sets of dolerite dykes which are of a Precambrian and early Tertiary age, respectively[3,4].

Du Toit and other writers have suggested that the Seychelles represent a fragment left behind during the break-up of Gondwanaland[5], and it is clear that this hypothesis is now in need of testing. Accordingly, in November 1963 (ref. 2) we collected 71 orientated specimens from 40 different dykes, sampling all the more accessible dykes shown on Mr. B. H. Baker's geological map[4] on the islands of Mahé, Praslin, Cousin, La Digne, Felicité and Marianne. The natural remanent magnetization directions of these specimens have been measured by one of us (T. A. R.) using an astatic magnetometer in Nairobi. The results are widely scattered and strongly suggest that the rocks are unstable. It is to be hoped that the a.c. demagnetization and thermal 'washing' techniques, which are now being tried, will reveal a significant grouping of the magnetic vectors.

This disappointing preliminary result would certainly not warrant publication in the normal course of events. However, the Seychelles are of current interest in connexion with the International Indian Ocean Expedition, and as this experiment is a particularly obvious one, we imagine that other groups are likely to consider going to these remote islands to collect palæomagnetic material. We wish them better luck than we have had.

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Heights of Central Absorbing Regions for a Strong Auroral Absorption Event at Lower Latitude

ABNORMAL ionospheric absorption of cosmic radio noise has been investigated at Moscow (46° 44′ N., 117° 00′ W.), Idaho, U.S.A., since September 1962. Observations are being made by three riometers operating at the frequencies of 20, 30 and 40·25 Mc/s.

The majority of abnormal absorption events obtained occurred prior to the International Years of the Quiet Sun. Five types of absorption were positively identified. They are sudden commencement absorption, sudden cosmic noise absorption, auroral absorption, sunset absorption and sunrise absorption.

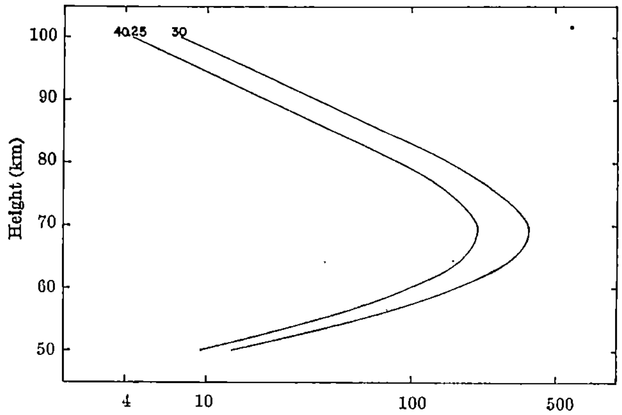

Fig. 1. Relaxation time as a function of height

One phase of the investigations included height determinations of the central absorbing regions for a strong auroral absorption event at lower latitude. This communication reports the heights of central absorbing regions for the strong auroral absorption events of September 22, 1963, which were deduced by utilizing the relaxation time method introduced by Ziauddin[1]. This method relies on the fact that the absorption (expressed in db) suffered by a radio wave in the lower ionosphere is proportional to the number of electrons encountered in the absorbing region and is a function of the collisional frequency of the electrons.

The electronic collisional frequencies, ν at various heights, were calculated from the following formula given by Nicolet[2]:

$$\nu = 5{\cdot}4 \times 10^{-10}\, nT^{1/2} \qquad (1)$$

Height distributions of n (number density of the atmospheric constituents) and T (atmospheric temperature) were adopted from the *U.S. Standard Atmosphere*, 1962 (ref. 3), which is an idealized, middle-latitude year-round mean over the range of solar activity between sunspot minimum and maximum. The calculated values of ν are given in Table 1.

Following Ziauddin's assumptions, the effective electron densities at various heights for the observing frequencies of 30 Mc/s and 40·25 Mc/s were determined from the following well-known equation:

$$A(\text{db}) = 0{\cdot}46 \int \frac{N\nu}{(w + w_2)^2 + \nu^2}\, dS \qquad (2)$$

The symbols used in equation (2) have usual meanings.

The effective electron densities at various heights were then combined with the corresponding recombination rates α given by Moler[4] to calculate the ionospheric relaxation times ($T = 1/2\alpha N$ sec) at various heights. The calculated relaxation times were plotted as a function of height in Fig. 1. Figs. 2 and 3 respectively show simultaneous strong auroral absorption events of September 22, 1963 (120° W.M.T.), observed at the frequencies of 30 Mc/s and 40·25 Mc/s. Heights deduced from Fig. 1 for 30 Mc/s events are in the range of 79–86 km, while for 40·25 Mc/s they are in the range of 72–78 km. The 40·25 Mc/s waves were absorbed at lower heights than the 30 Mc/s waves as predicted by magnetoionic theory. For 30 Mc/s events, heights of central absorbing regions are somewhat higher than those reported at higher latitudes[5]. For both 30 and 40·25 Mc/s events, there was a tendency for successive absorption onsets to progress toward lower heights. Ziauddin and Forsyth[5] also found a similar tendency.

It should be noted that the relaxation-time method is subject to certain criticisms, but for a first approximation

Table 1. THE ELECTRON COLLISIONAL FREQUENCY

Height (km)	50	55	60	65	70	75	80	85	90	95	100
ν (sec^{-1})	1·90^{8}*	1·03^{8}	5·48^{7}	2·90^{7}	1·45^{7}	6·91^{6}	3·01^{6}	1·19^{6}	4·77^{5}	1·91^{5}	8·14^{4}

* The form of $N \times 10^{m}$ is given in the form of N^{m}.

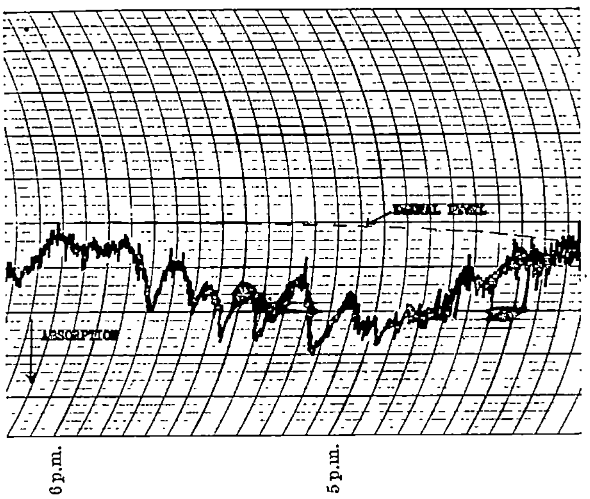

Fig. 2. The 30-Mc/s riometer record during the strong auroral absorption event of September 22, 1963 (120° W.M.T.)

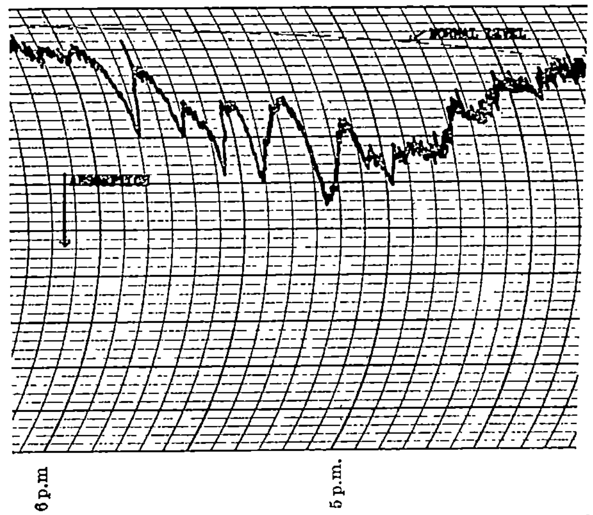

Fig. 3. The 40·25 Mc/s riometer record during the strong auroral absorption event of September 22, 1963 (120° W.M.T.)

it serves to yield information on heights of central absorbing regions for selected events.

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GEOCHEMISTRY

Content of Lead in the Earth's Initial Chondrite-like Oxide Mass and the Partition Coefficient of Lead for Crystallization of Silicate Melt

From the isotopic composition of lead in ores, the following empirical formula was obtained[1]:

$$\log (C_U/C_{204}) = 0{\cdot}503 \log (C_{Th}/C_{204}) + 0{\cdot}231 \qquad (1)$$

where C_U, C_{Th} and C_{204} stand for the weight concentration of uranium, thorium and lead-204 in the material of source from which common leads of ores were derived. The purpose of this communication is to treat this formula from the aspect developed in recent investigations[2-4] of the geochemistry of lanthanides and to extract some numerical information therefrom.

The same reasoning and mathematical treatment as presented in discussion[2-4] of lanthanide geochemistry will lead to the following theoretical formulæ by analogy.

$$\log C_{Th} = \log C^0{}_{Th} - (1 - k_{Th}) \log f_w \qquad (2)$$

$$\log C_U = \log C^0{}_U - (1 - k_U) \log f_w \qquad (3)$$

$$\log C_{Pb} = \log C^0{}_{Pb} - (1 - k_{Pb}) \log f_w \qquad (4)$$

where C_{Th}, C_U and C_{Pb} are the concentrations of thorium, uranium and lead in a liquid-type material such as basalt, $C^0{}_{Th}$, $C^0{}_U$ and $C^0{}_{Pb}$ are the concentrations of thorium, uranium and lead in average chondritic oxide mass which is assumed to be nearly equal to the source material of the Earth's oxide sphere, k_{Th}, k_U and k_{Pb} are the partition coefficients for thorium, uranium and lead, and f_w the value of liquid fraction for the concerned material. These equations (2), (3) and (4) are derived from a rather rough, but necessary, assumption that the partition coefficients of minor elements between the just-formed crystals and the coexisting silicate melt are constant during the whole process of solidification of melt. Therefore, the partition coefficients to be obtained from the foregoing equations should be regarded as mean values for the overall process.

From equations (2), (3) and (4), we get:

$$\log (C_{Th}/C_{Pb}) = \log (C^0{}_{Th}/C^0{}_{Pb}) - (k_{Pb} - k_{Th}) \log f_w \qquad (5)$$

$$\log (C_U/C_{Pb}) = \log (C^0{}_U/C^0{}_{Pb}) - (k_{Pb} - k_U) \log f_w \qquad (6)$$

Eliminating $\log f_w$ from equations (5) and (6) leads to[7]:

$$\log (C_U/C_{Pb}) = b \log (C_{Th}/C_{Pb}) + \log (C^0{}_U/C^0{}_{Pb}) - b \log (C^0{}_{Th}/C^0{}_{Pb}) \qquad (7)$$

where:

$$b = (k_{Pb} - k_U)/(k_{Pb} - k_{Th}) \qquad (8)$$

Comparison of these theoretical formulæ (7) and (8) with the empirical one (1) provides the equations :

$$0{\cdot}503 = (k_{Pb} - k_U)/(k_{Pb} - k_{Th}) \qquad (9)$$

$$0{\cdot}231 = \log (C^0{}_U/C^0{}_{204}) - 0{\cdot}503 \log (C^0{}_{Th}/C^0{}_{204}) \qquad (10)$$

As the partition coefficients k_{Th} and k_U have been estimated[5] to be 0·0733 and 0·182, respectively, the partition coefficient k_{Pb} is calculated to be 0·292 from equation (9). On the other hand, since $C^0{}_U$ and $C^0{}_{Th}$ are 0·0168 (refs. 5 and 6) and 0·048 p.p.m. (refs. 5 and 7), respectively, $C^0{}_{204}$ is estimated to be 0·00199 p.p.m. from equation (10). As the present-day isotopic fraction of lead-204 relative to the whole lead is about 1/72, and the atomic weight of lead is about 207, the concentration of whole lead in the Earth's initial chondrite-like oxide material in reference to the present time can be evaluated to be 0·145 p.p.m. (= 0·00199 × 72 × 207/204). It is intriguing that this value coincides with the average content of lead, 0·15 p.p.m. (ref. 6), in ordinary hypersthene-bronzite chondrites. But both carbonaceous chondrites and enstatite chondrites have much higher concentrations of lead, 1·5–4·2 p.p.m. (ref. 6). Since carbonaceous chondrites are inferred to contain nearly the cosmic abundances[8,9] of elements excepting highly volatile ones, the abundance estimated here will indicate that the major part of lead on the Earth has been incorporated into the Earth's core. If the initial overall content of lead for the Earth was comparable with that of ordinary chondrites, the foregoing result would suggest that any selective enrichment of lead in the Earth's metallic core did not occur.

Finally, a comment can be added in connexion with the partition coefficient obtained above, 0·292, for lead. The volcanic rocks belonging to the pigeonitic rock series[10] of Izu-Hakone area, Japan, were suggested to be tholeiites and their immediate derivatives, and the volcanic rocks of the hypersthenic rock series[10,11] of the same area to be the products from high-alumina basalt through contamination. At this point let us review the data on isotopic ratios and concentration[11] of lead in the rocks of these

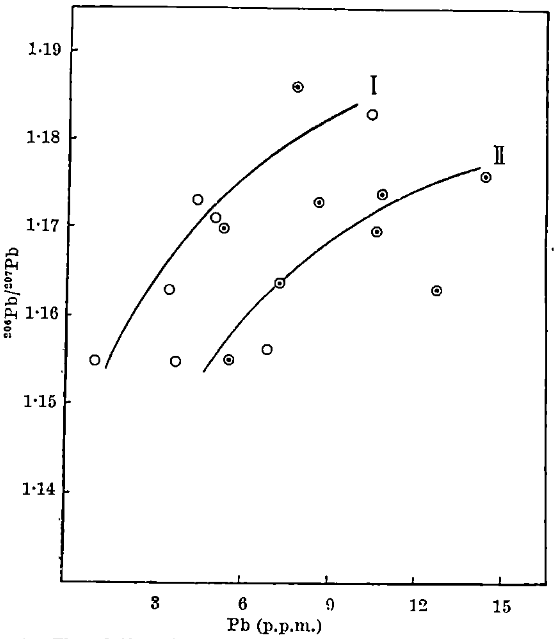

Fig. 1. The relationship between the isotopic ratio ^{206}Pb/^{207}Pb and the concentration of lead (ref. 11). ○, Pigeonitic rock series; ⊙, hypersthenic rock series

two series. It is noteworthy (see Fig. 1) that the concentration ratio of curve I to curve II for the same and smaller isotopic ratios ^{206}Pb/^{207}Pb appears to be roughly comparable with the partition coefficient estimated for lead. This suggests that the tholeiite and the high-alumina basalt could stand in the conjugate relation of solid and liquid (or crystal and melt) to each other, apart from the genetic sequence. Accordingly, this might give rise to the inference that the high-alumina basalt is a product of partial melting of the rock equivalent to tholeiite, or alternatively that the tholeiite is a product which was once separated as aggregate of crystals from the magma resembling a high-alumina basalt in composition. Since the thorium/uranium ratio, 4·0 (ref. 11), estimated from the isotopic composition of lead is nearly consistent with the ratios, 3·5–3·9 (ref. 12), actually observed in high-alumina basalts but in contrast to the corresponding ratios, 1·5–1·7 (ref. 12), observed in tholeiite, the latter inference gains support.

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Chlorine-36 produced by Neutron Capture in Meteorites

THE existence of the long-lived radionuclide chlorine-36 in iron meteorites, where it is produced by the spallation of the metal during its exposure to cosmic rays in space, is now well known. The measurements of cosmogenic ^{36}Ar and ^{36}Cl so produced permit one to calculate the so-called cosmic-ray exposure age of a meteorite. In this communication the results of experiments which show that in large iron meteorites the production of this nuclide also takes place via the reaction ^{35}Cl$(n,\gamma)^{36}$Cl on the lawrencite inclusions in the metal are presented. This result is of significance in deducing the history of meteorites from the investigation of cosmic-ray reaction products.

The experiments were carried out on a 2-kg slice of Canyon Diablo meteorite (U.S. National Museum No. 676) provided by Dr. E. P. Henderson. A 400-g piece from the metal was sawed off, cleaned, and dissolved in 8 M nitric acid in a closed system. ^{36}Cl was measured by techniques described earlier[1,2]. This measurement gave ^{36}Cl produced by both the spallation and the neutron-capture processes. For the next measurement, the main slice was cleaned in dilute nitric acid, and some chips were removed from it on a mechanical scraper using a tungsten carbide tool. These meteorite chips, weighing 248 g, were immersed in 1,000 ml. 0·5 M nitric acid and heated to boiling. The acid was decanted. Two more similar washings with fresh acid were carried out. From the combined washes silver chloride was precipitated and the ^{36}Cl was measured by the usual method.

Table 1. DATA ON ^{36}CL MEASUREMENTS IN CANYON DIABLO*

Specimen	Intact metal	Metal chips
Mass (g)	399·0	248·0
Natural Cl mass fraction (%)	0·0083	0·0357
Initial counts		
Chemical yield (%)	79·2	58·2
Background rate (c.p.m.)	0·228 ± 0·007	0·205 ± 0·007
Net sample rate (c.p.m.)	0·302 ± 0·019	0·085 ± 0·011
S (^{36}Cl) (d.p.m./kg)	3·21 ± 0·21	2·67 ± 0·34
Recycle counts		
Chemical yield (%)	52·6	49·2
Background rate (c.p.m.)	0·199 ± 0·007	0·182 ± 0·008
Net sample rate (c.p.m.)	0·183 ± 0·014	0·067 ± 0·014
S(^{36}Cl) (d.p.m./kg)	2·78 ± 0·21	2·45 ± 0·51
Average S(^{36}Cl) (d.p.m./kg)	3·0 ± 0·2	2·6 ± 0·3
Neutron-capture S(^{36}Cl) (d.p.m./kg metal)	0·6 ± 0·2	2·6 ± 0·3
Neutron-capture S_T(^{36}Cl) (d.p.m./g Cl)		7·3 ± 0·8
Spallation S(^{36}Cl) (d.p.m./kg metal)	2·4 ± 0·2	—

* S(^{36}Cl) = specimen specific ^{36}Cl activity.
S_T(^{36}Cl) = target-element specific ^{36}Cl activity.
c.p.m. = counts per minute.
d.p.m. = disintegrations per minute.

The experimental results are presented in Table 1. The standard deviations of the final results are somewhat larger than the statistical errors of counting because of various other sources of uncertainty; for example, non-uniformity of source deposition, etc.

The treatment of the metal chips with a total of 1·5 mol nitric acid should dissolve less than about 20 g of the specimen. The ^{36}Cl found in the washings of the chips is therefore not produced from the spallation of the metal. It is clearly associated with the easily washable natural chlorine, and is therefore produced in it by the (n,γ) process.

We assume that the production of ^{36}Cl by both the processes—spallation and neutron-capture—is uniform throughout our specimen, and calculate that the specimen specific activity of ^{36}Cl produced by spallation is $(2·4 \pm 0·2)$ d.p.m./kg metal, and that the ^{36}Cl produced by the neutron process is $(7·3 \pm 0·8)$ d.p.m./g Cl. Large variation in the ^{36}Cl activity within the slab is not expected, since the low specimen specific activity suggests that the specimen must have come from the deep interior of the meteoroid where the depth distribution of ^{36}Cl should be rather 'flat'[2].

Another radionuclide which is expected to be produced by the (n,γ) reaction is ^{59}Ni. Its target-element specific activity in our specimen is[3] $(0·30 \pm 0·07)$ d.p.m./g nickel. If both the nuclides ^{59}Ni and ^{36}Cl were produced by the capture of thermal neutrons, a value of 19 would be expected for the ratio $S_T(^{36}\text{Cl})/S_T(^{59}\text{Ni})$. The observed value is 24 ± 6. The agreement of the two numbers is probably accidental, since a complete thermalization of neutrons is not expected in even a moderately large meteoroid[4]. Detailed calculations on neutron production have been made for stone meteorites by Eberhardt, Geiss and Lutz[4]. These authors have also discussed the application of such investigations to the question of the size of the meteoroid and the problem of space erosion.

The presence of neutron-capture ^{36}Cl which can be washed away from the chips points to a possible source of uncertainty in calculations of the cosmic-ray exposure ages of iron meteorites. The experimental results that are used for those calculations are the ^{36}Ar concentration and ^{36}Cl specific activity in adjacent specimens. Generally the latter is measured by dissolving a compact mass and includes both the spallation and the neutron-capture ^{36}Cl. The ^{36}Ar is measured on a small metal chip after thorough cleaning. Any ^{36}Ar resulting from the decay of the neutron-capture ^{36}Cl would reside with the natural chlorine and would probably be removed during sample preparations. This would result in a low value for the ^{36}Ar concentration and hence in a low value for the apparent cosmic-ray exposure age of the meteorite.

I thank Prof. Truman P. Kohman for his advice. The meteorite was kindly donated by Dr. Edward P. Henderson. This work was supported by the U.S. Atomic Energy Commission contract *AT*(30–1)–844.

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METALLURGY

Oxygen Diffusion in Tungsten

Information concerning oxygen diffusion in tungsten is technologically valuable for many reasons. One such reason is its important role in the development of the structural properties of tungsten for high-temperature applications.

Because direct measurements are difficult, indirect assessments of oxygen diffusion in tungsten have recently been made, but the results seem somewhat confusing. For example, assuming the frequency factor Do as 10^{-2} cm²/sec, Stringer and Rosenfield[1] estimated the activation energy for oxygen diffusion in tungsten as 2·7 eV by using Cottrell's equation which was established on the basis of the Portevin–Le Chatelier effect. This value of activation energy agrees with those for oxygen diffusion in some transition metals in which the solubility of oxygen is smaller than 1 per cent. On the other hand, Jacobs[2] obtained a value of the frequency factor as 1·3 cm²/sec and the activation energy as 24,000 cal/mol for oxygen diffusion in tungsten by means of internal friction measurements. His results agree with those for oxygen diffusion in some transition metals in which the solubility of oxygen is greater than 1 per cent. Regardless of the empirical relation between diffusion and solubility for oxygen in transition metals, the two estimations yield serious disagreement on the rates of oxygen diffusion in tungsten.

The purpose of this communication is to deduce a diffusion coefficient of oxygen in tungsten at a convenient temperature from a direct measurement of the variation of oxygen concentration with time of diffusion and to compare with the estimations mentioned previously.

Examining the effect of the interstitial impurities on the recrystallization of tungsten, Allen, Maykuth and Jaffee[3] doped high-purity tungsten crystals with oxygen by means of a diffusion process. In their experiment, the boundary condition of the diffusion equation for diffusant from a constant surface concentration into an infinite cylinder is closely fulfilled. Although the experimental uncertainties due to the analytical procedure appeared for single crystalline specimens, the variation of the dopant concentration with time for polycrystalline specimens seems meaningful, that is, the average concentration of oxygen in tungsten is 33 p.p.m. for 6 h of diffusion at 1,700° C and 39 p.p.m. for 12 h at the same doping condition. Should the most reliable value of the solubility of oxygen in tungsten at 1,700° C be about 40 p.p.m.[3,4], the fractional saturation from the doping process is about 83 per cent for 6 h and 98 per cent for 12 h. Knowing the dimensions of the polycrystalline specimens, the diffusion coefficient of oxygen in tungsten can be estimated as 3×10^{-7} cm²/sec at 1,700° C. From the results of the single crystalline specimens, the diffusivity is calculated to have a value between 7×10^{-8} cm²/sec and 5×10^{-7} cm²/sec at the same temperature.

According to the Arrhenius relation for the temperature dependence of diffusivity, the diffusion coefficient of oxygen in tungsten at 1,700° C can also be assessed from the other two estimations of the activation energy and the frequency factor. The values are 3×10^{-3} cm²/sec and 1×10^{-9} cm²/sec from the internal-friction measurements and the Cottrell's equation respectively. Because of the serious disagreement of these two estimations, one should make an approximation based on the observations from the dosing experiment. Should 3×10^{-3} cm²/sec be the expected value, the time for saturation with oxygen at 1,700° C would take as short as 0·5 min. It is obvious that this value is too large for the diffusivity at the temperature. On the other hand, 1×10^{-9} cm²/sec would be too small for dosing to about 90 per cent of saturation within a few hours. It is therefore our opinion that the D value should be of the order of 10^{-7} cm²/sec at 1,700° C as estimated from the dosing experiment.

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Decomposition Pressure Hysteresis in the Zirconium-Hafnium-Hydrogen System

A recent investigation[1] of the Zr–Hf–H equilibrium system at pressures less than one atmosphere of hydrogen gas revealed the presence of hysteresis. Although this was not unexpected[2], it was somewhat disconcerting and demanded further elucidation. Results presented here will show that: (1) decomposition pressure hysteresis must be considered when dealing with equilibrium between gases and multi-component alloy systems; (2) hysteresis always involved a region of more than one phase and was most prominent at lower temperatures where diffusion was slowest; (3) no evidence for an interfering surface film was found; (4) internal factors such as stress and large surface area accompanying a martensitic reaction are prominent in the hysteresis reactions of the Zr–Hf–H system.

Hysteresis in metal–hydrogen systems has been found by a number of workers as far back as the early 1900's, and the subject has been reviewed in recent publications[3–7]. Most explanations involved internal solid-state properties of the hydrided alloys. On a pressure-composition plot different paths were followed on absorbing or desorbing hydrogen when crossing a multi-phased region because either: (1) the two paths represented different statistically predictable metastable states of long duration; or

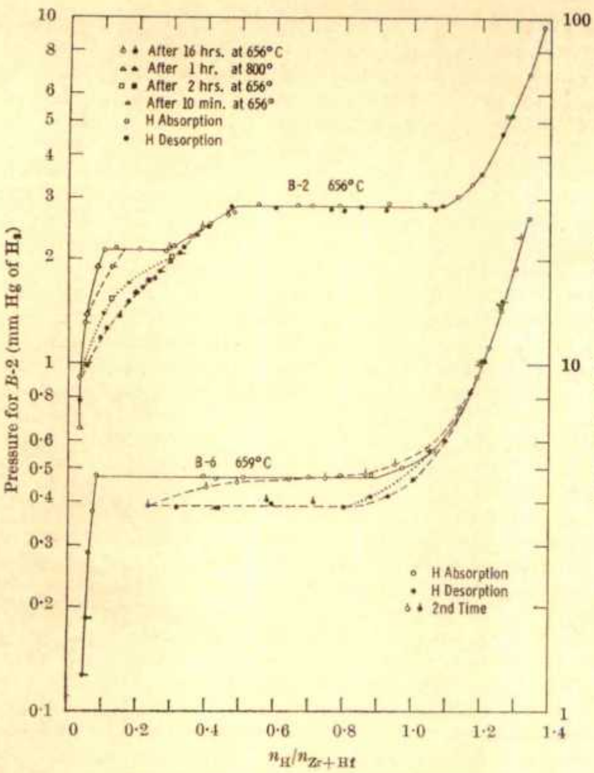


Fig. 1. Hysteresis in the Zr–Hf–H system

(2) the phase rule had introduced into it another variable, such as elastic restraint and long-range ordering; or (3) the repositioning of metal atoms particularly by diffusion was extremely and unobservably slow; or (4) the barrier to nucleation was very great; or (5) the presence of plastic deformation caused complicated volume changes which were irreversible. In addition, some results have shown that a surface film could form during absorption or afterwards so as to restrict the subsequent desorption of hydrogen from the metal. For the Zr–Hf–H system, approach to equilibrium curves was always rapid at first, tapering off exponentially, as expected; and, furthermore, no structural evidence was found for a limiting film on any of the processed specimens.

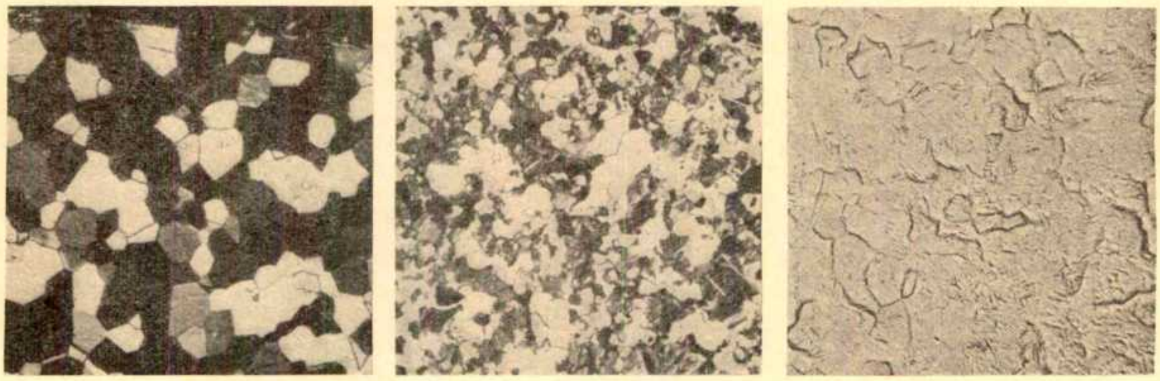
Fig. 1 shows the details for the two alloys *B*-2 (Zr + 37 w/o Hf) and *B*-6 (Zr + 73 w/o Hf) at approximately the same temperature. The aim of such a detailed investigation was to show that some inhomogeneity was present in the metal which could not be easily removed at the temperature of reaction with hydrogen. Open and closed, unmarked circles represent the first tracing of the isotherm with the previously unreacted alloy. Data points were obtained by the use of a high-sensitivity microgravimetric apparatus, previously described[2,8].

As seen in Fig. 1 for the *B*-2 alloy, removal of hysteresis was indeed dependent on time, being easier the higher the temperature and longer the time of pretreating the sample before rehydriding. Hysteresis then was essentially a memory-sensitive process where the effects of previous treatments could remain and influence future hydriding. The *B*-6 isotherm also showed the inability to retrace the original isotherm without some intermediate annealing treatment. Here, for the only time in the investigation, the descending pressure isotherm showed a hysteresis plateau.

Since hysteresis always involved a multiphase region, it was reasonable to assume that the larger metal atoms could not diffuse back after nucleation of the hydride(s) to the equilibrium positions they maintained on the ascending pressure curve. Hence, the hydride(s) in the β- or γ-regions which precipitated with increase in hydrogen content lost all their hydrogen on decreasing the pressure only if the hydride metal atoms redissolved into the lattice. The diffusion coefficients of zirconium and hafnium in the zirconium–hafnium alloys would then govern the appearance of hysteresis. With both hafnium and zirconium in solution in the hydride phase, as proved by electron microprobe analyses, the interactions between the two could well reduce the diffusion coefficients of either below that in the pure metal. This would make hysteresis more prominent in multiple metal atom–hydrogen alloys. An appreciable rise in temperature (150° C for *B*-2) increased the metal atom diffusion rate more than 2 orders of magnitude and restored the initial state of the lattice. The diffusion coefficient of hydrogen at the reactive temperature was already much greater than necessary to reach equilibration in the times used.

In an effort to determine the type of diffusion barriers which existed, a structural analysis of a hydrided and dehydrided *B*-6 alloy was conducted. This alloy was hydrided to one atmosphere of hydrogen pressure at 752° C and dehydrided through a hysteresis loop between the α- and γ-regions. The absorption isotherm was similar to *B*-6 in Fig. 1, but the desorption portion sloped with removal of hydrogen.

Fig. 2 shows the microstructural effects after complete dehydriding. The highly fragmented grains in (*b*) are seen as evidence for the previous coexistence of the hexagonal phases of the α-region and the hydrided colonies of the γ-region. In fact, not only had grain fragmentation occurred, but also a general grain roughening resulted as shown by the needle-like structure. Such a structure is reminiscent of martensitic transformations. The macro result was a specimen with a dull matt surface finish. Further evidence for a difference in initial and dehydrided specimen structures was the following: (1) the aforementioned dehydrided alloy, which was still reasonably



a *b* *c*

Fig. 2. **Structural** effects of hydriding. *a*, Etched sample of annealed, unhydrided *B*-6 alloy-polarized light (× *c* 140); *b*, same after hydriding + dehydriding – polarized light (× *c*. 140); *c* as *b*, normal light (× *c*. 350)

ductile, had a hardness of 150 Vicker's pyramid number compared with 115 Vicker's pyramid number for the initial material; (2) an X-ray powder pattern gave five lines for a face-centred-cubic of $a_0 = 4{\cdot}61$ Å although the alloy should have been entirely hexagonal. All 23 other lines of this pattern did index as hexagonal with c/a ratio nearly that of the parent B-6 alloy. Strain in the cubic phase was shown by a lattice parameter lower than the expected 4·72 Å from an equilibrium, fully hydrided, cubic B-6 alloy. The volume of the hydride with the smaller parameter was intermediate between the hexagonal B-6 lattice and the expected hydride, being additional evidence for a slow structural change of the hydrides back to the initial material.

In conclusion, it is seen that decomposition pressure hysteresis in the Zr–Hf–H system is intimately connected with a slow-diffusion process controlled by structural features, most likely of a martensitic nature. This connexion has been drawn recently for other than hydrogen systems, notably cobalt[9] and zirconia[10]. With an appreciation of the hysteresis phenomena, it was then possible to derive meaningful and consistent thermodynamic functions for the Zr–Hf–H system.

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CHEMISTRY

Hydroxyl Ions in the Mass Spectrum of Water Vapour

We have investigated the existence of stable primary OH^- ions in the negative ion mass spectrum of water vapour. Mann, Hustrulid and Tate[1] specially looked for OH^- ions in their mass spectrometric investigation of the ionization and dissociation of water vapour but found none. On the basis of this evidence Laidler[2] suggested extremely stable electronic configurations for the stable states of the OH^- ion which would be strongly repulsive to an approaching hydrogen atom. The hydrogen would thus play no part in the electronic excitation.

However, Cottin[3] has carried out a similar investigation and has found three types of OH^- ions; two of these are secondary products and are formed by electrons with energies between 5 and 12 eV. The ion which appears at 16 eV seems to be a primary ion. Cottin suggests that the three types of OH^- ions are produced in the following way:

$$H^- + H_2O \rightarrow OH^- + H_2$$
$$O^- + H_2O \rightarrow OH^- + OH$$
$$H_2O + e^- \rightarrow OH^- + H^+ + e^-$$

The pressure of water vapour in Cottin's work was about 10^{-2} torr in the ion source and the formation of secondary ions is readily understood. The presence of the primary ion could well have been due to collision stabilization of energy-rich states of OH^-.

We have converted an A.E.I. *MS2SG* mass spectrometer for the investigation of negative ions by reversing the appropriate electrical connexions and increasing the sensitivity by using a 100-kMohm grid leak in the electrometer detector instead of the usual 20 kMohm. Source pressures were about 10^{-4} torr to reduce any contribution from energy-rich states. OH^- ions appeared at 17·5 eV and the $OH^- : O^-$ ratio at 70 eV was 0·051. The negative ion mass spectrum of methanol has been investigated by Melton and Rudolph[4], and we repeated this work to check the mass spectrometer and aid the identification of the OH^- ions. The value for the ratio $OH^- : O^-$ was 0·213 and we found 0·235. However, our ratio $O^+ : O^-$ from O_2 was about 280 while theirs was about 43. Thus there seems to be much more discrimination against negative ions in our mass spectrometer. This is probably because negative ions are generally formed with appreciable kinetic energy and the mass spectrometer discriminates against them. This discrimination varies from instrument to instrument, and it may be the reason why Mann *et al.* were not able to detect any OH^- ions (particularly with pressures about 10^{-4} torr in the analyser).

The formation of OH^- means that the formation of O^- in the electron impact dissociation of water can still be due to the dissociation of OH^-. For the dissociation:

$$OH^- \rightarrow O^- + H$$

the appearance potential of O^- is given by the relation:

$$A(O^-) = D(OH^-) - EA(O) + \Sigma_E$$

where $D(OH^-)$ is the dissociation energy of OH^-, $EA(O)$ is the electron affinity of O and Σ_E is the energy contribution from the kinetic and electronic states of O and H. Now $D(OH^-) = D(OH) + EA(OH)$, where $D(OH)$ is the dissociation energy of OH and $EA(OH)$ is its electron affinity. Substituting this in the aforementioned relation and using the values of $D(OH)$, $EA(OH)$ and $EA(O)$ given in the literature[5–7], we get $A(O^-) = 4{\cdot}34 + 2{\cdot}65 - 1{\cdot}45 + \Sigma_E = 5{\cdot}54 + \Sigma_E$. Now $A(O^-)$ for one of the O^- species has been determined to be 7·4 eV. This leaves a not unreasonable amount of energy distributed between the different kinetic and electronic states of O^- and H.

The appearance of OD^- formed as a primary ion has been also observed by us. It appears at 18·1 eV and has an OD^-/O^- ratio of 0·065 at 70 eV.

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Disproportionation of Halogenated Alkyl Radicals

When 1,3-dichlorotetrafluoro acetone is photolysed, two CF_2Cl radicals and a molecule of carbon monoxide are formed. The CF_2Cl radicals combine to form 1,2-dichlorotetrafluoro ethane, and abstract chlorine atoms from the parent ketone to form dichlorodifluoro methane[1]; but, in addition to these products, a significant amount of 1,3-dichlorohexafluoro propane is found in the photolysis mixture. At first it was considered that this arose from a secondary photolysis of 1,4-dichlorohexafluoro acetone formed as a result of the chlorine abstraction reaction, but quantitative investigations[2] showed that it could not originate in this way.

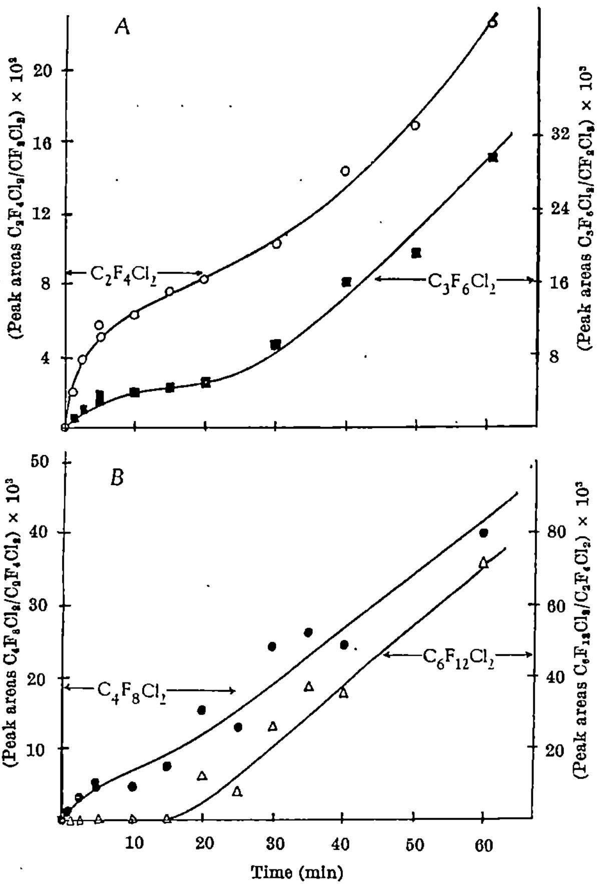

Fig. 1. Yields of products against time

It was suggested instead that the 1,3-dichlorohexfluoro propane was formed by the combination of CF_2Cl and CF_2CF_2Cl radicals. The CF_2CF_2Cl radicals were presumed to arise from a combination of CF_2Cl radicals with CF_2 radicals produced as a result of a disproportionation reaction:

$$2CF_2Cl \rightarrow CF_2 + CF_2Cl_2$$
$$CF_2 + CF_2Cl \rightarrow CF_2CF_2Cl$$
$$CF_2CF_2Cl + CF_2Cl \rightarrow ClCF_2CF_2CF_2Cl$$

There was little direct experimental evidence to support this suggestion other than the demonstration that the dichlorodifluoro methane detected in the system was formed by two distinct processes and the isolation of small quantities of 1,4-dichlorooctafluoro butane as a result of the dimerization of CF_2CF_2Cl radicals. In particular, no tetrafluoro ethylene was ever detected in the photolysis products, and it would have been expected to occur by the dimerization of CF_2 radicals.

Some support for the hypothesis was given by the demonstration by Gunning[3] of the occurrence of 1,3-dichlorohexafluoro propane in the products of the mercury photosensitization of dichlorodifluoro methane. In addition, the reactions observed by Mastrangelo[4] with CF_3 radicals could only be satisfactorily explained by assuming that CF_3 radicals also disproportionate.

Because of the ambiguous nature of the evidence from the mercury photosensitized decomposition of CF_2Cl_2, it was investigated in greater detail. The primary act was most probably the production of CF_2Cl radicals:

$$Hg^* + CF_2Cl_2 \rightarrow CF_2Cl + HgCl$$

since it has been demonstrated that mercury is consumed in the reaction. There is a possibility that 1,3-dichlorohexafluoro propane arises as a result of the photodecomposition of 1,2-dichlorotetrafluoro ethane produced by the combination of CF_2Cl radicals.

$$ClCF_2CF_2Cl + Hg^* \rightarrow CF_2CF_2Cl + HgCl$$
$$2\ CF_2CF_2Cl \rightarrow ClCF_2CF_2CF_2CF_2Cl$$
$$CF_2CF_2Cl + CF_2Cl \rightarrow ClCF_2CF_2CF_2Cl$$

If this were the case, then it would be anticipated that there would be a perceptible induction period in the formation of 1,3-dichlorohexafluoro propane, while sufficient dichlorotetrafluoro ethane accumulated in the system to compete with the CF_2Cl_2 for the quenching of excited mercury atoms. In Fig. 1*A*, the yields of 1,2-dichlorotetrafluoro ethane and 1,3-dichlorohexafluoro propane are plotted against irradiation time. It can be seen that there is no induction period and this behaviour can be contrasted with that illustrated in Fig. 1*B*. Here, 1,2-dichlorotetrafluoro ethane has been submitted to mercury photosensitization alone and the yields of the two main products formed are plotted against irradiation time.

$$ClCF_2CF_2CF_2CF_2Cl + Hg^* \rightarrow CF_2CF_2CF_2CF_2Cl + HgCl$$
$$CF_2CF_2CF_2CF_2Cl + CF_2CF_2Cl \rightarrow ClCF_2CF_2CF_2CF_2CF_2CF_2Cl$$

Here there is a long induction period in the formation of 1,6-dichloroduodecafluoro hexane due to the slow accumulation of 1,4-dichlorooctafluoro butane in the reaction system.

This suggests that the 1,3-dichlorohexafluoro propane obtained in the mercury photosensitization of CF_2Cl_2 is not formed by a secondary photosensitization of 1,2-dichlorotetrafluoro ethane, but arises as a result of some more rapid process such as the disproportionation of CF_2Cl radicals. In order to confirm this observation, varying amounts of 1,2-dichlorotetrafluoro ethane were added to CF_2Cl_2 before photolysis, and the amounts of 1,3-dichlorohexafluoro propane formed were measured. In Fig. 2, the rate of formation of 1,3-dichlorohexafluoro propane is plotted against the amount of 1,2-dichlorotetrafluoro ethane added. It can be seen that substantial

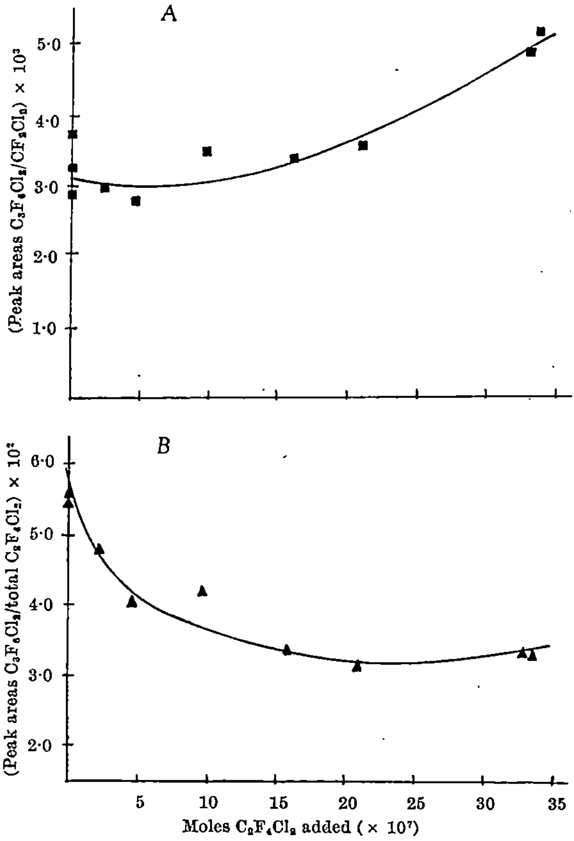

Fig. 2. *A*, yield of $C_3F_6Cl_2$ against moles $C_2F_4Cl_2$ added; *B*, ratio $C_3F_6Cl_2$ to $C_2F_4Cl_2$ produced + $C_2F_4Cl_2$ added against moles $C_2F_4Cl_2$ added

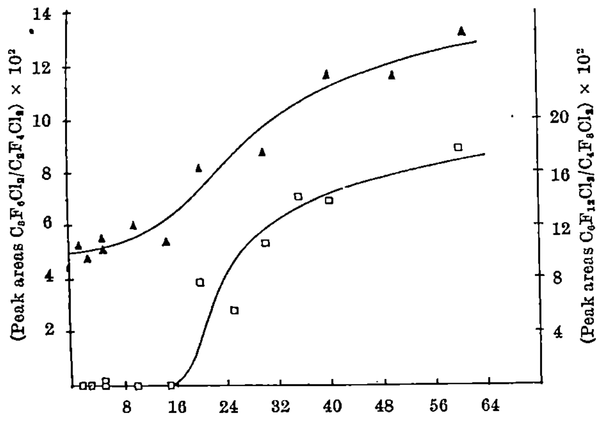

Fig. 3. Ratio of secondary to primary products against time. ▲, CF_2Cl_2 photolysis; □, $C_2F_4Cl_2$ photolysis

amounts must be added before any effect is perceptible, and these amounts are more than three times the concentration of 1,2-dichlorotetrafluoro ethane usually observed in the mercury photosensitized decomposition of CF_2Cl_2. In Fig. 2*B*, it is shown that the ratio of 1,3-dichlorohexafluoro propane to 1,2-dichlorotetrafluoro ethane actually decreases when extra amounts of 1,2-dichlorotetrafluoro ethane are added.

Finally, in Fig. 3, the ratios $[C_3F_6Cl_2]/[C_2F_4Cl_2]$ and $[C_6F_{12}Cl_2]/[C_4F_8Cl_2]$ are plotted against irradiation time, and it can be seen that the induction period, clearly visible for $C_6F_{12}Cl_2$ formation, is absent for $C_3F_6Cl_2$ formation.

It therefore appears probable that 1,3-dichlorohexafluoro propane, formed in the mercury photosensitized decomposition of CF_2Cl_2, arises by the interaction of primary radicals and does not involve a second excited mercury atom colliding with initially formed products. This does not rule out the possibility that CF_2 is formed in a single step:

$$Hg^* + CF_2Cl_2 \rightarrow CF_2 + HgCl_2$$

but this appears unlikely in view of the appearance of 1,3-dichlorohexafluoro propane in the photolysis products of 1,3-dichlorotetrafluoro acetone, where such a process cannot occur. Further, the ratio $[C_3F_6Cl_2]/[C_2F_4Cl_2]$ has the same value (0·04) in both systems.

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Reaction of Tetrafluoroethylene with Pentacyanocobaltate Ions and with Reduced Vitamin B_{12}

We have previously shown[1] that a cobalt–carbon bond can be formed in aqueous solution by the interaction of acetylene with the pentacyanocobaltate(II) anion, while recently[2] benzyl and alkyl halides have been similarly shown to give a Co–C bond.

We have found that tetrafluoroethylene is readily absorbed at atmospheric pressure by aqueous solutions of the hydridopentacyano-cobaltate(III) ion, $[HCo(CN)_5]^{3-}$, as well as by the pentacyano-cobalt(II) ion. The well-crystallized salts analyse correctly for $K_3[(CN)_5CoCF_2CF_2H]$ (*A*) [for example, Found: C, 20·8; N, 17·4; F, 18·0; Co, 14·2; K, 29·1. Required: C, 20·6; N, 17·2; F, 18·7; Co, 14·5; K, 28·8 per cent] and $K_6[(CN)_5Co(CF_2)_2Co(CN)_5]\cdot 2H_2O$ (*B*); the salts are stable in air and thermally up to about 300° C.

The nuclear magnetic resonance spectra support these formulations. Thus the H^1 spectrum for (*A*) is a triple triplet while the fluorine-19 resonance of the β-CF_2 is split into a doublet by the proton and further into a triplet by the α-CF_2 group; all these lines are sharp and well resolved. In contrast the α-CF_2 resonance is a broad single line; the broadening is presumably due to incomplete averaging of the spin coupling with the cobalt-59 nucleus produced by quadrupole relaxation of this nucleus. For the same reason, the fluorine-19 spectrum of (*B*) is only a broad single line.

The interaction of C_2F_4 with the hydrido-species is interesting in that it can provide a probe for the detection of transition metal to hydrogen bonds in aqueous media where detection by the high-field proton resonance line is difficult. Thus we have failed to observe such a high-field line in saturated aqueous solutions of the grey-green reduction product of cyanocobalamin. A search was made at 40 Mc/s several years ago[3], at the time of our first investigations of the hydridic nature of lower oxidation states, and, more recently, at 56·45 Mc/s. However, the reduced species immediately absorbs tetrafluoroethylene, and the resulting red product—dissolved in chloroform–phenol, in which it is much more soluble than in water—shows a ^{19}F resonance spectrum in which there is a doublet of splitting similar to that observed for the —CF_2H group of (*A*). While this suggests that reduced cyanocobalamin (B_{12s}) is a hydrido-species, we cannot rigorously exclude the possibility that it is a cobalt(I) species as recently suggested[4], since such a cobalt(I) species could react with C_2F_4 with subsequent hydrogen transfer from the solvent.

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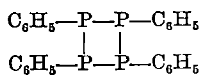
Molecular Structure of Phosphobenzene

The following polyphosphines of formula $(C_6H_5P)_n$ have been reported in the literature: (*A*) m.p. 149°–150° (refs. 1–9); (*B*) m.p. 190° (refs. 2 and 3); (*C*) m.p. 252°–256° (ref. 3); (*D*) m.p. 260°–285° (ref. 8). Compounds *C* and *D* are insoluble in organic solvents and have, presumably, a polymeric structure. The molecular weights of *A* and *B* in solution have not been established definitely. Several isopiestic, ebullioscopic and cryoscopic methods have been used by various workers and the following identities have been proposed. *A* = *B* = I[2]; *A* = I, *B* = II[3]; *A* = *B* = II[8]; *A* = II[4,5,9]. Recently it has also

$$C_6H_5-P=P-C_6H_5$$

I



$$\begin{array}{c} C_6H_5-P-P-C_6H_5 \\ |\quad\ \ | \\ C_6H_5-P-P-C_6H_5 \end{array}$$

II

been shown[8] that compounds *A* and *B* are tetraphenyl-*cyclo*-tetraphosphines (II) which differ in both the dissolved and solid states and are stereoconformers.

We have prepared compound *A* by the reaction of phenylphosphonous dichloride and phenyl phosphine[1,3-5] (equation 1):

$$nC_6H_5PCl_2 + nC_6H_5PH_2 \rightarrow (C_6H_5P)_n + 2nHCl \qquad (1)$$

and by a new method which involves the heating of phenylphosphine and a stoichiometric amount of phenylphosphonous bisdimethylamide at 170° for 1 h (equation 2); the yield is 83 per cent (ref. 10). This new method has also been used successfully for the preparation

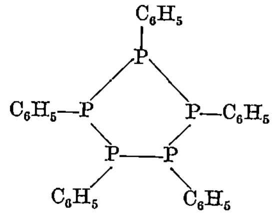
$$n\mathrm{C_6H_5P[N(CH_3)_2]_2} + n\mathrm{C_6H_5PH_2} \rightarrow (\mathrm{C_6H_5P})_n + 2n\mathrm{(CH_3)_2NH} \qquad (2)$$

of triphosphines (for example, 1,1,3,3,-tetraphenyl-2-methyltriphosphine m.p. 134°, equation 3) and biphosphines[11] (for example, tetramethylbiphosphine).

$$\mathrm{CH_3P[N(CH_3)_2]_2} + \mathrm{2HP(C_6H_5)_2} \rightarrow \mathrm{(C_6H_5)_2P{-}P(CH_3){-}P(_6H_5)_2} + \mathrm{2(CH_3)_2NH} \qquad (3)$$

The identity of the compound *A* prepared according to either equations 1 or 2 has been established by melting points, mixed melting points, infra-red spectra[12] and X-ray diffraction spectra. The molecular weight, determined cryoscopically in benzene, was found to be 439 ± 25; this corresponds to structure II, theoretical value 432·9: analysis $\mathrm{C_6H_5P}$ (108·07): calc., C, 66·67; H, 4·66; P, 28·66 per cent: found, C, 66·36; H, 4·65; P, 28·87 per cent.

Crystals of compound *A* (recrystallized from acetonitrile, m.p. of a single crystal 154°–156°), which has also been called phosphobenzene, are monoclinic, $P2_1/n$. The cell dimensions are a = 9·742, b = 10·139, c = 27·488 Å, β = 90·0°. The density, measured by flotation, is 1·324 g/c.c.; thus, assuming $Z = 4$, then M = 541. The calculated molecular weight for $\mathrm{(C_6H_5P)_5}$ is 540. In an attempt to throw some light on this discrepancy with solution measurements, an X-ray single crystal analysis has been carried out. The diffracted X-ray intensities were measured with the aid of a Hilger and Watts linear diffractometer; 2,614 of these intensities were assigned non-zero value, the remainder being ignored. The positions of the five phosphorous atoms in the asymmetric unit were obtained from a three-dimensional Patterson synthesis. From this point, the 860 observed planes with $\sin \vartheta/\lambda < 0{\cdot}35$ Å^{-1} were utilized in the calculations. The positions of the carbon atoms were determined from two successive three-dimensional electron density maps. Seven cycles of least-squares refinement reduced R from 0·35 to 0·10. The results show that, in the solid state, compound *A* is a pentamer, $\mathrm{(C_6H_5P)_5}$, with structure III.



III

We have now started to refine the structure with all 2,614 observed planes; the full results of the analysis will be published elsewhere.

Compound *B* was prepared according to the directions given in the literature[8]. From 4 identical preparations two yielded compound *B* exclusively whereas one preparation gave compound *B* in addition to a polymeric material melting at 215°–265°, and the other preparation gave compound *A* in addition to a material melting at 240°–275°.

A part from the crude compound *B*, m.p. 180°–185°, was recrystallized from benzene containing small amounts of acetonitrile. The compound was obtained in well-shaped crystals melting at 183°–186° in an open tube and at 229°–235° in an evacuated tube. The compound resolidified very slowly and then melted at 130°–155°. Analysis indicated that the compound contained one mole of benzene per 6 phenyl-phosphorus groups. The benzene could not be removed in the vacuum at room temperature; analysis $\mathrm{(C_6H_5P)_6.C_6H_6}$: calc., C, 69·42; H, 4·99; P, 25·58 per cent; found, C, 69·48; H, 5·09; P, 25·21 per cent.

Another part of crude *B*, recrystallized from tetrahydrofuran, was obtained in small needles melting at 189°–193° in an open tube and at 194°–198° in an evacuated tube.

Crystals of compound *B* recrystallized from benzene are rhombohedral, space group $R3$ or $R\bar{3}$. The cell dimensions are a = 10·12 Å, α = 77·574° or, when referred to hexagonal axes, a = 12·68, c = 20·96 Å. The density, measured by flotation, is 1·257 g/c.c.; thus, assuming $Z = 1$, the molecular weight is 737. The calculated molecular weight for $\mathrm{(C_6H_5P)_6.C_6H_6}$ is 726. The required molecular symmetry for $\mathrm{(C_6H_5P)_6}$ is 3 in the space group $R3$ or $\bar{3}$ in the space group $R\bar{3}$.

When recrystallized from tetrahydrofuran, crystals of compound *B* are monoclinic, space group $P2_1/c$. The cell dimensions are a = 12·31, b = 6·16, c = 24·86 Å, β = 117·64°. The symmetry of the $h0l$ reciprocal net is almost hexagonal, plane group p6m. The density, measured by flotation, is 1·275 g/c.c.; thus, assuming $Z = 2$, the molecular weight is 642. The calculated molecular weight for $\mathrm{(C_6H_5P)_6}$ is 649, and the required molecular symmetry for $\mathrm{(C_6H_5P)_6}$ is $\bar{1}$.

The cell dimensions are very similar to those found[13] for $\mathrm{(C_6H_5As)_6}$ which has a = 12·16, b = 6·22, c = 24·10 Å, β = 110·23°, space group $P2_1/c$.

Thus, although the possibility that compound *B* is a dimer or trimer cannot be excluded, it seems very probable that, in the solid state, it is a hexamer.

We thank Dr. H. H. Zeiss for his advice.

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A New Polymorph of Oxamide

IN his investigations at high pressure, Bridgman[1] found evidence of four polymorphs of oxamide, but he did not identify them. Two forms of oxamide have been obtained in an exploration of methods of preparation of the compound; either form may be prepared from the other by recrystallization.

Oxamide that is stable at room temperature is triclinic, and this form was described by Ayerst and Duke[2]; it is prepared by the hydrolysis of cyanogen by concentrated hydrochloric acid[3] or by thermal dehydration of ammonium oxalate[4]. An orthorhombic form of oxamide that is metastable at room temperature was obtained when oxamide was prepared by glow-discharge electrolysis of formamide[5] or when oxamide was crystallized rapidly from its solution in formamide. Only the triclinic form was obtained at any cooling rate from a hot aqueous solution of either form. Crystallization from 1,4-dioxane, dimethyl formamide, glycerol, or ethylene glycol also yielded only the triclinic form.

The orthorhombic form is obtained by rapidly cooling from 70° C to room temperature a solution of either form of oxamide in formamide. If the solution is cooled slowly (5° C/h), the triclinic form is obtained; at intermediate cooling rates, orthorhombic seed crystals develop

Table 1. POWDER X-RAY DIFFRACTION PATTERNS OF OXAMIDE

Orthorhombic form		Triclinic form	
d, Å	I	*d*, Å	I
5·57	8	—	—
5·15	22	5·15	35
4·72	17	4·72	65
3·60	22	3·62	11
3·36	11	3·35	17
3·22	6	3·22	15
3·04	100	3·02	100
2·55	49	2·57	44
—	—	2·53	29
2·38	18	2·40	10
—	—	2·35	17
—	—	2·18	6
—	—	2·16	3
2·01	5	2·01	5
1·81	14	1·81	8
—	—	1·71	2
1·67	4	1·67	6
1·64	5	1·65	8
—	—	1·61	4
1·55	4	1·57	4

Spectra obtained by J. P. Smith with a Norelco high-angle goniometer and copper K_α radiation ($\lambda = 1\cdot5405$ Å); intensities were read from X-ray goniometer tracings.

epitaxial overgrowths of the triclinic form or develop into monocrystal pseudomorphs of the triclinic form. At temperatures below 0° C, however, only triclinic oxamide is obtained at any rate of cooling.

Triclinic oxamide crystals in a saturated formamide solution showed no alteration after 4 weeks at 25° or 40° C. Orthorhombic crystals in a saturated formamide solution at room temperature developed a grainy texture from the growth of triclinic crystallites on the surface. In a saturated aqueous solution at 25° or 40° C, mixtures of the two forms showed a gradual decrease in the proportion of the orthorhombic form. Orthorhombic crystals that were crystallized from formamide, washed with acetone, air-dried, and stored in stoppered containers altered similarly and slowly formed triclinic monocrystal or oriented polycrystalline pseudomorphs.

Orthorhombic oxamide crystallizes as bipyramids flattened along N_γ and elongated along N_α, but more frequently as bevelled plates resulting from greater growth distortions along N_α and N_γ. Plate crystals, which result from rapid crystallization, commonly show penetration twinning. There is a moderately good cleavage plane parallel to N_β–N_γ in the plates. The crystals have class *mmm* symmetry and extinction positions are either parallel or symmetrical on principal views, in accord with orthorhombic symmetry. Refractive indices are $N_\alpha = 1\cdot430$, $N_\beta = 1\cdot685$, $N_\gamma = 1\cdot695$. The crystals are biaxial (–) with small $2V$, as shown by diffuse conoscopic figures; calculated $2V$ is 20°.

The X-ray diffraction spectra of the two modifications, Table 1, have many reflexions in common, suggesting a close structural similarity. The infra-red spectra of the triclinic[6] and orthorhombic forms are identical. Attempts to determine the unit-cell parameters of the orthorhombic dimorph were unsuccessful, however, because untwinned crystals of this dimorph large enough for single-crystal investigation could not be prepared. Satisfactory Weissenberg photographs were not obtained from even the largest twinned crystals. Further attempts will be made to determine the space-group symmetry of the orthorhombic dimorph.

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BIOPHYSICS

Use of Microwave Techniques for the Determination of Bound Water

ANY information about changes in the structure of molecular arrays which are reflected in changes of the water substance is useful to form a guideline for future work that tries to investigate the actual atomic and molecular basis of life. An accurate and relatively fast method has been developed which can assess the occurrence of a quantity called bound water. These bound-water molecules are defined by the fact that they are unable to follow the changing electric field of an applied microwave signal, indicating thereby that they are either bound to 3 or 4 other molecules of the same kind, or to various other types of molecules. The remaining water molecules (those bound to 2, 1 or no other water molecules) that are free to rotate with the impressed electromagnetic field absorb energy from the incident microwave signal. The difference between the two microwave signals can be interpreted to be an indication of the amount of water bound to a particular molecule.

Since water molecules have an appreciable dipole moment, they will tend to follow an applied electric field if not impeded by their environment. During this process of molecular re-alignment energy is expended and subtracted from the incident energy.

The sample, an aqueous solution of the substance to be investigated, is located at a certain point in a cavity the resonant frequency of which is in the microwave *X* band. In that frequency range most energy is absorbed by the water and only a negligible amount by the 'contaminating' substance. The response of the cavity to incoming microwave signals is then displayed as a function of their frequency on an oscilloscope or *X*–*Y* plotter and from that picture the dielectric constant of the sample material can be obtained. From the dielectric constant at microwave frequencies one may obtain in turn the static dielectric constant and plot that as a function of concentration of the solute. The initial slope of that graph then gives a quantity that is linearly related to the amount of water irrotationally bound to the solute molecules. Changes in that quantity can be recorded conveniently by photographing the oscilloscope trace (for fast changes) or by reduction of the response curve data to the desired quantity by means of an electronic computing device.

The rectangular cavity operates in the TE_{104} mode. The sample is contained in the glass tube which is located near a minimum of the electric field existing in the cavity. If one assumes that the change in the cavity characteristics due to the introduction of the sample is small, one may use the perturbation analysis developed by Slater[1] and applied to measurements of low loss dielectrics by Birnbaum and Franeau[2]. Our modifications change the expressions given by the latter authors to give the values of the dielectric constants in the form:

$$\Delta\varepsilon' = \varepsilon_w' - \varepsilon_s' = \frac{2c}{f_0}(f_s - f_w) \tag{1}$$

$$\Delta\varepsilon'' = \varepsilon_w'' - \varepsilon_s'' = C\left(\frac{1}{Q_s} - \frac{1}{Q_w}\right) \tag{2}$$

where $\Delta\varepsilon'$ or $\Delta\varepsilon''$ signifies the relative change in the dielectric constant of the material contained in the glass tube; the subscript (s) denotes properties pertaining to the solution and (w) to pure water. ($f_s - f_w$) is the shift in the resonant frequency of the cavity due to replacement of the pure water by an equal volume of solution, f_0 is the resonant frequency of the empty cavity, and C is a constant derived from cavity dimensions, given by:

$$C = V_0/4AV_T \tag{3}$$

where V_0 is the volume of the empty cavity, V_T the volume of the total dielectric material, and A is a correction factor for the position of the glass tube relative to $E_{\max}$.

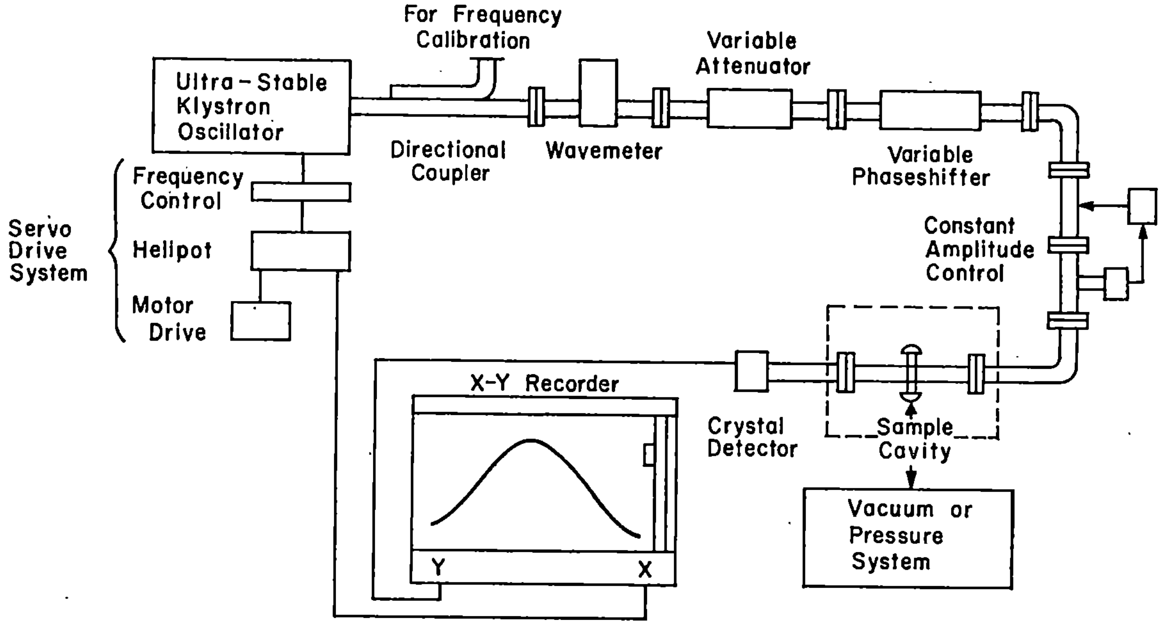

Fig. 1. Block diagram for dielectric constant measurement of aqueous solutions of microwave frequencies

The graph of ε_s, the static dielectric constant, as a function of concentration, is then used for further computations. The reason for the use of the static dielectric constant as an indicator of the amount of bound water can be explained by the following argument.

At the high electromagnetic frequencies used in the present method the protein or large solute molecule together with its shell of bound water may be considered as one unit, a cavity in the surrounding medium that has a relatively low dielectric constant; the bound water can only contribute to the static dielectric constant by virtue of its electronic and atomic polarization, which is small.

At low solute concentrations we assume with Haggis, Hasted, and Buchanan[3] that ε_s depends linearly on the concentration of solute so that:

$$\varepsilon_s = \varepsilon_{\text{water}} - \delta \cdot c \tag{4}$$

and:

$$\delta = \beta \frac{(\varepsilon_{\text{water}} - \varepsilon_{\infty\text{prot}})^{v} + (\varepsilon_{\text{water}} - \varepsilon_{\infty\text{water}})^{w}}{100} \tag{5}$$

where c is the concentration in g protein/100 ml. solution, v the partial specific volume of protein, $\varepsilon_{\infty\text{prot}}$ the high-frequency dielectric constant of protein, $\varepsilon_{\infty\text{water}}$ is the high-frequency dielectric constant of water, w is the weight of water irrotationally bound to 1 g of protein, and β is a parameter depending on the axial ratio of the spheroidal macromolecule.

Because of the uncertainty in estimates of some of the parameters in the foregoing equation, we only use the technique to measure relative changes in the water of hydration, either as a function of time during a given reaction, or as a function of concentration of some other substance that reacts with the given solute molecules.

Fig. 1 shows the schematic diagram of the experimental arrangement of the instruments. The Laboratory for Electronics Ultrastable X-band generator is used as a microwave source. The output is frequency-modulated by means of a potentiometer-monitored drive that produces a concurrent voltage signal to serve as the X signal on the X–Y plotter. Over the frequency range used (9·450–9·470 Gc/s) the output of the generator is amplitude-modulated to keep the intrinsic power variation of the klystron at less than 0·01 db. The amplitude modulation is accomplished by superimposing a lossy cavity characteristic and then taking a sample of the power, which is detected by a crystal, amplified, and fed back into a gyrator. A wavemeter, precision attenuator and phase-shifter precede the sample cavity and the detector crystal. The sample cavity is immersed in a constant temperature bath and the waveguide section containing it is kept at constant temperature and humidity by dried- and temperature-controlled air. Samples of solution are drawn into the cavity by gravity flow after the capillary section in the cavity is evacuated.

The foregoing outlined technique has been used[4] to determine changes in the water bound to hæmoglobin as xenon is introduced into the solution. Further experiments involving hæmoglobin, other proteins, and enzymes are in progress.

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BIOCHEMISTRY

A Suggested Role of Amino-acids in Deoxyribonucleic Acid

Several investigators have found amino-acids among the hydrolysis products of DNA's from many sources and some have suggested that small peptides may play a part in DNA structure and/or function. These have been reviewed by Bendich and Rosenkranz[1]. We have considered the possibility that such small peptides are concerned with the regulation of enzyme synthesis. For this reason we have examined the amino-acid residues found in samples of DNA from *Escherichia coli* grown in 'minimal' and in 'complete' media. Bacteria grown in minimal media must synthesize large numbers of compounds not made by those grown in complete media. Therefore, more enzymes should be formed and more DNA should be functional in bacteria grown in minimal media. If control of DNA function is exerted through firmly bound amino-acids

Table 1. AMINO-ACID RESIDUES IN SEVERAL *E. coli* DNA's

(μg amino-acid residue/100 mg DNA)

	Minimal media			Enriched media			
	No. 1*	No. 2†	No. 3†	No. 4*	No. 5†	No. 6*	No. 7†
Aspartic acid	212	116	150	27	27	32	31
Threonine	77	129	76	13	8	20	17
Serine	80	123	110	24	27	44	28
Glutamic acid	201	91	177	52	40	36	43
Proline	58	—	57	15	—	7	6
Alanine	129	73	56	19	19	16	13
Valine	115	54	86	13	—	20	22
Methionine	37	16	109	13	8	5	5
Isoleucine	82	44	111	9	7	13	12
Leucine	136	58	40	22	2	20	21
Tyrosine	85	37	46	13	4	14	15
Phenylalanine	77	38	38	12	4	13	12
Lysine	169			76			
Histidine	49			11			
Arginine	138			47			
Basic amino-acids	356			134			
Neutral and acidic amino-acids	1,288	779	1,056	230	146	241	225

* DNA was extracted from washed log phase *E. coli* B cells by the procedure of Kirby (ref. 2). An extra deproteinizing step was carried out just prior to dialysis. The DNA was dissolved in 10 per cent sodium chloride, deproteined twice with $CHCl_3$-i-AmOH, and reprecipitated with cold EtOH.

† DNA was extracted as described by Marmur (ref. 3). Samples of bacteria, 1–5, were kindly supplied by Dr. L. Kaplan, of this Institute. Samples 6 and 7 were purchased from General Biochemicals, Chagrin Falls, Ohio, and were in late log phase; all others were in mid-log.

No. 1: grown in an American Sterilized Co. Biogen, Davis (ref. 11) salt medium without citrate and 1 per cent glucose. Nos. 2 and 3: grown in Davis salt medium (ref. 11) and 1 per cent glucose in two independent batches. No. 4: grown in a biogen as No. 1 but with addition of 0·6 per cent yeast extract. No. 5: grown in 2·5 per cent Difco heart infusion broth. Nos. 6 and 7: batch grown with 4 per cent yeast extract, 2 per cent dextrose, 0·3 per cent KH_2PO_4, 0·1 per cent K_2HPO_4. No. 7 was treated with 50 mg/l. proflavine and irradiated with a 750-W lamp at 8 in. for 60 min. No. 6 was an untreated control for No. 7.

and peptides, then DNA from bacteria grown under these two sets of conditions should contain different amounts of amino-acids.

The bacteria were gathered and the DNA's were extracted by the use of either phenol[2] or sodium dodecylsulphate[3]. All samples were treated with RNase and then with chloroform and iso-amyl alcohol until no further protein was removed. The DNA's were characterized by the diphenylamine reaction[4] and by spectral shifts after treatment with DNase[5]. The DNA's were hydrolysed at 110° *in vacuo* with 6 *N* hydrochloric acid. The amino-acids were then determined with a Spinco Stein–Moore amino-acid analyser[6].

The results in Table 1 show that the total of the neutral and acidic amino-acid residues in the DNA from *E. coli* grown in minimal media ranged from about 8 to 12 hundred μg/100 mg DNA, while those from the bacteria grown in rich media were about 200 μg/100 mg DNA. The relative proportions of the individual amino-acids were about the same in each group. In one sample of each group, the basic amino-acids were determined. Essentially the same differences were observed as were seen with the neutral and acidic amino-acids. In one sample (No. 7), obtained from bacteria irradiated in the presence of proflavine, only 15 per cent of the DNA was extractable. In investigations with acridine orange, similar results were obtained by Smith[7], who postulated that the rest of the DNA was linked to protein. In view of the foregoing, it is interesting that this sample of DNA has the same amount of residual amino-acids as the unirradiated control (No. 6).

The possible significance of the bound amino-acids is emphasized by the finding that the same differences were observed in DNA samples prepared by the two quite different methods. Three different enriched and two minimal media were used. Despite these variations, the DNA from bacteria grown in minimal medium retained a much greater amount of bound amino-acids than the DNA from cells grown in enriched media. These differences support the suggestion that amino-acids may play a part in repression–derepression of enzyme synthesis, in addition to any structural function they may have[8].

If, as in the schemes of Jacob and Monod[9], a regulator gene in essence can act only to repress a structural gene (whether directly or indirectly), then blocking such action would lead to derepression. The data presented here are consistent with such a mechanism, that is, small peptides acting as rather firmly bound derepressors. (In this regard it is interesting to note that Gallant and Stapleton[10] have published data supporting the suggestion that repressors also are peptides.) Thus, we should expect bacteria grown in minimal medium to need many enzymes, and, therefore, their DNA to contain large amounts of derepressors. Such a mechanism of derepression might also explain control of enzyme function in differentiated tissue and in such dedifferentiated tissues as tumours.

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Inhibition of Glycolysis by Pyruvate in Relation to the Accumulation of Citric Acid Cycle Intermediates in the Perfused Rat Heart

RECENT investigations have established that the addition of metabolic fuels such as acetoacetate, fatty acids, lactate or pyruvate to hearts perfused with glucose and insulin inhibits the rate of glucose uptake and oxidation[1–4]. It has been proposed that during respiration of these substrates, glycolysis is inhibited by a common mechanism; namely, an indirect inhibition of phosphofructokinase as indicated by increased levels of hexose monophosphates and decreased levels of fructose-1,6-diphosphate[5]. Furthermore, phosphofructokinase activity has been shown to be depressed in hearts from diabetic rats which also have a decreased rate of glucose utilization[6]. It has recently been reported that citrate not only inhibits phosphofructokinase activity in cell-free system, but also accumulates in hearts from diabetic rats, and in isolated hearts perfused with long- and short-chain fatty acids[7–9]. These results have led to the suggestion that the rate of glycolysis may in some measure be controlled by the activity of the citric acid cycle. Williamson[10] has reported that the rate-limiting step of glycolysis in perfused hearts with pyruvate as substrate occurred not at the phosphofructokinase step, as proposed by Newsholme *et al.*[5], but at a site located between glyceraldehyde-3-phosphate and pyruvate. It was therefore considered of interest to examine the kinetics of the changes of the glycolytic intermediates after addition of pyruvate, in conjunction with the levels of certain citric acid cycle intermediates in both the intact heart and isolated heart mitochondria.

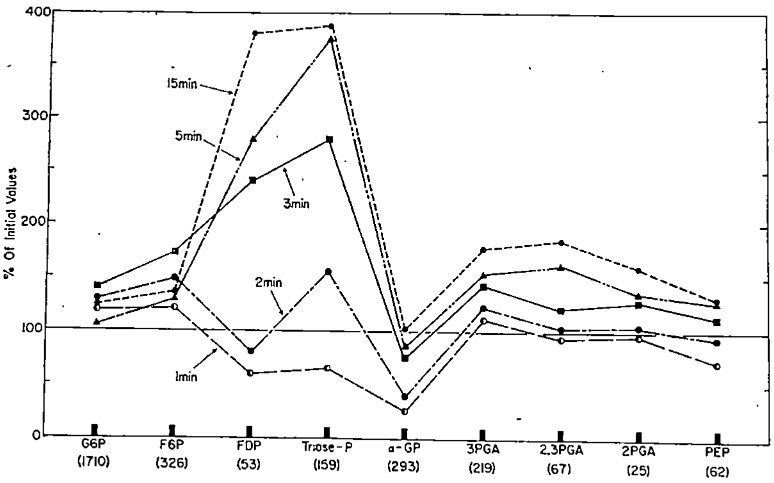

Fig. 1. The effect of pyruvate on the level of glycolytic intermediates in isolated, perfused rat heart. Hearts were perfused for the stated times with 5 mM glucose, 2 mU/ml. insulin, and 10 mM pyruvate. Control hearts were perfused with similar medium in the absence of pyruvate. Values for the glycolytic intermediates represent the mean of determinations on two hearts for each perfusion time, and results are expressed as a percentage of the initial control values. Control values for each intermediate are expressed in μmoles/kg dry wt. and are shown in brackets at the bottom of the figure. Dihydroxyacetone-P accounted for 83 per cent of the triose phosphate

Hearts from fed, male, Wistar rats were perfused as previously described[1]. All hearts were pre-perfused for 15 min with medium containing 5 mM glucose and 2 mU/ml. insulin before transference to recirculation circuits containing 15 ml. of the same medium plus 10 mM pyruvate. After different times of perfusion, the hearts were rapidly frozen by means of tongs cooled in liquid nitrogen, powdered, and extracted with perchloric acid. The glycolytic intermediates, citrate, α-oxoglutarate and malate, were estimated in an Eppendorf fluorimeter by minor modifications of standard enzymatic procedures[11]. Rat heart mitochondria were prepared and incubated as described by Chance and Hagihara[12]. The redox state of the mitochondrial pyridine nucleotides was investigated using the fluorescence techniques of Chance and Baltscheffsky[13].

The changes of the glycolytic intermediates in hearts at different times after exposure to pyruvate are shown in Fig. 1. It may be seen that glucose-6-phosphate and fructose-6-phosphate levels were elevated within 1 min after addition of pyruvate and remained elevated for the 15-min perfusion period. Fructose-1,6-diphosphate and triose-phosphate levels decreased within the first minute, but afterwards increased greatly above the control values, with fructose diphosphate lagging behind the triose phosphates. The intermediates between 3-phosphoglyceric acid and pyruvate also increased, but to a lesser extent than the triose-phosphates. The levels of α-glycero-P decreased by 75 per cent immediately after pyruvate addition and afterwards increased to the same values as those of the control hearts. The ratio of α-glycero-P to dihydroxyacetone-P decreased from an initial value of 2·2 to a plateau value of 0·5–0·6 2 min after the addition of pyruvate, indicating a change of the cytoplasmic pyridine nucleotides towards a more oxidized state.

The amounts of citrate, α-oxoglutarate and malate determined in the same experiment are shown in Fig. 2. All three compounds increased after 1 min, with citrate showing the most rapid initial increase, and after 15 min the values were, respectively, 15, 5 and 34 times the controls.

Experiments were performed with rat heart mitochondria to obtain information on the mechanism of citrate accumulation. With 5 mM pyruvate and 2 mM fumarate as substrate, the mitochondria respired rapidly and showed good respiratory control. On aerobic incubation with these substrates in the presence of phosphate acceptor, α-oxoglutarate, after a 1-min lag, was formed at the rate of 7·5 μmoles/g protein/min over a 12-min period. The initial level of α-oxoglutarate was 2 μmoles/g protein. The citrate content of the incubation medium remained constant at the initial value of 7 μmoles/g protein. Citrate also remained constant if the incubation with pyruvate and fumarate was carried out in the absence of phosphate acceptor. Addition of pyruvate (with or without fumarate) produced a rapid increase of reduced pyridine nucleotides, which was reversed by arsenite (an inhibitor of pyruvate oxidase) but not by malonate. Similar effects have been obtained with the intact beating heart[14], and NADH was found to be the major form of reduced pyridine nucleotide contributing to fluorescence increase, although NADPH also increased by 50 per cent.

The changes in the levels of the glycolytic intermediates after pyruvate addition illustrate the development of several different blocks along the glycolytic pathway. The addition of pyruvate is followed by an immediate rise of hexose monophosphates, citrate and α-oxoglutarate, and a fall of fructose-1,6-diphosphate and triose-phosphates. These changes are consistent with an initial inhibition of phosphofructokinase by citrate. The subsequent rise of triose-phosphates prior to fructose-1,6-diphosphate suggests the development of a more powerful inhibition at an enzymatic site between glyceraldehyde-3-P and 3-P-glyceric acid, even though citrate-levels continue to increase. However, the presence of a further block between 3-P-glyceric acid and pyruvate is indicated by the slow rise of the former intermediate immediately after pyruvate addition. The most feasible explanation for the observed changes of the glycolytic intermediates, therefore, involves primary inhibition of pyruvate kinase by the high concentration of pyruvate[15], and secondary inhibition of triose-P dehydrogenase by the build-up of its inhibitory product, 1,3-di-P-glyceric acid[16]. It is clear from these results that, under the special conditions of a high pyruvate concentration, phosphofructokinase is not the major rate-limiting enzyme of the glycolytic pathway and that there is no correlation between the citrate content of hearts and the levels of hexose mono- or di-phosphates. Some degree of inhibition of phosphofructokinase is indicated, however, by the increased levels of hexose monophosphates, and this could be mediated

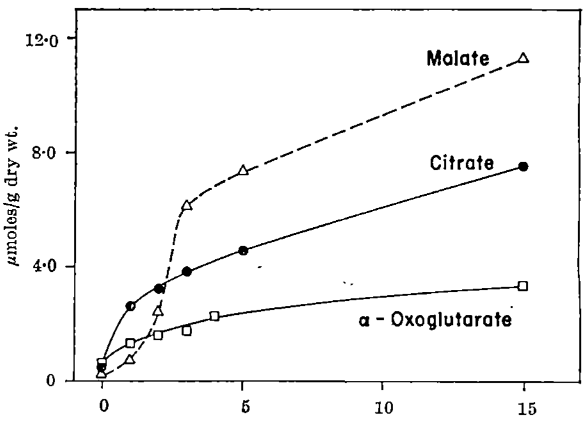

Fig. 2. The effect of pyruvate on the levels of malate, citrate and α-oxoglutarate in the isolated, perfused rat heart. The perfusion conditions were similar to those described for Fig. 1

by citrate. Possibly, initial inhibition of phosphofructokinase is reversed by the build-up of fructose-1,6-diphosphate, which has been shown to be a potent antagonist of citrate inhibition[9].

The lack of citrate formation in isolated mitochondria, despite its accumulation in the intact tissue, suggests the possibility that much of the citrate found in the intact tissue may be formed in the cytoplasm. This may be accomplished either by reversal of the isocitrate dehydrogenase reaction[17], or by the extramitochondrial condensation of oxaloacetate and acetyl coenzyme *A* (ref. 18). The increased formation of reduced pyridine nucleotides after pyruvate addition in both the intact heart and isolated mitochondria, and the reversal of this effect by arsenite, indicate that pyruvate is readily converted to acetyl coenzyme *A*, while the accumulation of α-oxoglutarate suggests that α-oxoglutarate oxidation may be limited by the availability of free coenzyme *A*. Isocitric dehydrogenase does not appear to be a rate-limiting enzyme under the conditions of these experiments.

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Staphylococcal Teichoic Acid Antibody in the Sera of Patients with Burns

THE sera of patients suffering from extensive burns have been found by one of us (J. K.) to contain precipitating antibodies to components of *Staphylococcus aureus* extracts. The presence of the antibodies was demonstrated by immunodiffusion methods from about two weeks after the first isolation of *Staphylococci* from the burned area. The more discriminating technique of immunoelectrophoresis showed that one of these numerous staphylococcal cell-components migrated rapidly towards the anode (its mobility similar to that of a phenol red marker). We have called this rapidly migrating component 'fast anodic antigen' (FAA); the antibody to it has only occasionally been found in normal human sera by the usual immunodiffusion methods. FAA is not an exotoxin but corresponds to a component of the cell wall.

Extracts containing FAA could be prepared by boiling or by autoclaving cells and also by perchloric acid or formamide extraction of whole cells. This suggested that FAA was probably a polysaccharide. For most of the present work, however, aqueous extracts were obtained from staphylococci (Glaxo strain 663) broken up in a modified Hughes press and subjected to brief ultrasonic treatment.

An antibody similar to FAA had been observed in the sera of rabbits immunized with whole-killed staphylococcal cells by a lengthy procedure (2 × 0·25 ml., 2 × 0·5 ml., 19 × 1·0 ml. doses intraperitoneally at 3-day intervals, 20,000 × 10^6 cells per ml.) designed to produce antisera to somatic antigens. Such antisera often showed by immunodiffusion in agar and on cellulose acetate the presence of at least eight distinct antibodies against whole-cell extracts, but by immunoelectrophoresis the one fast anodic arc could be clearly distinguished.

During immunochemical investigations of various staphylococcal cell fractions, we had observed that, among the active fractions, teichoic acid appeared to have an electrophoretic mobility similar to that of FAA when run on paper in barbitone buffer at *p*H 8·6. The teichoic acid extraction procedure of Baddiley *et al.*[1] from highly purified cell walls was used, although with our strains longer extraction times with trichloracetic acid (1 week) were required. The teichoic acid was finally freeze-dried, its identity being checked by chromatographic examination of acid and alkali hydrolysates.

We could confirm the observations of other workers[2,3] that teichoic acid can react with a specific serum antibody. We found teichoic acid to precipitate with antibody in antiserum from rabbits hyperimmunized with whole-killed staphylococcal cells. In spite of repeated attempts, however, we have been unable to immunize rabbits with pure teichoic acid and have concluded that it is not itself immunogenic and should therefore be designated as a haptene. The fact that whole-killed *Staphylococci* cells elicit a teichoic acid antibody response in rabbits points to some kind of structural association, involving teichoic acid within the cell wall, either as a higher polymer of teichoic acid than can be extracted with trichloracetic acid or as part of a moiety in which teichoic acid functions as haptenic determinant groups. In all probability the complex between teichoic acid and mucopeptide would fall into the latter class.

After these observations, we could demonstrate that teichoic acid on immunoelectrophoresis against both our potent rabbit antiserum and human burn serum gave a fast arc, suggesting that teichoic acid is serologically similar to FAA (Fig. 1). A further experiment—a linking immunoelectrophoresis run—showed confluent arcs, establishing beyond all doubt the identity of FAA and teichoic acid (Fig. 2). To the best of our knowledge this is the first time that a precipitating antibody to teichoic acid has been reported in human serum. Its presence was not surprising, as burn slough is almost invariably found to be heavily infected with *Staphylococci*, as well as with other organisms[5].

Recently, Mudd *et al.* have produced evidence of the part played by teichoic acid in inhibiting phagocytosis of *Staphylococci*[6]. They showed that normal human sera absorbed with teichoic acid had a reduced power to promote phagocytosis, presumably owing to combination of the teichoic acid with phagocytosis-promoting antibody. A precipitating antibody to teichoic acid would fit this picture reasonably well; the teichoic acid antibody would combine with the teichoic acid from the *Staphylococci*, leaving the phagocytosis unimpaired.

Work is in progress at present to determine the part played by teichoic acid in conferring immunity by examining various pathological human sera for the presence of antibody to teichoic acid. We are also extending the work on serum from burned patients; but it seems likely from our experience so far that high levels of this antibody can be attained in laboratory animals only after numerous injections over a long period and in man in special circumstances, such as those existing in burned patients.

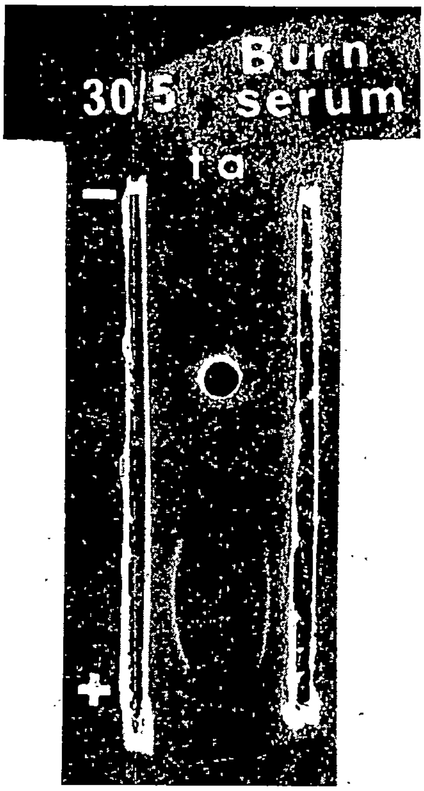

Fig. 1. Immunoelectrophoresis of staphylococcal fractions against serum from a burned patient and rabbit whole staphylococcal cell antiserum (30/5). *TA* represents wall ribitol teichoic acid. Adaptation of agar immunoelectrophoretic method (1 per cent washed 'Ionagar' on 3 in. × 2 in. slides, barbitone buffer pH 8·6. $\mu = 0·5$, voltage 10–12 V/cm)

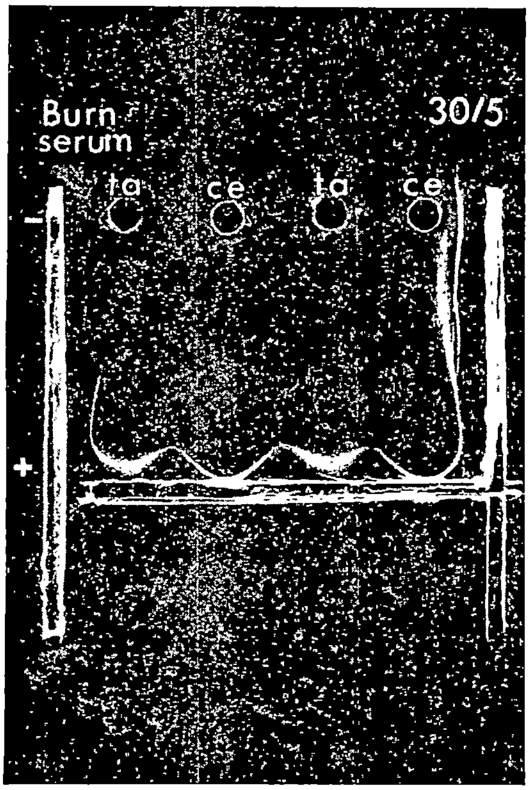

Fig. 2. Immunoelectrophoresis plate showing serological identity between the teichoic acid antibodies in serum from a burned patient and in rabbit whole-cell antiserum

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An Anomalous Effect of Ethionine on Ribonucleic Acid Synthesis

Besides giving rise to liver tumours[1], ethionine administration causes inhibition of protein synthesis[1,2] and also, in common with actinomycin D and puromycin, can reduce the extra synthesis of certain enzymes occurring in liver under the influence of 'inducing' agents[3]. Actinomycin D is known to suppress the synthesis of ribonucleic acid (RNA) possibly including 'messenger' RNA, *in vivo* or *in vitro*, and puromycin likewise suppresses RNA synthesis at least when administered to partially hepatectomized rats[4]. A fall in the incorporation of labelled precursors into RNA has been briefly reported to occur in liver after acute treatment with ethionine in large dosage[2,5], in accordance with the inhibiting effect on protein synthesis. Surprisingly, however, we have now encountered a converse effect on RNA synthesis in rats fed ethionine.

The rats were young male albinos, kept on a 20 per cent protein diet which, for the experimental rats, contained 0·25 per cent DL-ethionine (usually omitted on the day before killing). From the livers, excised after an overnight fast, there were prepared both a DNA-containing 'enzyme aggregate'[6] and, with the use of 2·2 M sucrose, highly purified nuclei[7]. Both types of preparation were assayed for the DNA-dependent RNA-nucleotidyl transferase ('RNA transcriptase', 'RNA polymerase'; Enzyme Commission 2.7.7.6)[6], with no addition of DNA but sometimes with addition of ammonium sulphate (to 0·4 M; cf. ref. 8).

In calculating *in vitro* results for the purpose of Fig. 1, activities were expressed on the basis of the actual amount of DNA in the sample assayed. For the controls, the mean values for μμmoles of ATP incorporated per mg DNA per min without (or with) ammonium sulphate were 2·9 (or 9·5) for enzyme aggregate and 13 (unchanged by ammonium sulphate) for purified nuclei; the values for UTP were similar. Whereas ethionine treatment has led in other laboratories to bile-duct proliferation and a rise in DNA/g liver, no such changes have now been found, the mean value for DNA being 1·75 mg/g both in control and in ethionine-fed rats. It may also be pointed out that for ethionine-fed rats the recovery of tissue DNA was similar to that in controls, both for enzyme aggregate (about 60 per cent) and for nuclei (about 40 per cent), and the total liver weight characteristically fell to 80 per cent of that in controls.

Both enzyme aggregate and nuclei, assayed in the absence of ammonium sulphate, showed a marked rise in activity in ethionine-fed rats, some rise being evident even with one day of feeding (Fig. 1). If ammonium sulphate were added to the enzyme aggregate system, and the comparison made with control preparations to

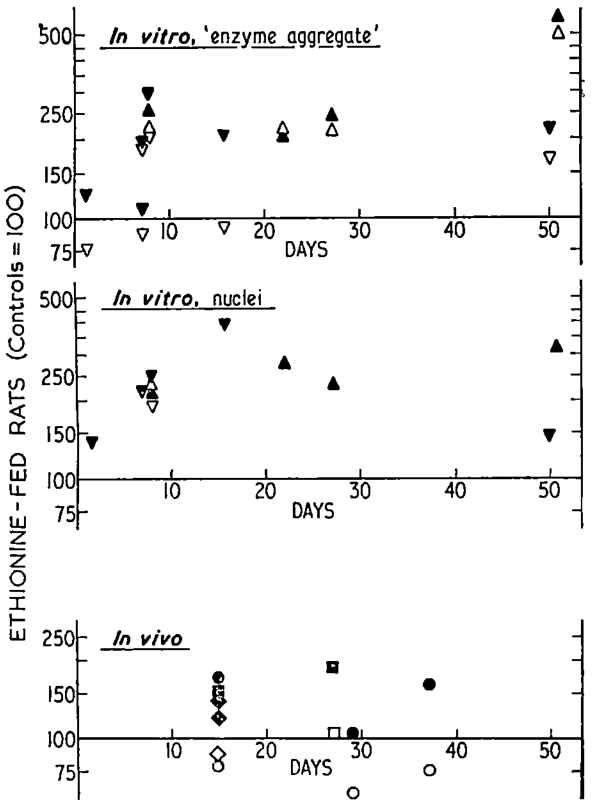

Fig. 1. RNA synthesis in rats fed ethionine (for the No. of days indicated on the axis), relative to that in controls (=100); see text for basis of calculation. Note log scale. *In vitro* results refer to the activity of RNA transcriptase, assayed on an 'enzyme aggregate' (top graph) or on purified nuclei (middle graph) as explained in the text. In one series of experiments (upright triangles) the precursor was ATP-8-^{14}C, and in a different series (inverted triangles) it was UTP-6-^{14}C. Assays without ammonium sulphate (solid triangles) and assays with ammonium sulphate added to 0·4 M (open triangles) were run side-by-side. *In vivo* results in the bottom graph refer to the recovery of radioactivity in whole-tissue RNA at different times after injection of orotate-6-^{14}C (solid symbols), and to the specific (not total) radioactivity of the uridine-nucleotide precursor pool (open symbols). Squares, circles and diamonds denote post-injection times of 30, 75 and 150 min respectively

which ammonium sulphate had likewise been added, the rise was reduced or abolished in some of the experiments with feeding periods of 16 days or less, but little reduced in most experiments (Fig. 1).

Measurements were also made of RNA labelling *in vivo* after injection of orotate-6-^{14}C (Fig. 1, bottom graph). With ethionine feeding the recovery of radioactivity in RNA was definitely enhanced, although less markedly than the incorporation *in vitro*. The enhanced recovery can validly be regarded as representing a rise in rate of RNA synthesis, since precursor measurements on the tissue suggested a fall rather than a rise in specific radioactivity (Fig. 1). Analyses on purified nuclei and on subfractions from the cytoplasm indicated that all cellular loci shared in the increase. Such analyses further showed that with ethionine feeding the amounts of RNA in purified nuclei and in the supernatant fraction were increased by up to 50 per cent.

There is, then, stimulation of RNA synthesis by feeding of ethionine, in contrast with the result obtained in acute experiments[2,5]. Preliminary experiments indicate that the stimulation found *in vitro* is not consistently, if at all, reduced by administration of actinomycin D or puromycin, but that (if account is taken of a tendency for precursor specific-activity to rise) the stimulation *in vivo* may be reduced by these agents or by adenine (the trial of which was prompted by its reported effect in reversing ethionine effects such as ATP depletion[1,2]). The tendency for the stimulation by ammonium sulphate to be reduced by ethionine, if fed for 16 days or less, suggests that there may be some increase in the availability of 'primer' DNA; but such a mechanism could evidently not account for the effect of ethionine when given for more than 16 days. Possibly the microsomal block in protein synthesis[2], while itself overcome with continued ethionine treatment[9], leads secondarily to a 'compensatory' increase in the output of messenger RNA, or else to a selective impairment of the formation of a 'repressor' governing the synthesis of RNA-synthesizing enzymes.

It should be emphasized that the qualitative agreement between the *in vitro* and the *in vivo* changes does not imply that RNA transcriptase is the sole enzymatic determinant of RNA synthesis. From the values for recovery of radioactivity in RNA *in vivo* and for precursor radioactivity, it would appear that the normal rate of RNA synthesis in liver is of the order of 3–8 mμmoles of UTP incorporated per mg DNA per min—a rate enormously greater than the *in vitro* rates already mentioned. Moreover, the turnover of RNA may also be governed by ribonuclease activity; in this connexion it is of interest that the acid–ribonuclease activity of supernatant fractions has been found in this laboratory to increase markedly in ethionine-fed rats.

In relation to the possibility that a rise in RNA synthesis is an important early step in heptocarcinogenesis, it should be pointed out that for carcinogenic azo dyes there is evidence of a rise in acute experiments[10], but with 2–5 weeks of feeding there is, in our experience, little change in RNA synthesis *in vivo* or *in vitro*. Moreover, there seems to be a striking rise in the rate of synthesis after prolonged feeding of α-naphthylisothiocyanate, which is non-carcinogenic but which (unlike ethionine as now given) causes bile-duct proliferation; but on addition of ammonium sulphate the activity *in vitro*, unlike that found after prolonged feeding of ethionine, becomes almost identical with that of controls likewise assayed in the presence of ammonium sulphate. In future work on the control of RNA synthesis, ethionine will evidently be a particularly useful tool.

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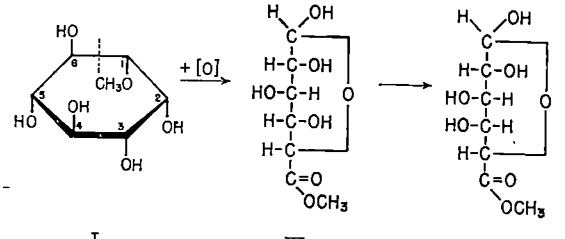
A Proposed Pathway for the Biosynthesis of Pectin and Methylated Uronic Acid Residues

MYOINOSITOL is readily converted into uronosyl and pentosyl monomers of pectin and related polysaccharides in higher plants[1,2]. During this conversion, myoinositol is cleaved between carbon atoms 1 and 6 and is oxidized at carbon 1 to yield D-glucuronic acid. The latter is further converted by the plant to a number of metabolic products including pectin and hemicellulose. It remains undetermined whether this conversion is an obligatory pathway of polysaccharide biosynthesis during cell wall formation, but certain theories may be advanced about the role of this process as a source of methylated uronosyl and pentosyl residues.

Four monomethyl ethers of myoinositol occur naturally[3]. They are L-bornesitol (1-*O*-methyl myoinositol) I, D-bornesitol (3-*O*-methyl myoinositol) II, D-ononitol (4-*O*-methyl myoinositol) III, and sequoyitol (5-*O*-methyl myoinositol) IV. (Hydrogen-carbon bonds are omitted in formulæ I, II, III, and IV.) If cleavage of the cyclitol ring occurs between carbon atoms 1 and 6 accompanied by oxidation of carbon 1 as observed in myoinositol, the product of 3-, 4-, or 5-*O*-methyl myoinositol will be 4-, 3-, or 2-*O*-methyl-D-glucuronic acid (V, VI, or VII).



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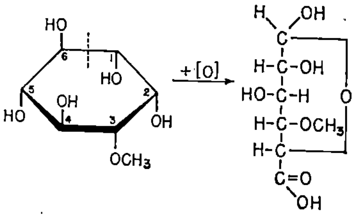
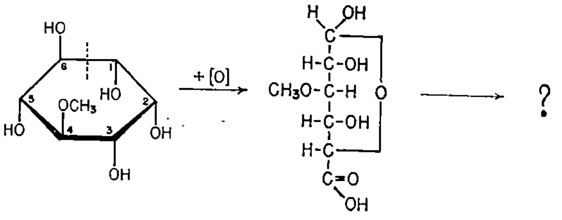


II V

4-*O*-Methyl-D-glucuronic acid is a common constituent of acidic polysaccharides found in hemicellulose[4], gums[5,6] and mucilages[6,7]. Characteristically, 4-*O*-methyl-D-glucuronosyl units occupy terminal, non-reducing positions, usually in short side-chains where they are attached to D-galactose or L-arabinose units or as single residues attached directly to the main chain of xylan. Presence of a 4-*O*-methyl group could control further chain enlargement and so have important consequences during polysaccharide biosynthesis[8]. Similarly, a 4-*O*-methyl group might also prevent decarboxylation[9] and so account for the widespread occurrence of 4-*O*-methyl-D-glucuronosyl units associated with pentosans. 3-*O*-Methyl-D-glucuronic acid has been reported in jute fibre hemicellulose[10]. No reports of naturally-occurring 2-*O*-methyl-D-glucuronic acid or its derivatives could be found although its decarboxylation product, 2-*O*-methyl-D-xylose VIII, has been isolated from plum leaf hemicellulose[11].



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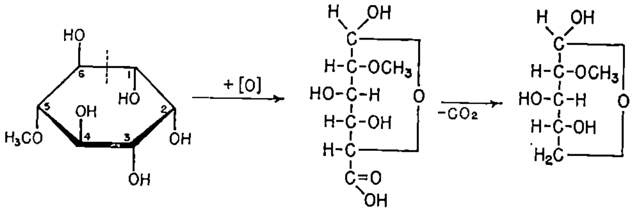

IV VII VIII

Now consider L-bornesitol. If this cyclitol is cleaved between carbon atoms 1 and 6 and carbon 1 oxidized, the product will be the methyl ester of D-glucuronic acid IX. This cleavage resembles an enzymatic reaction found in the microbial oxidation of camphor[12]. In the plant, the methyl D-glucuronate would have to be epimerized to methyl D-galacturonate *X* prior to its incorporation into pectin. Enzymatic steps that will catalyse this conversion as uronic acid nucleotides have been described[13], but the analogous conversion as methyl uronate nucleotides has not been explored. Incorporation of methyl D-galacturonate-U-^{14}C (methyl group unlabelled) into pectin has been demonstrated[14], but additional experiments are now needed to show that the methyl group accompanies uronic acid residues into polysaccharide during biosynthesis. Albersheim[15] has presented evidence that the rate of incorporation of myoinositol into pectin parallels the rate of incorporation of D-glucose and that in *Avena* coleoptile sections the effect of indolyl-3-acetic acid on incorporation of D-glucose and myoinositol into D-galacturonic acid residues and of methyl from methionine into methyl ester groups of pectin is similar.

The biosynthesis of myoinositol in plants proceeds from D-glucose without fragmentation of the carbon chain[16,17] by a cyclizing mechanism still not fully understood. Scholda, Billek, and Hoffmann-Ostenhof[18] have recently shown that myoinositol is the precursor of sequoyitol, D-inositol, and pinitol in leaves of *Trifolium incarnatum* infused with myoinositol-^{14}C. If so, it appears quite likely that L-bornesitol is also derived directly from D-glucose *via* myoinositol.

With considerations discussed here as a base, a hypothetical pathway of pectin biosynthesis is presented as follows:

D-glucose——→myoinositol——→L-bornesitol——→
methyl D-glucuronate——→methyl D-glucuronate
nucleotide——→methyl D-galacturonate nucleotide——→
pectin

Arrows between proposed intermediates do not necessarily indicate single step conversions. Indeed, in the plant all these conversions might well involve phosphorylated intermediates. The most important aspects of this new scheme are: (1) cyclization of hexose to a cyclitol; (2) methylation of the cyclitol; (3) oxidative cleavage of the cyclitol to the uronic acid methyl ester precursor of pectin, or the *O*-methyl glucuronic acid precursor of hemicellulose.

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Time-dependent Inhibition of Myogenesis by Actinomycin *D*

MUSCLE owes its morphological and biochemical identity to proteins such as actin, myosin, tropomyosin, etc.,[1] and it is reasonable to suspect that these molecules are somehow represented in the genome. Until rather recently, it was generally believed that all new proteins are manufactured by freshly made messenger RNA's of exceedingly short half-lives. However, recently it has become rather evident that there are at least a few systems in which genetic messages are sent many generations before they are used[2]. Is this the case with myogenesis, or is the necessary information for muscle differentiation sent shortly before it is called into play?

Actinomycin *D* seemed to provide an experimental approach to the question. The antibiotic complexes with DNA and thereby prevents synthesis of RNA. At the same time, already-formed templates continue in the manufacture of proteins and DNA replication occurs[3]. The myogenic system selected for investigation was regenerating mouse skeletal muscle. This choice was dictated primarily by the fact that there is a great deal of synchrony attending the reaction: during the second to third day after muscle injury a class of highly proliferative mononucleated cells appears in the wound[4,5], and on the fourth day newly developing muscle fibres are in abundance. If the mononucleated cells are arrested by colchicine new muscle fibres fail to appear on schedule[5]. Moreover, if the mononucleated cells incorporate tritiated thymidine, differentiating muscle fibre nuclei show radioactivity after autoradiography[6,7]. Thus, it would seem that in the 3–4-day interval after wounding many presumptive muscle cells are achieving the differentiated state.

Transections were inflicted in tibialis anterior muscle of female Swiss-Webster mice, weighing 25–30 g, under light methoxyflurane anæsthesia. Animals received a single intraperitoneal dose of 0·99 micrograms of actinomycin *D* (kindly donated by Merck and Co.) at 24, 48, 72 or 100 h after transection. Tissues were fixed for histological investigation five days after wounding. Day five was chosen because at this time there normally are scores of regenerating fibres in each section through the wound[1–6].

Large numbers of typical five-day regenerating myotubes were encountered in all sections of the 24, 48 and 100 h series (Fig. 1). In five of five specimens given actinomycin at 72 h, regeneration was severely depressed (Fig. 2) and was represented by only an occasional, single, immature muscle fibre. Although few in numbers, these fibres were normal morphologically.

The findings indicate that myogenesis depends on a new round of RNA synthesis (presumably messenger) in the interval immediately preceding the histological advent of muscle as a tissue. This, in turn, suggests that muscle is specifically represented in the genome and that the information for making it is communicated shortly before it is used. Along this same line, it may be noted that muscle regeneration can be prevented by X-irradiation, but sensitivity to the effective dose is lost somewhere on the third day; that is, after the genetic messages seem to have been sent[8].

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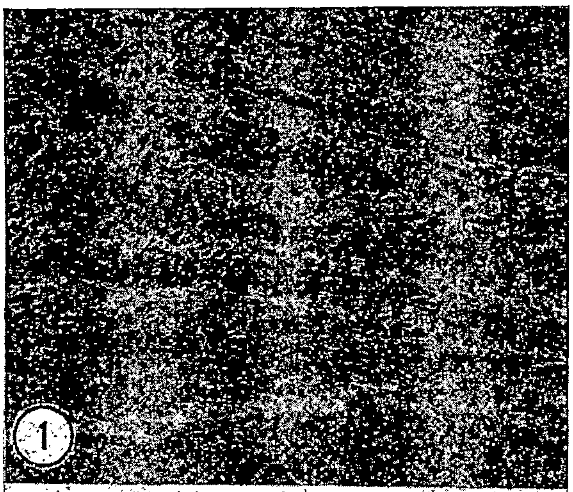

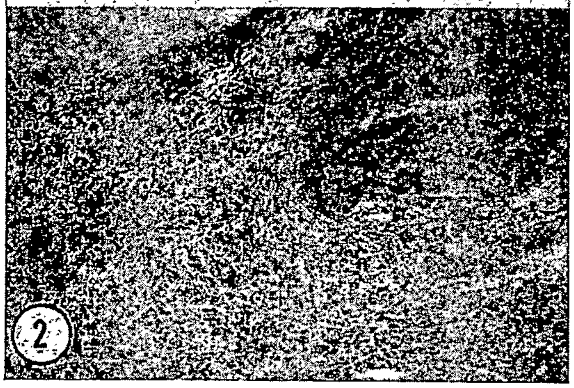

Fig. 1. Newly differentiating muscle fibres in five-day wound from animal injected with actinomycin *D* 24 h following muscle transection (× 200).

Fig. 2. Five-day wound coagulum from animal injected with actinomycin *D* 72 h after muscle transection. This representative field is devoid of newly differentiating muscle fibres. In normal regeneration and in cases injected with actinomycin *D* at 24, 48 and 100 h such sections exhibited scores of differentiating muscle fibres. (× 100)

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Occurrence of Homoserine in the Liver of Ethionine- or Methionine-treated Rats

INTRAPERITONEAL administration of L-methionine[1] as well as incorporation of DL-methionine[2] and DL-ethionine[3] in the purified diets of rats resulted in increased activity of rat liver cystathionase ('soluble' cysteine desulphydrase). Recently, I have also observed[4] that one injection of L-ethionine likewise caused, in 4 h, significant increase in the activity of the enzyme in rat liver. In an effort to determine the chemical basis for the effects of these amino-acids, analyses of the free amino-acids of the liver of control (non-treated) as well as of ethionine and methionine-treated rats were carried out.

For this purpose, male Wistar rats, weighing 90–100 g, were fed for 7 days a purified diet previously described[2], containing 18 per cent casein. The control animals were killed without any treatment; the ethionine- and methionine-treated animals were injected respectively with 54 mg L-ethionine and 48 mg L-methionine per 100 g body weight, by intraperitoneal route, 4 h before death. Another group was fed the purified diet containing 1·5 per cent DL-ethionine for a week. Free amino-acids were extracted from representative samples of 1 g of liver according to the procedure described by Awapara[5]. Chromatographic analyses of the extracts were performed on Whatman paper[4] (descending method), solvents: butanol/acetic acid/water (50:25:25) and phenol/water (80:20), on thin-layer silica gel (ascending method) using the same solvents, and in a Technicon autoanalyser after passing the extract through a column of 'Dowex 50', according to the method of Piez and Morris[6] as modified by Hamilton[7].

Presence of homoserine in the livers of ethionine or methionine-treated rats was indicated by paper chromatography and thin-layer silica gel chromatography. Furthermore, examination of the amino-acid profile obtained by the automatic recording apparatus revealed a peak in the position for homoserine, before that of glutamic acid, in the extracts of the ethionine- or methionine-treated rat livers. This peak was absent in the extracts of non-treated control animals' livers. Addition of DL-homoserine to the extract from control rat livers yielded a new peak located at the position identical with that occupied by the characteristic peak of the extracts from treated rats.

Demonstration of the accumulation of homoserine in the livers of ethionine- or methionine-treated rats is of particular importance, since administration of DL-homoserine itself[1], a substrate for cystathionase[8], induced an increase in rat liver cystathionase like methionine or ethionine. Matsuo and Greenberg[9] also reported the formation of radioactive homoserine in mice when injected with DL-methionine-2-C^{14}; however, the mechanism for the conversion of methionine to homoserine is not elucidated. According to Stekol *et al.*[10], methionine and ethionine follow the same metabolic pathway in the rat, but in the known pathway of the degradation of L-methionine[11], namely:

L-methionine → L-homocysteine → L-cystathionine → L-cysteine, the formation of homoserine could not be demonstrated, since the cleavage of L-cystathionine, the intermediate metabolite from which homoserine could be expected, gave rise only to α-ketobutyric acid and cysteine[6].

The elucidation of the mechanism of formation of homoserine in rat liver is under investigation.

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Presence of Imidazoleacetic Acid Riboside and Ribotide in Rat Tissues

THE products of histamine metabolism *in vivo* have been catalogued in investigations of the urine[1,2], but relatively little work has been done on metabolites present in tissues. Since excreted metabolites reflect only the composite outflow of terminal products and need not represent the metabolites present in a tissue, it seemed of interest to examine the radioactive compounds present in some tissues after the injection of labelled histamine and histidine. A special effort was made to demonstrate the presence of histamine adenine dinucleotide (HAD) and histamine adenine dinucleotide phosphate (HADP)[3,4].

Male rats (300–350 g) were injected intraperitoneally with (2-^{14}C)histamine (18 μc./μmole) or uniformly labelled (^{14}C)-L-histidine (240 μc./μmole) and decapitated after specified intervals. Organs were homogenized in 5 per cent trichloroacetic acid (TCA), centrifuged, and the supernatant solution extracted with ether. Each of the aqueous extracts was added to a 'Dow 1-Cl⁻' column and eluted with water (fraction 1-I), 0·001 N hydrochloric acid (fraction 1-II), 0·0025 N hydrochloric acid (fraction 1-III) and 4 N hydrochloric acid (fraction 1-IV). Fraction 1-III was then chromatographed on 'Dow 50-H⁺' and eluted with increasing concentrations of hydrochloric acid. Aliquots of various fractions were chromatographed on Whatman 3 *MM* paper in ascending systems containing ethanol–0·1 N acetic acid (50 : 50) and isopropanol–0·5 N hydrochloric acid (65 : 35) and descending in isopropanol–chloroform–ammonium hydroxide–water (55 : 25 : 10 : 10). These solvent systems separate the known metabolites of histamine, although some uncertainty exists in differentiating between methylated and non-methylated compounds.

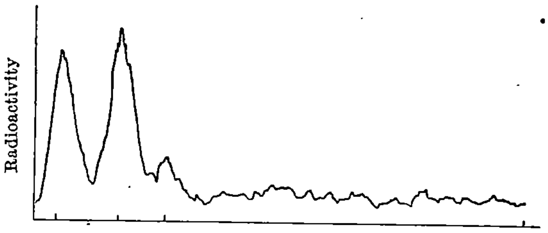

Fig. 1. Radioactive scan of chromatogram of TCA extract of kidney. The solvent system was isopropanol–CHCl$_3$–NH$_3$–H$_2$O

Histamine riboside, imidazoleacetic acid riboside, and imidazoleacetic acid ribotide were gifts of Drs. H. Tabor, H. Bauer and G. M. Crowley; histamine adenine dinucleotide phosphate (HADP) was a gift of Dr. S. G. A. Alivisatos. Labelled histamine adenine dinucleotide (HAD) and HADP were synthesized[3,4] with a pig brain DPNase[5].

Since kidney had the highest concentrations of radioactivity from injected histamine (Table 1), primary attention was given to that organ. Three major radioactive fractions were present on paper chromatography of the TCA extract (Fig. 1 and Table 2) of kidney from rats, each of which received a total of 35 μc. histamine (650 μg/kg) in three equal injections 120, 75 and 30 min before decapitation. On chromatography of the TCA extract on a 'Dow 1-Cl⁻' column, material that corresponded in all solvent systems with the radioactive peak at R_F 0·13 of Fig. 1 was eluted in fraction 1-I (Table 2), and radioactivity that chromatographed in all solvent systems as the peak at the origin of Fig. 1 was eluted in fraction 1-III (Table 2). All the radioactivity was eluted with water on chromatography of fraction 1-III on 'Dow 50-H⁺' (Table 2). The R_F values of the radioactivity in the chromatograms of these eluates corresponded with known imidazoleacetic acid riboside (fraction 1-I) and ribotide (fraction 1-III, 50-I) (Table 2). This identification was supported by hydrolysis of both 'Dow 1' eluates in 6 N hydrochloric acid for 64 h at 150° (ref. 6): chromatography of the hydrolysates showed that the radioactivity migrated in all systems with hydrolysed imidazoleacetic acid ribotide and with authentic imidazoleacetic acid. The third major peak in the chromatogram of the TCA extract (Fig. 1) did not correspond with any known metabolite tested (Table 2); hydrolysis of this material after elution from the chromatogram did not alter its R_F on chromatography. Similar chromatographic techniques showed the presence of imidazoleacetic acid ribotide in liver.

Since little radioactivity could be found in brain after the administration of labelled histamine (Table 1), it was necessary to inject labelled histidine to attain workable

Table 1. CONCENTRATION OF RADIOACTIVITY IN RAT TISSUES AFTER INJECTION OF LABELLED HISTAMINE AND HISTIDINE

Isotope injected	Time after infection (min)	Radioactivity in TCA extract of organs (c.p.m./g tissue) Kidney	Liver	Lung	Spleen	Brain
^{14}C-histamine 10 μc	30	280,000	73,000	16,000	16,000	1,000
	120	160,000	34,000	6,400	8,200	630
^{14} C-histidine 10 μc	30	32,000	58,000	6,400	16,000	7,30
	120	17,000	11,000	4,000	5,400	6,900

Table 2. R_F Values of Chromatographic Standards and of Radioactive Material from Tissues

		R_F values	
Compound or fraction	Ethanol-acetic acid	Isopropanol-	Isopropanol-$CHCl_3$-NH_3-H_2O
Histamine	0·74	0·43	0·62
1-Methylhistamine	0·78	0·50	0·71
Acetylhistamine	0·83	0·67	0·77
Imidazoleacetic acid	0·77	0·64	0·26
Methylimidazoleacetic acid	0·77	0·64	0·30
Imidazoleacetic acid riboside	0·74	0·57	0·13
Imidazoleacetic acid ribotide	0·67	0·54	0·00
Histamine riboside	0·86	—	0·24
Histidine	0·53	0·34	0·16
α-Methylhistidine	0·65	0·47	0·22
1-Methylhistidine	0·57	0·37	0·19
3-Methylhistidine	0·62	0·41	0·20
Urocanic acid	—	0·66	0·26
Dihydrourocanic acid	—	0·66	0·32
Carnosine	0·51	0·45	0·16
Formiminoglutamic acid	—	0·69	0·06
Glutamic acid	0·65	0·62	0·04
Anserine	0·60	0·39	0·17
HAD	0·36	0·08	0·03
HADP	0·60	0·42	0·31
Kidney:			
TCA extract	0·63	0·54	0·00
	0·66	0·57	0·13
	0·73		0·22
1–I	0·73	0·57	0·13
1–III	0·67	0·54	0·00
50–1	0·67	—	0·00
Eluate of unknown (Fig. 1)	0·63	0·57	0·22
Brain:			
1–I	0·52	0·35	0·13
	0·73	0·42	0·16
	1·0	0·57	0·58
		1·0	0·79
1–III	0·65	0·55	0·00
	0·82	0·89	0·34
			0·75
			0·79
1–IV	0·52	0·35	0·16
	0·76	0·64	0·34
		0·89	

levels of radioactivity. After the injection of 10 μc. of histidine both 120 and 30 min before decapitation (total histidine: 30 μg/kg), at least 80 per cent of the radioactivity migrated as histidine on chromatography of the TCA extract of brain. After chromatography on 'Dow 1-Cl⁻', eluate 1-I contained a small fraction of the total radioactivity that chromatographed in all solvent systems as imidazoleacetic acid riboside (Table 2) and which on hydrolysis with hydrochloric acid at 150° gave a product that migrated as imidazoleacetic acid. In addition, paper chromatography of fraction 1-III showed a radioactive peak corresponding to imidazoleacetic acid ribotide in all solvent systems (Table 2). The radioactivity incorporated into both the riboside and ribotide was less than 1 per cent of that in the total TCA extract, and of the same order of magnitude as the radioactivity identified as histamine and as imidazoleacetic acid. The radioactivity in these compounds was a function of the time between injection and decapitation and of the amount of isotope administered. Thus, when 30 μc. of histidine (45 μg histidine/kg) was injected into rats 120 min before decapitation, more than 5 per cent of the total radioactivity in the TCA extract was identified as imidazoleacetic acid ribotide on paper chromatography of fraction 1-III.

No evidence for the incorporation of radioactivity into HAD or HADP (histamine analogues of the nicotinamide adenine nucleotides) could be found after injection of labelled histamine or histidine into rats or guinea pigs 30 or 120 min before decapitation. To escape detection, less than 0·02 per cent of the total radioactivity in rat kidney or liver and less than 0·3 per cent in the rat brain (from histidine) could represent HAD or HADP. The dinucleotides may turn over too rapidly to be detected by these methods[7].

Although these experiments are only roughly quantitative and were not designed to measure steady-state levels in tissues, it is clear that tissue metabolites differ from urinary metabolites[2], which are imidazoleacetic acid riboside, 60–70 per cent; methylimidazoleacetic acid, 5 per cent; imidazoleacetic acid, 2 per cent; and acetylhistamine, 4 per cent. These results were obtained after a dose of histamine of 10 mg/kg, considerably higher than that used in the work recorded here. In kidney, multiple injections of histamine resulted in about half the label appearing in imidazoleacetic acid riboside with nearly as much in imidazoleacetic acid ribotide, not previously reported as a metabolite *in vivo*, and a smaller fraction in a third spot not coinciding with any of the urinary metabolites. In brain, after injection of labelled histidine, chromatographic data indicated the presence of imidazoleacetic acid riboside and ribotide, representing small fractions of the total tissue radioactivity, together with histamine, imidazoleacetic acid, and higher levels of unidentified metabolites.

The most likely pathway for synthesis of the ribotide is through condensation of imidazoleacetic acid with phosphoribosyl pyrophosphate a reaction demonstrated *in vitro*[8,9]; the riboside would then arise by dephosphorylation[9].

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PHYSIOLOGY

Acetylcholine Receptors in Muscle Membrane

Cæsium depolarization of muscle membrane is further increased when choline is added to the bathing cæsium-Ringer solution[1,2]. This cæsium-choline depolarization remains constant in the continued presence of cæsium, even when choline is removed from the Ringer. When cæsium is replaced by potassium following removal of choline, the cæsium–choline effect is reversed and the normal resting membrane potential is recovered[2]. It appears that choline reacts in specific fashion with membrane 'receptors', the result of this reaction depending on cæsium and potassium ions; in the presence of cæsium, a depolarization results; in the presence of potassium, none. Scanning data show that when muscles are treated with cæsium–Ringer, the entire muscle surface becomes sensitive to choline. The work presented here was designed to elucidate the action of acetylcholine (ACh) on the cæsium-treated muscle membrane, in comparison with the action of choline (Ch) previously described, and to determine whether: (1) an additional depolarization is evoked only when cæsium is added before acetylcholine; (2) D-tubocurarine and eserine have any effect on the additional depolarization produced by ACh.

The experimental work was carried out under conditions identical to those previously described[1,2]. Eighteen pairs of sartorius muscles from *Rana pipiens* were used in each experimental solution. Each point in the figures is the average of potential measurements on 170 cells. The *S.E.M.* for membrane potential measurements is less than 1 mV. Bathing solutions were made up by adding cæsium, potassium, choline, acetylcholine, eserine or

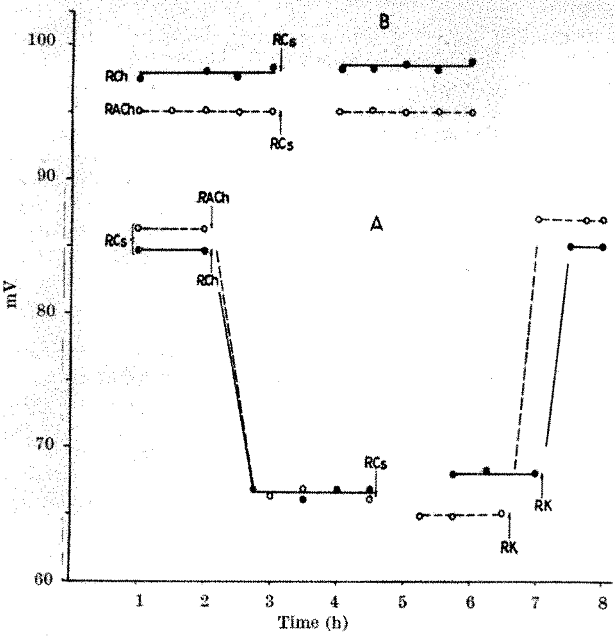

Fig. 1. Frog sartorius muscle membrane potential versus time after dissection. Bathing solutions indicated as follows: *R*.Cs = 2·5 mM cæsium–Ringer; *R*.Ch = 10 mM choline–Ringer; *R*.ACh = 10 mM acetylcholine–Ringer; *R*.K = normal (2·5 mM K) Ringer

D-tubocurarine in appropriate amounts to standard potassium-free Ringer (112 mM NaCl, 1·89 mM $CaCl_2$, 2·5 mM Na_2HPO_4, 0·5 mM NaH_2PO_4). Experiments with eserine were carried out in bicarbonate buffer. All experiments were performed at 25° C. The effects on the resting membrane potential of the various treatments are summarized in Figs. 1–3.

Fig. 1*A* indicates that 10 mM ACh or Ch evokes an additional depolarization in muscle fibres previously soaked in cæsium–Ringer. This additional depolarization is not reversed when muscles are washed with cæsium–Ringer free of Ch or ACh, but is reversed after washing with normal (2·5 mM potassium) Ringer. Equivalent amounts of either ACh or Ch evoke the same fractional depolarization, approximately 1·3 for 10 mM ACh or Ch. Neither substance produces this fractional depolarization in the absence of cæsium, or in normal Ringer (Fig. 1*B*). Addition of cæsium to cæsium-free Ringer containing ACh or Ch does not evoke the additional depolarization (Fig. 1*B*); the cæsium–choline or cæsium–acetylcholine effect is only observed when muscles are first equilibrated with cæsium.

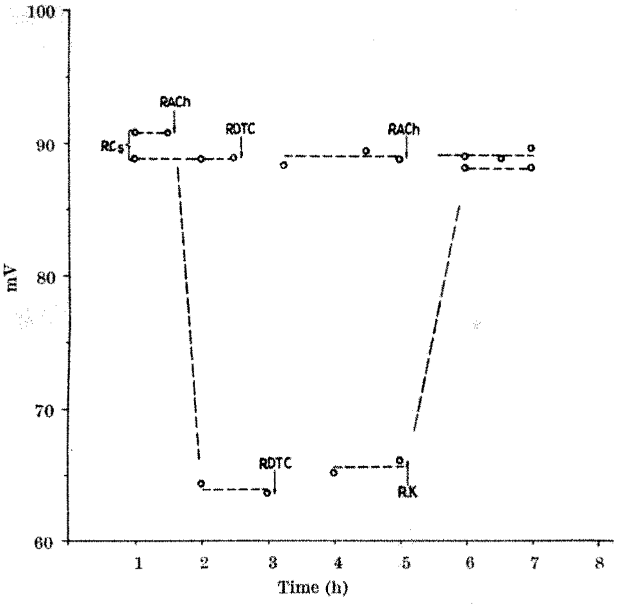

Fig. 2. Same as Fig. 1; *R*.DTC = D-tubocurarine–Cs–Ringer (1·4 × 10^{-4} M)

Fig. 2 shows that addition of D-tubocurarine to muscles previously soaked in cæsium–Ringer blocks the depolarizing effect of ACh or Ch added later. The depolarization produced by ACh or Ch added before D-tubocurarine is not altered when the muscles are afterwards washed with D-tubocurarine–cæsium–Ringer, but it is reversed when they are washed with normal Ringer, just as in Fig. 1*A*.

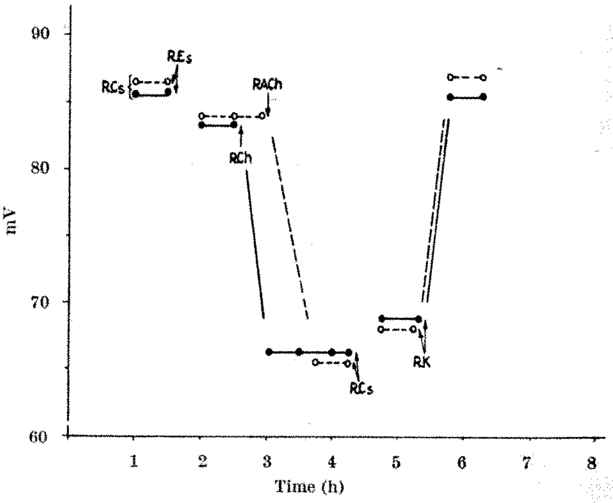

Fig. 3. Same as Fig. 1; *R*.Es = eserine–Cs–Ringer (1 × 10^{-3} M)

Fig. 3 demonstrates that the cholinesterase inhibitor, eserine, has no effect on the cæsium–ACh or cæsium–Ch depolarization, nor on its reversal by normal Ringer.

Results of scanning investigations under each of these experimental conditions showed no statistically significant regional differences in the average resting potential throughout the entire muscle cell surface, including end-plate regions[2].

The results obtained indicate that the stable depolarizing effect of Ch or ACh is only evoked when the membrane receptors are first 'sensitized' by cæsium ions. Cæsium does not sensitize these receptors if they have already been occupied by Ch or ACh. Evidence has been obtained that these receptors are located in the external surface of the muscle membrane[2]. Recent work has demonstrated that ACh reacts only at the external surface of the motor end-plate membrane of muscles fibre soaked in normal (2·5 mM K) Ringer[3]. The whole Ch–cæsium or ACh–cæsium-receptor complex is needed to produce the observed depolarization. It also appears that cæsium and potassium ions evoke different configurations in the receptor system, and consequently change its molecular complementarity to ACh or Ch in a different way. The restoration of the normal resting membrane potential by washing with normal Ringer after cæsium–ACh or cæsium–Ch depolarization suggests that potassium either releases ACh or Ch bound to receptors, or it restores the original configuration of the ACh- and Ch-receptor complex. (Experiments are in progress to determine differences in the rate of release of ^{14}C-labelled choline from muscles bathed in cæsium or potassium Ringer.) It has also been shown that the additional depolarization produced by ACh or Ch in cæsium–Ringer bears no relation to the external sodium ion concentration down to approximately one-third of the normal 112 mM sodium[1,4]. A direct and specific exchange between sodium and either Ch or ACh therefore seems most unlikely, as does the hypothesis that the depolarization is due only to a specific increase in sodium permeability.

D-Tubocurarine presumably prevented Ch or ACh from reacting with the cæsium-sensitized receptors, and consequently blocked their depolarizing effects. On the other hand, inactivation of acetylcholinesterase by eserine did not significantly affect the Ch or ACh depolarizations. This suggests that cholinesterase is not directly involved in these effects. It also suggests, but does not establish, that cæsium may inhibit cholinesterase under these experimental conditions.

Taken together, the D-tubocurarine and eserine effects, and the reversal of depolarization by washing with normal Ringer, lead us to conclude that Ch and ACh react similarly with cæsium-sensitized receptors, and that the depolarization resulting from this reaction does not require esterase activity for its reversal. D-Tubocurarine combines with the receptors in a way which prevents ACh or Ch from reacting, regardless of the presence of cæsium, but cannot displace ACh or Ch from prior combination with the receptors. ACh and Ch appear to react with the receptors in normal Ringer, but no depolarization results, and the receptor reaction with cæsium is blocked. On the other hand, the depolarizing effect of the ACh- and Ch-receptor reaction after receptor sensitization by cæsium is reversed by removing the cæsium. All these results together suggest very strongly an allosteric type of effect[7]; if one region or site on the receptor molecule reacts with ACh, Ch and D-tubocurarine, and another region or site reacts with cæsium and potassium, and if a change at either site changes the reactivity of the other, our results can easily be explained. Work in progress is aimed at testing this hypothesis.

The absence of any detectable localization of the cæsium–Ch and cæsium–ACh depolarization indicates that these receptors must be distributed over the entire muscle cell surface, not restricted to the motor end-plate region. It is well established that in the absence of cæsium, externally applied D-tubocurarine blocks ACh depolarization at the only place it occurs, the motor end-plate[3]. Our results suggest that receptors similar to those normally active in the motor end-plate are in fact present all over the surface, but are kept in a configuration which does not allow ACh-receptor reaction to lead to depolarization. The data presented lend new support to the view of the presence of the ACh system in conducting fibres[5,6]. This configuration is changed by the effect of cæsium so that depolarization does result from the ACh reaction. The nature of the substance(s) or mechanisms by which the ACh-receptors outside the motor end-plate regions are normally maintained in a configuration not permitting depolarization remains to be elucidated.

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Evidence for Sorption as a Mechanism of the Olfactory Analysis of Vapours

THE physiological basis for olfactory discrimination may involve at least two basic mechanisms which need not be mutually exclusive : (*a*) the receptors themselves may be more or less selectively sensitive to different groups of chemicals ; (*b*) the receptor sheet as a whole might separate chemical vapours by adsorption or absorption in a manner analogous to gas chromatography columns. There has been some supporting evidence for the first mechanism[1,2], and suggestive evidence for the second has recently been reported[3]. The evidence suggesting the second mechanism depends, in part, on sampling the activity from different regions of the frog olfactory mucosa by recording the discharges from two olfactory nerve branches (a lateral one and a medial one), which under a dissecting microscope appear to subserve different mucosal regions. That these branches do indeed subserve different mucosal regions is supported by recent electrical stimulation experiments. The antidromically conducted discharges in response to individual stimulation of the lateral and medial nerve branches were recorded by an electrode placed at several points on the epithelium. The discharge tracts for the two branches were quite separate and localized (Fig. 1).

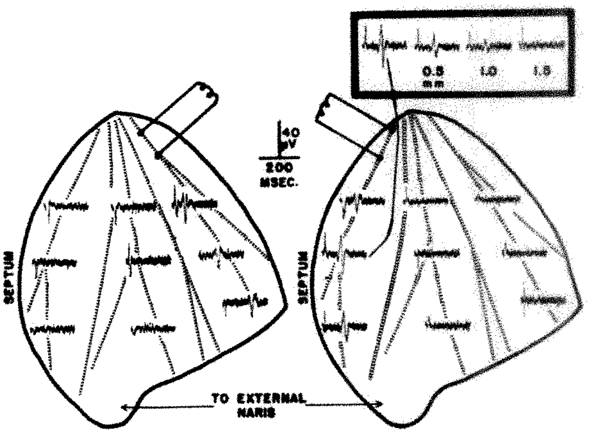

Fig. 1. Schematic diagram of the olfactory epithelium on the dorsal aspect of the olfactory sac of *Rana catesbeiana* showing the responses recorded at nine loci when the lateral nerve branch is stimulated (left) and when the medial nerve branch is stimulated (right). Hatched lines represent major olfactory nerve branches. The insert shows the reduction in response when the recording electrode is moved by 0·5 mm steps from the locus designated. Width of mucosa at the widest point is about 8 mm. Electrodes: bipolar, stainless-steel, 68μ diameter enamelled wire. Stimulator: Grass *S*4 with *SIU*4*A* isolation unit set for constant suprathreshold stimuli. This experiment was repeated in three animals with no appreciable difference in the results

I have observed[3] that the ratio computed by dividing the amplitude of the summated discharge recorded from the lateral nerve branch by that recorded from the medial nerve branch differed for different stimuli. In addition, the time lapse between the lateral nerve response and the medial nerve response (the latency difference) differed for different stimuli. These data demonstrated a space-time neural encoding of the analysis of the incoming vapours by the mucosa. In addition, since the latency differences could not easily be explained by selectively sensitive receptors, it was suggested that sorption along the mucosal sheet might also be involved in the stimulus analysis. Evidence to be reported here further supports this suggestion. This depends on the supposition that if sorption along the mucosal sheet played no part in the analysis and if selectively sensitive reactors were the only operative mechanism, the response amplitudes of the two nerve branches for any given chemical would not be appreciably changed in relation to each other by changing the direction of air flow through the nasal passageway. This would still hold even if the receptors were regionally grouped according to their selective sensitivity. If, on the other hand, the spatial separation of vapours by sorption were an important part of the analysis, the neural discharge pattern for any given stimulus would be considerably dependent on the direction of flow just as the region of greatest concentration at any given time in a physical sorption column is dependent on the point of entry of the gas.

These alternatives were tested by comparing the discharge magnitude of the lateral nerve branch to that of the medial nerve branch for several chemical stimuli under two conditions : (*a*) puffing the odorant into the external naris of the frog ; (*b*) puffing the odorant into the internal naris of the frog. The recording techniques, stimulating techniques, and data treatment have been described previously[3].

In all animals external naris stimulation with citral and geraniol yielded considerably larger responses on the medial nerve branch than on the lateral nerve branch, whereas internal naris stimulation with citral and geraniol yielded considerably larger responses on the lateral nerve branch and smaller responses or none at all on the medial nerve branch (Fig. 2). The shifts in response to *d*-limonene and octane were quantitatively much less but still in the same direction, although octane did occasionally show a slight shift in the opposite direction like that shown in Fig. 2. Thus, for citral, geraniol, and, to a lesser extent, *d*-limonene, the neural discharge patterns do indeed depend on the direction of air flow through the olfactory sac. Therefore, there is strong indication that sorption along the mucosal sheet is involved in the olfactory analysis of vapours.

It was also observed that internal naris stimulation yielded a shorter latency on the lateral nerve and a longer latency on the medial nerve than external naris stimulation, but this probably merely reflects the proximity of the mucosal regions supplied by these branches to each naris. However, no matter which branch responded first, when the chemicals were ranked according to the magnitude of the latency differences between their evoked responses on the two nerves, they fell into the same order regardless of the direction of air flow. This is not inconsistent with mucosal sheet sorption since the velocity of molecular movement through the olfactory passageway for each stimulus should not be expected to change with the direction of air flow *per se*.

It should be noted that because of vapour pressure differences, the stimulation with air bubbled through citral and geraniol was at a lower concentration than that with air bubbled through *d*-limonene and octane. Therefore the sorption mechanism and the resultant differential neural discharge may be analysing and encoding stimulus intensity rather than stimulus quality. This possibility is now being investigated.

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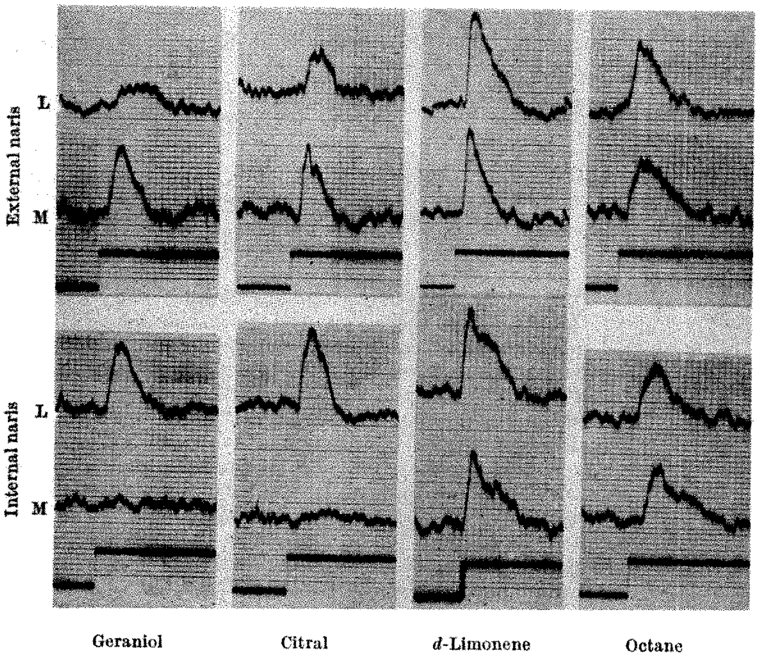

Fig. 2. Typical summated response records showing the differences in the response on the medial nerve branch (*M*) and lateral nerve branch (*L*) for different chemicals presented via the internal naris and via the external naris. The stimulus markers show only the stimulus onset. Frequency of the vertical time lines is 1/sec. In any given animal each stimulus was presented at least three times via each naris. The total experiment was repeated five times in four different animals (in one animal both sides of the nose were used). The results stated in the text did not differ significantly from experiment to experiment except as stated

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HÆMATOLOGY

Slow Alpha$_2$-Globulin of Rat Serum

By immunological techniques, Darcy[1] has shown, in rats bearing a Walker's tumour, the appearance of a serum protein absent in normal rat serum.

Recently, several authors have demonstrated by starch-gel electrophoresis the appearance under certain conditions of a slow α_2-globulin in rat serum. This slow α_2-globulin has been found during certain periods of physiological activity : neo-natal period, gestation[2,3] and in animals submitted to various experimental conditions such as tumoral growth, surgical exeresis[2,4,5].

We tried to get some information concerning the biological significance of this protein by studying the variations of the level of the slow α_2-globulin during different states of normal growth, tissue regeneration and tumoral proliferation.

So, the level of rat slow α_2-globulin seems to reflect physiological and pathological growth processes. On the other hand, several authors have shown an increase of the hepatic mitotic index during different kinds of growth processes[6–8].

Since the liver seems to play the most important part in the synthesis of serum proteins, we thought that it might be interesting to compare the appearance of slow α_2-globulin with the variations of the hepatic mitotic index.

Two different strains of ascitic hepatoma have been examined in rats—*C* and *D* zajdela hepatoma (Institut du Radium)[9,10]. Sera and ascitic fluids of these rats have very high levels of slow α_2-globulin on the 7th day after grafting of the tumour.

In starch-gel electrophoresis[11] using continuous as well as discontinuous buffer system[12], slow α_2-globulin moves as a narrow band which has a more cathodic position than α_1-*M* (ref. 13) of rat serum (Fig. 1). This α_1-macroglobulin has a sedimentation rate of 18·3 *S* and it is found in the first fraction during filtration on 'Sephadex *G* 200'[14,15]. By repeated freezing and thawing as well as by ageing this protein

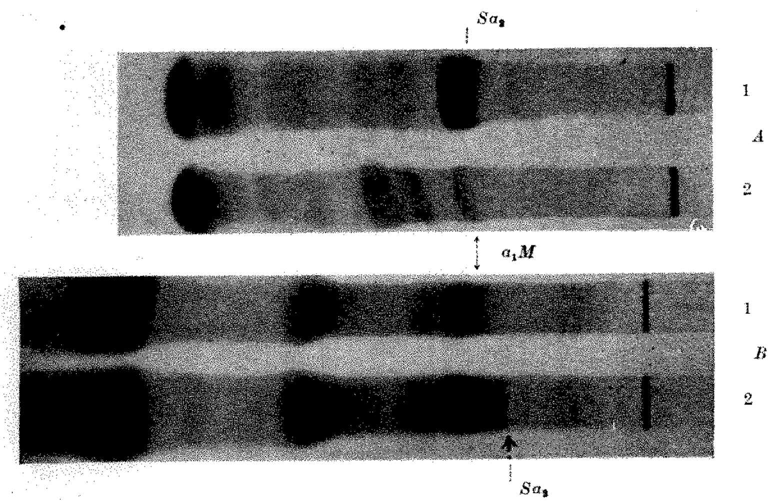

Fig. 1. Starch-gel electrophoresis, naphthalene black staining. *A*, discontinuous buffer system: (1) serum of hepatoma-bearing rat; (2) normal rat serum. *B*, Borate buffer: (1) normal rat serum; (2) serum of hepatoma-bearing rat

undergoes transformation in two constituents as revealed by starch-gel electrophoresis. The same behaviour was observed with the slow α_2-globulin. However, antigenic determinants of the two proteins under consideration are different.

Slow α_2-globulin is absent from normal rat serum as revealed by starch-gel electrophoresis. Furthermore, normal rat serum submitted to immunoelectrophoresis[16] using a specific rabbit anti-rat slow α_2-globulin serum[17] shows no trace of this protein. However, we always found this protein in the sera of rats bearing zajdela hepatoma (*C* and *D* strains) as well as in the sera of hepatectomized rats performed by the technique of Higgins and Anderson[18], as after nephrectomy or after laparotomy. Finally, we identified slow α_2-globulin in new-born rats during fifteen days after birth. The identity of slow α_2-globulin observed during the neo-natal period with the protein observed in the various experimental conditions described here has been proved by double diffusion testing.

The growth of young rats is quite regular during the first forty days of life and their body weight is generally doubled in intervals of nine days (Fig. 2). Liver weight increases following the same rhythm. The hepatic mitotic index of animals aged from one week to one month has been evaluated to 1·5–2 parts per thousand. This experimental work has been done on 5μ liver slices fixed in neutral, salted formol, included in paraffin and coloured by hæmatin–eosin–saffranin.

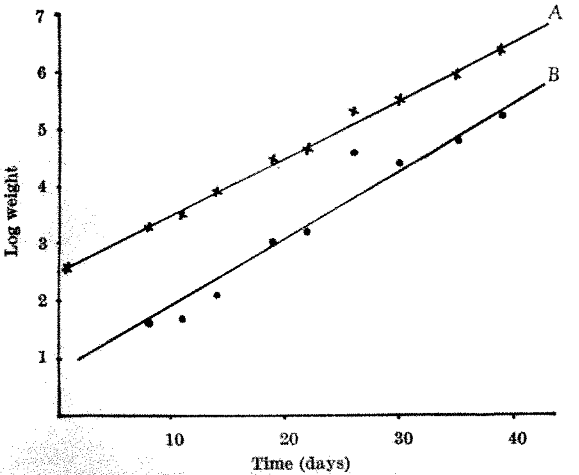

Fig. 2. Comparative diagrams showing the evolution of body weight and liver growth in rats. *A*, body weight of animals (g); *B*, liver weight (dg)

After the first month of life, the hepatic mitotic index decreases progressively until it reaches 0·05 parts per thousand in the adult animal. Slow α_2-globulin is present at a high level at birth time; it is then decreasing progressively and this protein is no more detectable under our experimental conditions around the fifteenth day of post-natal period.

Partial hepatectomy has been carried out in rats aged 6–10 weeks. A few hours later the cells of the remaining hepatic fragment show nuclear swelling and hypertrophy of the nucleoli. This is followed by a marked increase of mitosis. The mitotic reaction starts earlier in animals hepatectomized after six weeks of life (12–24 h) than in rats already ten weeks old (24–48 h). The highest level (10–15 parts per thousand) is reached after about 48 h. It returns to normal 7–10 days after hepatectomy. So far as slow α_2-globulin is concerned, it can be detected about 12 h after hepatectomy in animals 6 weeks of age and after 24 h in animals 10 weeks old. The titre of the slow α_2-globulin is highest after about 48 h; it then decreases and this protein is no longer detectable 10 days after hepatectomy.

The serum of rats hepatectomized three days after the grafting of ascitic hepatoma shows a very high level of slow α_2-globulin.

If unilateral nephrectomy is performed in rats 10 weeks old, the weight of the remaining kidney increases by about 60 per cent 10 days later. Practically no mitosis is seen in the removed kidney. Only a few mitoses are seen in the hypertrophic remaining kidney. However, slow α_2-globulin is present about 12 h after the removal of one kidney, but its level remains rather low. Hepatic mitotic index in these animals increases weakly to about 0·16 parts per thousand.

Simple laparotomy gives rise to the transient appearance of slow α_2-globulin between the 24th and the 48th hours.

Slow α_2-globulin appearing in rat serum during various states of physiological and induced growth, as well as after transplantation of ascitic hepatoma, has proved to be identical by immunological methods. So it became sure that this protein is not exclusively related to tumoral processes.

The existence of a very small quantity of slow α_2-globulin in adult male rats cannot be excluded. Indeed, we observed that certain sera of apparently healthy animals may absorb rabbit anti-slow α_2-globulin serum.

The results of our experiments do not corroborate the theory of direct interrelationship between hepatic mitotic index and the rate of synthesis of slow α_2-globulin. Indeed, the slow α_2-globulin appears before the elevation of the mitosis rate in hepatectomized animals. This protein is present after laparotomy and after nephrectomy in spite of a low mitotic index (0·16 parts per thousand). Slow α_2-globulin is absent in rats one month old whereas these animals show a ten-fold increase of the hepatic mitotic index as compared with the preceding cases. Although this protein is found during pregnancy, tumoral proliferation as well as during tissue regeneration and in the post-natal period, it is not in direct connexion with general tissue growth processes. Indeed, the evolution of slow α_2-globulin is not proportional to the variations of the hepatic mitotic index.

The real physiological meaning of the slow α_2-globulin remains unknown. Work is in progress, based on the use of fluorescent antibodies, to localize the cellular site of protein production.

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Inhibition of Thrombin and of Fibrin Polymerization, Two Activities derived from Plasmin-digested Fibrinogen

As was previously shown in our laboratory[1], the clotting of fibrinogen initiated by one proteolytic enzyme (thrombin) is specifically inhibited by the products resulting from the action of another proteolytic enzyme (plasmin) on the same substrate.

Although this phenomenon has aroused considerable interest, the exact mechanism of the inhibitory action of these fibrinogen degradation products is still the subject of controversy. Some results indicated that fibrinogen degradation products affect mainly the first enzymatic step of the fibrinogen–fibrin conversion[2–4], another showed their predominant influence on polymerization–gelation steps of this conversion[5]. Recently, evidence has been presented that fibrinogen degradation products act solely as an inhibitor of the polymerization–gelation phase leading to the formation of a defective fibrin clot[6–8].

The results of the experiments described here indicate that in the course of plasmin-catalysed fibrinogen proteolysis at least two distinctly different activities can be found among a variety of degradation products.

The effect on the overall fibrinogen–fibrin conversion of fibrinogen degradation products formed at various times of fibrinogen digestion with plasmin is shown in Fig. 1. As can be seen from this graph, the prolongation of the thrombin time in the presence of fibrinogen degradation products reaches a maximum and then decreases to a constant level. A sharp decrease of the peak of the inhibitory activity to a constant level indicates that at least two active fragments may be derived from fibrinogen, activity of the first ('early' products) being, in contradistinction to the second ('late' products), destroyed by plasmin. It is not possible, however, to say now whether the second active fragment is the degradation product of the first or whether it is formed independently. Moreover, it should be pointed out that the activity of 'late' product(s) remains on a constant level, although the proteolytic reaction still proceeds.

Table 1. INFLUENCE OF 'EARLY' AND 'LATE' FIBRINOGEN DEGRADATION PRODUCTS (FDP) ON THE CLOTTING TIME OF FIBRIN MONOMER (*A*) AND FIBRINOGEN WITH THROMBIN (*B*)

	Clotting time (sec)	
	A	*B*
With saline	5	10
With 'early' FDP	20	80
With 'late' FDP	38	40

0·1 ml. (1·4 mg) of fibrinogen or fibrin monomer solution (both in 1 M sodium bromide *p*H 5·3) was mixed with 0·05 ml. of saline or FDP (0·25 mg) and immediately afterwards clotted by the addition of 0·7 ml. of 0·05 M phosphate buffer *p*H 7·6 to (*A*) or the same buffer containing 2·5 units of thrombin to (*B*).

Table 2. AMOUNT OF TCA-SOLUBLE PEPTIDES RELEASED FROM FIBRINOGEN, FDP, OR MIXTURES OF BOTH INCUBATED FOR 5 MIN AT *p*H = 7·8 WITH 0·024 N.I.H. UNITS OF THROMBIN

After addition of TCA up to 7·5 per cent final concentration, peptides in the supernatant were determined according to Lowry *et al.* (ref. 11)

Reaction mixture (4 ml.)	TCA soluble peptides in μg
(1) Fibrinogen (16·0 mg)	17·2
(2) 'Early' FDP (16·0 mg)	8·0
(3) Fibrinogen (16 mg) + 'early' FDP (0·8 mg)	14·7
(4) Fibrinogen (4·0 mg)	6·3
(5) Fibrinogen (4·0 mg) + 'early' FDP (0·8 mg)	4·3

It should also be noted that the difference in the effect of 'early' and 'late' fibrinogen degradation products on the thrombin time disappears at *p*H below 7. This problem will be discussed elsewhere[9].

Since the assay of the thrombin time does not enable us to decide which step of the fibrinogen–fibrin conversion is affected by fibrinogen degradation products, the comparison between the clotting time of a thrombin–fibrinogen mixture and fibrin monomer was made. The latter system makes it possible to measure the polymerization–gelation step alone, excluding the first enzymatic step. The results shown in Table 1 indicate that the 'late' fibrinogen degradation products affect the clotting time of both systems to the same degree. By contrast, a very distinct prolongation of the clotting time by the 'early' fibrinogen degradation products is observed in the system containing the thrombin–fibrinogen mixture, while the polymer-

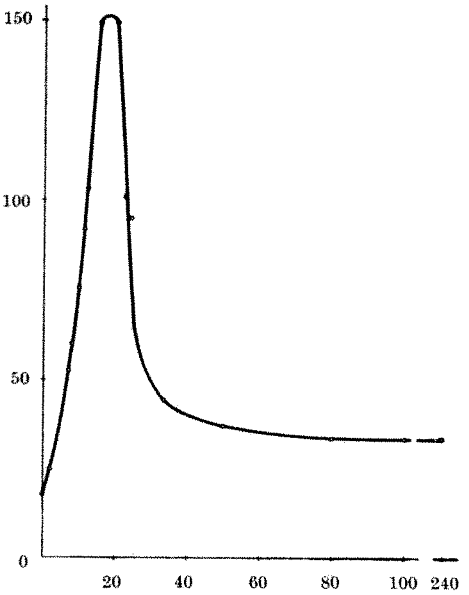

Fig. 1. Clotting time of fibrinogen by thrombin, in the presence of FDP formed in the course of fibrinogen proteolysis by plasmin. Bovine fibrinogen (96 per cent clottable) was incubated with human plasmin (90 cas. units/mg protein), the ratio being 100 : 3 respectively. The reaction was carried out at 37° C in *p*H-stat and *p*H = 7·8 was maintained with 0·1 N NaOH. To stop further action of plasmin, soybean trypsin inhibitor was added to the samples of the incubation mixture withdrawn at various time intervals. 0·5 mg of fibrinogen was mixed with 0·3 mg of FDP and clotted with 1 N.I.H. unit of thrombin at *p*H 7·6. Abscissa, time of fibrinogen digestion by plasmin (min); ordinate, clotting time (sec)

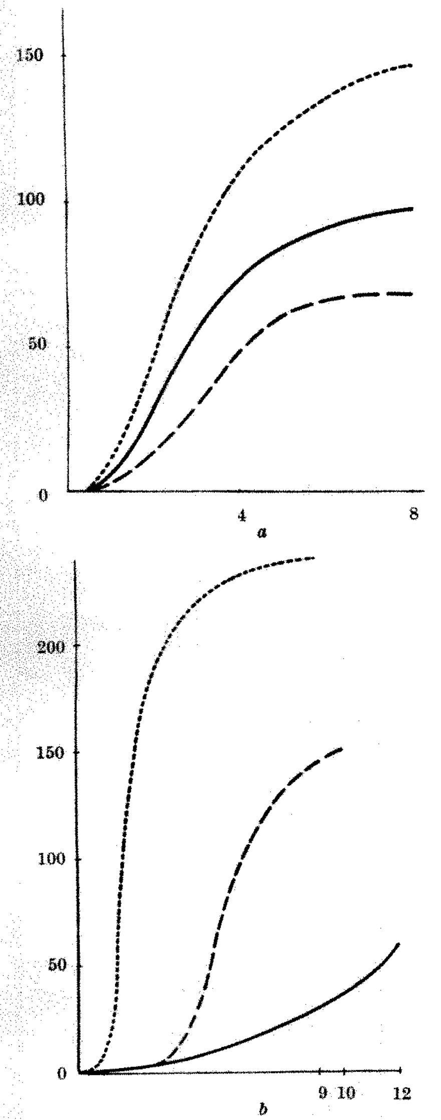

Fig. 2. Influence of 'early' and 'late' FDP on optical density changes during clotting of fibrin monomer (*a*) and fibrinogen with thrombin (*b*). Ordinates give absorbance at 350 mμ and abscissæ time (min). Final concentrations of fibrin monomer and fibrinogen were 0·45 mg/ml. and 0·55 mg/ml. respectively; concentrations of both 'early' and 'late' FDP were 0·12 mg/ml. Reaction *p*H = 7·5, phosphate buffer molarity 0·05; temperature 20° C. For experiments with fibrinogen 0·02 N.I.H. u./ml. of thrombin was used. Readings were performed every 15 sec. Upper dotted line—control (without FDP), solid line—with 'early' FDP, and broken line—with 'late' FDP

ization–gelation step, as tested by fibrin monomer clotting time, is much less affected.

In the experiment presented in Fig. 2 the effect of fibrinogen degradation products on both systems was followed by the previously described spectrophotometric assay[10]. The results presented in Fig. 2 are compatible with those of the experiments shown in Table 1. As can be seen from Fig. 2*a*, 'early' fibrinogen degradation products exert a much smaller inhibitory effect on fibrin monomer polymerization than 'late' fibrinogen degradation products. By contrast, when the overall reaction including the enzymatic step is followed by means of optical density recording, the reverse phenomenon is observed (Fig. 2*b*). This implies that 'early' fibrinogen degradation products affect mainly the first enzymatic step of the fibrinogen–fibrin conversion, whereas 'late' fibrinogen degradation products act as inhibitors of the polymerization–gelation step. Additional evidence for antithrombin activity of 'early' fibrinogen degradation products is provided by the assay of the amount of TCA-soluble peptides released by thrombin from fibrinogen in the presence of these products. The results displayed in Table 2 indicate that thrombin splits off less peptide material from the mixture of 'early' fibrinogen degradation products with fibrinogen as compared with the equivalent amount of fibrinogen alone.

All this indicates that among a variety of fragments released from the fibrinogen molecule after plasmin digestion two definitely different activities can be distinguished. One of them contained in 'early' products interferes with the enzymatic step of the fibrinogen–fibrin conversion and is plasmin susceptible. The other activity bound with 'late' products is plasmin resistant and interferes with the polymerization of fibrin monomer. It is not known whether the observed inhibition of the polymerization–gelation step by the 'early' fibrinogen degradation products is due to the action of the 'early' fibrinogen degradation products themselves or to the presence of the concurrently formed 'late' fibrinogen degradation products. Nevertheless, the originally proposed term 'antithrombin VI'[12] seems to be appropriate for the 'early' fibrinogen degradation products activity, whereas the term inhibitor of fibrin polymerization should be reserved for the 'late' fibrinogen degradation products activity.

The significance of the biologically active products of plasmin-catalysed fibrinogen degradation in the pathogenesis of acute fibrinolytic stages has been well recognized. Recently, the appearance of a severe hæmorrhagic diathesis in such cases has been ascribed mainly to the action of fibrin polymerization inhibitor(s) derived from fibrinogen[6,7]. The evidence displayed here suggests, however, that antithrombin activity of 'early' fibrinogen degradation products may, in fact, play an equally important part in the development of this coagulation defect.

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Lack of Anti-A Hæmagglutinin Response to Influenza Infection

McDonald and Zuckerman have reported[1] an excess of blood group O and a corresponding deficiency of group A among patients with influenza A_2 infection. Blood group A-like antigens have been found in commercial influenza vaccines[2], and injection of non-dialysable material from such vaccines has given 4- to 15-fold increases in anti-A titres[3].

It is not known whether influenza virus *per se* contains A-like antigens capable of affecting the isoagglutinins; the reported increase in anti-A might be due to stimulation by antigens acquired from the chicken eggs in which the virus is grown. Investigations of the isoagglutinins during

Table 1. Isoagglutinin Changes during Influenza Infection

Influenza A_2							
Anti-A							
No. of two-fold dilution steps	+3	+2	+1	0	−1	−2	−3
No. of cases	0	2	31	51	19	5	0
Anti-B							
No. of two-fold dilution steps	+3	+2	+1	0	−1	−2	−3
No. of cases	1	4	36	104	48	4	1
Influenza *B*							
Anti-A							
No. of two-fold dilution steps	+3	+2	+1	0	−1	−2	−3
No. of cases	1	0	7	16	18	0	0
Anti-B							
No. of two-fold dilution steps	+3	+2	+1	0	−1	−2	−3
No. of cases	1	1	10	25	23	2	0

Table 2. Distribution of ABO Blood Groups

		A	O	B	AB	Total
Influenza A_2	Observed	105	93	15	8	221
	Expected	106	88	18	9	221
Influenza *B*	Observed	28	37	7	2	74
	Expected	36	30	6	2	74

influenza infection might therefore shed light on the antigenic content of this virus.

We have followed the isoagglutinin titres in paired sera collected from 221 patients with influenza A_2 and 74 patients with influenza *B* during 1961–64. The diagnostic criteria have been clinical symptoms accompanied by a 4-fold or greater increase in influenza antibody titres (complement fixation and/or hæmagglutination inhibition tests).

Our observations are summarized in Tables 1 and 2. Sixteen paired sera showing two or more titre-step difference from first to second sample have been retitrated. By this check only 3 showed the same difference, namely a 4-fold decrease in anti-A in one case and 4-fold decreases in anti-B in two cases.

It seems therefore justified to conclude that influenza infection does not produce any change in isoagglutinin titres.

The distribution of ABO blood groups among our patients with influenza A_2 corresponds to that of a normal control population, whereas the number of influenza B patients is too small for definite conclusions.

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PATHOLOGY

Effects of Adenine and Guanine on Hepatic Glucose Release and on the Action of Insulin on the Liver

We have recently obtained information from work on normal subjects and mild and severe diabetics indicating that insulin has an increasing effect on hepatic glucose metabolism in untreated diabetics with progressively elevated fasting blood sugar-levels and glycosuria implying greater degrees of catabolism and gluconeogenesis[1]. There is also evidence of a hepatic action of insulin when diabetic patients are treated with phenethylbiguanide[2]. In view of the similarity between the ethylbiguanide side-chain and the nucleotides adenine and guanine, we wished to test the effect of these compounds on hepatic carbohydrate metabolism and on the action of insulin on the liver.

Using the rat liver perfusion technique developed by one of us[3,4], the release of glucose over two successive periods of 1 h has been investigated. Control levels were measured during perfusions with glucose-free tyrode solution and the effects of added adenine (in two concentrations, designated as Adenine, 0·25 γ/ml., or adenine, 0·025 γ/ml.), guanine (designated as Guanine, 0·25 γ/ml., or guanine, 0·025 γ/ml.) alone or with insulin (in two concentrations designated as Insulin, 20 milliunits/ml., or insulin, 0·2 milliunits/ml.) on hepatic glucose release expressed in mg/h have been examined so far.

Table 1

Perfusion	No. of expts.	Hepatic glucose release (mean ± *S.D.*) 1st h	Hepatic glucose release (mean ± *S.D.*) 2nd h
Control	12	4·7 (1·2)	4·7 (2·9)
Insulin	8	3·7 (1·0)	3·0 (1·2)
adenine	8	5·5 (1·8)	5·1 (2·1)
Adenine	10	10·4 (2·7)	8·3 (4·0)
Adenine + Insulin	8	8·3 (1·8)	2·5 (1·1)
Adenine + insulin	8	3·6 (1·2)	3·9 (1·6)
guanine	8	4·4 (2·7)	2·7 (1·8)
Guanine	10	8·3 (1·7)	8·3 (2·0)
guanine + Insulin	8	4·2 (2·6)	1·7 (0·9)
Guanine + insulin	8	4·6 (2·5)	3·1 (2·3)

The nucleotides guanine and adenine caused statistically significant increases in hepatic glucose release. Insulin, in concentrations which in previous work[3] failed to reveal a statistically significant action in controlled circumstances, suppressed the glucose-releasing action of the nucleotides, even at the lower dose-levels, suggesting that its action is hormonal rather than stoichiometric.

The results (Table 1) appear to be in close harmony with, and provide some degree of explanation for, the clinical findings here. If we consider the state of affairs in the fasting untreated diabetic, increasing demands for gluconeogenesis to offset losses through glycosuria will be associated with increasing protein catabolism. It seems probable that the concentrations of nucleotides, no longer involved in protein synthesis, might increase in the tissues, diffuse into the blood stream and perfuse the liver as well as accumulating locally from the cells in that organ. From the present evidence, it would appear that in low concentrations nucleotides may increase hepatic glucose release and that, in such circumstances, insulin, in levels which approach the physiological for the portal circulation, has a powerful action restraining hepatic glucose release.

Further work on the role of these phenomena on glucose homeostasis is being carried out.

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Preparation of Antigens Specific of Human Breast Carcinoma by an Immunochromatographic Method

In spite of a rapidly mounting literature on the problem of specific antigens in human neoplasms, there have been few attempts to separate these antigens in amounts permitting biochemical and immunological analyses of the same. Such analyses not only would shed light on carcinogenic mechanisms, including possibly the serological back-tracking of eventual biological agents, but would make possible a much-desired immunological classification of tumours. Work from this laboratory has been concentrated on such efforts.

In previous reports we have described methods to extract potential antigenic proteins from human tissues, both normal and neoplastic[1-3]. Even though immunodiffusion and direct cytotoxicity tests have indicated amply the existence of specific precipitinogens and cytotoxinogens in neoplastic cells, the antibodies hitherto produced on a large scale in large animals were not always totally devoid of activity against normal antigens from normal cells.

The production of large amounts of hyperimmune γ-globulins required the use of large animals and hence the need for increasing amounts of antigens. These requirements automatically limited the possibilities of inoculating separate animals with separate tumours and compelled us to work with large pools of different tumours and of normal tissues.

Departing now from antibodies produced against pooled tumour antigens and pooled normal tissue antigens, we investigated a more homogeneous pool consisting of 4 adenocarcinomas of the breast.

As reported previously[2], a major step introduced by this laboratory in the separation of tumour-specific antigens is the elimination of normal antigenic components from the tumour antigen complement by absorption with antibodies produced against a large pool of normal tissues obtained from a pool of serum from four different horses.

The dynamics of this absorption in a system of soluble antigens and soluble antibodies was previously described and all the inherent difficulties of this totally unknown system discussed. The recent introduction in our methodology of a form of insolubilized immune γ-globulin[4] greatly simplified and improved the absorption of normal components from the tumour antigen complement, free of residual horse γ-globulin.

Diazotization of horse hyperimmune anti-normal human tissue γ-globulin results in cross-linking of the globulin to an insoluble form (polyglobulin) which can be used as a chromatographic column bed[4,6]. This communication describes the use of such a column for the separation of tumour-specific antigens from breast carcinoma.

Sources of breast carcinoma: 1, 2 and 3: 60 g each of tumour tissue (adenocarcinoma of the breast, grade II) obtained from surgical specimens (mastectomies); 4: 60 g of a liver totally and diffusely infiltrated by cells of adenocarcinoma of the breast.

A total of 240 g of clean tumour tissue was homogenized in glycine buffered saline, *p*H 10·4, and fluorocarbon as described elsewhere[1] and a solution containing 3·5 g per cent of protein obtained. This will here be referred to as unabsorbed breast antigen.

Equine hyperimmune anti-tumour γ-globulin (*HTU GG*, Lot. No. 27 GG33) with a precipitin titre, in single-gel diffusion, of 2^{-5} against the inoculating antigen. This antigen was obtained by fluorocarbon treatment of a large pool of solid tumours in which breast adenocarcinomas were largely represented[2].

Equine hyperimmune anti-normal γ-globulin (*NHGG*, Lot. No. 4,5 GG21) with a precipitin titre of 2^{-7} against the inoculating antigen which was obtained by fluorocarbon treatment of a pool of nearly every normal tissue in the human body[2].

Copolymerized NHGG: Insolubilization of the anti-normal γ-globulin by cross-linkage with tetrazobenzidine bridges was obtained as described elsewhere[4].

A chromatographic column of this insoluble *NHGG* was prepared with 30 g of γ-globulin. This column was equilibrated with 0·1 M phosphate buffered saline, *p*H 7·2, at 2° C. This temperature is maintained by a jacket of crushed ice surrounding the column.

An aliquot of 180 ml. of unabsorbed breast tumour antigen containing 6·3 g of protein in glycine buffered saline *p*H 10·4 was placed over the column and allowed to soak through by removing 180 ml. of phosphate buffered saline (fraction 0). The column was then incubated for 1 h at 2° C and several fractions of 100 ml. each collected. Phosphate buffered saline was added in amounts of 100 ml. to promote descent of each new fraction.

The size of the γ-globulin column was calculated to exceed five times the weight/weight ratio of the precipitin titre previously obtained in single-gel diffusion hæmatocrit tubes[5].

Each fraction was tested in single gel diffusion for quantitative precipitation against *HTUGG* and *NHGG* and in double gel-diffusion for identification of reactants. Protein concentrations were determined by the absorption at 280 mμ wave-length.

Table 1 and Fig. 1 summarize the results obtained. In them it is apparent that: (1) The unabsorbed tumour antigen complement consists of 8 antigens, 2 of which are in common with normal tissues (Fig. 1, No. 1). (2) Antigens reacting against anti-normal γ-globulin do not appear in any of the collected fractions. (3) Increase in protein concentration results in increase in the number of tumour antigens while normal antigens remain undetected. (4) The largest number of tumour antigens (that is, 6) equals the number of antigens in the unabsorbed original

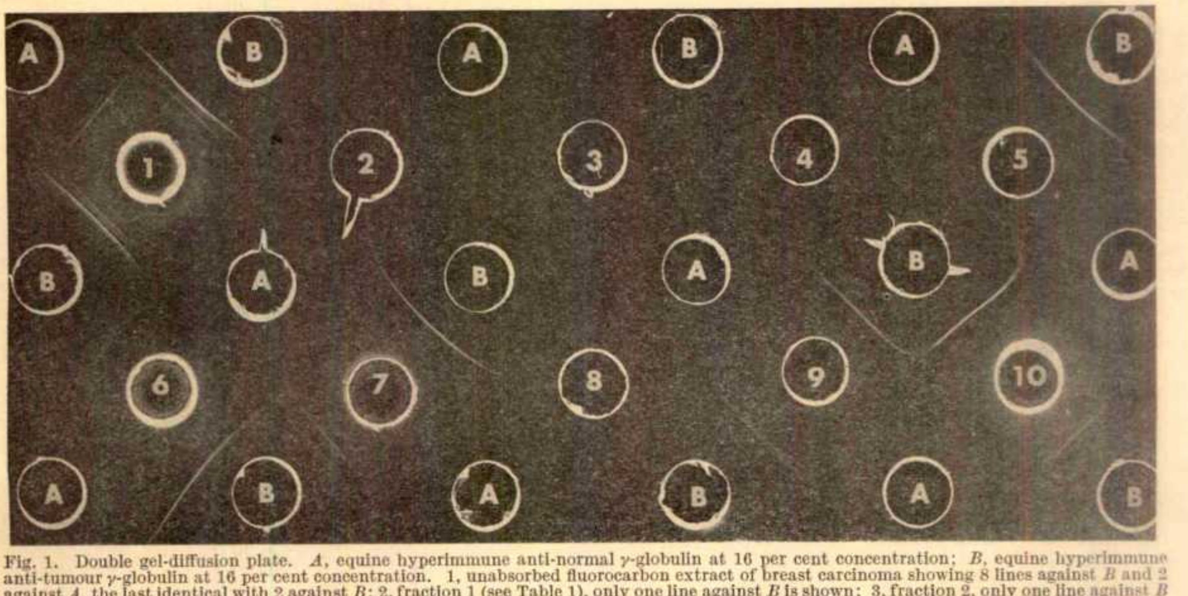

Fig. 1. Double gel-diffusion plate. *A*, equine hyperimmune anti-normal γ-globulin at 16 per cent concentration; *B*, equine hyperimmune anti-tumour γ-globulin at 16 per cent concentration. 1, unabsorbed fluorocarbon extract of breast carcinoma showing 8 lines against *B* and 2 against *A*, the last identical with 2 against *B*; 2, fraction 1 (see Table 1), only one line against *B* is shown; 3, fraction 2, only one line against *B* is shown; 4, fraction 3, a faint line which could be seen in the plate did not reproduce in the photograph; 5, fraction 4, two lines against *B*, only; 6, fraction 5, six lines may be seen against *B*, none against *A*; 7, fraction 6, at least 4 lines may be counted against *B*, none against *A*. Other numbers are not relevant to the context of this communication

Table 1. IMMUNOCHROMATOGRAPHIC SEPARATION OF BREAST CARCINOMA ANTIGENS ON A COLUMN OF CROSS-LINKED HORSE ANTI-NORMAL (HUMAN) γ-GLOBULIN

Fraction	Titre in single gel-diffusion		Protein concentration g%	No. of lines in double gel diffusion (Fig. 1)	
	Against *NHGG*	Against *HTUGG*		Against *NHGG*	Against *HTUGG*
Unabsorbed tumour antigens	2^{-7}	2^{-5}	3·5	2	8
0 (1st 100 ml.)	0	0	0·0	0	0
1 (2nd 100 ml.)	0	2^{-3}	0·070	0	1
2 (3rd 100 ml.)	0	2^{-3}	0·062	0	1
3 (4th 100 ml.)	0	2^{-3}	0·051	0	1
4 (5th 100 ml.)	0	2^{-4}	0·480	0	2
5 (6th 100 ml.)	0	2^{-4}	1·680	0	6
6 (7th 100 ml.)	0	2^{-5}	0·600	0	4

NHGG, equine hyperimmune anti-normal γ-globulin; *HTUGG*, equine hyperimmune anti-tumour γ-globulin.

extract (that is, 8) which react with anti-tumour globulin (Fig. 1, No. 1 and *B*) minus the antigens in the same (that is, 2) reacting with antinormal globulin (Fig. 1, No. 1 and *A*). Analysis of the protein complement of breast tumour tissue reveals only antigenic acquisition but no antigenic deletion or deviation, 70 per cent of the protein being of normal antigenic nature.

These results seem to indicate that this method resulted in the resolution and large-scale preparation of specific antigens from human adenocarcinoma of the breast.

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Effect of Early Thymectomy on Development of Mammary Tumours in Mice

IT is now well established that the thymus plays an important part in the development of immunological competence in mammals. For example, in the mouse, removal of the thymus within the first 24 h after birth results in a serious impairment of the capacity to produce circulating antibody and the ability to reject allogeneic grafts of normal or neoplastic tissues. In addition, these mice also develop a wasting syndrome ending in early death[1-7]. Surgical ablation of the thymus at 6 and up to 35 days of age also produces a state of immunological impairment, which is more severe when thymectomy is performed earlier in life. These animals do not develop the wasting syndrome and have a life-span comparable with that of non-thymectomized controls[8-10].

Since development of mammary tumours in susceptible strains of mice depends at least in part on the presence of an infectious agent transmitted by the mother to the progeny via the milk (Bittner's virus), it was considered of interest to ascertain whether or not thymectomy performed at 6 days of age in mice of a high cancerous strain would affect the spontaneous development of mammary tumours in these animals.

Two groups of female mice of the *C3H/Bi* strain were used. One group of animals was thymectomized 6 days after birth by the technique routinely used in this laboratory[11], and the other left as non-thymectomized controls. After surgery, mice were raised by their own mothers and weaned at 30 days of age, at which time the thymectomized and non-thymectomized mice were housed in plastic cages in groups of 4–5 female mice per cage. One normal male of the same strain was introduced into each cage and the mice were allowed to breed. Breeding behaviour was assessed by the number of litters born from each mother in both groups (Table 1). The mice at all times had free access to 'Purina Laboratory Chow' and tap water.

The incidence of spontaneous mammary tumours in both groups was determined by weekly inspection of each individual mouse. The age of the mouse was recorded at the time a tumour appeared.

Table 1. INCIDENCE OF SPONTANEOUS MAMMARY ADENOCARCINOMA IN THYMECTOMIZED AND NON-THYMECTOMIZED FEMALE MICE OF THE *C3H/Bi* STRAIN KEPT AS BREEDERS

Group	No. of mice	Breeding behaviour. No. of litters born (mean)	No. of mice with cancer	%	Mean cancer age (days ± *S.D.*)
C3H normal	73	4·4	69	94·5	276 ± 5·04
C3H thymect. at 6 days of age	26	4·6	15	57·6	335 ± 16·2

The results of these experiments (Table 1) demonstrate that, in the group of *C3H* mice thymectomized at 6 days of age, 57·6 per cent of the females developed spontaneous mammary tumours as compared to 94·5 per cent in the group of non-thymectomized controls. This difference is statistically significant at the 1 per cent level. Furthermore, thymectomized mice developed tumours significantly later than non-thymectomized controls. In the former group the average cancer age was 335 ± 16·2 and in the latter 276 ± 5·04 days.

Although the interpretation of these results must remain a matter of conjecture at the present time, a few pertinent comments are in order. According to Bittner[12] development of breast cancer in mice is dependent on the concurrent effect of three factors, namely, inherited susceptibility, proper hormonal stimulation, and the presence of the mammary tumour 'agent' or virus which is transmitted from the mother to the offspring by the milk. Since the inherited susceptibility for tumour development is genetically controlled and presumably does not change after thymectomy, it might be that the mechanism operating in cancer prevention by early thymectomy, as reported herein, could be related to an alteration of the hormonal stimulation for tumour development. Another possibility is that the thymus may be essential early in life for proper growth and multiplication of the mammary tumour virus. Finally, the development of mammary tumours could be related to the immunological responsiveness of the host animal to the virus. It has been shown recently that neonatal thymectomy in mice greatly reduces the mortality resulting from inoculation with lymphocytic choriomeningitis virus (LCM), and this reduction in mortality is the result of decreasing the host response to the virus[13,14]. Perhaps the development of spontaneous mammary tumours in mice is also in part dependent on a similar mechanism.

Experiments designed to investigate these and other possibilities are in progress.

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RADIOBIOLOGY

A Cellular Component of Thymic Function

THYMECTOMY in the new-born[1,2] or in the adult irradiated mouse[3] leads to a long-lasting suppression of the ability to respond to primary antigenic challenge. It has been shown in both instances that thymus grafting is effective in restoring immunological competence[2,4,5] and, furthermore, when using a cytologically marked thymic graft, it has been possible to detect cells derived from the graft in the spleen and lymph nodes[2,5]. It has, therefore, been suggested[5] that it may be these donor cells which are directly responsible for the recovery of the primary immune response in thymus-grafted animals.

In order to test this possibility, six *CBA* male mice were thymectomized[6], irradiated (850 r.) and given 5 million syngeneic bone marrow cells. On the day of irradiation, each mouse was grafted under the kidney capsule with a single thymus lobe from a 1–3-day-old *CBA/T6T6* mouse. (The cells of *CBA/T6T6* mice are characterized by a pair of small marker chromosomes.) Twenty-eight days later half the mice were given an injection of sheep red cells (0·2 ml. of a 20 per cent suspension in Alsever's solution). Three days after challenge the animals were killed and cytological preparations were made following the methods of Ford and Hamerton[7] and Rothfels and Siminovitch[8]. The slides were analysed and the results are shown in Table 1.

Table 1. CYTOLOGICAL ANALYSIS OF SPLEENS FROM *CBA* MICE BEARING A CYTOLOGICALLY MARKED (*CBA/T6T6*) THYMUS GRAFT

Uninjected mice				Mice injected with sheep cells			
Mouse No.	No. of cells scored	% *CBA*	% *CBA/T6T6*	Mouse No.	No. of cells scored	% *CBA*	% *CBA/T6T6*
1	38	89·5	10·5	4	50	66·0	34·0
2	46	100	0	5	50	80·0	20·0
3	47	93·6	6·4	6	50	86·0	14·0

There is a significant increase ($P < 0·05$) in the number of marked donor cells found in the spleens of the injected mice. This would indicate that cells, presumably derived from the thymic graft, are capable of dividing in response to antigenic stimulation. It is not, therefore, unreasonable to suppose that the thymus graft restores immunological function, at least in part, by itself providing appropriate cells. The implications of this preliminary finding are the subject of further investigation.

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Removal of Radiocæsium from Beef

Two of the long half-life nuclides of major interest in food and feedstuffs which result from fall-out are radiostrontium and radiocæsium. Cæsium is almost always associated with the other alkali metals and particularly resembles potassium. Hood and Comar[1] demonstrated that ^{137}Cs is absorbed and distributed rapidly in the soft tissues and the body fluids, including milk. In distribution investigations, these authors found that the greatest accumulation of radiocæsium occurred in the muscles as compared with other body tissues. Ekman[2] reported that radiocæsium accumulated more rapidly in the physiologically active muscles than in inactive muscles; however, ultimately the inactive muscles were reported to have a higher content.

Using four human subjects, Richmond, Furchner and Langham[3] found that radiocæsium had a biological half-life of about 135 days. Other workers have reported half-lives varying from 100 to 150 days. Combined with the fact that radiocæsium has a considerable biological half-life in man and that the two most important nuclides, ^{134}Cs and ^{137}Cs, have physical half-lives of approximately 2·8–30 years, respectively, it becomes increasingly important to consider the significance of radiocæsium intake from the point of view of health.

In the inland arctic regions, caribou and reindeer are the main sources of meat and milk for the Eskimos and the Lapland tribes. In the arctic these herbivorous animals subsist essentially on lichens which are known to concentrate radiocæsium from fall-out. Meat from these areas has been reported to contain significant levels of radiocæsium. Whole-body counting investigations of human beings by Palmer *et al.*[4,5] and Liden and Andersson[6] in Alaska, Finland and Sweden have demonstrated that these arctic inhabitants have high radiocæsium body burdens as compared to other peoples. In northern regions of the world, some uncomplicated treatments for removing ^{137}Cs and ^{134}Cs from muscle may now be of value. In the temperate zones also, if cattle and other domestic animals grazed on pasture contaminated with heavy amounts of fall-out and then had to be utilized for human consumption, knowledge of some removal techniques would be valuable. Meyer *et al.*[7] reported that the loss of ^{134}Cs from cooked beef muscle was related to the amount of water used and the exposed surface area. Average activity removed was 19 per cent from oven roasts, 43 per cent from braised steaks, and 53 per cent from stew. Additional treatments prior to cooking may be of value to reduce the level of radiocæsium in the tissue. Data are reported here on the removal of radiocæsium from raw beef muscle from a heavy steer which had received intravenously a 10-mc. dose of ^{134}Cs.

Since radiocæsium accumulates in the soft tissue, it must be to a degree associated with the muscle and plasma proteins in a manner similar to the relationship sodium and potassium ions have with these proteins. If radiocæsium associates with proteins, then the degree of association might be affected by changes in *p*H. Cæsium-134 was more easily leached from cubed samples of longissimus dorsi (L.D.) at the isoelectric point of rigor, *p*H 5·5, than from samples at *p*H 6·4 and 5·7 removed from the steer carcass at earlier post-mortem times. After subjecting the leaching solutions to ultrafiltration[8] and counting portions of the filtrate and residue, it was found that the soluble protein fraction had the same c.p.m./ml. as did the filtrate; thus this experiment failed to demonstrate any association between ^{134}Cs and the leachable protein.

Variable volumes of leaching solution (0·85 per cent sodium chloride) had little effect on the total amount of activity removed from meat cubes over a given period of time. The most effective removal treatments incorporated agitation and replacement of the leaching solution. In four changes of saline solution and a total of 2 h agitation, 63 per cent of the ^{134}Cs was removed from 2·5-cm slices of L.D. muscle. Twenty-eight per cent of the ^{134}Cs was removed from the steaks during the first 30-min period of agitation (Fig. 1). When ground chuck was treated similarly, 75 per cent of ^{134}Cs was leached from the meat

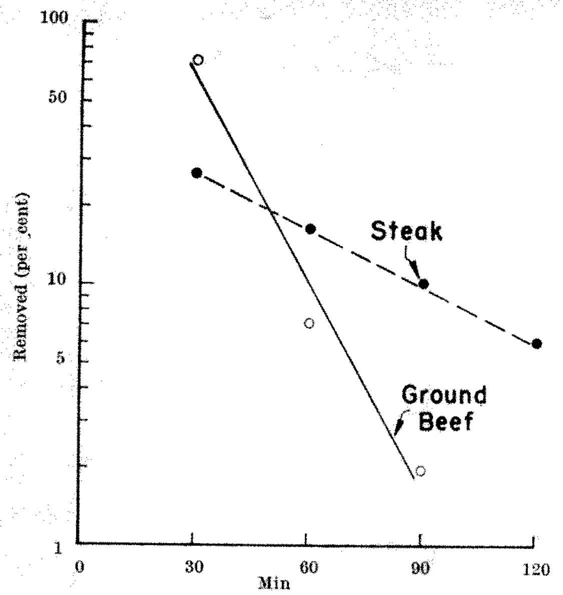

Fig. 1. Removal of ^{134}Cs from steak and ground beef during successive 30-min periods

in the initial 30-min period of agitation. The ground chuck lost 33 per cent of its total nitrogen in three 30-min treatments. The steaks lost 6 per cent total nitrogen when given 4 half-hour periods of agitation and salt solution changes.

The resulting ground beef had a very pale greyish-tan colour. The surface of the slices of L.D. had some faint pink areas while their interior had a red though faded colour increasing in intensity towards the centre. Steaks and ground beef from animals not dosed with ^{134}Cs and given the same agitation-leaching treatment were edible. After grilling, ground-beef patties had little flavour and were very light in colour; however, the steaks retained some of the delicate beef flavour and had a light colour similar to that of cooked pork.

In tests with flowing tap-water, approximately 81 per cent of the ^{134}Cs was removed from one-inch cubes of round in 12 h and over 99 per cent in 60 h. Freezing had little effect on the ^{134}Cs content of chuck. After accounting for drip loss and sampling, the average c.p.m./g were the same in thawed chuck roasts as in fresh.

In contrast to cæsium, strontium nuclides concentrate in the skeletal tissue. Strontium-89 and ^{90}Sr can be eliminated almost completely by boning the animal carcass[9]. Cæsium radionuclides can be removed in part from beef by cooking if the drippings and juices are discarded and by the treatments described in this communication. In areas of the world where cooked meat is eaten, a series of treatments including boning, a precooking treatment of leaching and agitation, and cooking would result in edible meat with reduced protein content and with approximately a 90 per cent reduction in radiostrontium and radiocæsium.

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BIOLOGY

Mechanism of Apical Dominance in Plants

THE phenomenon of apical dominance in plants has been the subject of considerable research over the past thirty years (reviewed by Audus[1]), yet there is still no general agreement as to the nature of the mechanism involved. Much of the work on this problem has been based on the assumption that the inhibiting influence of the apex is mediated either directly or indirectly by auxin. There is, however, considerable evidence that nutritional factors are also involved and that the expression of apical dominance is at least partly determined by the nutritional status of the plant.

This effect of nutrition was well illustrated in a recent investigation of the influence of the nitrogen supply on the growth and development of *Agropyron repens* (L.) Beauv. In this investigation, which is described more fully elsewhere[2], it was found that the inhibiting influence of the rhizome apex on the growth of the lateral buds could be quite precisely controlled by varying the nitrogen level. At low nitrogen levels bud growth was completely inhibited but could be induced to occur by removing the rhizome apex; whereas, at a high level of nutrition, the onset of dormancy was eliminated, all the buds developing as lateral rhizome branches on the intact plant.

This nutritional effect is similar to that reported by Gregory and Veale[3], who showed, in experiments with flax, that the degree of apical dominance was largely dependent on the nitrogen and carbohydrate nutrition of the plant. While these workers concluded that nutrition was the main factor in apical dominance they also postulated, on the basis of anatomical observations, that auxin produced in the stem apex may inhibit the buds indirectly by preventing the formation of their vascular connexions with the main axis, thus depriving them of an adequate nutrient supply. This hypothesis is but a slight modification of that proposed earlier by van Overbeek[4], who suggested that auxin may impede the flow of nutrients to the buds by causing some kind of blockage of the vascular supply.

Neither of these hypotheses, however, can claim any direct experimental support. Indeed, there is now considerable evidence that auxin plays an essential part in the differentiation of vascular tissue[5], and may, at least in certain species, be a limiting factor in its formation[6]. When this function of auxin is considered in relation to Gregory and Veale's hypothesis, it would seem that the development of vascular connexions to the buds is more likely to be limited by the supply of auxin from the bud itself, rather than to be suppressed by auxin from the stem apex. Thus, the variation observed by these workers in the development of the bud vascular connexions at different nodes of the stem may well reflect differences in the amount of auxin produced by the buds prior to the onset of dormancy. It is, therefore, suggested, merely as a working hypothesis, that the initial stimulation of bud activity when the stem apex is removed is due directly to the increased availability of nutrients. This stimulation of growth and metabolic activity might be expected to result in an increase in auxin production which, in turn, may enhance the flow of nutrients to the bud by promoting a greater development of the vascular connexions.

This hypothesis is supported, first, by van Overbeek's[4] observation that, in *Pisum sativum*, decapitation of the stem is followed within 12 h by a rise in the auxin content of the axillary buds; and, secondly, by the work of Moreland[7], who noted in experiments with *Phaseolus vulgaris* that within 48 h of removing the stem apex, and before the cotyledonary buds had increased appreciably in size, the vascular supply to the buds had been strengthened. Moreover, the cyclical nature of this postulated mechanism, in which nutrient supply, growth, auxin production and vascular tissue formation are regarded as interdependent, and the role attributed to irreversible anatomical changes agree well with the observation that bud growth, once established, cannot easily be arrested[1,4,8].

It is also assumed, on this hypothesis, that the auxin produced by the stem apex performs a similar function and thus, by controlling the differentiation of the vascular tissue in the stem, may determine, at least to some extent, the capacity of the apex to monopolize the nutrient supply. While such a mechanism may serve to maintain the dominance of the apex, the fact that the stem apex is initiated before the lateral meristems in the ontogeny of the plant may well provide it with a sufficient initial advantage in competing for nutrients to account for the dominance which it later achieves as the pattern of growth is established.

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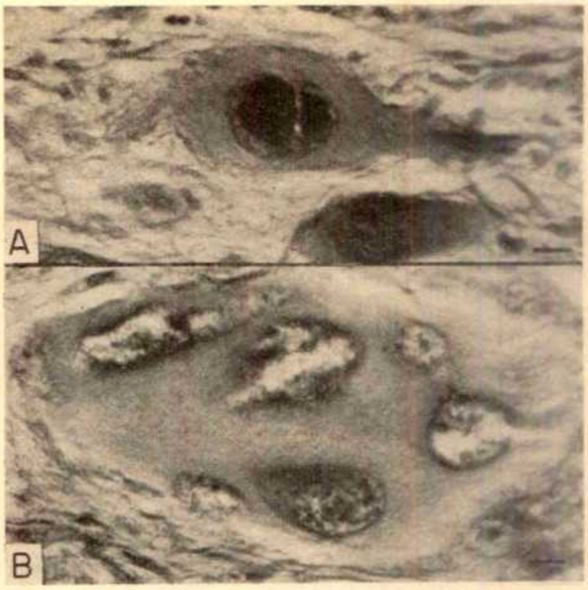

Fig. 1. *A*, caudal neurosecretory neurone with two intensely stained intranuclear cytoplasmic invaginations from a 'pond' *Albula*. *B*, Large caudal neurosecretory neurone with a multilobate nucleus from an open sea *Albula*. Acid violet stain. Scales = 10μ

Secretion Masses in the Nuclei of the Caudal Neurosecretory Cells of the Teleost *Albula vulpes*

'NUCLEAR secretion' has been reported by a number of students of neurosecretory cells, especially in fish hypothalamic neurones[1-7]. Neurones exhibiting similar characteristics have been described in the caudal neurosecretory system of two isospondylous teleosts: *Albula vulpes* and *Elops hawaiensis*[8,9]. The caudal neurones of many teleosts may possess various kinds of nuclear inclusions, generally less prominent than those of *Albula* and *Elops*, and not necessarily considered as 'secretion'[10-12]. Inasmuch as the actual production of proteinaceous secretory product within the nucleus is not consonant with the present conception of the protein-secretory cell, we undertook a re-examination of the caudal neurosecretory neurones in *Albula vulpes*, the Hawaiian 'oio' or bonefish, with the electron microscope.

The nuclei of the caudal neurosecretory neurones of *Albula* are highly polymorphic. In light-microscope preparations stained with acid violet[13] densely staining 'inclusions' are seen in many nuclei (Fig. 1*A*); the axon terminals around capillaries in the neurohæmal organ of this system—the urophysis—are also intensely coloured. Other cells show cytoplasmic invaginations, some of which appear to be surrounded completely by the nucleus. Although some of the inclusions are not obviously connected with the perinuclear cytoplasm, a canal is probably present. If the canal happens to be in the plane of section, it would be indiscernible (see Fridberg[11]).

On the ultrastructural level, the nature of the nuclear 'inclusions' previously interpreted as secretion masses soon became apparent (Fig. 2). The masses are intranuclear cytoplasmic invaginations (*ICI*) and are characterized by the presence of the usual cytoplasmic organelles: rich endoplasmic reticulum with dilated cisternæ, mitochondria, Golgi membranes, and ovoid lysosome-like bodies. Elementary neurosecretory granules were also present, occasionally associated here, as in the perinuclear cytoplasm, with Golgi membranes[14,15]. The elementary granules were present in higher concentration in the 'intranuclear' cytoplasm than in the more peripheral

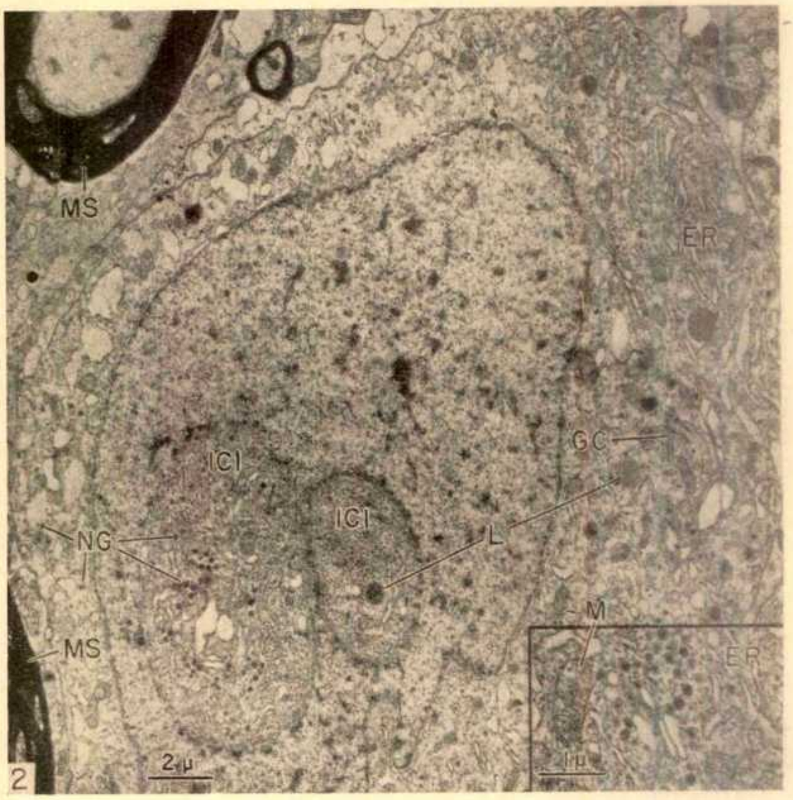

Fig. 2. Electron micrograph of portion of caudal neurosecretory neurone with two intranuclear cytoplasmic invaginations (*ICI*). Inset: intranuclear cytoplasmic invagination with concentration of neurosecretory granules (*NG*); *ER*, endoplasmic reticulum; *GC*, Golgi centre; *L*, possible lysosomes; *M*, mitochondria; *MS*, myelin sheaths

cytoplasm (Fig. 2); this might account for the intense staining with acid violet.

Whether all varieties of previously described intranuclear materials, especially the colloid droplets[1,4] in hypothalamic neurosecretory neurones, are intranuclear cytoplasmic invaginations is still uncertain. Colloid droplets are seen extracellularly in the caudal neurosecretory system of various fishes (for example, *Paralichthys californicus*) and may represent products of cell degeneration.

The intranuclear cytoplasmic invaginations were most prevalent in animals taken from sea-water 'ponds'. Fish caught in the open sea had fewer intranuclear cytoplasmic invaginations, generally located in small cells close to the urophysis. The more anteriorly located large cells usually possessed extensively multilobate nuclei (Fig. 1*B*). Some nuclei were obviously intermediate stages between those with intranuclear cytoplasmic invaginations and the multilobate types. The cytoplasmic regions bordering on intermediate nuclei stained strongly with acid violet. One group of adult females taken from the open sea showed no sign of intranuclear cytoplasmic invaginations, but all caudal neurosecretory cells contained complexly lobate nuclei. The variable nuclear morphology in *Albula* may be correlated with habitat, but also possibly with age and/or sexual maturity. The open sea fish were larger and had better developed gonads than the 'pond' fishes.

In view of the still elusive functions of the caudal neurosecretory system and the urophysis[8,16] the cytological alterations seen in *Albula* may be indicative of actions unrelated to its suggested ionoregulatory role[17] and merit further investigation.

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Synchronization of Hatching in American Bobwhite Quail (*Colinus virginianus*)

IT is known that chicken embryos show great variation in their rate of development. This is due to three main factors: variations in the latent period, variations in the environmental factors of incubation and the inherent variations of the embryos themselves[1,2]. Some, or possibly all, of these factors are likely to occur under natural conditions within a single clutch of eggs, and particularly in species which incubate large clutches.

Given these variations, it seems remarkable that the young of many species of birds hatch at the same time. Among British game birds this is reported for the common quail (*Coturnix coturnix*), the common partridge (*Perdix perdix*), the pheasant (*Phasianus colchicus*) and the red grouse (*Lagopus scoticus*)[3]. Similarly it has been found here that when a clutch of the American bobwhite quail (*Colinus virginianus*) is incubated artificially, the eggs will hatch within an hour or two of each other. This synchronization of hatching (emergence of the chick from the shell) occurs in spite of considerable variability in the previous easily detectable stage of 'pipping'; that is, the appearance of one small hole in the shell indicating that pulmonary respiration has already begun. In the bobwhite quail eggs normally pip about two days before hatching.

The experiment described here shows that it is possible not only to shorten the normal incubation period (23 days) by incubating embryos in contact with others 24 h older, but also, by doing so, to synchronize the final stages of development so that all embryos hatch at the same time.

Eggs were taken from two pairs of quail, each one being removed from the parents soon after laying and stored at a temperature of approximately 18° C for up to 14 days. Clutches were incubated in a 'Curfew' observation incubator run at 40·5° C. The eggs were incubated in clutches of 2, 3, 4, 5, 10 and 11 eggs, progeny from the two pairs being kept separate. Seven eggs were incubated singly. Inside the incubator clutches were separated by distances of at least 4 in., but within each clutch eggs were in contact with each other. In all clutches one unincubated egg was placed within the clutch 24 h late. The eggs were observed regularly from the 20th day onwards and notes were made of the approximate times of pipping and hatching of each egg.

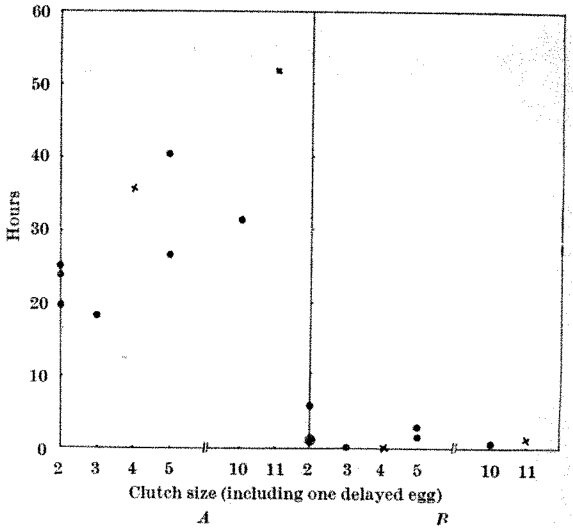
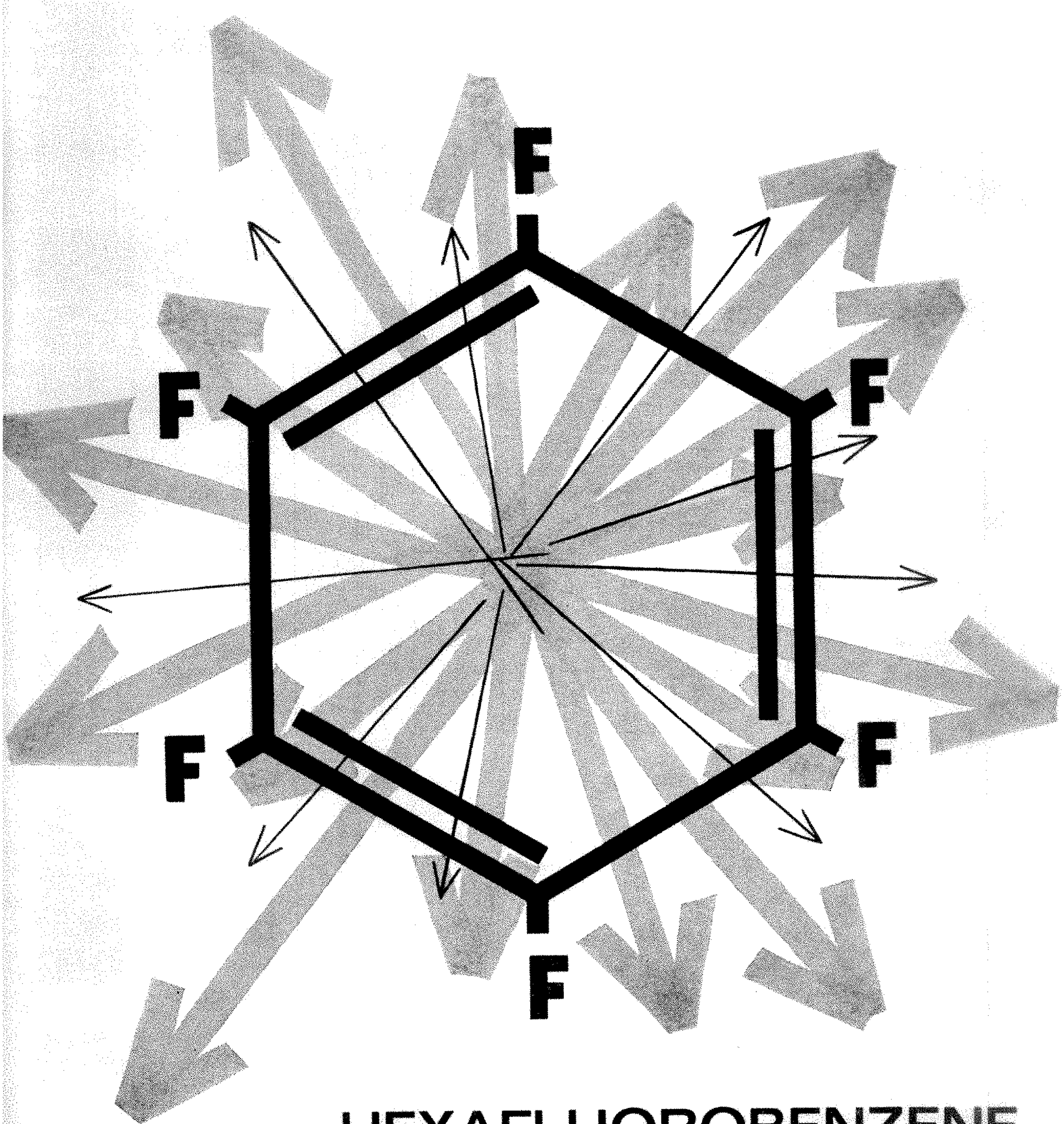


Fig. 1. *A*, Interval between pipping of first and last eggs; *B*, interval between hatching of first and last eggs. ●, Eggs from pair *A*; ×, eggs from pair *B*

Results are given in Fig. 1. Fig. 1*A* shows the time-interval between the pipping of the first and last eggs in each of the nine clutches observed. The last egg to pip was, in all cases except one, the delayed egg. This time interval varied from 18 to 52 h, suggesting that each egg pipped at approximately its 'normal' time, that is, on the 21st or 22nd day of incubation. Fig. 1*B* shows, however, that the time-interval between the hatching of the first and last eggs in these same nine clutches was never more than 6 h. The mean number of hours between pipping and hatching was 23 for the nine delayed eggs and 44 for the seven eggs incubated singly. This, considered in conjunction with Fig. 1, suggests, therefore, that the delayed embryos accelerated their development between



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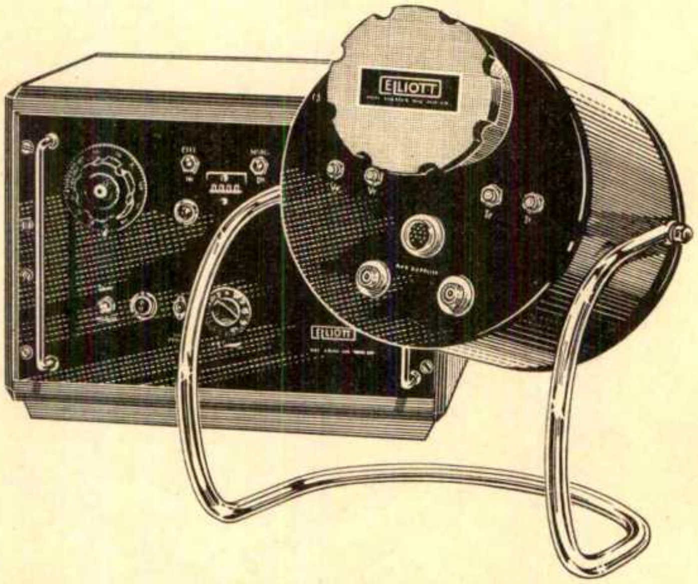

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pipping and hatching, and that this shortening of the incubation period is due to the proximity of the more advanced eggs in each clutch.

These results are consistent with the view that there is, in this species, a mechanism acting between eggs which tends to synchronize the embryos' development towards the time of hatching, by speeding up the retarded ones. The nature of this mechanism is under consideration.

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✓ Mammalian Intrafusal Muscle Fibres

RECENT histological investigations of cat muscle spindles[1-3] have established the presence of two types of muscle fibre, the large nuclear-bag fibres and the smaller and more numerous nuclear-chain fibres (Fig. 1*A*). Boyd[3] maintains that each type of muscle fibre receives its own pattern of innervation, there being, in particular, a segregation in the size distribution of the fusimotor supply. However, earlier work by one of us[4] described spindles in the quadriceps femoris of the rabbit which did not show this structural duality, since they were composed of nuclear-bag fibres only. We wished to ascertain the validity of this observation, and to determine the extent to which the muscle-fibre content of cat spindles represents the typical mammalian condition.

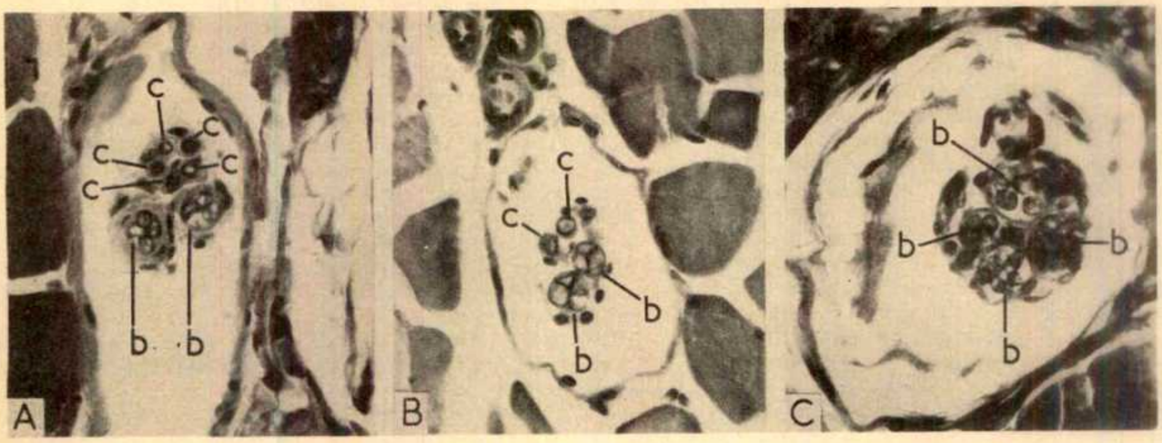

Fig. 1. Transverse sections through the equatorial region of spindles from cat soleus (*A*), rat rectus femoris (*B*), and rabbit vastus intermedius (*C*) muscles. Nuclear-chain muscle fibres (*c*) outnumber nuclear-bag muscle fibres (*b*) in the cat (2 bag : 5 chain fibres); are present in equal proportion in the rat (2 bag : 2 chain fibres); and are absent in the rabbit (4 bag fibres only). (10μ paraffin sections stained with hæmatoxylin and eosin; × *c*. 410)

Fourteen spindles from rabbit hind-limb muscles were traced from end to end in serial transverse section, 3 from vastus intermedius, 3 from tenuissimus, and 8 from soleus. All spindles possessed nuclear-bag fibres only, the most frequent number being 4 (Fig. 1*C*). This was also the case in 3 spindles from the vastus intermedius of a hare, and in 3 out of 6 spindles from a rabbit fore-limb muscle (extensor digitorum communis). The other spindles in this sample, however, possessed 3 bag fibres accompanied by 1 or 2 chain fibres.

The typical fibre content of the rat spindle proved to be 2 bag and 2 chain fibres (Fig. 1*B*); 8 spindles from rectus femoris and 6 from soleus had this composition In a further 3 spindles from soleus the proportion of bag to chain fibres was 2 : 1, 3 : 1, and 2 : 3. Samples of 3 spindles taken from a thigh flexor muscle of a hedgehog, a tree shrew, and an armadillo had bag-to-chain fibre ratios of 2 : 2, 2 : 2, and 2 : 4 or 5, respectively.

Cooper and Daniel[5] found chain fibres to be the most numerous in the majority of human spindles sampled from neck and lumbrical muscles, but a few contained bag fibres only.

Nuclear-bag fibres are therefore a basic component of the mammalian spindle, whereas nuclear-chain fibres, though usually present in various proportions, may be lacking. A 'chain only' composition has been observed[2] in part of a cat tandem spindle, but this condition is probably aberrant. Evidently most spindles in a particular mammal possess a similar type of fibre composition, though other types may occur. Thus the lagomorph spindle is typically 'bag only', but combinations with chain fibres also occur; human spindles may lack chain fibres, though typically they are present in excess.

In the cat spindle the secondary endings lie predominantly on the nuclear-chain fibres[3]; in the rabbit spindle they lie on the nuclear-bag fibres mainly in the myotube regions[4] (hence the descriptive term 'myotube ending' suggested by Granit[6]). Both myotube and nuclear chain offer a similar substratum for the ending, and it is significant that the myotube regions in the rabbit are typically three to four times longer than those in the cat. The 'chain dominant' human spindle resembles the cat spindle in these respects[5]. As observed at or near the level of spindle entry, the motor innervation of the 'bag only' rabbit spindle is derived from both large and small fusimotor fibres (Barker[4]; Barker and Cope[7]; Ip, unpublished observations) as in the cat[3,7]. Matthews[8] has suggested that in the cat spindle these large and small motor fibres may represent functionally distinct types controlling the dynamic and static responses of the primary ending, respectively. The same correlation applied to the typical rabbit spindle would necessitate postulating a mechanism whereby this control could be achieved through the contraction of bag fibres only.

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Dry Matter Production by Detached Ears of Wheat

Cereal yields have been shown to depend more on assimilation in the ears and flag leaves than on translocation into the grain of compounds manufactured or stored in other green parts of the plant[1]. Quantitative estimates of the relative contributions from the ears, the flag leaves or other green parts have been made by shading and defoliation methods[2–6], by enclosing ears in a transparent chamber supplied with $^{14}CO_2$ (ref. 7), or by following changes in the carbon dioxide content of air-streams entering and leaving such a chamber[8]. Such methods have been criticized for various reasons[9,10]. Buttrose and May[9] claimed that their technique with regulated floret numbers is free of many of these disadvantages, but Thorne[8] has shown that one of the assumptions on which the method is based is not valid and Lupton[11] has demonstrated that different results can be obtained if different numbers of florets are left on the ear. Obviously, this method of measuring net assimilation of cereal ears is also open to criticism.

Where it is necessary to measure the absolute rate of net assimilation by a cereal ear it is difficult to suggest any method better than those previously mentioned. In some situations, however, it may be enough to demonstrate that the net assimilation in the ears and flag leaves of one variety is relatively greater than in another. For this purpose the changes in dry weight of detached ears have been followed. The ears are taken shortly after anthesis from plants lifted and removed to the laboratory early in the morning while still fully turgid. The stems are cut several inches below the flag leaf node and trimmed under water to leave one inch of stem below this node. The experiments have been conducted out of doors with the ear preparations placed in batches of 72 in polythene-lined boxes (12 in. × 18 in. × 6 in.) covered with a hardboard lid pierced by holes 0·25 in. in diameter through which the stems pass. The boxes are filled with quarter-strength Long Ashton complete nutrient solution which is changed at three-day intervals. Since ear size accounts for much of the varietal differences in grain yield in wheat, conscious selection for uniformity of ear size in the detached ear preparations may, unwittingly, remove any varietal differences in ear assimilation that do exist. In these investigations the detached ear samples of each variety were, therefore, sorted into several size grades and equivalent numbers of each size grade were included in each sampling unit in the same proportion as that found to be typical of each variety.

Five experiments have been carried out with detached ear preparations from three winter and six spring wheat varieties in 1961 and 1962. The results of a 1962 experiment with the three winter wheat varieties 'Cappelle', 'Professeur Marchal' and 'Milfast' are given here. The detached ear preparations were made on July 11, 16 days after ear emergence. Twenty-four preparations from each variety were placed in each of four boxes and the flag leaf laminæ removed from twelve of them. This arrangement gives four replicates of a 3 × 2 split plot design. Two gatherings, each of six ears, were taken after 10 and 33 days, on July 22 and August 13. In addition, an initial sample of thirty ears of each variety was taken, and at the close of the experiment a further sample of thirty ears was collected from intact plants growing at the original sites in the field.

After 33 days it was found that the ear dry weights of the detached ear preparations with flag leaves were 1,508 mg for 'Professeur Marchal', 1,334 mg for 'Cappelle' and 1,164 mg for 'Milfast' (*L.S.D.* 129, $P = 0·05$). Ears of the same varieties from plants grown in the field weighed 2,067 mg, 1,600 mg and 1,483 mg, respectively, at the same date. Detached ear preparations have, therefore, produced 50 per cent less ear dry weight, an average increment of 491 mg, than field grown ears, average increment 863 mg, but still show varietal differences of the same size as those found for field grown ears. Similar results have been found in the other experiments. The dry weight contributions attributable to the flag leaf or to the ear plus peduncle have been calculated and these figures are given in Table 1 for the two sampling intervals and for the full 33-day period of the experiment.

Table 1. Contributions to ear dry weight (mg) from flag leaves (*F*) and ears plus peduncles (*E* + *P*) of detached ear preparations of 3 winter wheat varieties, Upperwood Farm, Reading, 1962

	Period 0–10 days		Period 11–33 days		Period 0–33 days	
	F	*E* + *P*	*F*	*E* + *P*	*F*	*E* + *P*
'Professeur Marchal'	191	107	36	273	227	378
'Cappelle'	137	114	62	198	199	312
'Milfast'	126	66	30	141	150	207
Mean of all varieties	149	96	43	204	192	299

L.S.D. ($P = 0·05$)	0–11 days	Period 11–33 days	0–33 days
(*a*) Between means of all varieties	11	18	20
(*b*) Between sub-treatment means within varieties (horizontal comparisons)	28	41	50
(*c*) Between treatment means between varieties (vertical comparisons)	20	29	35

Over the 33-day period the flag leaf contributions (192 mg) averaged only 64 per cent as much as the ear plus peduncle contributions (299 mg). Differences between these contributions were significant in each variety. Between varieties the flag leaf contribution of 'Milfast' (150 mg) was significantly lower than those of 'Cappelle' and 'Professeur Marchal' (199 and 227 mg respectively), which were not significantly different. The ear plus peduncle contribution of 'Professeur Marchal' (378 mg) was significantly greater than that of 'Cappelle' (312 mg), which in turn was significantly greater than that of 'Milfast' (207 mg). During the first 11 days of this period the flag leaf contributions (149 mg) averaged 155 per cent more than the ear contributions (96 mg), a trend evident in all varieties, but the difference between the contributions in 'Cappelle' was not significant. During this period the flag leaf contribution from 'Professeur Marchal' (191 mg) was significantly greater than those from 'Cappelle' and 'Milfast', which were similar (137 and 126 mg respectively), while the ear plus peduncle contributions of 'Professeur Marchal' and 'Cappelle' were similar (107 and 114 mg respectively) and both significantly greater than that of 'Milfast' (66 mg).

Over the 11–33-day period the flag leaf contributions were much reduced (43 mg), averaging only 21 per cent of the ear plus peduncle contributions (204 mg), the differences between the contributions being significant in each case. Observations at the time bear out the suggestion from these figures that flag leaves began senescing shortly after the eleventh day and were completely dried out by about the eighteenth day. Differences in the flag leaf contributions of the three varieties were slight, but the ear plus peduncle contribution of 'Professeur Marchal' (273 mg) was significantly greater than that of 'Cappelle Desprez' (198 mg), which, in turn, significantly exceeded that of 'Milfast' (141 mg). It may thus be concluded that the flag leaf contributes some 13·5 mg/day during the earlier part of the ear growth period, falling after the eleventh day to a value of 2·0 mg/day. The ear contribution was less than that of the flag leaf to begin with, averaging 8·7 mg/day, and, on the figures available, this was maintained at 9·3 mg/day during the last 22 days. Observations suggest that the actual rates would be higher than this for the first half of this period and lower for the last half as ear senescence became marked.

It is obvious that much of the physiology of these detached ear preparations requires to be investigated before it will be possible to consider them physiologically comparable to ears growing on an entire plant. The results of these investigations suggest, however, that such preparations may prove a convenient means of comparing the assimilatory capacity of the flag leaves and ears of different varieties and species of cereals. In addition they may prove a useful research tool in other subjects of physiological interest such as grain dormancy, the mineral requirements and water relations of the cereal ear and biochemical factors determining quality factors in wheat grains.

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ENTOMOLOGY

Danger of Spraying Wooden Insect Cages with Melamine Resins

THE original cages used by us for rearing *Glossina austeni* were made of deal (coniferous softwood) by the local carpenter; the wood was left untreated. A precision wood-working firm was commissioned to make similar cages in large numbers. On their advice, mahogany plywood, exterior grade, was used, and the cages surface-sprayed with a melamine resin which would seal the wood against absorption of fæces and facilitate the washing of dirty boxes.

Twenty new, melamine-sprayed cages were first kept for three days in a room at 33° C so as to expel any residual solvent. Batches of about 25, 4-day-old, newly fertilized females were housed alternately in melamine-sprayed or deal cages; survival rates were recorded at days 14, 30 and 60 after fertilization (Table 1).

Table 1. TOXICITY OF MELAMINE RESINS

A comparison of survival rates among female *G. austeni* at days 14, 30 and 60 after mating when 3–4 days old

Cage type	Total No. of flies	Day 14 No. survived	Day 14 Survival %	Day 30 No. survived	Day 30 Survival %	Day 60 No. survived	Day 60 Survival %
Untreated deal cages	484	390	81	310	64	198	41
Melamine-sprayed plywood cages	476	363	76	262	55	140	29

The survival rate was lower in the new, melamine-sprayed cages, and, further, the differences at each age became progressively greater at 5, 9 and 12 per cent, suggesting that the melamine resin has slow-acting toxic properties. The difference at day 14 is not significant ($\chi^2 = 2 \cdot 65$; $0 \cdot 2 > P > 0 \cdot 1$). The difference at day 30 is significant ($\chi^2 = 8 \cdot 1$; $P < 0 \cdot 01$), as is the difference at day 60 ($\chi^2 = 13 \cdot 9$; $P < 0 \cdot 001$).

Melamine resin is a condensation product of melamine and formaldehyde, so presumably the formaldehyde is the toxic component. Since all surfaces of the mahogany plywood had been thoroughly sprayed with melamine resin, there can be no question of harmful emanations from either the mahogany or the bonding material used in plywood construction.

Since phenol-formaldehyde resins are now used as the bonding agent for plywood, a small test was undertaken to see if survival in untreated mahogany plywood boxes is worse than in deal boxes. The results suggest that survival is worse in the mahogany plywood boxes for the first seven weeks after manufacture, but not thereafter, whereas the melamine-sprayed cages are still toxic after 10 weeks.

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✓ Effects of Brain and Corpus Cardiacum Extracts on Hæmolymph Trehalose of the Cockroach, *Periplaneta americana*

THE first report relating hormonal factors in insects to the regulation of a particular hæmolymph sugar was that of Steele[1]. He demonstrated that saline extracts of the corpus cardiacum of the cockroach *Periplaneta americana* (L.) cause about a 100 per cent increase in hæmolymph trehalose within 1 h after injection and the effect persists for several hours. The level of reducing sugar (glucose?) was generally lowered in these animals. Later, Steele reported that the extract accelerated the release of inorganic phosphate and that as hæmolymph trehalose was elevated fat-body glycogen declined[2]. Nerve cord glycogen also declined, but gut and muscle glycogen were unaffected. The interpretation is that the hormone appears to increase the activity of phosphorylase and produces a glycogenolic effect. More recently Bowers and Friedman[3] reported finding that in the South American cockroach, *Blaberus discoidalis* (Serville), administration of aqueous extracts of the corpus cardiacum significantly elevate both trehalose and glucose in the hæmolymph. They also found a decrease in fat-body glycogen 5 h after injection and extracts of the corpus allatum were also tested and found to be without effect. They note also that animals with heads tied off respond to the active extract, indicating that the effect is not mediated through other cephalic glandular material.

We wish to report here our observations which generally confirm and supplement these reports. A portion of this work has been briefly reported on elsewhere[4]. Adult male and female cockroaches, *Periplaneta americana* (L.), were examined. Tissue extracts were prepared from cockroaches that were rapidly anæsthetized by carbon dioxide gas and then chilled. The heads were removed and dissected in cold Hoyle's solution[5]. The corpus allatum (*CA*), corpus cardiacum (*CC*), brain or supraœsophageal ganglion (*B*), and subœsophageal ganglion (*S*) were placed on homogenizer pestles and frozen until used. The tissues were homogenized in Hoyle's solution, one tissue per 0·02 ml. Recipient animals were deprived of food for at least 18 h prior to injection. Six to ten animals constituted the experimental groups and each animal received 0·01 ml. of one of the tissue extracts (equivalent to one-half tissue). Control animals received Hoyle's solution only. After 1 h the animals were quickly immobilized by carbon dioxide, thoroughly chilled in a freezer, and their hæmolymph extracted (as a group) by the centrifugal method[6]. A 0·1 ml. portion of hæmolymph was then analysed for glucose (glucose oxidase–peroxidase method[7]) and trehalose (anthrone method of Wyatt and Kalf[8]).

Some of the results obtained during this work are shown in Tables 1 and 2. For analysis, only experiments in which all four tissues were tested simultaneously are shown here. A total of 25 experiments were conducted, but in the

Table 1. EFFECTS OF TISSUE EXTRACTS ON HÆMOLYMPH SUGARS; MALE DONORS AND MALE RECIPIENTS

(Values are in mg per cent)

		Glucose					Trehalose				
	Extract	*CC*	*B*	*CA*	*S*	Control	*CC*	*B*	*CA*	*S*	Control
Experiments	I	82	30	80	101	43	2,097	1,145	948	938	562
	II	564	236	47	281	36	3,008	797	703	347	529
	III	126	156	121	64	205	2,386	949	977	960	926
	IV	325	143	64	333	94	959	894	462	268	813

Table 2. Effects of Tissue Extracts on Hæmolymph Sugars; Male Donors and Female Recipients

(Values are in mg per cent)

		Glucose					Trehalose				
	Extract	*CC*	*B*	*CA*	*S*	Control	*CC*	*B*	*CA*	*S*	Control
Experiments	I	73	134	68	19	80	1,080	498	727	454	569
	II	106	71	62	37	117	1,082	783	404	669	824
	III	92	142	145	491	334	1,190	650	566	426	260

majority of them only two or three tissues were tested. The results from all of them tend to fit the same general patterns as discussed here.

The significant elevation of trehalose following cardiacum administration confirms the observations of both Steele[1] and Bowers and Friedman[3]. The results in Table 1 for trehalose values were subjected to non-parametric analysis using rank order and yielded a coefficient of concordance of 0·75. After a continuity correction was applied, the order: $CC > B > CA > S >$ control, was significant ($P < 0{\cdot}01$). The comparable data of Table 2 show a rank order starting with *CC* and then *B*, but it is just significant at the 10 per cent level. The observation that the brain extract was generally effective also in elevating trehalose levels is noteworthy. Steele[1] tested the effects of brain, among other tissues, for this effect in 2 experiments and dismissed the slight increase in trehalose he found as probably due to traces of cardiaca tissue remaining attached to the brain. In our dissections the brains were carefully cleaned of any cardiaca tissue and we believe the hyperglycæmic effect observed is real. This could be interpreted to mean that the active factor of the cardiacum is produced by neurosecretory cells of the brain, if the result is viewed in the framework of the anatomical studies which suggest such a neuroendocrine relationship[9].

The results of glucose determination are too variable for statistical analysis so it is impossible to know if the cardiaca extracts tended to elevate glucose, as was the case in the results of Bowers and Friedman[3], but generally it was not depressed, as suggested by Steele[1].

Males given cardiacum extract usually had more hæmolymph trehalose than females. In 10 experiments in which males were tested with cardiaca extract, 7 groups had trehalose-levels greater than 1,000 mg per cent, whereas only 3 out of 9 groups of females had so high a level. Both groups were given extracts from male tissue. Thus, there may be a sex difference in responsiveness to the cardiaca factor.

A series of five experiments on the effects of starvation on the response to cardiacal extract showed that male animals starved for 8 days prior to injection fail to make any response to the hormone. Allatal extracts are also without effect. Furthermore, the levels of circulating trehalose in the control and experimental groups all were about 400 mg per cent, that is, about two-thirds the normal value.

The glucose values found in this work were higher than Steele's[1] and the trehalose values generally lower. The reason for these differences is probably relatable to strain differences and diet factors.

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VIROLOGY

Inactivation of *MHV*-3 Virus by Intestinal Content and Intestine of Normal Mice

In previous work we reported on certain biochemical changes in experimental (*MHV*-3 virus)[1] and in human viral hepatitis[2,3]. This communication deals with the behaviour of *MHV*-3 virus[4], eliminated with bile in the intestine of infected mice. Because of evidence of virus inactivation in the intestine, experiments were also carried out on the effect of intestinal content and intestine from normal mice on *MHV*-3 virus.

Twenty mice of *NMRI*-stock, weighing about 10 g and fed on 'Altromin' diet, were inoculated intraperitoneally with 100 LD_{50} of *MHV*-3 virus. From mice killed 72 h after inoculation, samples were taken of the liver, gall-bladder bile, intestinal content of the first 5 cm of the small intestine as well as of the last 5 cm, fæcal content of the last section of the colon and, finally, fæces expelled. After addition of antibiotics the LD_{50} of the aforementioned materials was determined by the method of Reed and Muench[5].

The results given in Table 1 show a gradual decrease in the level of the virus from liver to expelled fæces. A striking difference in virus-levels can be observed, particularly between the bile and the first portion of small intestine and between the first and last portions of the small intestine. This finding, suggesting a possible inactivation of the virus during its pathway in the intestinal lumen, led us to perform the following experiments.

Table 1. Levels of Virus in Different Materials from Mice infected with 100 LD_{50} of *MHV*-3 Virus

Materials	LD_{50}
Liver	$10^{-7 \cdot 5}$
Bile	$10^{-6 \cdot 1}$
Smallintestine content (first 5 cm)	$10^{-2 \cdot 4}$
Smallintestine content (last 5 cm)	$< 10^{-1}$ *
Colon content (last section)	$< 10^{-1}$ †
Expelled fæces	$< 10^{-1}$ ‡

* At 10^{-1} dilution 4 mice of 10 used died.
† At 10^{-1} dilution 2 mice of 10 died.
‡ At 10^{-1} dilution no mortality was observed.

Mice were injected intraperitoneally (0·1 ml.) with 10-fold dilution of each material from 10^{-1} to 10^{-9} (10 mice for each dilution). The experiment reported in Table 1 is one of three which gave similar results.

Six mice of the *NMRI*-strain were used. From four of them, killed by decapitation, the whole intestinal content was taken and the intestine itself was thoroughly washed to remove any fæcal debris. From the two remaining mice the intestine was taken together with its content. These three materials (intestine, intestinal content and the two together) were carefully homogenized and tested for their inactivation power against *MHV*-3 virus. The virus, stored at −70° C as a 10 per cent suspension ($LD_{50} = 10^{-7 \cdot 6}$), was used in 10-fold dilution from 10^{-1} to 10^{-9}. Three series of these dilutions were prepared to contain a homogenate of the three different materials in final concentrations of 10 per cent. A fourth series was the control one (no homogenate added). All samples were incubated for 90 min at 37° C and frequently shaken. After incubation, virus-levels were determined in each sample as already indicated above[5].

The results presented in Table 2 show that virus-levels are much higher in the control series than in the presence of the different materials tested. This seems to show that virus can be highly inactivated by intestinal content and intestine. Liver and brain tissues from normal mice were used in the same experimental conditions to test whether

Table 2. Effect of Intestine and Intestinal Content on *MHV*-3 Virus Infectivity

Materials	LD_{50}
Virus	$10^{-6 \cdot 6}$
Virus + intestinal content	$10^{-2 \cdot 8}$
Virus + intestine	$10^{-2 \cdot 5}$
Virus + intestinal content and intestine	$10^{-2 \cdot 6}$

For details see text and Table 1.

they, too, had an inactivating power against *MHV*-3 virus; no effect was found.

From our results a certain ability to inactivate *MHV*-3 virus may be attributed to intestinal content and intestine of normal mice. Investigations on the mechanism of this inactivation and on the nature of the compound or compounds involved are now in progress.

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Inhibition of Localized Virus Lesions by *N*-Dimethylaminosuccinamic Acid

PREVIOUS investigations have shown that the application of 2,4-dichlorophenoxyacetic acid or its salts or certain phenoxyacetic acid analogues greatly altered the number of virus lesions or the amount of virus present in tobacco ringspot (TRSV) inoculated plants[1,2]. This confirmed the findings of several other workers[3,4].

Applications of solutions containing the growth suppressant (2-chloroethyl)-trimethylammonium chloride (CCC) reduced the amount of tobacco mosaic virus in floating leaf disks[5].

This communication describes the response of tobacco ringspot virus inoculated plants of the 'SR' cultivar[6] of *Vigna sinensis* (Torner) Savi to foliar applications of the plant growth retardant chemical *N*-dimethylaminosuccinamic acid (DMAS)[7].

Seeds of the test plants were sown in 3-in. plastic pots containing two parts soil, two parts peat moss, and one part sand. In 8–10 days the primary leaves were sufficiently expanded to permit inoculation. Ten-plant plots were dusted with 400 mesh silicon carbide and inoculated with TRSV juice expressed from inoculated tobacco plants showing good symptoms of the disease. The inoculum was diluted approximately 1 : 250 with 0·01 M neutral phosphate buffer. This inoculum containing 1 per cent silicon carbide was applied by means of a 1-in.-wide, round, T-head acrylic plastic inoculator[8]. The DMAS solutions were applied approximately 45 min after inoculation when the inoculated leaves had dried. In some cases it was mixed with the inoculum. Characteristic, large, susceptible-type, localized lesions (typically preceding systemic necrosis) appeared and were counted 3–5 days after inoculation.

The results of four experiments are illustrated in Fig. 1. Application of an aqueous solution of 5,000 p.p.m. of DMAS to leaves of *Vigna sinensis* 45 min after inoculation reduced the virus local lesions by 90·1–92·9 per cent (Table 1). The solution containing 2,500 p.p.m. of DMAS was almost as effective as the stronger solution, and that containing 1,250 p.p.m. reduced lesions 66·4–83·6 per cent. Progressively less inhibition of virus lesions was caused by solutions containing 625, 312 and 156 p.p.m. of DMAS.

Table 1. INHIBITION OF TRSV LESIONS ON BLACK COWPEA (*Vigna sinensis*) BY *N*-DIMETHYLAMINOSUCCINAMIC ACID

	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
DMAS (p.p.m.)	Lesion No.*	Inhibition (%)	Lesion No.	Inhibition (%)	Lesion No.	Inhibition (%)	Lesion No.	Inhibition (%)
0 (control)	18·5	0	23·8	0	21·3	0	109·2	0
156	16·2	12·4	21·6	9·2	20·1	4·7	—	—
312	12·7	31·4	13·7	42·4	13·1	38·5	—	—
625	8·0	56·8	10·4	56·3	10·2	52·1	52·8	51·7
1,250	3·4	81·6	8·0	66·4	3·5	83·6	20·4	81·3
2,500	1·3	93·0	3·1	87·0	2·2	89·7	0·4	99·6
5,000	1·4	92·4	1·7	92·9	2·1	90·1	0·0	100·0

* Means of 20 leaves.

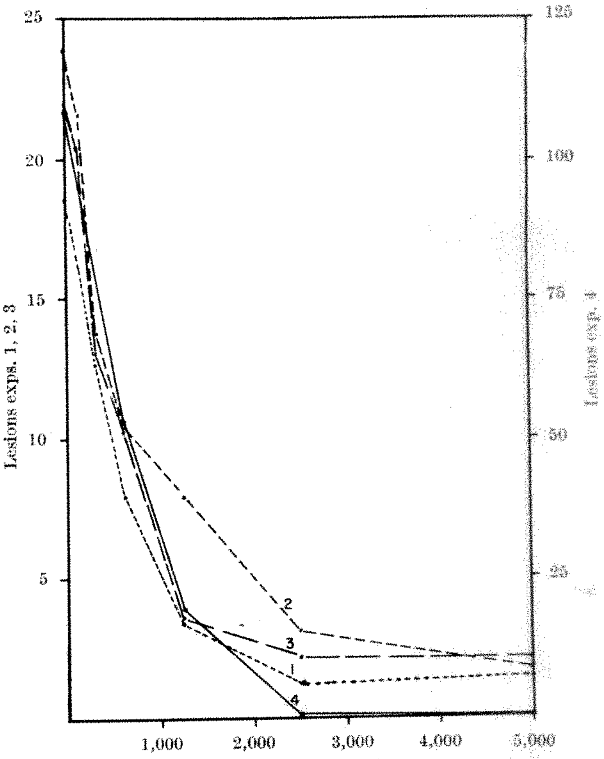

Fig. 1. Reduction of local infection of tobacco ringspot virus (TRSV). on black cowpea (*Vigna sinensis*). Foliar application of DMAS 45 min after inoculation or water spray (control, no DMAS) in exps. 1, 2, 3. Application simultaneously with inoculation (exp. 4)

Extrapolated values for zero concentration fall remarkably close to the actual values for the non-treated (water spray only) control. Lesion inhibition is consistently related to concentration of DMAS over a wide range in concentration. In some cases plants treated with solutions containing 2,500 p.p.m. or more of DMAS had irregular, small, necrotic patches. These patches probably do not represent the interference with lesion formation reported herein since they were also observed on leaves of non-inoculated plants and on non-inoculated parts of inoculated leaves. Inhibition of lesions was pronounced on many leaves with no evidence of chemical injury.

The observed response may be the result of inoculum inactivation, cell injury due to DMAS, altered host susceptibility, or viricidal action. Elucidation of the mechanism of action, however, must await the outcome of further more definitive research now in progress. Other methods and sites of application, the effects of related chemicals, and analysis of treated plants are being investigated.

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PSYCHIATRY

Variations of Urinary Creatinine and its Correlation to Tryptamine Excretion in Schizophrenic Patients

In the course of investigating the effect of diet on behaviour and excretion of urinary constituents in schizophrenic patients a remarkably wide variation in the daily output of creatinine was found in several subjects. We also observed a high degree of correlation between the excretion of creatinine and urinary tryptamine. Both phenomena are shown in Fig. 1, where the daily excretions of creatinine and tryptamine along with urine volume and daily tryptophan intake are plotted over 18 consecutive days for the patient who showed the greatest association of these two variables. During the period of observation the creatinine excretion varied from 0·90 to 1·78 g/24 h with a corresponding range of tryptamine excretion of 45–182 μg/24 h. The correlation coefficients between urinary tryptamine and creatinine as well as that of tryptophan intake are presented in Table 1. Both Fig. 1 and Table 1 show that the variations of tryptamine in the urine are not related to the tryptophan intake, which was maintained between 0·53 and 1·04 g/24 h. A highly significant positive correlation is, however, associated with the excretions of tryptamine and creatinine. During the observation period, the patient received a standardized diet containing 1·0–1·5 g of tryptophan/24 h. Exact records were kept of uneaten food and the actual daily intake of each major food constituent calculated by difference. The same standardized diet was given for two months prior to the collection period in order to stabilize the tryptamine excretion in regard to tryptophan intake. No psychotropic medication had been given during 8 months previous to the experiment.

Also shown in Fig. 1 are the close relationships between the excretions of creatinine and tryptamine and the total daily urine volume. The association of urine volume with creatinine and tryptamine was observed only in this patient, while positive creatinine–tryptamine correlations were also found in other instances without large daily fluctuations in urine volume. Although it may appear that this patient was unable to concentrate urine, specific gravities ranging from 1·003 to 1·028 were found which varied inversely to urine volume. Repeated determinations of blood urea, non-protein nitrogen and serum creatinine were consistently found within normal limits. These data therefore rule out gross failures of kidney function. It may rather indicate that other humoral changes occur along with the variations of creatinine and tryptamine excretion to alter the daily urine volume.

The association of creatinine and tryptamine excretions was observed in this patient on another occasion of 16 consecutive days ($r = +0·64$) and twice in another patient for periods of 20 days each ($r = +0·56$ and $+0·33$, respectively). In addition, suggestive positive correlations were observed in a larger group of 9 patients on whom 5-day pooled urine collections were made over a 6-month period of continuously controlled tryptophan intake. For two other indole derivatives, total indolyl-3-acetic acid and 5-hydroxyindoleacetic acid, weaker but still positive correlations were obtained.

Since the classical studies by Folin[1,2] on creatinine excretion in man it has generally been accepted that the daily urinary excretion of creatinine in a given individual is quite constant and rather independent of variations of other physiological measures such as urine volume, exercise or diet[3]. Later reports, however, have indicated that considerable spontaneous variations in creatinine excretion are encountered in certain individuals[4,6]. Our findings demonstrate that the daily creatinine excretions need not remain constant but can fluctuate markedly in schizophrenic patients who are otherwise in good health and are receiving an adequate diet.

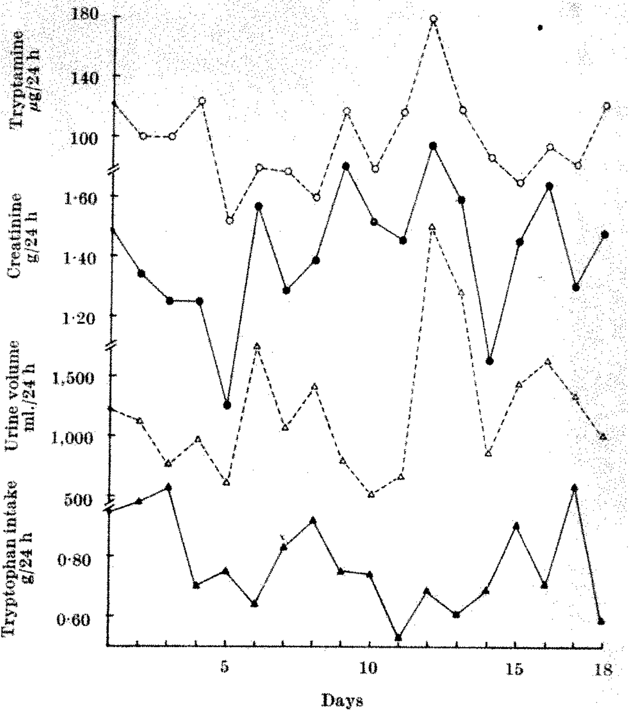

Fig. 1. Daily excretions of tryptamine, creatinine and urine volume in a schizophrenic patient for 18 consecutive days

Vestergaard and Leverett[5] have stressed the point that creatinine excretion over short periods of time should not be used to calculate excretion ratios for other urinary constituents. We should like to extend this to include the use of 24-h creatinine excretions in the case of schizophrenic patients. Numerous investigators have attempted to associate abnormalities of tryptophan metabolism with schizophrenia. Many of the reports are negative or conflicting. Some of the apparent discrepancies in the literature may be related to the fact that the creatinine excretion is usually assumed to be constant in this disease and biochemical findings are often reported as amount of substance excreted per mg or g of creatinine. Our results show that this assumption is not warranted in certain schizophrenic patients and that the excretion of tryptamine and possibly other indoles may be linked in some manner to the excretion of creatinine.

The association of tryptamine excretion with creatinine output is remarkable and may represent a metabolic finding of importance for this disease. This is indicated by the fact that the most marked daily fluctuations of creatinine and tryptamine occurred when the patient showed worsening of his psychotic symptoms, such as thought disturbances as well as delusional experiences accompanied by hyperactivity and hostility[7].

Table 1. Correlation (r) between the Daily Excretion of Tryptamine, Creatinine and Daily Tryptophan Intake

No. of daily collections	Correlation coefficient r * between urinary tryptamine † and creatinine ‡	p §	Correlation between urinary tryptamine and tryptophan intake ¶	p
18	+0·60	0·005	−0·32	n.s.

* r Correlation coefficient according to the formula by Pearson for a linear regression.

† Tryptamine was determined by the method of Sjoerdsma *et al.* (ref. 9).

‡ Creatinine was determined by the alkaline picrate method (ref. 10).

§ p is given for $t = \sqrt{\frac{r2\ (n-2).}{(1-r2)}}$

¶ Tryptophan was calculated on the basis of protein content of the food as total nitrogen.

These episodes were occasionally preceded by a reduced food intake accompanied by losses of weight. In this patient the increased creatinine output appears to be related to weight fluctuations brought about by self-imposed starvation. This observation contrasts with reports in the literature that creatinine excretion decreases during prolonged starvation in man and animals[6,8]. The possible implications of this complex clinical and biochemical pattern in relation to mechanism of periodic behavioural exacerbations in schizophrenic subjects has been discussed previously[7]. Complete details of the association of creatinine and tryptamine excretions will be reported elsewhere.

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PSYCHOLOGY

Effects of Different Terminal Sounds on Short-term Memory for Initial Consonant Sounds

IF more items are presented than are to be recalled, the additional items produce a large amount of 'stimulus interference' in the recall of the remaining items[1]. This is true under conditions where subjects have complete knowledge of the items that are not to be recalled, this knowledge being possessed by the subjects before, during, and after presentation of the list. Even though subjects know which items to ignore, they cannot ignore them completely, and therefore the extra presented items interfere with the memory trace for the items to be recalled.

The purpose of the present experiment is to investigate stimulus interference in short-term recall under conditions where the number of items presented is constant, but the items in some lists contain more phonemes or 'longer' phonemes than the items in other lists. As in the previous experiment, it is important to control for response interference by holding constant the amount to be recalled. In this experiment subjects are to recall the six initial consonant phonemes of a list of six artificial items, each item consisting of a consonant followed by a vowel (CV) or a consonant followed by a vowel and another consonant (CVC). Within any one list the terminal vowel or terminal vowel and consonant are the same for each item in the list. Some of the terminal vowels and consonants are 'longer' than others in temporal duration in ordinary English speech. The experiment described here is designed to determine: (1) whether items that consist of three phonemes (CVC items) produce more stimulus interference in the recall of the initial consonant of the items than items that consist of two phonemes (CV items); (2) whether temporally longer terminal phonemes produce more stimulus interference in the recall of initial consonants than shorter terminal phonemes.

Subjects listened to a 'ready' signal, followed after 1 sec by a list of six artificial items, followed immediately by recall of the six initial consonant phonemes. Correct recall consisted of filling-in six boxes with the correct consonants in the correct order. Presentation of the artificial items was at a rate of two items/sec, and 15 sec was provided for recall. There were six conditions, each with four sub-conditions. The six conditions are six different types of lists, each type of list being a list consisting of one type of item: (SV) consonant followed by a short vowel ('a' as in father, 'e' as in let, 'i' as in bit, or 'u' as in but), (LV) consonant followed by a long vowel ('ā' as in made, 'ē' as in eat, 'ī' as in bite, or 'ō' as in hope), (SV+k) consonant followed by a short vowel followed by the short consonant 'k', (LV + k) consonant followed by a long vowel followed by the short consonant 'k', (SV + z) consonant followed by short vowel followed by the long consonant 'z', (LV + z) consonant followed by long vowel followed by the long consonant 'z'. A typical list of items in condition (SV + k) was as follows: pak, dak, gak, vak, bak, kak. The four subconditions in each condition are the four possible vowels that can be used in each condition. The order of presenting the 6 × 4 = 24 conditions was random in blocks of 24, and there were 5 blocks in the experiment, making a total of 120 experimental lists. Each experimental list contained six different initial consonants chosen from the following set of 13 consonants: b, d, f, g, k, l, m, n, p, s, t, v, z. Scattered at random through the experimental lists were 15 lists with some initial consonants appearing in more than one item in the list: 5 with one item repeated once, 5 with two items repeated once, and 5 with three items repeated once. The purpose of this was to prevent any strategy based on a belief that no initial consonant was ever repeated. Subjects were 39 M.I.T. undergraduates taking psychology courses who participated in the experiment as a part of their course requirements.

As given by Fletcher[2] the duration of the different short and long vowels and consonants is as follows: a (0·306 sec), e (0·219 sec), i (0·211 sec), u (0·280 sec), ā (0·305 sec), ē (0·341 sec), ī (not given), ō (0·325 sec), k (0·075 sec), z (0·230 sec). The average duration for these short vowels is 0·254 sec and for the long vowels is 0·324 sec. Obviously, the absolute duration of each sound depends on the rate of talking; what is important for our purpose is the relative durations of the sounds.

Scoring each item in each list as one opportunity for error yielded the ordered recall error rates for each condition that are reported in Table 1. The results are quite clear. Neither terminal vowel duration nor terminal consonant duration had any effect on recall. Increasing the number of terminal phonemes from two to three by adding a single terminal consonant consistently increased the error rate for initial consonants. The error rate for each condition was computed for each subject, and differences between any two conditions were tested using the Wilcoxon Matched-Pairs Signed-Ranks test applied to the 39 difference scores. The error rate in condition SV was significantly less than that in SV + k ($P<0{\cdot}05$) and less than that in SV+z ($P<0{\cdot}02$). The error rate in condition LV was significantly less than that in LV+k ($P<0{\cdot}001$) and less than that in LV+z($P<0{\cdot}001$). The

Table 1. ERRORS IN ORDERED RECALL OF INITIAL CONSONANTS (%)

Type of terminal	Terminal phonemes			
Vowel (V)	V	V+k	V+z	Mean
Short (SV)	41·5	44·2	45·3	43·7
Long (LV)	39·2	47·5	45·3	44·0
Mean	40·3	45·8	45·3	43·8

Table 2. 'k' AND 'z' INTRUSIONS (% OF OPPORTUNITIES FOR ERROR)

Type of intrusion	Terminal phonemes					
	SV	LV	SV+k	LV+k	SV+z	LV+z
'k'	2·3	3·2	2·6	3·1	2·7	3·5
'z'	2·7	1·4	2·2	3·3	2·5	1·6

overall difference between conditions with and without a terminal consonant was significant at well beyond the 0·001 level.

One possible explanation of the foregoing result might be that in the lists with terminal consonants the terminal 'k' or 'z' keeps 'popping into mind' during recall of the initial consonants and greatly increases the frequency of 'k' or 'z' intrusions in 'k' or 'z' lists, respectively. Table 2 shows that this is definitely not the case. The tiny increase in the rate of 'k' and 'z' intrusions from SV and LV to SV+k, LV+k, SV+z, and LV+z amounts to about 0·8 per cent, whereas the total error rate increases by about 5·2 per cent. Furthermore, the increase in 'k' and 'z' intrusions is not specific to 'k' and 'z' conditions, respectively. There is no significant difference in the 'k' or the 'z' intrusion rate between 'k' and 'z' conditions. It is clear that the stimulus interference of the terminal consonant is not reflected primarily in intrusions of that consonant.

Even though subjects know they are to ignore the terminal consonant, they cannot do so completely. The terminal consonant may weaken the memory traces for the initial consonants, or it may establish its own memory trace which competes with the traces for the initial consonants at the time of recall, or both. It is not possible to decide between these possibilities at the present time. However, it is clear that it is the presence of an extra consonant phoneme, not its temporal duration, that produces the interference.

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Perception of Pattern and Colour in the Stabilized Retinal Image

OBSERVATIONS of coloured areas using the stabilized retinal image (an image which remains stationary on the retina despite eye movements) have produced reports of gradual desaturation of colours[1], or of colour fusion if bipartite coloured fields are used[2]. When black and white geometrical shapes are examined, a marked loss of contrast occurs, with ultimate failure of perception over parts or the whole of the visual field[3]. The examination of targets in which colour gradations and major pattern variations occur simultaneously has, however, not been reported; and it is not easy to predict the phenomena which might be expected to occur in these conditions.

Plates of the Ishihara test for colour blindness have been used as targets for this investigation. They were photographed as colour transparencies and viewed through two types of stabilizing apparatus; either that described by Pritchard[4], in which a miniature collimator is mounted directly on the contact lens, or through the equipment described by Clowes[5]. In this the target is viewed using a complex optical system, which, although cumbersome, permits rather more versatility in the manipulation of the target material.

In normal usage at the recommended viewing distance, Ishihara charts subtend a minimum angle of 6·5°; when viewed through the collimator they subtend 10° on a homogeneous buff background extending to 35°, and through the optical system they subtend 2° on a 10° background. In stabilized vision the targets were symmetrically disposed about the fovea and were in sharp focus. Ishihara charts provide only a crude test of colour vision, suitable for rapid screening of major defects; but they are ideally suited for the purposes of this experiment, which is to explore the gross changes of form and colour perception in stabilized vision.

Eight subjects, all of whom had previously observed black and white targets in stabilized vision, reported their subjective experiences when viewing the Ishihara charts. The following observations were made reliably and repeatedly by all subjects.

(1) In normal vision no abnormalities of colour perception were detected in any of the subjects.

(2) Fade-out of perception seemed less marked, and was certainly slower in occurring than with simple black and white material.

(3) The coloured numerals or convoluted lines desaturated and ultimately vanished as patterns, merging with a neutral-coloured background. The apparent colour of the residual image varied somewhat with the target used, and ranged from grey to dull brown or green.

(4) The background detail itself, that is, the series of tiny circles of which the target is composed, was highly stable and rarely faded out. Desaturation of colour was always more prevalent than loss of contour and the Ishihara plates often appeared as a set of uniformly bright monochromatic circles.

(5) Interruption of the light source for periods as short as 0·5 sec produced regeneration of the colours, though these quickly seemed to fade out again.

The following observations were made repeatedly by two subjects, who were the only ones to experience the experimental conditions described.

(6) Continuously flickered illumination in the frequency range 4–8 c/s (which has previously been shown to be beneficial in maintaining the visibility of a stabilized retinal image) did not prevent the colour desaturation effects described here except in the case of red lines on a grey background in which good visibility of the target and freedom from colour fusion were obtained over extended viewing periods.

(7) Side-to-side oscillation of the target through 2 min arc at frequencies in the range 4–8 c/s prevented colour fusion in targets having orange lines on a blue ground but otherwise produced no major effects.

The results of these experiments indicate that hue discrimination and form perception are mediated by separate mechanisms, for in these tests the two types of perception fail independently; disappearance of the form of the background circles was never observed to occur before colour desaturation had taken place, however.

It should be noted that failure of colour perception always produced characteristic effects which could not be confused with dichromatic vision, such as might be engendered by precise foveal fixation.

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An Auditory Illusion of Distance

We have previously described experiments showing that the apparent position of a sound source in space can be moved about the horizontal plane if sound is additionally fed by a single headphone to one ear[1]; the magnitude of this apparent movement depends largely on the frequency and intensity relations between the loudspeaker and headphone sounds. As we investigated this phenomenon, which we regard as an example of binaural interaction, we noticed that if two headphones were used instead of one a single sound image was perceived the apparent distance of which from the listener could be controlled by changing loudspeaker-to-headphone loudness relations. The work recorded here examines this illusion of distance more closely.

A diagram of the experimental situation is shown in Fig. 1. A listener, wearing headphones, sat in a semi-reclining position facing a loudspeaker 320 cm away at the end of a cloth-covered arc 26 cm wide. Four red lights were spaced along the arc at a distance of 320, 250, 150 and 50 cm from the listener's head. Ten msec bursts of band-pass filtered noise (1·5–6 kc/s) were delivered at a repetition rate of 6 per sec to both loudspeaker and headphones. The headphone stimulus was delayed electronically so that the speaker and headphone pulses would reach the listener simultaneously.

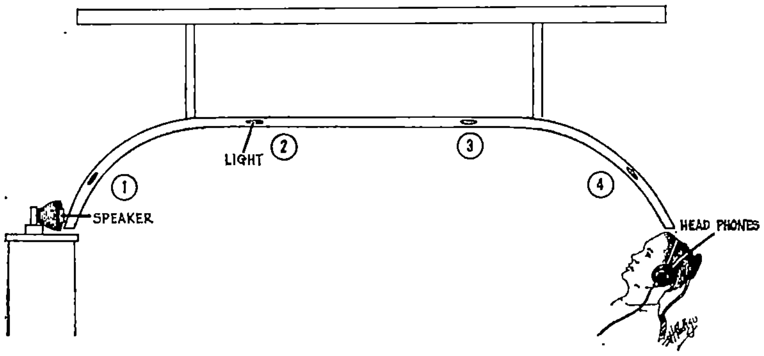

Fig. 1. Diagram of listening situation showing relation between loud-speaker, light positions Nos. 1–4 and listener

Six listeners participated. The tests were conducted in a sound-treated room the only illumination of which was that provided by the lights mounted on the arc. Headphones were placed on the listener and thresholds were then determined for the speaker and for the headphone stimuli. Next, with the speaker stimulus presented at 15, 20 or 25 db above threshold, the listener was instructed to adjust an attenuator controlling the headphone stimulus (2 db steps) until the perceived auditory image was coincident with a previously specified light. As soon as the listener had accomplished this, his attenuator reading was recorded. The listener was then asked to bring the sound image to coincide with a different light and the new attenuator reading was recorded. This scaling procedure was repeated six times, and median attenuator settings were calculated for each of the four lights at each of the three sensation-levels of the speaker stimulus.

To recapitulate: in this first or scaling session the listener was given control over one of the attenuators and was asked to adjust it so as to move the sound image to particular locations. In the next five sessions the listener had no control over the stimuli; the attenuator settings he had previously arrived at were reproduced and he was asked to identify the light positions from which the sounds appeared to be originating. As soon as he responded the 'correct light' was brightened briefly to tell him whether his choice was consistent with judgements made during his scaling session. Listeners were given six trials per session with each of the twelve possible combinations of speaker/headphone sensation-levels. Order of presentation was varied from day to day.

Table 1. MEDIAN PER CENT CORRECT IDENTIFICATIONS OF ILLUSORY AND REAL DISTANCES

	Light positions			
Distances	1	2	3	4
Illusory	84	77	85	97
Real	48	55	81	88

Table 1 shows the median per cent 'correct' identifications for all test sessions. No significant improvement in performance could be detected as testing progressed, suggesting that listeners were not learning to recognize apparent sound sources on the basis of any qualitative differences between sounds. Several persons who took no part in these formal tests nevertheless listened to the stimulus combinations; nearly all volunteered that the distance illusion was striking.

We were interested in finding out how well a listener would perform if the sound was actually emanating from one or the other light position; in other words, using 'real' rather than 'illusory' distance. Five additional sessions were conducted with a small loudspeaker placed next to each light. No headphones were worn on these occasions. Thresholds were taken for the loudspeaker at light position No. 1 and the auditory stimuli delivered by each of the speakers was set to 15, 20, or 25 db above this threshold. Median per cent 'correct' identifications for this condition are also shown in Table 1. A comparison of these figures with those obtained earlier clearly shows that over-all performances were superior when listeners were asked to judge illusory rather than real distances.

When other cues for distance are minimal, a listener judges the distance of a sound primarily by its loudness; that which is louder appears nearer[2]. Our listeners performed poorly in judging real distances presumably because there was an overlap of loudness between adjacent speakers and hence the loudness cues were ambiguous. There was also loudness overlap between many of the combination stimuli used in the illusory distance test; but listeners performed reliably and were able, in the majority of instances, to identify illusory distance correctly. In fact, the illusion of distance can be compelling even when the normal loudness cues are reversed ; thus we have observed that as the intensity of the loudspeaker is increased (increasing the total perceived loudness) a sound image previously located in the head can be moved away. These observations indicate that whatever the basis for this illusion of distance it certainly concerns more than loudness. In the experiments so far described the intensities at the two ears have been equal; however, if a right-left intensity differential is introduced and suitably varied, the apparent sound source in space now moves laterally.

In these types of listening situations, the two auditory inputs (loudspeaker and headphones) each acting alone produce an auditory image; one is externalized while the other appears to be in the head. When the two inputs are presented simultaneously only a single image is perceived. Our results suggest that the apparent location of this fused image is determined by the relative dominance of the two separate images.

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MATHEMATICS

Gravitational Collapse

In a recent letter[1] Prof. H. Bondi states that it is impossible to determine the number of particles contained in a static distribution of matter and radiation by means of an integral over that configuration. In so doing he denies to a local observer the capability of measuring the number of particles contained in a small but finite volume, and thereby denies the possibility of assigning a physically meaningful particle density at every point in the configuration.

If, on the other hand, one asserts that an observer can determine a local number density, it is easy to show that the total particle content of any configuration is a well-defined invariant quantity.

The infinitesimal integral:

$$\int_{\Delta} \sqrt{-g}\, \mathrm{d}x^1\, \mathrm{d}x^2\, \mathrm{d}x^3\, \mathrm{d}x^4$$

is a known invariant[2]. It is always possible to choose the co-ordinate system in such a way that $g_{\mu 4} = g_{4\mu} = \delta_{4\mu}$. Hence:

$$\int_{\Delta'} A^4 \sqrt{-g}\, \mathrm{d}x^1\, \mathrm{d}x^2\, \mathrm{d}x^3$$

where A^4 is the fourth component of an arbitrary vector, is also an invariant. In a local Cartesian frame of reference, $g_{11} = g_{22} = g_{33} = -1$, $g_{44} = 1$ and $\mathrm{d}x^1\, \mathrm{d}x^2\, \mathrm{d}x^3$ represents an infinitesimal proper volume element. If A^4 is chosen as the local proper particle density, q, then:

$$\mathrm{d}N = \int_{\Delta'} q\, \mathrm{d}x^1\, \mathrm{d}x^2\, \mathrm{d}x^3$$

represents the invariant number of particles contained in the chosen volume element. Transforming to a co-ordinate system commonly used for spherically symmetric systems:[3]

$$(A^4)' = \frac{\partial(x^4)'}{\partial x^{\nu}}\, A^{\nu} = \frac{\partial(x^4)'}{\partial x^4}\, A^4$$

$$= e^{-\nu/2}\, q$$

$$\sqrt{-g} = e^{\nu/2}\, e^{\lambda/2}\, r^2 \sin\theta$$

and, finally:

$$N = \int_{\text{entire system}} q\, e^{\lambda/2}\, r^2 \sin\theta\, \mathrm{d}\theta\, \mathrm{d}\varphi\, \mathrm{d}r$$

gives the total invariant particle content of the system.

In this same co-ordinate system:

$$M = \int_{\text{entire system}} \rho\, r^2\, \mathrm{d}r \sin\theta\, \mathrm{d}\theta\, \mathrm{d}\varphi$$

where ρ is the proper energy density, represents the total gravitational mass of the system. It is clear that N and M are linearly independent unless very stringent and non-physical limitations are placed on the relationship between the particle density, energy density, and pressure distributions.

The particular choice of q (as well as of ρ and pressure), which has physical meaning, must, of course, involve further considerations. If the static configurations are to represent stages in the evolution of a real body losing energy, then considerations of energy flow within the body place restrictions on the relation between the pressure and temperature distributions which, in addition to a physically reasonable choice of equation of state, serve to specify uniquely the relation between the ρ, q, and pressure distributions at different stages in the evolution of the system.

Bondi appears to confuse the meaning of indistinguishability owing perhaps to his failure to recognize the distinction between energy density and particle density. Two static configurations with identical energy density and pressure distributions may certainly contain different numbers of particles, as Bondi himself states. But if we can attribute different nucleonic content to the two configurations, we have clearly distinguished them from one another, despite the fact that they may possess the same gravitational mass (that is, total energy content).

In the example which Bondi cites, the two systems initially have total mass $M_1(0)$ and $M_2(0)$ and particle number $N_1(0)$ and $N_2(0)$. Let m_1 and m_2 represent the rest masses of particles contained in 1 and 2. Since, in the chosen initial states, energy density and (particle-mass × particle-density) are identical, $m_1 N_1(0) = M_1(0)$ and $m_2 N_2(0) = M_2(0)$. Bondi demands that, after a time t, $M_1(t) = M_2(t)$, $M_1(t) = M_1(0)$, and $M_2(t) < M_2(0)$. In so doing he has simply assigned different physical properties to the matter-radiation field in the two cases. His particular choice of the relationship between energy density, pressure and particle density is such that, since $M_2(0) > M_1(0)$, $N_2(t) m_2 = N_2(0) m_2 > N_1(t) m_1 = N_1(0) m_1$. This conclusion, to which Bondi also subscribes, does not demonstrate the impossibility of determining particle number from a relativistic integral over a static configuration; on the contrary, it is reached only by considerations, implicit or otherwise, based on the existence of such an integral.

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[3] Iben, I., *Astrophys. J.*, 138, 1090 (1963).

I am afraid Dr. Iben and I have been misunderstanding each other. In my communication I pointed out that an integral involving only the density and pressure of the material could not reveal the particle number. I correctly referred to a contrary statement by Eddington; but I mistakenly also referred to Dr. Iben's paper, which I had misunderstood. I now appreciate that no such error does in fact occur in his paper. On the other hand, Dr. Iben has misunderstood my communication. I stressed at the beginning that I was discussing integrals involving only the density and pressure of the material, and used the words 'relativistic integral' further on merely as a shorthand for this. In particular, I was thinking of Eddington's identification of the energy invariant as particle number density.

I trust that Dr. Iben agrees fully with me that no term or combination of terms of the energy tensor has any connexion with the particle number. The particle number may be put into the theory as is done in Dr. Iben's own work, but in no way can it be deduced from the metric.

H. BONDI

Department of Mathematics,
King's College
(University of London),
Strand, W.C.2.

I agree with Prof. Bondi that only energy density and pressure are included explicitly in the energy tensor. However, both energy density and pressure are related to particle density and any application of relativistic theory to a physically realistic description of a massive body in a highly condensed state requires a choice as to this relationship.

ICKO IBEN, JUN.

California Institute of Technology,
Pasadena.

FORTHCOMING EVENTS

Wednesday, September 16

Bone and Tooth Society (at the Royal National Orthopaedic Hospital, 34 Great Portland Street, London, W.1), at 4.30 p.m.—Annual General Meeting followed by Casual Communications.

Royal Aeronautical Society, Graduates' and Students' Section at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Film Show.

Thursday, September 17

Mineralogical Society (in the Geology Department of Bristol University, Queen's Building, University Walk, Bristol), at 2.30 p.m.—Scientific Papers.

Institution of Mining and Metallurgy (at the Geological Society' Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. A. W. Fletcher, Dr. D. S. Flett and Mr. J. C. Wilson: "Solvent Extraction of Ferric Iron by a Carboxylic Acid"; Mr. R. Healey and Mr. V. E. Clifton: "Some Recent Developments in the Construction of Vertical Zinc Retorts at Avonmouth".

Friday, September 18

Association of Clinical Biochemists, South Region (at the Middlesex Hospital Medical School, London, W.1), at 7 p.m.—Discussion Meeting on 'Hospital Laboratories in Denmark and Sweden". Speakers: Mr. P. M. G. Broughton, Dr. A. B. Anderson and Dr. Clifford Riley.

Television Society (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. E. J. Gargini: "Colour Television by Wire".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Senior Lecturer or Lecturer in Soil Science in the Department of Crop Husbandry and Horticulture, University of Ghana—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1 (September 15).

Assistant Lecturer or Demonstrator (with medical qualifications registrable in the United Kingdom) in Anatomy—The Registrar, The University, Manchester 13 (September 19).

Lecturer or Assistant Lecturer (preferably with an interest and research experience in electrical and magnetic ceramics) in Ceramics, in the Division of Ceramics, Houldsworth School of Applied Science—The Registrar, The University, Leeds, 2 (September 19).

Chair in Brewing and Applied Biochemistry; and Chair in Civil Engineering—The Principal, Heriot-Watt College, Edinburgh (September 21).

Research Assistant (honours graduate in zoology, biochemistry or genetics) in Genetics, to participate in investigations into the physiological genetics of melanotic tumours in the fruit-fly *Drosophila*—The Registrar, The University, Leeds 2 (September 21).

Research Assistant in the Department of Geography—The Registrar, The University, Leeds, 2 (September 21).

Senior Lecturer in Education; a Senior Lecturer in Physics; Lecturers (2) in Chemistry; a Lecturer in Education, a Lecturer in Electrical Engineering; a Lecturer in Geography; a Lecturer in Physics; a Lecturer in Psychology; and Lecturers (2) in Zoology at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 21).

Special Research Assistant to Professor M. M. Cole in the Department of Geography—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (September 21).

Chair of Mathematical Economics or Econometrics—The Registrar, The University of Warwick, Coventry (September 25).

Chair of Psychology in the Faculty of Science—The Registrar, University of Birmingham, Birmingham 15 (September 25).

Veterinary Surgeon (man or woman aged 35 or over, M.R.C.V.S., with considerable professional experience, including experience of care of laboratory animals) in London, for duties which include inspection of premises registered for experiments, and advising on applications for licences or certificates—The Civil Service Commission, Savile Row, London, W.1, quoting Ref. 5956/64 (September 25).

Lecturer or Assistant Lecturer (with a good honours degree in chemistry or pharmacy) in Pharmacy—The Registrar, The University, Manchester, 13 (September 26).

Reader in Microbial Genetics—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, 2 (September 26).

Research Fellow or Assistant in Psychology at Queen's College, Dundee, to work on a research project on the bisensory presentation of information—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (September 26).

Assistant Curator (with a degree and experience in medieval and later archæology, including excavation supervision) in Archaeology—The Director, City Museum, Bristol (September 28).

Scientific Officer (with a first- or good second-class honours degree in physics, or alternatively in engineering, and preferably some research experience in a branch of classical physics)—The Director, Agricultural Research Council Unit of Soil Physics, School of Agriculture, The University, Cambridge (September 28).

Pfizer Research Fellow for work in the Department of Pharmacy under the direction of Prof. J. B. Stenlake on the metabolism of drugs—The Registrar, The University of Strathclyde, George Street, Glasgow, C.1 (September 30).

Lecturer in Oral Pathology in the School of Dental Surgery—The Registrar, The University, Liverpool, quoting Ref. CV/261 (October 3).

Technical Officer (with a university degree or an equivalent technical qualification) in the Computing Laboratory, Department of Mathematics, Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (October 3).

Assistant Lecturer (with an honours degree in sociology) in Sociology at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 5).

Lecturer or Assistant Lecturer in the Biochemistry Department, University of the West Indies—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (October 5).

Assistant Lecturer in the Chemistry Department—The Secretary, Royal Free Hospital School of Medicine (University of London), Hunter Street, London, W.C.1 (October 9).

Chair of Electrical Engineering—The Secretary, The Queen's University, Belfast, Northern Ireland (October 10).

Lecturer (graduate in science or medicine) in Pharmacology at the University of Western Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 10).

Senior Lecturer or Lecturer (preferably with interest in morphology or physiology) in Botany at Ahmadu Bello University, Northern Nigeria—The Secretary Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 10).

Reader in Medical Physics at the Postgraduate Medical School of London—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 12).

Senior Lecturer in Mathematics at the University of Western Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 17).

Chair of Numerical Analysis—The Registrar, The University, Liverpool (October 30).

Laboratory Steward in the Department of Physics, The Victoria University of Wellington, New Zealand, to be responsible for the general care and efficient operation of the laboratories and equipment—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (November 6).

Adviser (honours graduate between 30 and 50, national of the United Kingdom or the Republic of Ireland, with experience in cocoa research, genetics, breeding and agronomy) on Cocoa Research, Colombia, to be responsible for cacao research with special regard to obtaining improved varieties or hybrids, and efficient methods of cultivation—Appointments Officer, Department of Technical Co-operation, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/43/02.

Agronomist (with a degree in agriculture, and preferably field experimental experience) in Swaziland, to conduct field experimentation into soil fertility and fertiliser problems—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC213/169/07.

Animal Husbandry Officer (national of the United Kingdom or the Republic of Ireland, with a degree in agriculture, and extensive experience in practical animal husbandry, preferably with tropical livestock) in Sarawak, to develop the livestock industry—The Appointments Officer, Department of Technical Co-operation, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/155/06.

Assistant Lecturer or Lecturer in Experimental Physics—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, marking envelope "Application".

Biochemist, Basic Grade (graduate in chemistry, biochemistry, chemistry with physiology or biology or Grad.R.I.C.) for the Biochemistry Section of the Pathology Department—The Secretary, Hope Hospital, Salford, 6.

Biochemist (with interest in the central nervous system) for work on biochemical and electron cytochemical aspects of demyelinating diseases—The Director, M.R.C. Research Group of Demyelinating Diseases, 13 Framlington Place, Newcastle upon Tyne, 2.

Chair of Plasma Physics—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, marking envelope "Confidential".

Field Assistant (with a degree in zoology, or zoology and botany, a strong interest in ecology and conservation, and fit enough for continuous field work)—The Warden, Slapton Ley Field Centre, Slapton, Kingsbridge, Devon.

Isotope Technician to join a team engaged in investigating and treating thyroid disorders with radioactive iodine—The Hospital Secretary, New End Hospital, London, N.W.3.

Junior Research Associate (with a good physics honours degree) in Physics, for research work on microwave resonance in semiconductors to be carried out under the direction of Dr. E. E. Schneider, Reader in Solid State Physics—The Registrar, The University, Newcastle upon Tyne, 1.

Laboratory Technician for work in the medical application of radioactive isotopes—The Secretary, Institute of Nuclear Medicine, Middlesex Hospital Medical School, London, W.1.

Mathematician to teach mathematics to all levels—The High Master, Manchester Grammar School, Manchester, 13.

Microbiologist (with at least a B.Sc. degree with microbiology as a major subject or the equivalent, and several years experience) in the Microbiological Section of the South African Bureau of Standards, Pretoria, to assist in an actively developing microbiological section, whose functions are concerned mainly with industrial microbiology, for example, testing of germicides, food products, antibiotic potencies, maintenance of stock cultures, conducting microbiological factory surveys of food processing plants, etc.—The Attaché (SABM), South African Embassy, South Africa House, Trafalgar Square, London, W.C.2.

Medical Laboratory Technician (A.I.M.L.T. or equivalent); and a Scientific Officer (with a science degree) for interesting work of a specialist nature—The Administrative Officer, North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middlesex.

Physicist, Basic Grade (with a good degree) for the Physics Laboratory—The Secretary, Lambeth Hospital, Brook Drive, London, S.E.11.

Reader (preferably with a higher degree and actively engaged in research in fluid mechanics and thermodynamics) in the Department of Aeronautical and Mechanical Engineering—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST 64/90.

Research Assistant (with a good honours degree or diploma in archæology) in Archæology at the University of Ibadan, Nigeria, to assist the Research Prof. of Archæology—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1.

Research Assistant for an investigation involving histochemistry and tissue culture—The Secretary, Pathology Department, St. George's Hospital Medical School, London, S.W.1.

Research Assistants (2) (science graduates) in the Department of Histology and Electron Microscopy—The School Secretary, Royal Dental Hospital of London, School of Dental Surgery, Leicester Square, London, W.C.2.

Research Student for the development of new techniques in stress determination by use of brittle lacquer and strain gauging—Prof. W. A. Tuplin, Applied Mechanics Department, The University, Sheffield.

Research Students in Organic and Organometallic Chemistry—The Professor of Chemistry, University of Sussex, Brighton, Sussex.

Scientific Officer (young graduate physicist or physical chemist, preferably with two or three years research experience) in the Physics Laboratory, for work concerned with the application of physical measurements to baking problems—The Secretary, British Baking Industries Research Association, Baking Research Station, Chorleywood, Rickmansworth, Hertfordshire.

Senior Physicist (Senior Grade); and an Assistant Physicist (Basic Grade) in the County Medical Physics Department, St. George's Hospital, Lincoln—The Group Secretary, Lincoln No. 1 Hospital Management Committee, County Hospital, Sewell Road, Lincoln.

Senior Physicist to be in charge of a group concerned with the application of electronics in medicine—The Chief Physicist, Regional Medical Physics Department, Sheffield Regional Hospital Board, 21 Claremont Crescent, Sheffield, 10.

Senior Technician I (with experience in biochemistry) to work under the direction of the Biochemist—The Consultant Pathologist, Hereford County Hospital, Hereford.

Senior Technician (with good general histological experience and appropriate qualifications) to take charge of a histological research laboratory —Dr. W. P. Dallas Ross, The Medical School, The University, Birmingham, 15.

Technician for clinical research in the Respiratory Function Laboratory —The Assistant Secretary, University College Hospital, Gower Street, London, W.C.1.

Veterinary Officers (male, M.R.C.V.S. or equivalent) in Northern Rhodesia, for general veterinary work, including control and prevention of livestock disease, supervision of vaccination campaigns and mass treatments —The Appointments Officer, Department of Technical Co-operation, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 217/132/02.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 741, Vol. 247 (16 July 1964): The Middle Pleistocene of North Birmingham. By M. R. Kelly. Pp. 533–592 + plate 9. (London: The Royal Society, 1964.) 24*s.*; 3.60 dollars. [148

Department of Scientific and Industrial Research: National Chemical Laboratory. The Chemistry of Complex Cyanides: a Literature Survey. Pp. vi + 93. (London: H.M. Stationery Office, 1964.) 27*s.* 6*d.* net. [148

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County Council of the West Riding of Yorkshire: Education Committee. Don Valley Institute of Further Education. Adwick-le-Street Centre—Prospectus 1964–65. Pp. 12. Armthorpe, Rossington and Tickhill Centres—Prospectus 1964–65. Pp. 14. Bentley and Askern Centre—Prospectus 1964–65. Pp. 15. Edlington and Sprotborough Centres—Prospectus 1964–65. Pp. 13. (Doncaster: Don Valley Institute of Further Education, 1964.) [148

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The Empire Cotton Growing Corporation. Progress Reports from Experiment Stations, Season 1962–63. Tanganyika Coastal Regions. Pp. 12. 2*s.* 6*d.* Tanganyika Lake Regions. Pp. 26. 2*s.* 6*d.* Northern Nigeria. Pp. 22. 2*s.* 6*d.* Uganda. Pp. 48. 2*s.* 6*d.* Western Nigeria. Pp. 10. 2*s.* 6*d.* (London: The Empire Cotton Growing Corporation, 1964.) [148

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Mauritius Sugar Industry Research Institute. Annual Report 1963. Pp. 153 + xxxii. (Reduit: Mauritius Sugar Industry Research Institute, 1964.) [147

The Theory of Spherically Symmetric Space-Times. By Hyoitiro Takeno. (Scientific Reports of the Research Institute for Theoretical Physics, Hiroshima University, No. 3.) Pp. viii + 287. (Takehara, Hiroshima-ken: The University, 1963.) [147

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Records of the Dominion Museum, Wellington. Vol. 4, No. 21 (26 March, 1964): An Occurrence of the Swimming Crab, *Portunus pelagicus* in New Zealand. By R. K. Dell. Pp. 303–304. Vol. 4, No. 22 (26 March, 1964): Notes on the Species of Lepidoptera taken by Light-Trapping at Wellington between November, 1962, and November 1963. By D. E. Gaskin. Pp. 305–309. Vol. 4, No. 23 (26 March, 1964): The Female Reproductive Organs of Layard's Beaked-Whale *Mesoplodon layardi* (Gray). By Charles McCann. Pp. 311–316. (Wellington, N.Z.: Dominion Museum, 1964.) [147

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Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 63–35: The Geology and Ceramic Properties of Shale from Bacon Point, Prince Edward Island. By V. K. Prest and J. G. Brady. Pp. 20. 75 cents. Paper 64–31: Anomalous Molybdenum in Stream Sediments and Soils in the Vicinity of the Nicholas Denys Granite, Bathurst Area, New Brunswick. By M. Tauchid. Pp. 4. 35 cents. (Ottawa: Queen's Printer, 1964.) [147

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BRITAIN'S CONTRIBUTION TO EDUCATION OVERSEAS

FOLLOWING the memorandum relating to the Commonwealth Education Conference meeting at Ottawa during August–September, issued in June by the Council for Education in the Commonwealth, Political and Economic Planning has published a valuable broadsheet, *Teachers for the Commonwealth—the British Contribution**. That contribution is appraised and criticized in the light of the wider context of education in the developing countries generally. Written by M. Elphick and R. Manley, the Broadsheet, like Mr. Guy Hunter's *Education for a Developing Region* (see *Nature*, 203, 677 ; 1964), centres largely on a study of East African problems. In this case, however, the research on which it is based was concerned with East African students in Britain. Both studies were assisted by grants from the Ford Foundation, and, like Mr. Hunter in his introduction and that part of the book which discusses policy issues, the Broadsheet illumines the way in which Commonwealth problems are related to wider issues and emphasizes the real dangers and difficulties which confront African Governments to-day in dealing with educational issues.

It is somewhat curious, therefore, that neither Mr. Hunter nor the Broadsheet refers to Dr. S. Dedijer's percipient article "Underdeveloped Science in Underdeveloped Countries", published in *Minerva* last autumn (see *Nature*, 201, 1153 ; 1964). Both seek, within the specific but scarcely more limited field of education, to stress the basis of education policy, the dangers which the Governments of developing countries should seek to avoid, and the points to which countries providing educational assistance should give particular care and attention. The memorandum from the Council for Education in the Commonwealth was particularly concerned that full use should be made of the opportunity afforded by the Ottawa Conference and that principles underlying the existing schemes for Commonwealth exchanges of teachers and students should be viewed in their wider context and also in relation to such questions as: (1) whether the training given in Britain and other advanced countries actually constitutes a serious drain on the developing countries because their scholars never return; (2) whether their own universities and postgraduate schools are losing their best talent; (3) whether the courses for overseas students are designed to make the maximum contribution to the economic and social development of these countries. The form in which educational aid can most usefully be given calls for consideration, as well as the manner in which liaison should be achieved between Commonwealth programmes and those of international organizations, like Unesco, which are attempting to co-ordinate educational development in the developing countries as a whole.

The Commonwealth Prime Ministers' Meeting in July took note of the scope which exists for co-operation between the Governments and peoples of the Commonwealth in social as well as economic development. It expressed warm wishes for the success of the Ottawa Conference and made known its appreciation of the British Government's offer to increase to an average of £5 million a year, over the 5 years starting in 1955-56, the capital assistance which it already provides for higher education in developing Commonwealth countries. However, the communiqué issued by the Meeting on July 15 did not reflect the imagination for which the Council for Education in the Commonwealth is looking, nor did the debate on the communiqué in the House of Lords on July 29—though the significance of Voluntary Service Overseas was recognized by the Bishop of Chester and the Duke of Devonshire. In all fairness, it should be noted that the communiqué refers to the Prime Ministers' belief that it might be valuable to supplement the existing arrangements for promoting the study of administration and development planning throughout the Commonwealth. It should also be recorded that the Prime Ministers considered that there might be advantages in making arrangements which could include the formation of a new institute to provide facilities for specialized training and research for senior administrators concerned with development problems in relation to the needs of new countries. Moreover, they agreed that arrangements for additional training should include the strengthening of existing institutions. The context of this section of the communiqué indicates that to some extent the Prime Ministers were aware of the implications of Dr. Dedijer's argument about the planning and execution of development projects. The reference to the desirability of establishing a Commonwealth Foundation to increase interchange between Commonwealth organizations in professional fields certainly shows some imagination, but it may well require more generous contributions from Commonwealth Governments than is visualized in the communiqué, and support from both public and private quarters may also be desirable.

At Ottawa this year the agenda included three new items for discussion : adult illiteracy ; mass media ; and co-operative arrangements for the planning and development of educational institutes. These were welcomed by the Council for Education in the Commonwealth, though its memorandum, in the main, is limited to reviewing the existing schemes. It urges the need for some shift in emphasis in co-operation towards building up institutions in the developing countries of the Commonwealth. The representations of the Council in this memorandum generally follow the pattern of the report on the second Commonwealth Education Conference at Dublin. The Council urges that under the Commonwealth Scholarship and Fellowship Plan there should be a substantial programme of new awards, particularly in professional and technical subjects, and emphasizes the importance of more students from Britain and the other advanced countries taking up the scholarships now being offered in the developing countries. It is seriously concerned over the training of teachers. Because only about 3 per cent of overseas students in Britain are training to be teachers, it is also concerned to ensure that the teachers when trained return home to serve as teachers in their own countries. The justification for the whole Commonwealth bursary scheme would largely disappear if a high proportion of those being trained as teachers took up other jobs on their return home. The Council also suggests that the Commonwealth Education Liaison Committee should supply an annual report on training awards similar to that provided by the Commonwealth Scholarship and Fellowship Plan, and it recommends co-operative Commonwealth planning for developing teacher training institutions in the developing countries, especially in finding staff.

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* *Planning*, 30, No. 482 (August 17, 1964). *Teachers for the Commonwealth—the British Contribution.* By Michael Elphick and Roy Manley. Pp. 243–282. (London: Political and Economic Planning, 1964.) 5s.

Another important recommendation is that the Conference should, in future, re-assess the long-term needs of the developing countries and propose plans for satisfying these needs. It is in this connexion that the memorandum refers to Unesco projects such as the Addis Ababa Plan, which revealed future needs far beyond those discussed at the first two Commonwealth Education Conferences. Only in such a context is it reasonable to expect that recruitment difficulties will be minimized, if not eliminated, and the foundations laid for a career service mutually satisfactory to the teacher and the developing country. It would also help to disclose the attitude of the developing countries to any fresh proposals for the development of a non-Commonwealth career service as visualized by Political and Economic Planning. The memorandum also recommends that the Conference should consider how the establishment of regional English language centres, as recommended at Delhi, could be accelerated, and the Council is even more concerned about the poor response to Britain's offer of bursaries for overseas students training to become technical teachers. This, and difficulties in the way of obtaining industrial experience, call for investigation both by the Conference and by the British Government.

Other recommendations addressed to the Ottawa Conference are concerned with the place of Governments and voluntary bodies in social education and the place of social education in Commonwealth co-operation. The British Government is asked to consider, in consultation with British publishers, how best to help establish indigenous publishers, printers and booksellers in the developing Commonwealth countries where they do not already exist. It is also asked to look into ways of giving financial assistance to voluntary organizations in Britain which help with training programmes for Commonwealth countries in university education. Clearly, too, the Commonwealth Education Liaison Committee can scarcely be expected to undertake the new duties envisaged in the memorandum, or even to implement really effectively those placed on it by the Delhi Conference, unless it is more adequately staffed and financed, with wider terms of reference.

This is recognized by the Council for Education in the Commonwealth, and the appropriate action recommended in its memorandum is in line with the concern of the Prime Ministers' Conference to strengthen a Commonwealth Secretariat, and with the criticism that the complexity and diversity of Britain's present administrative structure is a hindrance to educational co-operation which is expressed in the Broadsheet from Political and Economic Planning. Equally in line with the findings in that Broadsheet is the conclusion, in the Council's memorandum, that the main gap in Britain's present financial arrangements is the lack of regular provision to help independent Commonwealth countries in building up their institutions. Moreover, the funds devoted specifically to schemes of Commonwealth educational co-operation at Oxford and Delhi have not been large, and a substantial part of Britain's own allocation of £6 million is unlikely to have been spent by 1965; actual money transfers for educational purposes have not been made under the Commonwealth Educational Co-operation arrangements.

The Broadsheet is concerned essentially with only one aspect of educational co-operation—that of the British contribution to training teachers. However, it derives much of its value from the way in which Commonwealth co-operation in this field is seen in the light of the three massive Unesco plans for educational development, in Asia (the Karachi Plan of 1960), in Africa (the Addis Ababa Plan of 1961, followed by the Tananarive Conference in 1962, dealing with higher education, for which Sir Alexander Carr-Saunders directed a study *Staffing African Universities* †) and in South America (the Santiago Plan of 1962). All three of these plans stress the necessity for co-ordination and planning, both in general educational development and in the provision and acceptance of external assistance. A meeting in Tokyo in 1962, following up the Karachi Conference, also asked for a substantial increase in external assistance. A White Paper on the progress of the Department of Technical Co-operation, in 1962, stressed the importance of international co-ordination, and the Chairman of the Development Assistance Committee of the Organization for Economic Co-operation and Development emphasized the increasing complexity of the flow of assistance to developing countries.

Britain's contribution to educational assistance has recently been described independently in a survey by Peter Williams for the Overseas Development Institute (see *Nature*, 203, 439 ; 1964) and it is unnecessary here to recapitulate the detail, but Mr. Williams's reflexions anticipate some of the comments and conclusions of the Broadsheet. Although developing countries all over the world are gravely short of teachers, the Broadsheet recognizes that it is logical that Britain's help should go mainly to the Commonwealth, within the framework of a wider international effort. This is especially so since the Commonwealth has a special place in Britain's educational technical assistance and the member countries share a common administrative background and educational history and are in a particularly favourable position to help each other. It is recognized, too, that Britain's administrative arrangements in this field are necessarily affected by her present educational structure, and the consequent voluntary nature of the procedure in recruiting teachers with a constant emphasis on short-term secondments. Further, these circumstances have led to grave problems in the receiving countries and in the recruiting agencies. These problems, impeding harmonious and efficient co-operation, have lowered Britain's ability to supply enough teachers to meet the demand.

The Broadsheet makes three main criticisms of Britain's contribution: it is *ad hoc* and short-term in nature; attempts at long-term planning seem to have been neglected, and executive procedures in recruitment need simplifying. Another impediment lies in the belief that the future demand is unlikely to be sustained, and a third is the shortage of teachers at all levels in Britain and the consequent reluctance of universities, and of local education authorities, which have a statutory obligation to provide adequate educational services in their areas, to make matters worse for themselves by sending their teachers overseas.

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† *Staffing African Universities*. By Sir Alexander Carr-Saunders. Pp. 26. (London: The Overseas Development Institute, 160 Piccadilly, W.1, 1963.) 2*s*. 6*d*. See also *Nature*, 198, 1024 (1963).

An obvious first step, as the Council for Education in the Commonwealth recognizes, is to obtain more accurate estimates of future needs for teachers. Countries such as Nigeria and Tanganyika are already beginning to attempt such forecasts, and Britain could help by building up her technical and advisory staff in the overseas High Commissions and by a greater emphasis on detailed planning on the spot. To meet requirements over a long period, however, calls for an all-out planned effort by British agencies and for co-ordination by the Government, whether through the present Department of Technical Co-operation and the Foreign, Colonial and Commonwealth Relations Offices, or through a Ministry of Overseas Development, as envisaged by the Labour Party. In any event, the Government must revise its entire approach to technical assistance in education, and must recognize that long-term demands, as well as more immediate needs, have to be met.

The Broadsheet stresses the importance of securing the closer co-operation of the various bodies interested in this field. It recommends that the Commonwealth Education Liaison Committee and its Unit should be strengthened and its authority increased as an instrument for channelling education assistance to Commonwealth countries in collaboration with international agencies and with other donor countries. Beyond this the Broadsheet criticizes the Department of Technical Co-operation for rejecting the recommendation of the Select Committee on Estimates for the establishment of a Commonwealth Advisory and Technical Service, based on a pool of officers in the permanent employ of Britain and seconded to overseas Governments. On the contrary, it finds powerful arguments for a small Commonwealth Education Service, which the authors of the Broadsheet believe would attract teachers of high calibre. The demand would guarantee employment for its members in a variety of posts for a long time to come, and the periods spent in Britain between postings would provide opportunities for professional refreshment through secondment to local education authorities, schools, colleges and universities. The Service could have its own internal structure and its members could be assured of increasing emoluments, with prospect of eventual absorption into the administrative and educational offices of the Ministry or into Britain's teaching service. The Broadsheet questions whether a supply of teachers from Britain can be guaranteed, unless there is a regular service from which teachers can be posted, and it recommends that the Government should urgently consider forming such a Commonwealth Education Service. In the meantime, the Government should materially encourage education authorities at all levels to be fully aware of the part which they can play in Britain's contribution to Commonwealth education.

Mr. Williams's survey of educational assistance for the Overseas Development Institute convinced him also that Britain's administrative arrangements were too complicated and that her aid was not always deployed to the best advantage. While specifically recommending the setting up of a Commonwealth Educational Service, he does not suggest that all decisions and making of policy could be concentrated in a single functional department. He, too, emphasizes the need for fresh and imaginative thinking on administration and finance by the Government—this should not be a matter of party politics. This is also underlined by Mr. Guy Hunter, who is at some pains to stress not merely the opportunities of educational aid and the vital importance for Britain herself that she should take a full part in this work, but also the way her capacity to participate is affected by the structure and character of her own educational institutions. His preoccupation with East Africa does not lead him to overlook the needs of Africa and Asia generally.

All these appraisals of Britain's effort in educational assistance and of the opportunities which confronted the Ottawa Conference are fair and well balanced, nor do they forget that education is only one aspect of technical assistance, though perhaps the most vital. Nevertheless, those other aspects sometimes require special consideration in the context of education, and alone among the surveys mentioned here Sir Alexander Carr-Saunders's paper touches on the question of research. This is a factor which can be as important in attracting university teachers from Britain to teach in overseas institutions as promoting the development of flourishing academic institutions in the developing countries. Here Britain has much to give and there has so far been lamentably little evidence that the British Government is alive to the opportunities or the importance of the issues. The decision to dissolve the Overseas Research Council is only the latest evidence of an indifference that verges on folly. It would seem probable that the Government's attitude is responsible for so few scholarships offered in developing countries being taken up at present from Britain. Moreover, this accusation could well be levelled at other developed Commonwealth countries. No Commonwealth Educational Service which covers university teachers should be allowed to neglect the relation between teaching and research. It is to be hoped that some searching enquiries will be brought to bear in the near future on research opportunities overseas, especially on the functioning of the Commonwealth Scholarship and Fellowship Plan and the supply of teachers overseas.

FUEL CELLS

Fuel Cells

Edited by Will Mitchell, jun. (Chemical Technology: a Series of Monographs, Vol. 1.) Pp. xv + 442. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 107*s.* 6*d.*

For more than a hundred years a practical solution to the problem of obtaining electrical energy directly from oxidizable fuels has been the subject of many enquiries. These have, of recent years, become more active under the stimulus of various national interests, and the problems which have to be solved before a satisfactory economic 'fuel' cell can be constructed have in consequence become more clearly defined.

It is fitting that the important subject should be dealt with in the first volume of a new series of monographs on *Chemical Technology*, and Mr. Will Mitchell, jun., is to be congratulated on his success in collecting together the views and work of no less than fifteen of the more active workers in this field, nearly all being citizens of the United States. There is included, however, a good deal of the work of the committee on fuel cells of the English Electrical Research Association, for the investigations of this committee included not only the hydrogen oxygen cell which culminated in the development of porous electrodes and the Bacon cell, but also the fused salt hydrocarbon air cell made operative at Sondes Place by the utilization of effective silver zinc electrodes as well as suitable membranes and eutectic salt mixtures. In addition, less successful cells were developed on the utilization of Nernst type solid electrolytes at Birmingham and oxy-redox cells, some of which showed promise, in London.

While the electronic mechanism of a hydrogen electrode is now well understood, the mechanism of ensuring a high rate of transfer of gaseous hydrogen to the chemisorbed state at the catalytic electrode electrolyte interface so as to ensure a high current density is not readily solved while the oxygen electrode mechanism is still a matter of controversy. The relatively high cost of hydrogen renders the hydrocarbon and particularly the carbon monoxide cell especially attractive, and it may be that a technical battery along these lines may be the ultimate solution.

The book contains a wealth of information which will save future investigators much labour. It suffers, one supposes, unavoidably by the fact that as each chapter is written by a different author, there is a great deal of repetition both in historical as well as theoretical material. This is compensated for in part at least by the somewhat different points of view adopted. It is said that an ill wind blows nobody any good, but the interest in the fuel cell cannot fail to stimulate electrochemists who have at their disposal readily available supplies of free radicals generated at will at the electrodes to venture more daringly into the field of preparative organic chemistry.

ERIC K. RIDEAL

SYMPOSIUM ON PERSONNEL DOSIMETRY

Personnel Dosimetry Techniques for External Radiation Their Application in Nuclear Installations. (Proceedings of the Madrid Symposium, 1–5 April, 1963.) Pp. 510. (Paris: Organization for Economic Co-operation and Development, European Nuclear Energy Agency; London: H.M. Stationery Office, 1963.) 32 F.; 47*s.* 6*d.*; 8 dollars.

THIS record of the symposium on "Personnel Dosimetry Techniques for External Radiation" contains 28 papers, 20 in English and 8 in French with abstracts in both languages, presented at 7 scientific sessions. The symposium was introduced by Dr. A. S. McLean, of the U.K. Atomic Energy Authority, with an address entitled "Perspectives in Radiation Dosimetry".

The first scientific session, with Prof. R. M. Sievert (Sweden) as chairman, dealt with protection norms and photographic emulsions. H. Jammet (France) compared the biological aspects of radiation protection with health requirements for protection against occupational diseases. L. S. Taylor (United States) considered social and legal problems in applying norms and the inflexibility of numerical values in legally binding codes. Margarete Ehrlich (United States) spoke on physical considerations of film dosimetry including side effects, and evaluation procedures.

Film dosimetry was continued in the second session, under the chairmanship of Dr. A. S. McLean. F. Duhamel and G. Soudain (France) reviewed the present-day practice of the Commissariat à l'Energie Atomique. Radiation workers in Britain will be particularly interested in the description, by M. J. Heard and B. E. Jones (United Kingdom), of the new film holder with six filtered and one unfiltered areas developed by the Atomic Energy Authority and the Radiological Protection Service. The third paper, which is not listed in the table of contents, was by H. Dresel (Germany) and described the universal film holder for neutrons and mixed γ-, β-, and X-rays designed at the Radiologisches Institut der Universität, Freiburg i. Br. The session concluded with an interesting paper on biological methods in personnel dosimetry by Prof. E. H. Graul (Germany). Electronic instruments have been used for the counting and size measurement of formed blood elements before and after ultrasonic resistance tests and for rapid resistance measurements on erythrocytes and leucocytes. Results obtained after various radiation exposures were presented.

The third session, under the chairmanship of Dr. C. Polvani (Italy), dealt with physical methods of dosimetry and dosimetry of neutrons. W. Hanle and A. Scharmann (Germany) described solid-state dosimeters. Prof. R. M. Sievert emphasized the importance of dose-rate in addition to integrated dose and gave details of personal dose-rate meters. J. A. Dennis (United Kingdom), and A. H. W. Aten, jun., G. Samson, G. A. Brinkman, and J. W. Dalenberg (The Netherlands) presented papers on activation techniques for neutron dosimeters, considering in particular the use of phosphorus-31, sulphur-32, gold-197 and indium-115.

Neutron dosimetry was continued in Session 4, under the chairmanship of Prof. E. H. Graul, when three papers were read on recoil proton emulsions. G. Portal (France) discussed the problems of rapid analysis in mass employment of track films. Absolute spectrometry was described by P. Amadesi, A. Cavallini and O. Rimandi (Italy), and V. Mejdahl and Miss C. R. McCarthy (Denmark) reported on the use of induced fading to reduce the γ-ray blackening without seriously affecting the density of the proton tracks. Two papers followed on dosimetry of very high-energy radiations with F. P. Cowan (United States) reporting on the experience at the Brookhaven National Laboratory and J. Baarli and J. Dutrannois on that at CERN.

Session 5, with Dr. L. S. Taylor (United States) as chairman, was devoted to new developments. D. H. Peirson (United Kingdom) described the semi-automatic densitometer and track-counting equipment at Harwell. The densitometer gives an immediate print-out of the equivalent γ-ray dose, and provides a punched tape for subsequent automatic computation of the true dose. The track counter has been developed with glass-backed emulsions and detects track-lengths down to 5 μm, which is equivalent to a neutron energy of 0·5 MeV.

J. H. Schulman, F. H. Attix, E. J. West and R. J. Ginther (United States) dealt with thermo-luminescent dosimeters as attractive replacements for film badges. They are linear in response from 10^{-3} to 10^5 rads, dose-rate independent from 10 mr./min to at least 7,000 r./min, and, with appropriate filtration, independent of γ-ray energy from 40 keV to more than 1 MeV. M. Ladu, M. Pelliccioni, and E. Rotondi (Italy) then described a γ-insensitive fast neutron detector with good angular response and the ability to measure 1/10 m.p.l. G. Cowper (Canadian not American, p. 364) reviewed advances in dosimetry at Chalk River. The session concluded with remarks (not listed in the table of contents and with 'mrad' instead of 'Mrad' in the last equation, p. 367) by W. Minder (Switzerland) on chemical dosimeters which are not yet sufficiently sensitive for personnel dosimetry.

In Session 6, under the chairmanship of Prof. L. Bugnard (France), H. Joffre (France) and K. Becker (Germany) spoke on some of the problems, capabilities, and limitations of methods of personnel dosimetry. A number of spelling and other mistakes appear in the latter paper. N. Adams (United Kingdom) then presented an important paper (with an error in the ordinate scale of Fig. 1) directly relating absorbed dose in an organ to the apparent exposure dose given by the quick assessment of a film badge. A. Lafontaine (Belgium) made a plea for an interpretation of the biological and medical significance to accompany each statement of the physical dose.

The final session of the symposium, under the chairmanship of Dr. S. Halter (Belgium), heard four reviews of dosimetry practices of the national atomic energy organizations of the United States (D. M. Ross), Britain (W. A. Langmead), France (P. Candes, J. M. Lavie, and M. Bricka), and Spain (E. R. Rodriguez and R. G. del Campo).

The book is a useful collection of papers and its value is increased by the adequate reporting of the discussion which followed each presentation. D. Hughes

PROGRESS IN OCEANOGRAPHY

Progress in Oceanography
Vol. 1. Edited by Dr. M. Sears. Pp. vii+383. (Oxford, London, New York and Paris: Pergamon Press, 1963.) 100*s*.

PROGRESS in Oceanography is intended to be the first of an annual series of volumes with which it is hoped to keep oceanographers, and others interested in the sea, up to date with the rapidly expanding field of oceanography. The approach of the publishers is two-fold; to present reviews of the present status of currently important fundamental aspects of the subject, and to provide a medium for the publication of papers on original research of wide interest, particularly those which are longer than those generally published in scientific journals. In addition, it is hoped to publish preliminary accounts of work in which new methods or approaches are used. Chapters lying within each of these headings are included in the present volume.

In the first chapter, E. Seibold describes recent work carried out at the University of Kiel on the transport of near-shore sand. A well-documented review of the methods which have been used in the investigation of this problem is presented. An outline is also given of the procedures developed at Kiel for the study of sand transport; these include the examination of the structures and internal structures of the sediments and their petrology. The results obtained by the application of these new techniques to the Baltic and North Seas will be of interest to both geologists and those engaged in coastal engineering.

The longest chapter in the book (124 pages) is essentially the Ph.D. thesis presented by D. C. Blanchard at the Massachusetts Institute of Technology. It is devoted to a rather lengthy and highly speculative quantitative study of the electric charge separating at the surface of the sea, and seems out of place in a book of this type.

The total suspended organic matter in the upper layers of the sea (in terms of carbon) appears to average about one-tenth of the total dissolved organic carbon. Zooplankton and fish represent only a small proportion of it (c. 2 per cent and 0·2 per cent respectively). The major part of the suspended matter (80 per cent) consists of detritus mainly derived from dead phytoplankton, together with lesser amounts of fæcal pellets and organic material derived from the land. Phytoplankton constitutes the remaining 20 per cent, amounting to an annual production of organic carbon in the hydrosphere of c. 3×10^{10} tons. The importance of the role of the suspended matter in the sea in the marine food chain is only now being appreciated. Since much of the recent work on this subject has been carried out at the Nanaimo Biological Station of the Fisheries Research Bureau of Canada, the excellent review of this topic by T. R. Parsons is particularly timely. A very useful and thorough account is presented of the determination and chemical composition of both phytoplankton and detritus. In a further section the geographical distribution of these types of particulate matter and of bacteria, fungi and yeasts is described. The chapter ends with a summary of the literature on the utilization of all these organic materials as sources of food for secondary producers. Our knowledge of this important topic is at present very fragmentary but is rapidly expanding, and it is to be hoped that the publishers will persuade Dr. Parsons to review the subject again in a few years time.

In a chapter entitled "The Salinity Problem", Cox has given an authoritative and critical, but rather irritatingly chatty, account of methods for the determination of chlorinity and salinity which will be of interest to all oceanographers. The section on conductivity salinometers is of particular value as it embodies an account of the author's personal experience with many types of these instruments, and gives a useful assessment of their precision.

The final chapter of the book is by F. C. Fuglister; it describes a comprehensive investigation of the Gulf Stream system undertaken in 1960 by the Woods Hole Oceanographic Institution. The larger part of the chapter is taken up with diagrams of sections and with tables of oceanographic station data which seem rather out of place in a book of this type.

The book is attractively produced and stoutly bound and should be in the library of every oceanographic institution. However, its contents are so varied in subject that it is doubtful whether any individual would wish to spend £5 on it for the one or two chapters which may be of interest to him. The appearance of other volumes in this series will be awaited with interest. J. P. Riley

DEVELOPMENT OF MODERN DIURETICS

Diuretics
Chemistry and Pharmacology. By George deStevens. (Medicinal Chemistry: a Series of Monographs, Vol. 1.) Pp. xiii + 186. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 7 dollars.

OVER the past seven years important advances in medicinal chemistry have made available for clinical use a number of diuretics which are well tolerated and effective when given orally. In the previous thirty-five years, the mainstay of diuretic therapy had been the group of organic mercurial compounds of which mersalyl was probably the most popular in this country. Chance observation by Vogl, a third-year medical student in Vienna, had directed attention in 1919 to the diuretic potential of a mercurial drug which was being used for the treatment of syphilis. In spite of extended investigations, only a limited number of organic mercurials came into clinical use and among them none was entirely satisfactory when given other than by injection. While there were many miscellaneous medicinal substances which could provoke a diuresis and some were active by mouth, there was no drug available other than an injectable organic mercurial that could be relied on for a potent and sustained diuretic action without more or less serious undesirable effects.

The discovery of the inhibitory action of sulphanilamide on carbonic anhydrase and the rational explanation of its acidotic effect led to the clinical testing of its diuretic activity in 1949. Krebs had meanwhile published an account of the carbonic anhydrase inhibiting activity of a number of cyclic sulphonamide derivatives. Further extensive work in several laboratories resulted in the marketing of acetazolamide, which deStevens calls "the first clinically effective, non-mercurial, orally active diuretic drug". Until about 1958, this drug was used as a reasonably satisfactory substitute for organic mercurial compounds, when a moderate diuresis was required. Its use was subject to the limitations that its effectiveness waned as the metabolic acidosis developed and that excessive potassium loss might occur during its use.

Other sulphonamides were investigated by Sprague and Beyer and their colleagues. This work led to the *m*-benzene disulphonamides including dichlorophenamide and 5-chloro-2,4-disulphamylaniline, which had diuretic properties closer to those of the mercurials and differing from acetazolamide. Their action in man is characterized by a greater loss of chloride rather than bicarbonate ions and a significantly lower incidence of acidosis and refractoriness. Novello and Sprague then described the preparation of chlorothiazide by ring closure of 5-chloro-2,4-disulphamylaniline. At about the same time, hydrochlorothiazide was synthesized by deStevens and his colleagues. These were the forerunners of a series of thiazides of which several have found extensive clinical application.

The major part of this monograph is devoted to the sulphonamide and benzothiadiazine series of diuretics. The historical background of developments in these series of drugs makes interesting reading. The account of structure-activity relationships is of special value. Another important chapter is that dealing with substances which either antagonize the action of aldosterone or interfere with its synthesis. Other substances which have been used as diuretics are dealt with briefly, as befits their relative unimportance in therapeutics.

This is a valuable review which emphasizes particularly the chemical aspects of the subject. The treatment of the physiology of renal function is starkly simple and some errors have crept in. Thus, on p. 48, sodium carbonate is said to be formed by the union of bicarbonate ions with sodium ions in the tubular cells. There is also a very brief account of the pharmacological evaluation of diuretic action. Chapter 9 includes an assessment of the place of diuretics in the treatment of hypertension. The coverage of the biological activities of the diuretics is not comprehensive. The actions of thiazides—and of more recently introduced drugs such as triamterene—on urea, uric acid and glucose metabolism, are not mentioned. However, since the main emphasis of the monograph is on chemical aspects, especially structure-activity relationships, of diuretic drugs, these deficiencies are less serious. There is an adequate index and the book is well produced. The titles announced for further volumes in this series of monographs (some have already appeared) suggest that it will be a very valuable venture. D. R. WOOD

PSYCHOLOGIST'S BEDSIDE BOOK

Readings in Psychology

Edited by Prof. John Cohen. Pp. 414. (London: George Allen and Unwin, Ltd., 1964.) 52*s*. 6*d*. net.

THE collection, under one cover, of papers previously published elsewhere can be justified on the grounds either that it gives access to papers of historical merit, or that it brings together the contribution of experts in a particular field. But when the chapters are not classics and when the spread of specialities is broad, justification is harder to find. That is the situation with *Readings in Psychology*. Of its twenty-three chapters, only six appear to have been written especially for the book. The remainder are reprints of papers, chapters in textbooks, and presidential addresses to various learned societies.

The best indication of the book's coverage can be obtained from a list of its chapters: Cohen, a perspective for psychology; Oldfield, experiment in psychology—a centenary and an outlook; Burt, Francis Galton and his contributions to psychology; Meredith, models, meanings and men; Inhelder, some aspects of Piaget's genetic approach to cognition; Mace, homœostasis, needs and values; Zangwill, physiological and experimental psychology; Steinberg, aspects of psychopharmacology: drug-induced changes in emotion and personality; Vernon, attention and visual perception; Drever, perceptual organization and action; Humphrey, 'fifty years' experiment on thinking; Bowden, thought and machine processes; Mackay, information theory in the study of man; Summerfield and Legge, information theory and perception; Cohen, contact between minds; Hunter, the organization of memory; Vernon, the psychology of intelligence and *g*; Fraisse, the sources of emotion; Broadbent, response to stress in military and other situations; Hearnshaw, temporal integration and behaviour; Glover, research techniques in psychoanalysis and in general psychology: an essay in contrasts; Rodger, the effective use of man-power; Thorpe, some concepts of ethology.

Is there a hidden thread of continuity under this apparent hotch-potch? Prof. Cohen, in his foreword, points out that the sequence of chapters is methodical, but by this he appears to mean merely that the first couple of chapters are concerned with historical issues, the next few are theoretical, the next physiological and so on—a clump of chapters dealing with one set of topics is followed by another clump dealing with another set, but the ordering of these clumps is arbitrary and could be altered without affecting the merit of the book. Such a minimal degree of ordering gives no sense of continuity to the reader, who, if a non-psychologist, would be well advised to go elsewhere if he is seeking general information about the subject; from this volume he will only obtain an impression of an enormously wide, fragmented discipline, if discipline at all.

What then is the object of such a book? In view of the choice of authors—all except two are British—it would seem that one purpose is to publicize the contributions of modern British psychologists. As a corrective to the parochialism of much American writing, such a step is to be welcomed, but it is unfortunate that it had to take this form. How much better if these same contributors would combine forces to write a general textbook.

Readings in Psychology has to be dipped into, and, approached in this manner, it could be both instructive and enjoyable. Many of the chapters are clearly written—possibly a result of their earlier delivery as spoken papers. Some report recent research but one or two are quite out of date although written within the past ten years. It is equally irritating to be confronted with a chapter which has not been revised at all as with one which has a postscript pointing out its present-day inadequacies. The authors of these chapters should have been asked to contribute new articles or at least to remedy the deficiencies in the originals.

Perhaps the book can best serve psychologists as a bedside book. D. W. FORREST

DIAGNOSTIC FEATURES OF THE DIGENETIC TREMATODES

Keys to the Trematodes of Animals and Man.

By K. I. Skrjabin and others. Translated by Raymond W. Dooley. Translation edited by Hisao P. Arai. Pp. xvi+351. (Urbana, Ill.: University of Illinois Press, 1964.) 10 dollars.

FOR more than fifteen years (1947–62), Academician K. I. Skrjabin and his staff at the Helminthological Laboratory of the Academy of Sciences of the U.S.S.R. laboured to produce twenty volumes with a total of 14,000 pages entitled *Trematody Zhivotnykh i Cheloveka* (Trematodes of Animals and Man), an enormous compilation of taxonomic data on digenetic trematodes which still requires four volumes for completion. Skrjabin's initial idea of producing eight to ten volumes of information about genera became an ambition to define by means of keys and descriptions all the known species of Digenea and to include lists of hosts, data on distribution and some information about life histories.

Helminthologists unable to read Russian cannot utilize this fund of information and, because a complete translation could not be envisaged, the decision was made in certain quarters to do the next best thing and translate abstracted essentials for presentation in the compendium under review here. The book is divided into nearly equal halves, one containing keys to taxonomic groups and lists of references and the other more than nine hundred line diagrams and a subject index. The selection and translation of essential data made a formidable task for Hisao P. Arai and Raymond W. Dooley of the University of Illinois, who used Whitfield's (1958) transliterated form of the Cyrillic alphabet and worked to a rigorous discipline. We have to remember that many descriptions of species are excluded and that only those citations to the original literature which are rendered in Cyrillic in the major work are presented, which means that readers refer to the twenty volumes for particulars about numerous non-Russian references.

The constituent compilations of the original volumes were published as they became available, so that they are not in any conventional order, and this disorder has been emphasized by abridgment. Moreover, the taxonomic scheme is incomplete, so that the reader cannot examine the classification of the Digenea as a whole and, indeed, is irritated and frustrated in his efforts to do so. On p. 3 the major groups are indicated. The Digenea is divided into the sub-subclasses Gasterostomatoinei and Prosostomatoinei and the former group is not mentioned again, relevant subsequent references being to the subfamilies of Bucephalidae and literature concerning an order named as Bucephalida (pp. 178–179). Similarly, the Prosostomatoinei is divided into the superorders Aspidogasteriformes and Fascioliformes, but the former receives no further mention, although the suborder Aspidogastrata is divided into its two families on p. 50. The Fascioliformes is divided into ten orders (the Alcicornata, Didymozoata, Fasciolata, Faustulata, Heronimata, Notocotylata, Paramphistomatata, Sanguinicolata, Schistosomatata and Strigeata), but only two of these (Notocotylata and Paramphistomatata) are completely resolved into families. On p. 44 the Schistosomatata is named as a suborder and divided into two superfamilies Sanguinicoloidea (already designated as an order) and Schistosomatoidea, which receives no further mention, the following key referring to subfamilies of the family Schistosomatidae (p. 47). The reader will be disappointed often in this way and may conclude that the entire taxonomic scheme was established with pre-ordained classes, subclasses, sub-subclasses, superorders, orders, suborders, superfamilies, families and lower groups some of which remained unresolved.

In conjunction with the primary disarray, this lack of finality gives the impression of bewildering muddle where clarity might have prevailed. Some dissatisfaction may be felt also about the use of keys which were soon outmoded because of subsequent revision or outdated by the appearance of new species. One great drawback of keys is that they become unusable when new species appear and must be completely redrafted. The Russian keys are often very detailed but, like others, are not infallible. They have been duplicated for both subfamilies and genera of Echinostomatidae (pp. 8–10 and 101–102) and then remade (pp. 102–103) with new inclusions, as also for Dinuridae (pp. 81 and 150), Lecithasteridae (pp. 82 and 118) and Lecithochiridae (pp. 87 and 140). The reader is somewhat bewildered by the apparently belated promotions and, sometimes, unexplained omissions.

When examined carefully, the book indicates clearly the somewhat alarming impression of a rapidly expanding classification of the Digenea, alarming because promotion to family rank is sometimes from the status of genus, where taxonomic characters used often fail to receive universal acceptance. We realise how difficult trematodes are to classify only when we take into consideration certain facts: they exist, when alive, in a semi-liquid state with a minimum of skeletal structure, are very extensile and contractile, continue to grow and sometimes alter their bodily proportions long after attaining sexual maturity and, indeed, throughout life, show variability according to various phases of sexual life, appear sporadically and often in small numbers and, above all, are liable to show morphological artefacts on account of preservation and mounting for microscopical inspection. In reviewing the recent taxonomic scheme of Yamaguti[1], my analysis showed that out of 102 families, fifty-nine contained no more than four genera and thirty-one only a single genus. Skrjabin recognizes 125 families, but the discrepancy between the two schemes is much greater than these figures indicate. Forty-two families named by Skrjabin are not recognized by Yamaguti, although one (Elytrophallidae) is mentioned in a footnote and another (Megasolenidae) is regarded as a synonym. Two other families (Aerobiotrematidae and Stichocotylidae) are not even represented by genera in Yamaguti's scheme, and seven others (Faustulidae, Lecithochiriidae, Liocercidae, Microscaphiidae, Opisthoporidae, Ratziidae and Xenoperidae) are represented only by genera. The remaining thirty-one families are represented by Yamaguti as subfamilies: seven are promotions from the Allocreadiidae, six from the Paramphistomatidae, three from the Hemiuridae, two from the Opisthorchiidae, two from the Troglotrematidae and eleven respectively from the families Acanthocolpidae, Brachycoeliidae, Callodistomatidae, Cyathocotylidae, Diplostomatidae, Heterophyidae, Microphallidae, Plagiorchiidae, Pronocephalidae, Protorodiplostomatidae and Strigeidae.

On the other hand, twenty-one families (six of them new) which are named by Yamaguti do not appear in Skrjabin's scheme. Three of them (Allocreadiidae, Bhaleraoidae and Mesometridae) are unrepresented by genera, nine of them (Anenterotrematidae, Aporocotylidae, Botulidae, Brahamputrotrematidae, Eumegacetidae, Ommatobrephidae, Pachypsolidae, Paragonimidae and Rhopaliasidae) are represented only by genera, and nine of them (Apocreadiidae, Brachycoeliidae, Hasstilesiidae, Leucochloridiidae, Moreauiidae, Nasitrematidae, Pleorchiidae, Telorchiidae and Waretrematidae) are represented by subfamilies. Readers may rightly infer that in the four Russian volumes still to appear some of these families and possibly others will be named.

Another source of bewilderment for the reader is provided by the lists of literature which are introduced frequently into the text. Innumerable references which have value in the series of original volumes are useless in this book because they have no bearing on the diagnosis of digenetic trematodes. Altogether, these references take up about one-fifth of the text. On pp. 17–20, the first five of a long list of papers on fascioliasis concern liver fluke in an eleven-year-old girl, distomatiases of children in Turkestan, methods of 'de-helminthization' of manure, the treatment of sheep with carbon tetrachloride and methods of introducing medicinal substances into small animals. Even more incongruous are references to allergic skin reactions (p. 50), heredity and variability (p. 58), anoplocephalid tapeworms (p. 59), migrations of fishes (p. 77) and nematodes of poultry (p. 157), as well as many items cited "no title given". Space has been far better spent on figures, which are miniatures of those in the Russian volumes and might have been augmented, but indications of size are not consistently given in either illustrations or text and would have been of great advantage to the diagnostician. The book will not be popular but will have many dour users, who will peruse it just as grimly as I did, until I noticed the misprinted reference to "the helminth fauna of sand-papers".

BEN DAWES

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International Review of Connective Tissue Research

Vol. 1. Edited by David A. Hall. Pp. xiii+401. (New York and London: Academic Press, 1963.) 14.00 dollars.

THE first volume of *International Review of Connective Tissue Research*, a new periodical, contains eight review articles. It is not idly called 'international' since the authors come from seven different countries—Scotland, Holland, Denmark, Hungary, India, Australia and England.

The past decade has seen a great increase in real knowledge of connective tissues and their components, and any method of assembling this is potentially valuable. There are now a number of examples of this type of reviewing periodical, which would be most valuable if it could give a reasonably complete running account of the subject. However, in practice they usually contain a heterogeneous collection of articles of variable interest and value. For every one you want you must buy and carry perhaps two or three others, and often more. This seems to be inevitable, and judgment must therefore be based on the best articles. In this volume there are certainly two which should be of wide interest and value, on the structure of collagen by G. N. Ramachandran and its metabolism by D. A. Lowther. The articles are complementary and have tables in common. There is a useful article on calcification of skeletal tissues by S. M. Weidmann. I also found J. Balo's article on atherosclerosis fresh and interesting.

In short, there is useful material in this volume, mainly for the specialist who is already familiar with some aspect of the subject. It would be more generally useful if the bibliographies gave titles, even if some of the articles had to be shortened and 17 pages of author index left out. There is a subject index, but it is only 5 pages long.

The Mushroom Hunter's Field Guide

By Prof. Alexander H. Smith. Revised and enlarged edition. Pp. 264 (188 figures). (Ann Arbor: University of Michigan Press; London: The Cresset Press, Ltd., 1963.) 45*s*. net.

LIKE its predecessor, *The Mushroom Hunter's Field Guide* is written for the amateur in non-technical style and has been considerably enlarged to include accounts of 188 species as against 124 in the original book. However, since Prof. A. H. Smith has altered the selection of species described in the first edition by deleting some and adding others, it follows that the book, as revised, covers an appreciably different range of fungi. The text has been modified, although the format remains much the same, with data grouped under the headings "Identification marks", "Edibility" and "When and where to find it". Furthermore, while each species is illustrated with black-and-white photographs of an extremely high standard, many of those previously used have been replaced. Another innovation is that 89 species are also illustrated in colour. Here one should note that the numbers of the captions refer to the species number in the text and are not in a running sequence.

In general the descriptions of the species, although of necessity brief, are adequate. However, just occasionally brevity has resulted in omission of important information. Thus I would have expected some reference to the fact that *Agaricus placomyces* becomes bright yellow on bruising, especially as this reaction clearly separates it from *A. silvaticus*—a species with which it is liable to be confused according to Prof. Smith (p. 197).

As to the addition of coloured plates, these will undoubtedly help to increase the appeal of this book. In the preface Prof. Smith writes: "Illustrations in color, however, are the most difficult aspect of a publication on mushrooms. To be of help rather than to be misleading, the color work must be accurate". I share these sentiments, but I'm not sure that Prof. Smith has altogether succeeded in his aim; although a number of the coloured photographs are very good, others are bad. Thus on Plate 124*a* *Amanita muscaria* is shown as blue, whereas it must be assumed that the plate represents the white form of the species. Likewise *Lactarius rufus* is depicted as bright pink, whereas this fungus should be rich reddish-brown. Similarly the illustrations of *Lycoperdon pyriforme* and *Armillaria mellea* are so poor as to be scarcely recognizable. Nevertheless, taken as a whole, the coloured plates are superior to many which have been used to illustrate mycological works in recent years.

Apart from these relatively minor criticisms, I have no hesitation in recommending this excellent book to those living in North America who wish to obtain a knowledge of the larger fleshy fungi, be it for general interest or from the point of view of gastronomy. Furthermore, those who have used *The Mushroom Hunter's Field Guide* will want to purchase the revised edition since it covers an appreciably increased range of species. For those with an interest in mycology outside North America, this work will naturally have a more restricted appeal, but since it is attractively produced, reasonably priced, and of very good value, they too may wish to add it to their bookshelves.

D. A. Reid

Les Maladies des Plantes Maraichères

Vol. 1. By C. M. Messiaen et R. Lafon. Pp. 153. (Paris: Institut National de la Recherche Agronomique, 1963.) 21 F.

THIS practical little manual of vegetable crop diseases is largely based on the experience of the authors in southern France. After brief but adequate introductory chapters, describing the agents of disease in plants and the principles of plant disease control, come chapters devoted to diseases of the following crops: tomato; egg-plant and capsicum; cucurbits; haricot beans; peas and broad beans; celery; and parsley. Symptoms are illustrated by simple but effective line drawings, supplemented by a few photographs.

R. W. G. Dennis

Underwater Acoustics

Proceedings of an Institute sponsored by the Scientific Affairs Committee of the North Atlantic Treaty Organization and conducted by the Pennsylvania State University at the Imperial College of Science and Technology of the University of London, July 31–August 11, 1961. Edited by V. M. Albers. Pp. xiii + 354. (New York: Plenum Press, 1963.) 12.50 dollars.

UNFORTUNATELY the main title of this book, and the one it is likely to be known by, is a little misleading. It does not pretend to be a text-book on underwater acoustics but is a compilation of the papers presented at a symposium, sponsored by the North Atlantic Treaty Organization, in London in 1961. Nevertheless, the standard of the papers given is high and the book may be used for specialized reference in certain fields of underwater acoustics.

Papers include work on piezoelectric transducers, explosive sources, directivity and scanning, internal waves, scattering, ambient noise, flow noise and model propagation studies. Certain important areas of the field are not covered, notably: controlled propagation experiments in the sea and their theoretical interpretation, non-piezoelectric transducers, and the design of transducer arrays including reference to the interaction problem between elements.

It is clear that this is a difficult subject to present adequately since the scientific and engineering matter covered by the title *Underwater Acoustics* is very large; and the problem is complicated by the various grades of security imposed by national and international organizations. The organizers of this symposium are to be congratulated on pioneering such a meeting in the United Kingdom.

J. S. M. Rusby

OBSERVATION OF THOMSON AND CO-OPERATIVE SCATTERING OF RUBY LASER LIGHT BY A PLASMA

By A. W. DeSILVA*, D. E. EVANS and M. J. FORREST

Culham Laboratory, U.K. Atomic Energy Authority, Abingdon, Berks

WHEN monochromatic light is directed on to a plasma, a fraction of the light undergoes scattering on the free electrons, the motion of which introduces Doppler broadening. A number of theoretical papers, for example, Dougherty and Farley[1], Fejer[2] and Salpeter[3], have dealt with the problem of the resulting wave-length spectrum and have shown that it can be characterized by a single parameter:

$$\alpha = \frac{\lambda}{4\pi D \sin \theta/2}$$

where λ is the wave-length of the incident light, D the plasma Debye length and θ the angle to the incident beam at which the light is scattered. Theory shows that for $\alpha < < 1$ the spectrum takes the form of a gaussian the width of which is given by the average velocity of electrons at a temperature T_e, decreased by a geometrical factor, sin $\theta/2$. This has been called incoherent or Thomson scattering. For $\alpha > 1$ the electrons behave collectively and their motion is determined in part by the motion of the ions. The spectrum is expected to consist of a central feature the width of which depends on the sound velocity in the plasma, together with two peaks shifted on either side of the centre by an amount given by the electron plasma wave velocity.

We have designed an experiment in which the correct choice of plasma parameters allows us to detect both the ($\alpha < < 1$) mode and the collective ($\alpha > 1$) mode in the same plasma merely by altering the directions from which scattered light is observed. The upper part of Fig. 1 illustrates the experimental arrangement for observation of the $\alpha < < 1$ feature. A Q-switched ruby laser produces a 2·5-MW pulse of light at 6935·5 Å having a spectral width of 0·05 Å (ref. 4) which is introduced into the plasma through a suitably baffled entry tube and is carried away through an exit tube which terminates in an efficient light trap.

The plasma is a pulsed hydrogen arc formed in 200 m torr of hydrogen between a pair of stainless steel electrodes 50 cm apart. An axial magnetic field of 5 k.gauss maintains the plasma for about 350 μsecs during which a peak current of about 20 k.amp flows. Stark broadening measurements of the $H\beta$ line have given an electron density of 9×10^{14} cm^{-3} and subsequent measurements of the width of the $\alpha < < 1$ feature gave an electron temperature $kT_e = 2{\cdot}2$ eV. This means $\alpha < 1$ for $\theta > 16°$. Observations were made at 170° ± 1·5° and 10° ± 1·5° for which $\alpha = 0{\cdot}15$ and $\alpha = 1{\cdot}7$ respectively. In both cases the detection optics were based on a conical lens, placed concentric about the laser beam, which accepted all the light

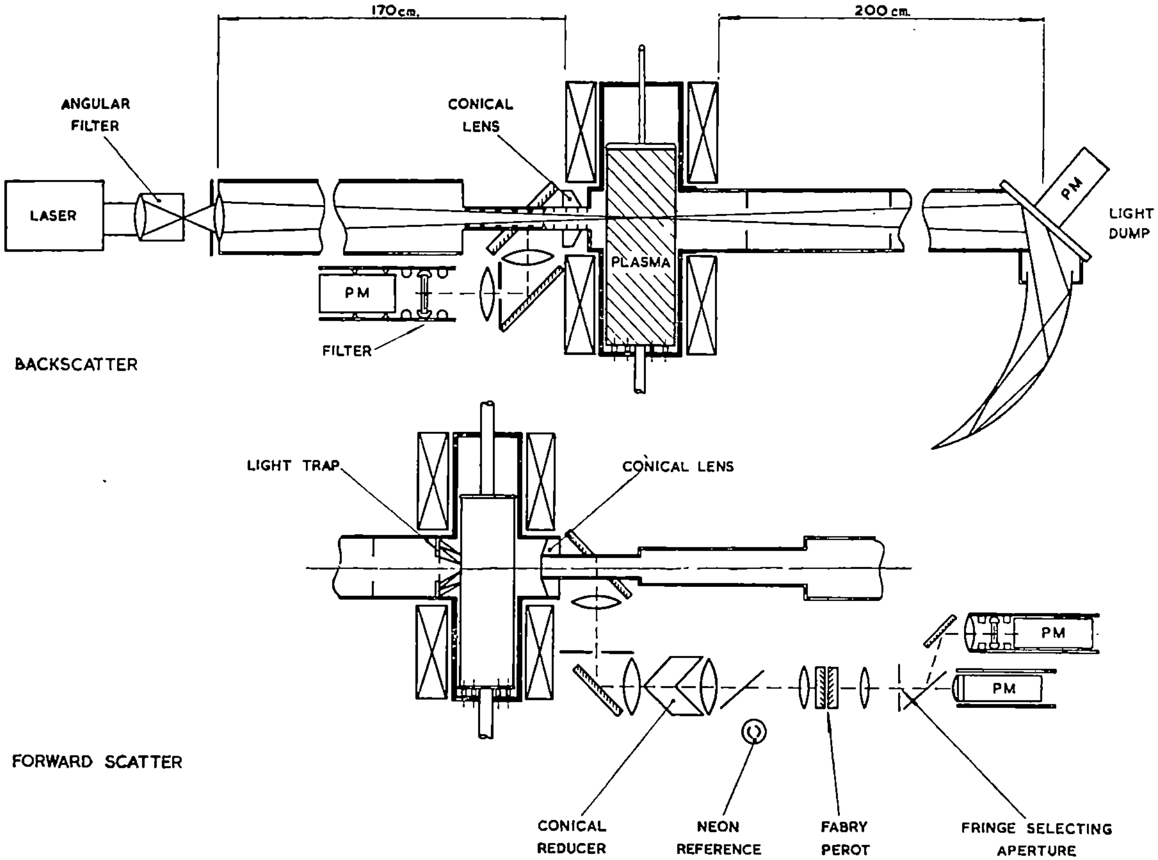

Fig. 1. Schematic diagram of experimental arrangement

scattered at the preselected angle from the plasma. In the back-scatter experiment, spectral resolution was obtained by tilting a nominally 3 Å band pass interference filter centred at 6943 Å. An E.M.I. 9558*A* photomultiplier with an S_{20} (trialkali cathode) response detected the light transmitted by the filter. The lower part of Fig. 1 shows the experimental arrangements adopted for forward scattering measurements. The optical train and detector are essentially the same as before, but since the acoustic feature was expected to have a width of about 0·2 Å, the tilting filter was abandoned in favour of a scanning Fabry-Perot interferometer.

Fig. 2 shows the results of the scattering experiment at 170°, and displays the total scattered signal as a function of wave-length. It therefore includes stray scattering of the main beam from the walls of the vessel, etc., and this appears as an anomalous peak at the centre of the distribution. Measurements made in the absence of plasma (black circles) indicate that the stray light has been reduced to a manageably low level. The stray light spectrum, which is simply the instrumental shape of the filter and the detection optics, has been subtracted from the total signal and a Gaussian curve (half half-width 35 Å) has been fitted, from which it appears that the electron temperature $kT_e = 2{\cdot}2$ eV.

Fig. 3 displays the signal observed in the forward scatter position as a function of wave-length. In this case no correction for stray light was required since an observation made without plasma in the vessel showed that stray light and Rayleigh scattering on neutral hydrogen were undetectable. The spectral resolution of the system is restricted by the absolute light intensity available which has set a practical limit of 2 Å on the setting of the interferometer. The experimental points are consistent with an instrumental profile 2 Å wide, and one can regard this as an upper limit to the width of the feature being observed.

Nitrogen at a pressure of 0·25 atmosphere was substituted for plasma in the chamber and the intensity of light at the detector due to Rayleigh scattering was measured. The ratio of intensities of Rayleigh scattered light and light scattered by the plasma was $S_r/S_t = 8{\cdot}8$. The expected value of this ratio was calculated assuming the plasma-scattered light to be associated with a cross-section equal to about 0·5 the Thomson cross-section, as predicted by the theory of co-operative scattering. Rayleigh cross-sections were taken from George *et al.*[5]. The theoretical Rayleigh cross-section led to $S_r/S_t = 9{\cdot}0$ and the experimentally determined cross-section gave $S_r/S_t = 21$. We conclude that the intensity of the signal observed in the forward scatter experiment is consistent with scattering by the acoustic wave.

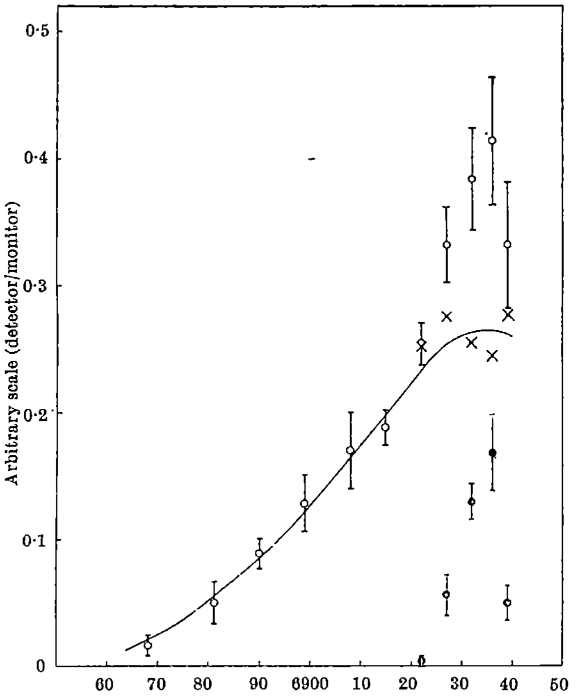

Fig. 2. Spectrum of light scattered at 170°. Laser wave-length 6935·5 Å. O, Scattered + stray light; ●, stray light alone; ×, scattered light alone

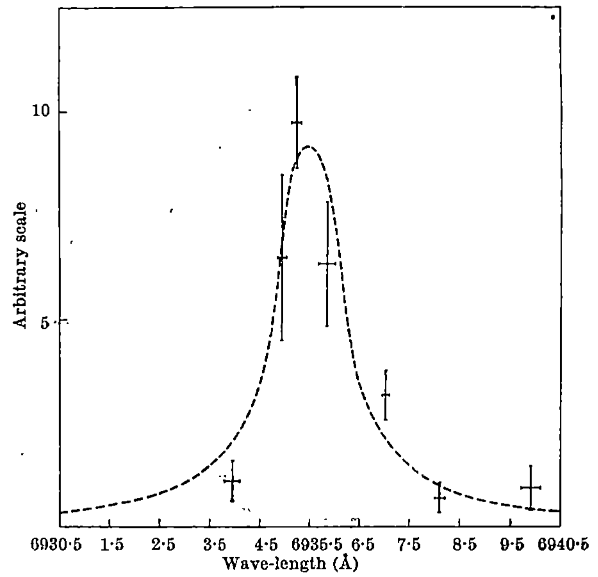

Fig. 3. Spectrum of light scattered at 10°. Laser centred at 6935·5 Å. Ion resonance $a = 1{\cdot}7$; - - -, instrumental line shape; stray light negligible

The width (< 2 Å) is also consistent with acoustic wave scattering, and at the same time rules out the possibility that the light scattered at 10° is due simply to Thomson scattering, for if this were so a width of $\dfrac{\sin 10°/2}{\sin 170°/2} \times 70$ Å $= 6$ Å would be expected.

It remains possible that there is a contribution to the observed forward intensity due to some effect apart from co-operative scattering. Photo-ionization of neutral hydrogen followed by immediate radiative recombination could produce spurious photons, but there are unlikely to be many, owing to the very short relaxation time (10^{-10} sec) compared with both the laser pulse length (2×10^{-8} sec) and the recombination time (10^{-3} sec). The intense electromagnetic field of the laser light in the plasma will produce photons at the laser frequency through stimulated emission of radiation. These will, however, be in phase with the stimulating field, and so will travel in the same direction as the principal laser beam. Again, only a negligible number are expected to reach the detector.

Work is proceeding to improve the strength of the forward scattered signal with the aims of measuring the width of the central peak and of detecting the satellites at the plasma frequency.

We thank Dr. R. Wilson and Dr. D. J. Bradley for their help and advice. One of us (A. W. DeS.) gratefully acknowledges the support of the National Science Foundation during a major part of the work.

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EVOLUTION AND PREVENTION OF DRUG-RESISTANCE

By Prof. M. G. SEVAG and BARBARA ASHTON

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THE capacity exhibited by all organisms to develop tolerance for new environmental conditions has been an important factor in the continuing existence of life on Earth. It has also had adverse effects on some creatures. To man, one of the most disturbing of these effects is being manifested through the resistance certain microbes develop for the 'wonder' drugs he uses to counteract microbial diseases.

The peculiar capacity of pathogenic micro-organisms to develop strains which are resistant to drugs which on initial application are lethal to most individuals—and eventually to thrive on those drugs—did not come into clear focus until Paul Ehrlich *et al.* made their famous chemotherapeutic researches during the first decade of this century. Over the years, concern for these effects intensified as a greater variety of drugs was produced and wider use was made of them. With the advance of modern chemotherapy, microbial resistance to sulphonamides and antibiotics became a frustrating medical and scientific problem. More recently we have learned that similar adaptability occurs among the cells causing cancerous growth during treatment by radiation and chemicals, and that viruses may also develop this same kind of tolerance and thereby interfere with the therapy of viral infections.

Because of the danger of these effects, a large research effort is being made to understand and control them. Experiments are proceeding with a wide variety of antibiotics, individually and in combination; investigations are in progress on the mode of action of antibiotics; and comparisons are being made of the biochemical and genetic differences between normal and resistant strains. These investigations have not yet produced sure-fire measures for preventing the emergence of microbes or other micro-organisms that are resistant to antibiotics. The synergistic combination of antibiotics or drugs, by trial and error, is beset with uncertainties or may result in an antagonistic action. These trials have not produced a guiding principle to cope with the phenomenon of drug-resistance.

Prevention of the Emergence of Drug-resistant Strains*

In previous communications[1,2] it was reported that spermine in a combination with an antibiotic prevented the emergence of drug-resistant strains from *Staph. aureus*, *Escherichia coli* and *Aerobacter aerogenes*. In the work recorded here the antimalarial drug atabrine ('Quinacrine') is used as a substitute for spermine. In using atabrine we were guided by the fact that, like spermine and streptomycin, it complexes stably with nucleic acids and DNA is known[3] to be the carrier of the code for the resistance character.

We therefore entertained the idea that atabrine complexing with DNA could prevent the resistance-inducing action of an antibiotic on normal bacteria. This working hypothesis is supplemented by the following results.

The results with atabrine and various antibiotics are presented in Table 1. It is to be seen that chloromycetin, tetracycline, erythromycin, novobiocin, penicillin, sulphathiazole and streptomycin, each together with atabrine, exercise bactericidal action; in the absence of atabrine a staphylococcal strain of greater resistance emerges. Likewise, as shown in Tables 2 and 4, no resistant strain of *E. coli* emerges in the combined presence of streptomycin or sulphathiazole and atabrine. In Table 3, the results show that *E. coli* resistant to streptomycin are not prevented from growing in a combination of streptomycin and 250 μg of atabrine. On the other hand, the same resistant strain is killed by a combination of as little as 1 μg of sulphathiazole and 100 μg of atabrine; the contents of the system plated out on nutrient agar showed no colony growth.

Of special consideration is the effect of *p*-aminobenzoic acid (PABA) on sulphathiazole inhibition with and without atabrine (Table 1, systems 27–29). In the absence of atabrine sulphathiazole inhibition is either absent or readily antagonized by PABA. In the slower rate of antagonism by PABA of the sulphathiazole inhibition in the presence of atabrine, the specific preventive effect on bacteria of the latter two substances is eventually nullified.

A bacterial strain which had been sub-cultured once in streptomycin grew readily in the combination of this antibiotic and spermine or atabrine. In other words, once the resistance character is acquired by a strain a combination of atabrine or spermine and the antibiotic is ineffective. Indeed, two cells from a population which had grown for the first time in 100 μg streptomycin/ml., added to 100 million sensitive normal cells, grew freely in the presence of both streptomycin and spermine or atabrine. The results show that our system selectively eliminates all the 100 million sensitive cells and permits the multiplication of as few as two actually resistant exogenous cells. Had there been, therefore, as few as only two naturally occurring resistant cells in the inoculum of 100 million normal cells, our system would have selectively permitted their multiplication in a combination of the antibiotic and spermine or atabrine. On the other hand, we must take note of the fact that a resistant strain emerges from 100 million normal bacteria used alone as inoculum in a growth medium containing only the antibiotic. One may conclude, therefore, that these normal bacteria must have contained certain mutants which, possessing the potentiality of becoming resistant, were catalysed by the antibiotic to take on the resistant character. This mediation by an antibiotic is prevented from becoming an actuality in a combination of atabrine or spermine and the antibiotic.

Determination and Prevention of the Emergence of Cross-resistant Strains

As already discussed here, atabrine or spermine is ineffective in combinations with an antibiotic to which a bacterial strain has become resistant by one or more previous sub-cultures. However, atabrine or spermine is highly effective in combination with another antibiotic to which the bacterial strain has not been exposed before. In the absence of spermine or atabrine, a strain resistant to an antibiotic can become resistant also to a second or third antibiotic. Customarily, the ability of the penicillin-resistant strain to grow in streptomycin would be attributed to an unspecific cross-resistance against the latter antibiotic. On the basis of the current 'selection theory' of antibiotic resistant strains, the above facts may be interpreted to indicate that, either the penicillin-resistant

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* The experimental procedures used throughout this investigation were the same as those used in the previous investigations[1,2].

Table 1. Prevention of the Emergence of Antibiotic-resistant Strains from the Sensitive Population of *Staphylococcus aureus* 3A

Growth system (additions μg/ml.)	Growth turbidity readings at hours of: 0	14–18	43–46	65–90	114–118	138–148	168–186	209–312
1. None	3	276	248	—	—	—	—	—
2. Atabrine 80	8	230	214	—	—	—	—	—
3. Atabrine 160	5	194	204	—	—	—	—	—
4. Chloromycetin 7	8	26	38	37	—	232	—	—
5. Chloromycetin 8	3	17	22	19	—	20	150	—
6. Chloromycetin 7 + atabrine 160	0	16	23	23	—	24	31	—
7. Chloromycetin 8 + atabrine 160	6	21	23	25	—	27	33	—
8. Tetracycline 1·0	5	18	16	16	—	235	232	—
9. Tetracycline 2·5	3	16	18	18	—	21	22	250
10. Tetracycline 1·0 + atabrine 160	2	16	20	17	—	20	24	20
11. Tetracycline 2·5 + atabrine 160	3	15	21	20	—	26	28	30
12. Erythromycin 2·5	2	15	13	98	—	226	—	—
13. Erythromycin 2·5 + atabrine 80	0	13	14	14	—	20	—	25
14. Erythromycin 2·5 + atabrine 160	8	26	26	26	—	26	26	26
15. Novobiocin 5	7	14	20	214				
16. —— Atabrine 100	8	216	220	—	—	—	—	—
17. Novobiocin 5 + atabrine 100	9	18	28	27	—	25	24	—
18. Penicillin 0·2 units (inoculum $0·5 \times 10^8$/5 ml.)	7	153	132	—	—	—	—	—
19. Penicillin 0·5 units	9	62	152	—	—	—	—	—
20. —— Atabrine 100	7	218	216	—	—	—	—	—
21. Penicillin 0·2 units + atabrine 100	3	—	—	5	5	5	—	11
22. Penicillin 0·5 units + atabrine 100	0	—	—	2	2	4	—	8
Sulphathiazole (inoculum 25 cells/5 ml.)								
23. —— PABA 5–40	0	32	256	—	—	—	—	—
24. —— Atabrine 100	0	0	0	5	—	27	180	—
25. —— Atabrine 100 + PABA 5–40	0	0	0	3	—	157	158	—
26. Sulphathiazole 100	0	0	37	157	215	—	—	—
27. Sulphathiazole 100 + PABA 5	0	47	236	—	—	—	—	—
28. Sulphathiazole 100 + atabrine 100	0	0	1	8	—	15	22	—
29. Sulphathiazole 100 + atabrine 100 + PABA 5	0	3	8	72	—	185	193	—

Growth medium: Salts-glucose + 1 per cent casein hydrolysate + tryptophan 20 μg + 1 μg thiamine + 1 μg nicotinamide/ml.
Inoculum: 1×10^8 cells/5 ml. medium collected from a growth in 0·5 per cent glucose broth (Sevag and Barbara Ashton).

cells used as inoculum were double mutants, or they contained a certain number of double mutants, which were selected by streptomycin, yielding a population of cocci resistant to both antibiotics. However, because of the fact that the streptomycin-resistant cells are not susceptible to the combined action of streptomycin and spermine or atabrine, and because of the fact also that the penicillin-resistant cells were killed by the combination of streptomycin and spermine or atabrine, it would be reasonable to conclude that the penicillin-resistant strain was neither a doubly resistant mutant, nor possessed an unspecific cross-resistance to streptomycin prior to exposure to it. On the basis of the above facts, one may therefore be permitted to assume that the gradual emergence of a streptomycin-resistant strain from a penicillin-resistant strain results from an acquisition of a new resistance character. This acquisition also is prevented in the presence of spermine or atabrine[1].

Table 2. Prevention of the Emergence of a Drug-resistant Strain from the Normal Strain of *Escherichia coli* Strain *B* in the Combined Presence of Streptomycin and Atabrine

Growth system (additions μg/ml.)	Growth turbidity readings at hours of: 0	21	45	117
1. None	7	167	—	—
2. Streptomycin 10	13	143	—	—
3. Streptomycin 30	8	23	162	
4. —— Atabrine 40	11	98	156	—
5. Streptomycin 10 + atabrine 40	9	6	6	4
6. Streptomycin 30 + atabrine 40	15	14	16	15

Inoculum: 2×10^8 cells/5 ml. of salts-glucose 1 per cent casein hydrolysate medium. Growth in glucose broth is one-half of the above control values. The effects of the drugs on the growth are, however, of the same degree (Sevag and Barbara Ashton).

Table 3. Failure to prevent the Growth of the Drug-resistant Strain of *Escherichia coli* Strain *B* in the Combined Presence of Streptomycin and Atabrine

Growth system (additions μg/ml.)	Growth turbidity readings at hours of: 0	4	22	47	118
1. None	0	41	61	67	74
2. Streptomycin 1,000	5	38	61	68	78
3. —— Atabrine 250	10	33	74	91	100
4. Streptomycin 100 + atabrine 250	8	27	57	61	70

Inoculum: $1·0 \times 10^8$ cells/5 ml. of 0·5 per cent glucose-nutrient broth (Sevag and Barbara Ashton).

In hospital laboratories, this system offers a dependable and practical method to determine whether or not a strain carries cross-resistance to other antibiotics. By the use of this system one can also determine which antibiotic together with spermine or atabrine is the most effective to prevent the emergence of a strain with additional resistance characters from an already resistant strain.

Prevention of the Emergence of Resistant Strains in Systems Excluding 'Selection'

A direct experimental illustration of the specific effects of a drug and atabrine on the sensitive strain of *Staphylococcus aureus* is provided by the results presented in Fig. 1. In these experiments the possibility of selecting mutants as resistant strains assumed to occur naturally by the 'selection theory' is excluded. The growth rate was determined by colony counts. As inoculum 20–25 cells/5 ml. medium were used. Colony counts of the growth in the liquid medium were made at 0, 18, 24, 42 and 72 h. In the system with 100 μg sulphathiazole or 100 μg of atabrine, at no period was there a decrease in the number of cells. In sulphathiazole together with atabrine, the cell number remained constant during the first 24 h, then decreased gradually, and no colony grew at the 72-h period.

The fact that in sulphathiazole alone, or atabrine alone, the cell numbers remained constant for several hours before they began to multiply shows clearly that none was killed and there were no selections of the assumed 'naturally occurring mutants' from the initial inoculum of 4–5 cells/ml. Such a possibility is excluded also by the fact that all the cells were killed in the combined presence of sulphathiazole and atabrine, as would be expected if all were normal sensitive cells. The fact, however, that the normal cells grew in sulphathiazole after a short delay indicates that the drug exercised an action on the metabolism of the cells whereby the bacteriostatic action of sulphathiazole was nullified. The conclusions from these investigations are corroborated by the results of our previous work with Drs. Ishii[10] and Yokota[11], showing that in sub-lethal concentrations of sulphathiazole in which the cells survive from 50 to 82 per cent (determined by colony count), the mass of cells experience an increment of the folic acid synthesizing enzymes; thereby the inhibitory action of the drug is counteracted.

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Table 4. Prevention of the Emergence of Sulphathiazole-resistant Strains from Sensitive and Streptomycin-resistant Strains of *Escherichia coli* in the Combined Presence of Sulphathiazole and Atabrine

Growth system (additions μg/ml.)	Sensitive strain *B*						Resistant to 1,000 μg SM*/ml.					
	Growth turbidity readings at hours of:											
	0	18	42	71	137	160	0	18	42	71	137	160
1. None	10	161	197	—	—	—	4	160	144	—	—	—
2. ST 1	1	85	140	—	—	—	4	160	124	—	—	—
3. ST 10	4	8	7	6	—	157	8	12	15	112	222	—
4. —— Atabrine 50	9	113	174	—	—	—	8	105	127	—	—	—
5. —— Atabrine 100	2	61	184	—	—	—	0	0	17	87	154	—
6. ST 10 + Atabrine 50	0	0	0	0	—	4	0	1	1	0	6	7
7. ST 1 + Atabrine 100	1	2	2	2	—	13	0	2	1	1	8	11

Inoculum: about 1×10^6 cells/5 ml. of salts-glucose + 1 per cent casein hydrolysate medium (Sevag and Barbara Ashton).
* SM = streptomycin; ST = sulphathiazole.

Another more recent unexpected observation, to be reported elsewhere, is the profound change staphylococci experience by one or two sub-cultures in sulphathiazole alone. This experience renders staphylococci mortally sensitive to atabrine *per se*. As shown in Fig. 1, normal cells without such an experience survive atabrine with no difficulty.

Discussion

It has been variously reported that DNA is the genetic seat of the drug-resistant character of bacteria[3]. We thought, therefore, that an agent capable of complexing stably with DNA should in some manner influence the development of resistance behaviour of bacteria. Streptomycin[4], spermine[5], and atabrine ('Quinacrine')[6], and diamino acridines, such as acridine orange[7], complex with nucleic acids. DNA possesses acidic phosphate groups; spermine contains two secondary and two primary amino groups; atabrine contains, in addition to the acridine nucleus, one secondary and one tertiary basic amino groups in the aliphatic side-chain of the molecule, and streptomycin contains two guanido and one tertiary basic amino groups. The acidic groups of DNA and the basic groups of the other substances interact, yielding various complexes of different stability. Streptomycin–DNA and spermine–DNA and atabrine–DNA complexes form precipitates. Spermine and streptomycin complexed with DNA are non-dialysable[8] through a 'Cellophane' dialysis membrane, indicating that these complexes are not readily dissociable.

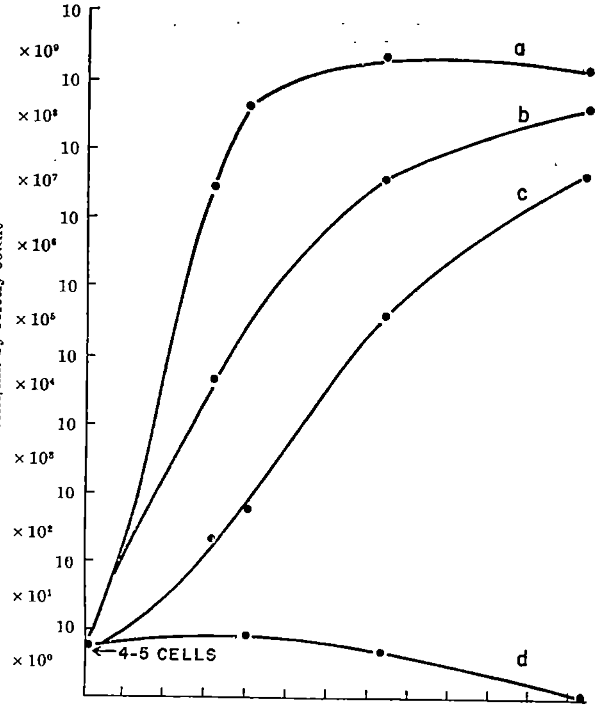

Fig. 1. *Staphylococcus aureus*, 3*A*. Growth rates in liquid media determined by colony counts by plating out aliquots at various periods of growth. *Inoculum size at 0 h:* 4–5 cells/ml. liquid medium. Curve *a*, control; curve *b*, in 100 μg/ml. sulphathiazole; curve *c*, in 100 μg/ml. atabrine; curve *d*, in the combined presence of 100 μg of sulphathiazole + 100 μg/ml. atabrine

Chemical nature of competition among the reactants. In view of the common basic groups in spermine, atabrine and streptomycin, competition among them for the acidic groups in DNA and in bacterial structure must be held in view. In the ribosomal system of *E. coli*, the incorporation of ^{14}C-valine is inhibited competitively by spermine and streptomycin[8]. The incorporation of ^{14}C-valine is inhibited 36 per cent by 100 μg of spermine and 92 per cent by 100 μg of streptomycin. The inhibition by streptomycin *per se* is reduced to 44 per cent if spermine is also present. This reduction of inhibition is independent of the order of the addition of the two competitors. The results may, perhaps, be interpreted to indicate that spermine has sufficient affinity for the common reactive sites of the system to be capable of reducing the inhibition by streptomycin to the level of its own inhibition. Relating this observation to the effect of their combined action on sensitive bacteria, one would ask the question whether or not streptomycin could displace spermine competitively from its complex with bacterial DNA and thereby nullify their combined action in the prevention of the emergence of resistant strains. Such an event, which would be expected to occur if streptomycin could have displaced spermine from its reaction sites in bacteria, was not observed.

Spermine with two secondary and two primary basic amino groups is very active. Spermidine with one secondary and two primary basic amino groups is less active. In contrast, putrescine and cadavarine with only two primary amino groups and agmatine with one guanido and one primary amino group are inactive. Generally, the basic property of the alkylamines is greater than that of ammonia and increases with the number of alkyls introduced. On this basis, the tertiary amino group will combine with the acidic groups of DNA more tightly than the secondary, and the latter more tightly than the primary, alkyl-amino groups. The inactivity of putrescine, cadaverine and agmatine is possibly due to their weaker binding and they are therefore readily displaceable by streptomycin. Supporting evidence is provided by Felsenfeld and Huang[9], who found that spermine binds more tightly to nucleic acids than putrescine.

The reports regarding the competitive displacement by streptomycin of acriflavine and acridine orange, from their combination with DNA[7], are of limited relevance to the results obtained with atabrine. Since the two primary amino groups in acriflavine and the two dimethylamino groups in acridine orange are attached directly to the aromatic nucleus, they may be considered as weaker bases than the primary, secondary and tertiary aliphatic amines. This difference arises from the basic character of the aliphatic radicals in contrast to the acidic character of the aromatic radicals. Their degree of affinities for DNA would, hence, be expected to be of different order. Streptomycin would readily displace aromatic amines but fail to displace the aliphatic amines or displace them with greater difficulty. Acriflavine or acridine orange would therefore compare to putrescine, cadaverine and agmatine in their failure to prevent the emergence of resistance. In contrast, atabrine, like spermine, because of its secondary and tertiary amino groups in the branched aliphatic side-chain, would be expected to complex DNA more tightly. In both the salts–glucose–casein hydrolysate or glucose broth medium no evidence of atabrine displacement by streptomycin with *E. coli* was observed. Nor were any observed with staphylococci growing in glucose broth. Occasionally, however, staphylococci grew in the salts–glucose–casein hydrolysate medium containing streptomycin and atabrine and 3 per cent phosphate salts. On reducing the phosphate concentration of the medium from 3 to 1 per cent growth failed to occur in the presence of streptomycin and atabrine during an incubation period of 163 h. Thus, streptomycin fails to displace atabrine in these systems.

An examination of the structures of the other antibiotics listed in Table 1 shows that none of them possesses amino groups comparable with those of streptomycin. They are therefore not expected to displace atabrine competitively. In fact, each of them together with atabrine prevented the emergence of resistant strains from the sensitive population of staphylococci.

When we consider DNA as the site of streptomycin action for the mediation of the resistance phenomenon we must consider also the question of whether or not the other drugs listed in the Tables function in a manner comparable with streptomycin. At present we do not have a direct answer to this question. However, on whatever site in the bacterial structure these drugs exercise an antibacterial effect, the fact remains that their resistance-inducing effects are registered lastingly in DNA. One may therefore conclude that the structural parts of a cell are in close communication with each other and exchange effects with DNA. (For supporting experimental data, see refs. 10 and 11.)

As stated previously on the possible role of spermine[2], atabrine by complexing with DNA prevents the resistance-inducing catalytic function of the drugs since neither spermine nor atabrine has any effect on the resistant state once it has come about.

Reduction of bacteria to a 'common status' by atabrine or spermine and the double role of a drug. Spermine or atabrine acting on various species of bacteria reduces them to a 'common status' with respect to the two major roles of drugs; first, as a positive catalyst, a drug mediates the emergence of the resistant strains from individual cells which possess potentiality for acquiring a resistance character; secondly, as a negative catalyst, a drug exercises an antibacterial role according to its specific mode of action. Spermine or atabrine, responsible for the production of the 'common status', prevents the first role of a drug. The abolition of the first role by atabrine or spermine lays open the normal cells and those with the potentiality to become resistant to the antibacterial action of drugs. This relationship may be better understood if we note the fact that when sulphathiazole was antagonized by PABA (Table 1, systems 28 and 29) its bacteriostatic or its atabrine dependent antibacterial role (Fig. 1) was eliminated, whereby a strain more resistant to sulphathiazole than the parent bacteria failed to emerge.

The above interpretation appears to find support in the fact that once an antibiotic, such as streptomycin, mediates the establishment of the resistance phenomenon in the atabrine- or spermine-free system, afterwards, the action of an antibiotic together with atabrine or spermine is no longer effective. In other words, the resistance-inducing role of an antibiotic must be blocked by atabrine or spermine before the bactericidal action of the antibiotic can take full effect on all the bacterial cells.

In the situations considered here, the role of spermine or atabrine may perhaps be compared with the inhibition of DNase action on DNA by atabrine, blocking the substrate regions to the active sites of the specific enzyme[6]. Thus, atabrine, by combining with DNA, renders it unyielding to changes by its specific enzyme. It may therefore be postulated that atabrine combining with bacterial DNA renders it unyielding to changes by an antibiotic, whereby the first role of the antibiotic fails to materialize and the second, the bactericidal role, takes full control of the situation, preventing the emergence of a resistant strain.

Conclusion

Three pertinent facts follow from the results reported here: (*a*) at the concentrations of drugs used, resistant strains emerge from an inoculum of 100 million normal cells; (*b*) the emergence of resistant strains from such an inoculum is prevented from occurring in the combination of a drug and spermine or atabrine; (*c*) the growth of the strains which are sub-cultured once in a drug *per se* is not inhibited by the combination of atabrine or spermine and that drug.

According to the tenets of the 'selection theory', a drug has no function in the catalysis of normal cells, or in causing them to experience the phenomenon of drug-resistance; it is said, also, not to have any action on the resistant cells which are assumed to be present in a normal population. The cells which have multiplied for the first time in the presence of a low dose of a drug must therefore have the same resistance character as those assumed to be present to begin with in an inoculum of normal bacterial population. On these bases the combination of a drug and atabrine, being incapable of preventing the multiplication of resistant bacteria, should permit the multiplication of the drug-resistant cells assumed to be present. The experimental data presented in the previous[1,2] and present investigations do not support these teachings of the 'selection theory'. Hundreds of inocula, each containing 100 million normal cells and capable of yielding resistant strains in the presence of drugs alone, failed to yield resistant strains in the combined presence of drugs and spermine or atabrine. On the basis of these facts, in the absence of spermine or atabrine, the drugs must have selectively catalysed the cells possessing potentiality into the resistant state. Furthermore, the results reported previously[10] show that sulphathiazole brings about mass transformation in the enzymatic composition of bacteria. The results with 25–50 cells as inoculum show also that this drug produces the resistant phenomenon (Fig. 1) in the absence of a selective process.

Atabrine has previously been shown to be an anti-malarial agent, and, since its very recent clinical trials in combination with antibiotics did not produce any undesirable effects, it is hoped that we are, perhaps, on the road to being able to cope with the medical aspect of the drug-resistance problems. It is also suggested that the principle outlined in this investigation may find application to non-bacterial systems, such as cancerous and viral systems.

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MORE FOSSIL PRIMATES AND OTHER MIOCENE MAMMALS FROM NORTH-EAST UGANDA

By Dr. W. W. BISHOP
Curator, Uganda Museum, Kampala

THE existence of fossiliferous Miocene strata in Uganda was first recorded in 1958. Since that year various investigations have been carried out and short accounts of the fossiliferous sediments and their contained fauna have appeared in *Nature*[1–3] and elsewhere[4,5].

In December 1963 and January 1964, with financial assistance from the Boise Fund of the University of Oxford, an expedition was organized, based on the Uganda Museum, Kampala. The aim was to carry out further detailed investigations of various sites at the two principal fossiliferous localities associated with the extinct and eroded volcanoes of Napak (34° 17′ E., 2° 5′ N.) and Moroto (34° 45′ E., 2° 32′ N.) in Karamoja District, north-east Uganda.

The expedition was fortunate to have the services for three weeks in January 1964 of Mrs. Sonia Cole. Dr. René Lavocat, Directeur de l'École des Hautes Études, Paris, also joined the field party for the same period. His experienced field identifications and enthusiastic pursuit of fossil rodents and primates contributed greatly to the success of the expedition. Field assistance was given by Mr. J. Nzabonimpa and other staff of the Museum and by Mr. F. Onyi and Mr. S. Rhemtulla, students at Makerere University College. I thank all these people, the Commissioner of the Uganda Geological Survey, Mr. and Mrs. Stevenson of Iriri, Karamoja, and others, for their assistance.

The following is a brief statement of the results of the expedition, placing on record a note of the fossils recovered, based on preliminary field identifications. A considerable fauna and a large corpus of stratigraphical information are now available in the Museum on the Uganda Miocene, and a monograph is in preparation which will include full details of the 1963–64 expedition and of earlier work.

Sediments

Table 1 records all the finds of fossils made by the expedition at the two main groups of localities which were investigated.

Group *A* embraces the sites: Napak I, IV, V, IX and X, from which the bulk of the mammalian fauna was recovered. As suggested previously[2], the excellent state of preservation of the fossils at these sites is the result of burial in calcareous, sub-aerial pyroclastic deposits, which are usually medium to fine tuff in grade.

Group *B* includes the sites Napak II and Moroto I and II. Here, owing to the deposits consisting primarily of gritty sediment resting on or near a surface of Pre-Cambrian rocks, the preservation is much less perfect. 'Small fauna' is virtually absent and a rather different ecological setting from that of Group *A* is suggested by those species which are preserved as identifiable fossils.

One of the aims of the expedition, in addition to adding to the faunas from different sites, was to relate the fossils as clearly as possible to their parent deposits by undertaking archæological-type excavations. From these, details of the lithologies and the mode of occurrence of the fossils were obtained with the view of reconstructing from the stratigraphical and palæontological evidence the nature of the local environment at each of the faunal sites.

As an adjunct to such palæo-ecological investigations it was hoped to establish a reason for the 'fossiliferous patches' which occur within the tuffs. Three such bone concentrations were noted during the 1961 expedition[2], and a further 'patch', Napak IX, was located in 1962 by the Brathay Exploration Group expedition to Uganda[6]. Again during the present expedition the bulk of the pieces of mammalia collected came from the four Group

Table 1. DETAILS OF FOSSILS FOUND BY EXPEDITION, 1963–64

	Group *A*						Group *B*			
	Napak I	Napak IV	Napak V	Napak IX	Napak X	Totals Group A	Totals Group B	Napak II	Moroto I	Moroto II
Total mammalia	345	1,410	522	205	1	2,483	1,021	565	198	258
Indeterminate bones	162	1,009	379	144	—	1,694	919	520	183	216
Better bones	139	188	85	35	—	447	27	11	6	10
Skulls, teeth and jaws	44	213	58	26	1	342	75	34	9	32
	Skulls, teeth and jaws									
Rodent	33	158	23	4	—	218	2	1	—	1
Proboscidea	2	1	—	16	—	19	33	16	1	16
Artiodactyl	6	14	18	4	1	43	6	1	2	3
Perissodactyl	—	5	—	—	—	5	3	3	—	—
Carnivore	—	6	3	—	—	9	2	1	1	—
Insectivore	—	2	—	—	—	2	—	—	—	—
Primate	—	18	7	1	—	26	2	—	—	2*
Unidentified	3	9	7	1	—	20	27	12	5	10
	Non-mammalian fossils									
Reptile	10	65	35	2	—	112	684	359	316	9
Bird	—	4	1	2	—	7	1	—	—	1
Fish	—	—	—	—	—	—	3	3	—	—
Millipede	1	4	—	—	1	6	—	—	—	—
Gastropod	5	74	49	29	5	162	1	1	—	—
Fruit	—	—	2	—	22	24	—	—	—	—
Coprolite	—	—	—	—	—	—	147	90	57	—

* Two individuals represented by 56 fragments.

A sites, Napak I, IV, V and IX, which range from 50 ft. to 150 ft. in diameter. Few fossils were found from the outcrops of the tuffs which are the lateral equivalent of those at the fossil sites, despite deliberate and careful search over the mile or more of exposure revealing apparently identical lithologies. The problem was to try to establish whether the concentrations represented a biocœnosis, or life association, of the animals recorded by the fossil evidence, or a death assemblage (thanatocœnosis).

In a pyroclastic deposit it is tempting to invoke some occurrence recalling the destruction of Pompeii or suggesting the former existence of a fumarole emitting poisonous gas. However, the virtual absence of complete skeletons or partially articulated limbs as revealed by excavation makes this unlikely. In addition the occurrence of signs of gnawing and chewing on many of the fossil bone fragments, together with the very high proportion of skulls, teeth and jaws in relation to post-cranial fragments (an average of 14 per cent of total finds for all the Group *A* sites (Table 1), suggests the action of predators in devouring the carcases and scattering the inedible remnants prior to their gentle stratification by ash from the next explosive phase of volcanic activity.

The life association might possibly have arisen from concentration of animals as dictated by the presence of some necessity such as a water-hole or salt-lick. With a high percentage of primates it was just conceivable that, even in rocks as early as Miocene, the fossil concentrations represented bone accumulation following a successful hunt. The results obtained have shown that although the fossils originate as part of natural death assemblages their secondary concentration in patches arises from different causes.

The method of approach was to clear surface vegetation and then to rake and brush all loose detritus on the fossil sites into small heaps. This unconsolidated material was then passed through one or more of three grades of large sieve. The meshes used were 1 mm, 2 mm and 4 mm. The yield of fossils from the sieves reflected the richest parts of a site much as in geochemical prospecting.

Trenches were then opened at the most promising localities, to expose the sediments and fossils *in situ*. The excavations revealed that the fossils in the rich patches were not derived from a single 'bone bed' but in every case from several thin and sparsely fossiliferous layers separated from each other by a few feet of barren tuff. These breaks in deposition showed various degrees of soil formation and frequently had casts of horizontally ramifying roots of fossil vegetation. At Napak I six such horizons occurred within 35 ft. of tuff. Although the yield of fossils from each horizon was small, identifiable and apparently identical rodents were recorded from all the levels. Again, at Napak IV, where the most extensive excavations were carried out, three similar discrete horizons, which yielded fossils consistently yet sparingly, were located within 25 ft. of sediment.

An important factor contributing to the patches appeared to be the presence of a suitable present-day catchment area in association with the fossiliferous layers to enable a secondary concentration of fossils to be built up through gradual etching-out by processes of weathering and erosion acting over a long period of time.

At Napak IV the catchment area is provided by a basin-like dry valley with a restricted outlet. At Napak I, V and IX (and also at X where few Mammalia but numerous fruits were obtained) the catchment area is provided by flat benches or berms, underlain by horizontal resistant strata, occurring on the ends of spurs. The specimens derived from the thin fossiliferous horizons on the steep slopes are concentrated on the 'flats' at their bases.

One other variable which seems to contribute to the resistance of the fossils to weathering, which is essential for the natural but gradual etching-out of well-preserved specimens, is the immediate chemical environment or sedimentary matrix of the fossil.

In thin section the tuffaceous matrix in which the fossils are embedded can be seen to possess a calcite cement. Because of recrystallization it is virtually impossible to be certain of the initial composition of the ash which blanketed the original bones. However, chemical analyses show that at present the fossil bones, tusks and teeth have a CaO content ranging from 44 to 49 per cent, while the tuffs forming the matrix range from 37 to 40 per cent. A thin layer of calcite frequently encrusts the fossils and binds them together as coherent specimens even when the bone or tooth enamel itself exhibits numerous cracks.

At all the fossiliferous localities veins of secondary calcite occur and in places the tuffs have been sufficiently calcified to warrant their being referred to loosely as 'limestones'. Some joint planes are marked for short distances by bands of calcareous tufa up to three-quarters of an inch in thickness. At Napak IV immediately above the fossil site and centrally situated in relation to the fossiliferous area, a former spring-eye is marked by a 'motor tyre' structure of travertine. There seems little doubt from the nature of the upper surface of the travertine that the spring was a recent feature. However, as a result of movement of calcium-rich surface and ground water near the spring-eye, the tuffs are intensely calcified and all joints and cracks are filled with calcium carbonate.

It seems probable that the deposition of travertine at Napak IV and possibly the action which gave rise to the bands of tufa at other localities, although of comparatively recent age, gave to the fossils that extra durability which enabled them to survive on erosion to become secondarily concentrated in their respective catchments. A few of the fossils which were recovered by excavation had escaped secondary calcification. When this was the case the bones were exceptionally fragile and required very careful removal and preservation owing to their cracked condition. Such fragile specimens would become comminuted while in the soil instead of surviving to lie on the surface.

It is suggested that, in the absence of secondary calcification plus a suitable catchment, the tuffs would appear to be largely unfossiliferous. The fossiliferous patches reflect not only a suitable medium for burial and initial fossilization but also a suitable chemical environment and physical catchment to ensure the re-emergence and concentration of recognizable specimens for the palæontologist.

Fauna

Mammalia. During six weeks' field work a total of 3,504 mammalian specimens was collected, of which 417 pieces were parts of skulls, teeth and jaws. Although many of the post-cranial pieces recorded as 'better' bones in Table 1 are well preserved, the extent to which they are assignable to genera or species is variable. Thus comparison of numbers of different groups present at each site has been limited to the more readily and consistently identifiable skulls, teeth and jaws.

Although 1,021 mammalian specimens were recovered from the Group *B* basal grits, only 75, or 7 per cent, were fragments of skulls, teeth and jaws. Of these, broken-up fragments of molars and tusks of Proboscidea, chiefly representing *Trilophodon angustidens* with a few fragments of *Dinotherium*, account for almost 50 per cent. Teeth of other mammals occur only sparingly at these sites, but the presence of rhinocerotids and anthracotheres is of interest as both are rare at Group *A* sites. Carnivore remains have not been recorded previously from the Group *B* localities, but a tooth from Napak II recovered during the recent work appears attributable to *Pterodon*, which in the form of *Pterodon africanus* has been recorded from Napak I. The small fauna of primates, insectivores and rodents is virtually absent from Group *B* although single rodent incisors were recorded for the first time at Napak II and Moroto II. The remains of large primates recovered from Moroto II are discussed below.

In sharp contrast to the assemblages at the Group *B* sites is the overwhelming abundance of rodents at the Group *A* sites where they comprise 218, or 64 per cent, of the total of 342 skulls, teeth and jaws collected. However, there is considerable individual variation in the percentages of rodents from site to site. At Napak IV the 158 rodents represent 74 per cent of the total, and at Napak I 75 per cent, but at Napak V the percentage drops to 40 per cent and again to 16 per cent at Napak IX.

Artiodactyls also show considerable variation in relative abundance with 32 per cent of the total at Napak V falling to 16 per cent at Napak IX, 14 per cent at Napak I and 7 per cent at Napak IV. Where the total number of skulls, teeth and jaws collected at a site is small, some doubt may exist as to whether the observed differences are significant. However, if reference is made to the 1961 collections from the same sites a remarkably similar picture is presented. At that time rodent percentages were also highest at Napak IV with 84 per cent and artiodactyls at Napak V with 34 per cent of the total.

Proboscidea are more difficult to assess, as both tusks and molars are easily fragmented on exposure to weathering. Thus a single individual may be represented in the collections by numerous fragments. This appeared to be the case at Napak IX, where 16 fragments of proboscidean teeth were collected in a total of 26 pieces. At Napak I, where numerous fragments of Proboscidea have been collected previously, only two more were found in a total of 44 pieces. By contrast another 33 rodents were recovered, presumably because the smaller rodent fragments are more difficult to see. Rodents as a whole are so numerous that they offer the best basis for comparison between localities differing slightly in age or ecological setting, with histograms illustrating the nature of each fauna as in micropalæontology.

Primates, which are normally reticent candidates for fossilization, have been consistently present at four of the Group *A* sites. At Napak IV they were next in abundance to rodents and the 18 specimens constituted 8 per cent of the total. At Napak V, 7 more specimens were recovered comprising 12 per cent of the total compared with 14 per cent in 1961. The primate finds are dealt with in more detail below.

The mammalian fauna, so far identified from all the Group *A* sites at Napak, bears a remarkable similarity to the genera and species occurring in the assemblages from Rusinga and Songhor in Kenya, although the relative abundance of many of the species may later be shown to differ.

Lower Vertebrates and Invertebrates. A total of 1,147 non-mammalian fossils was collected comprising reptiles, birds, fish, millipedes, gastropods, fruits and coprolites. The numbers in each group vary tremendously as shown in Table 1, with reptiles by far the most numerous with 796 pieces and land snails next in importance with 163 individuals. The non-mammalian fossils help to emphasize differences in the nature of the assemblages and in the type of preservation at the two groups of sites. Reptiles are most abundant at the Group *B* sites, where they consist chiefly of chelonian scutes, while the land snails are entirely restricted to Group *A*.

At the Group *A* sites reptiles are comparatively insignificant in numbers compared with mammals (a total of 112 pieces compared with 2,482). At these sites the principal reptile remains are snake vertebræ, lizard scutes and chelonian fragments. The millipedes and fruits are few in number and restricted entirely to Group *A*, as for the most part are the bird remains, while fish and coprolites have been recorded only from Group *B*.

There seems little doubt from the Group *A* faunal evidence of the sub-aerial nature of the tuff environment. The Group *B* fauna suggests a waterside setting.

Primates. Between 1958 and 1963 nineteen fossil fragments identifiable as primate were recovered from the Napak Group *A* localities and eight fragments from Moroto II [1-3]. The success of the 1963–64 expedition in this field can be seen from the fact that another 28 individual specimens representing primates (26 parts of skulls, teeth and jaws (Table 1)) were collected at Napak. At Moroto II, 56 fragments were recovered representing parts of probably not more than two individuals.

The majority of the finds at Napak were from site IV, which yielded a total of 18 pieces of skulls, teeth and jaws. With the exception of *UM.P.* 64–01, a right upper M^1 of *Proconsul major*, the majority of the finds were of small primates[6].

The most complete specimen from this site *UM.P.* 64–02 is an undeformed palate and snout which seems attributable to a small species of the hylobatine genus *Limnopithecus* or *Pliopithecus* (Simons has recently proposed that the genera *Limnopithecus* and *Pliopithecus* can no longer be considered distinguishable[7]).

The specimen *UM.P.* 64–02 is the most complete single specimen so far recovered of a small representative of this genus. Although still not fully developed from its block of matrix it can be seen to retain on the left side M^1, M^2 and M^3 and the root of the canine, and on the right side, P^3, P^4, M^1, M^2 and M^3. The face is present as far as the lower portion of the left orbit. The remaining site IV finds are a variety of small fragments and isolated primate molars, canines and incisors of varying sizes. Some are larger and some smaller than appropriate teeth of the *Limnopithecus* specimen *UM P.* 64–02 referred to here.

Although most of these specimens were recovered by sieving the surface detritus, seven specimens were found *in situ* in two small excavations. The density of primate remains is remarkable as the whole of Napak site IV measures only 45 ft. by 50 ft., while the excavations are 4 ft. by 9 ft. and 5 ft. by 8 ft., respectively, and were dug into bands of tuff less than 1 ft. in thickness.

Napak V yielded seven specimens all from the surface detritus, including five single teeth of small primates, a lower premolar of *Proconsul*, and an upper right lateral incisor with broken crown, which is also ascribed to *Proconsul*. Napak IX yielded two specimens, a fragment of right mandibular ramus of *Limnopithecus* containing M_2 and M_3, and the head of a femur possibly of the same genus. The four Napak sites have now yielded in 6 years a total of 45 parts of primate skulls, teeth and jaws representing fossil Pongidae, Hylobatinae, Galagidae and possibly Cercopithecidae.

By contrast with the variety of forms and the number of individuals recovered from Napak, the site at Moroto II is remarkable for the fact that although 64 primate fragments have been recovered they probably represent not more than three individuals of the single species *Proconsul major*.

A brief history of the finds at Moroto II is as follows: In August 1961 at the end of an expedition to Uganda from the University of Glasgow, Bishop and Whyte found the new site Moroto II which yielded, following only a brief investigation, two primate fossils. These were part of a left maxilla without teeth (*UM.P.* 62–11), and a fragment of a massive right mandibular ramus (*UM.P.* 62–10).

In December 1961 Prof. D. B. Allbrook was able to visit the new Moroto site with a party of students from the Department of Anatomy, Makerere University College. They found a large piece of the right maxilla and two adjoining fragments of the original specimen *UM.P.* 62–11, together with a single left upper canine (*UM.P.* 62–12), and two small mandibular fragments probably part of

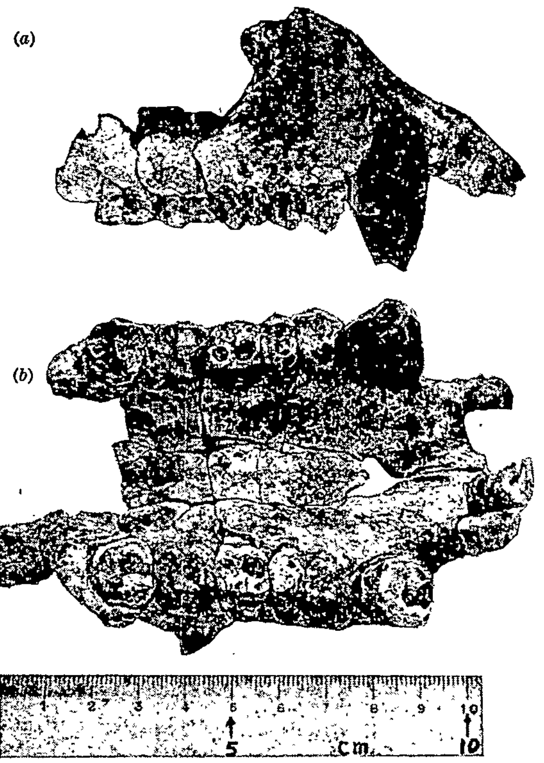

Fig. 1. The new palate *UM.P.* 62–11 from Moroto II. (*a*) Lateral view; (*b*) palatal view

UM.P. 62–10. All this Moroto II primate material and another seven pieces found between 1958 and 1962 at Napak (*UM.P.* 62–03 to *UM.P.* 62–09) have been attributed by Allbrook and Bishop to the species *Proconsul major*[3].

Despite a time-lag of 2·5 years since the discovery of the first primate fossil at Moroto II the recent expedition has now recovered, by sieving and excavation at the original site, 56 additional pieces, of which 49 seem to belong to the palate *UM.P.* 62–11 and a further 7 with the fragmentary mandible *UM.P.* 62–10.

It is not proposed to describe the new material in any detail here as a full account of all the Uganda primate fossils is in preparation. However, as the palate *UM.P.* 62–11 is the most complete upper dentition known of a 'Lower' Miocene ape, apart from the classic Rusinga skull of *Proconsul africanus* discovered by Dr. L. S. B. and Mrs. Leakey, a preliminary account of the Moroto II specimen is given here.

When originally described, the specimen only had the crowns of three teeth, right P^4 and M^1 and left M^2 (ref. 3, Fig. 1). An additional 14 pieces have now yielded at least parts of the crowns of another 12 teeth.

Details of the teeth are given in Table 2. The molar–premolar series is now complete on both sides of the palate although a small chip of enamel is missing from the anterior/lingual corner of the right M^2 and another chip from the lingual portion of left P^3. The left canine is broken off and missing just above the base of the enamel, but the right canine is almost complete although reconstructed for about 7·0 mm above the alveolus and lacking the tip of the crown (Fig. 1*a*). Both left incisors are entire, but the right lateral incisor although complete in the crown is lacking a portion of the root for 3·0 mm above the alveolus. The sixteenth tooth, the right medial incisor, was lost during the life of the creature and the alveolus in the fossil is partially filled with bone. Both lateral incisors show elliptical wear facets from contact with the lower canines.

Table 2. TEETH OF SPECIMEN *UM.P.* 62–11

	Left			Right		
	A.P. (mm)	*Tr.* (mm)	*Ind.*	*A.P.* (mm)	*Tr.* (mm)	*Ind.*
M^3	12·7	13·5	106	13·0	13·7	105
M^2	12·9	13·3	103	13·0	13·4	103
M^1	11·7	12·0	103	11·0	12·0	109
P^4	7·7	14·3	186	7·5	14·0	187
P^3	9·8	13·9	142	9·9	13·9	140
C	19·0	15·5	82	18·0	15·0	83
I^L	9·7	8·2	85	9·7	8·1	84
I^M	8·7	10·3	118	—	—	—

Eleven more fragments of the palate and lower face have been added to the specimen, while another seven fragments comprising the nasal area as far as the base of the orbit have been reconstructed into one piece. This specimen joins on to the reconstructed snout and palate at one point but is not included in Fig. 1*a*. The complete specimen has been reconstructed from a total of 36 fragments. Another 17 pieces undoubtedly belong to the same specimen but cannot be fitted into place.

Although the specimen has been reconstructed from so many fragments the bones are massive and all the breaks are sharp and unabraded, which suggests that the specimen had only just eroded out and that it broke up immediately on doing so. The jagged edges of adjoining pieces fit together with a perfection which prevents errors in reconstruction. As the specimen is free from post-mortem deformation the profile of the muzzle and the morphology of the palate can be seen perfectly and recall strongly those of maxillary specimens of *Proconsul africanus* from Kenya, although the Moroto II specimen is much larger. (Average length of cheek teeth row in *P. africanus* 35 mm, and in *UM.P.* 62–11, 54·6 mm.)

Specimen *UM.P.* 62–10 is now represented by ten fragments although only two can be joined together. There is little hesitation in associating them as originally part of one individual, as the bone has a characteristic purple-brown patination and the fragments were all recovered from a surface area 8 ft. in diameter. This location is 40 ft. away across an erosion gully from the spot at which the parts of *UM.P.* 62–11 were found but lies on the same stratigraphical horizon.

Four of the specimens are only broken pieces of bone and three others retaining parts of the roots of teeth are too fragmentary and abraded to warrant further comment here. One piece shows the broken-off root of a large canine tooth still surrounded by massive but rather worn bone. The portion of canine is 27·0 mm in length and measures 17·0 mm by 13·0+ mm at its widest part.

The fragment of massive right mandibular ramus *UM.P.* 62–10, described by Allbrook and Bishop[3], has now been joined to another mandibular fragment containing the very broken crown of M_1 and completing the anterior portion of M_2. The measurements of the shattered crown of M_1 are *A.P.* 10·0 mm, *Tr.* 10.0 mm, and of the root of M_2, *A.P.* 11·4 mm, *Tr.* 12·7 mm.

Although the identifiable fragments of *UM.P.* 62–10 and 62–11 represent lower and upper dentitions respectively of a large fossil ape, the separation of the two finds into two subsites in the same gully, their different preservation, and the fragmentary nature of *UM.P.* 62–10 make it doubtful if they represent parts of one individual. It is suggested that two individuals are possibly involved with the isolated upper canine *UM.P.* 62–12 probably representing a third.

Throughout this paper the Moroto II specimens have been assigned to the species *Proconsul major* as defined by Le Gros Clark and Leakey[6]. The reasons for the original designation of *UM.P.* 62–11 as belonging to this species were set out at some length by Allbrook and Bishop[3].

However, it may be added that Simons[7] has recently directed attention to the proliferation of genera in Miocene primates and has suggested that the existing material should be assigned to only three or four genera. *Proconsul* and *Dryopithecus* are included in these four, but it is worthy of note that Simons makes reference to "the probability that *Proconsul* cannot be separated generically from *Dryopithecus*".

In the face of such views, together with such considerations as the variability of dentition and skull morphology in living pongids, and the range of variation in fossil specimens owing to sexual dimorphism, it seems premature to do more than refer the specimen to the existing species *P. major* at this stage. It is unfortunate that the bulk of the Kenya specimens of *P. major* are mandibular fragments or from lower dentitions, with the exception of two isolated upper second molars, while the majority of the Uganda specimens are by an odd coincidence maxillary fragments or upper teeth. The similarities between the two upper second molars from Kenya (5·381′49 and *CMH.* 35(5)) and the relevant teeth from Napak and Moroto have been referred to previously[3].

The mandible fragment (*UM.P.* 62–10) from Moroto II is remarkably similar to the relevant fragment of *Proconsul major* recovered from Songhor in Kenya. Although it has already been shown that the upper and lower dentitions from Moroto II cannot safely be considered as belonging to the same individual, their general similarity in size makes it probable that they belong to the same species. Until it can be shown beyond doubt that two apes of almost identical size and similar morphology which lived and died close together at Moroto II nevertheless belonged to separate species, it seems preferable to apply Occam's Razor and assume that they were two individuals of the same species.

A similar argument can be applied at Napak, as molars morphologically identical to those of *UM.P.* 62–11 from Moroto II have been recovered from Napak I, Napak IV and Napak V. Table 3 compares details of the upper first and second molars recovered from Napak and Moroto. The additional fragments of the canines from the palate *UM.P.* 62–11 confirm in size and morphological similarity the comparison which was made by Allbrook and Bishop[3] between the six upper canines from Uganda. These are *UM.P.* 62–11 and 62–12 from Moroto II and from Napak I (*UM.P.* 62–03) and Napak V (*UM.P.* 62–04 and 05). The broken right lateral incisor from Napak V has dimensions *A.P.* 9·8 mm, *Tr.* 7·9 mm and an index of 81 which compares closely with the lateral incisors of the Moroto II specimen. It also has a similar elliptical wear facet.

Table 3. VARIOUS UPPER FIRST AND SECOND MOLARS FROM UGANDA

Details			*A.P.* (mm)	*Tr.* (mm)	Index
Moroto II	*UM.P.* 62–11	Left M^1	11·7	12·0	103
Moroto II	*UM.P.* 62–11	Right M^1	11·0	12·0	109
Napak V	*UM.P.* 62–07	Right M^1	11·6	13·0	112
Napak IV	*UM.P.* 64–01	Right M^1	11·7	13·5	115
Moroto II	*UM.P.* 62–11	Left M^2	12·9	13·3	103
Moroto II	*UM.P.* 62–11	Right M^2	13·0	13·4	103
Napak V	*UM.P.* 62–08	Left M^2	15·2	17·2	113
Napak I	*UM.P.* 62–09	Left ? M^3	15·0	—	—

Conclusion. Fossil remains of *Proconsul major*, an ape exhibiting slight variations in dentition and morphology, similar in range to those to be observed in the modern gorilla, have now been recovered from four separate sites in Uganda. These are Moroto II (2 or 3 individuals), Napak I (2 or more individuals), Napak IV (1 individual) and Napak V (at least 2 individuals). From Kenya the species is known from Songhor and Rusinga, and the general faunal similarity of the Napak assemblage to the faunas of these two sites has already been commented on.

Simons suggests that detailed comparisons may show material virtually identical with the species *Proconsul major* of East Africa to occur among the Miocene 'Siwalik' apes of India and Pakistan.

In ending this article on the new material from Uganda perhaps one cannot do better than to echo part of a sentence from the pen of Prof. T. N. George. It is to be hoped that more new genera and species of Miocene primates will not be established which are based on fossils "differing morphologically in only minor ways from specimens already described"[9].

Thus the Moroto II palate is ascribed to *Proconsul major*. With the other new finds from Uganda it should greatly assist comparative investigations of East African, European and Siwalik material. Until these are carried out, *Dryopithecus* and *Proconsul* seem best viewed, again as suggested by Prof. George, as variable "collateral representatives of the generalized radical from which both the pongids and hominids sprang".

Radiometric Dating

During the 1961 expedition a series of specimens collected from Napak and Moroto was sent for ^{40}K/^{40}Ar dating to Drs. J. F. Evernden and G. H. Curtis, of the University of California, Berkeley, and Dr. P. Damon, of the University of Tucson, Arizona.

I thank Dr. Damon for details of the first determination. This is for biotite from a sample of tuff within the fossiliferous sediments at Napak I. The apparent age obtained was 19 ± 2 million years. (Full details of this determination together with results for other samples will be given as soon as available.)

Dr. Damon writes: "The potassium content indicates that the biotite has not been seriously perturbed by ion exchange and the retentivity should be good in the absence of subsequent reheating. The biotite flakes and books were ragged in some cases, rounded in others and of different sizes. The possibility has to be borne in mind of contamination by older biotite from basement rock. A large error is assigned because there was only enough biotite for a single analysis and the air correction was two-thirds of the total argon".

The age of 19 ± 2 million years would fall within the Burdigalian which agrees with the generally accepted faunal age of the East African 'Lower Miocene'. It would also be in keeping with the ^{40}K/^{40}Ar age of 14 million years quoted for the Fort Ternan fauna[10]. However, an age of 15·3 ± 1·5 million years has been recorded by Evernden and Curtis as their best date for tuffs on Rusinga Island[7]. As this date refers to horizons older than those which yielded the 'Burdigalian' fauna from Rusinga a problem is raised by the short period of time available for the development of a virtually new series of forms at Fort Ternan in all the faunal groups, compared with those occurring in the 'Lower Miocene' at Rusinga, Koru and Songhor and at Napak.

All the differences between the typical 'Lower Miocene' fauna and the contrasting assemblage at Fort Ternan are unlikely to be ecological, and it would seem that the Napak date appears more realistic at present from the palæontological evidence. However, it must be emphasized that these are merely the first results from radiometric dating of fossiliferous Tertiary strata in East Africa, while faunal correlations between Europe and Africa, based on land mammals, are somewhat speculative.

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NATURAL RUBBER RESEARCH

A CONFERENCE to mark the silver jubilee of the Natural Rubber Producers' Research Association (NRPRA) was held at St. John's College, Cambridge, during April 7–9, and was attended by more than 150 delegates from the universities and industries of many countries.

The Association was formed in 1938 to meet the need for research into the nature and properties of natural rubber. Together with its sister organization, the Rubber Research Institute of Malaya, it is financed by and operates under the ægis of the Malayan Rubber Fund Board in Kuala Lumpur. It thus represents an unusual, if not a unique, variation in the present pattern of technical assistance, being a scientific foundation supported by a developing member of the Commonwealth in the territory of an industrial member—to the reciprocal benefit of both. In setting up the Association it was recognized that rubber technology needed to be based on a firmer scientific foundation and that the time had come when it was necessary to do something about it. These times were recalled by Sir Harry Melville in a plenary address in which he traced the achievements of the Association over the past twenty-five years. Sir Harry pointed out that doubts existed in the 1930's as to whether rubber was really a high molecular weight polymer at all and that then there were still supporters of the 'micellar' or association theory of natural polymers, according to which the polymers consisted of small molecules associated by unusually strong residual valency forces into aggregates or micelles.

The problem that faced the British Rubber Producers' Research Association, as it was then, was the introduction of a rationale into the technology of making satisfactory rubber materials and components. To achieve this end it set out on investigations which were to bring clearly into focus the molecular make-up of natural rubber and similar polymers and the physical background of their unique mechanical properties. In describing the prominent contribution of NRPRA to the development of polymer science, Sir Harry directed particular attention to the mechanism of vulcanization, oxidative degradation and rubber elasticity.

Stimulating plenary addresses were also given by Prof. Herman Mark (Brooklyn) and Prof. James Bonner (California). Prof. Mark described recent advances in polymer chemistry with special reference to new types of co-polymers, including 'ladder' polymers. He stated that the recent advent and commercial production of so-called 'stereo' rubbers had led to great interest in, and fuller understanding of, the mechanism of ionic polymerization. The use of ionic catalysts offered the possibility of producing a much wider range of materials than could be made with free radical catalysts and unusual combinations of monomers had now become possible. Of special interest were co-polymers which exhibit flexibility and stability at extremely high temperatures, and Prof. Mark demonstrated the outstanding high-temperature properties which could be achieved with ladder polymers.

Prof. Bonner discussed some of the problems likely to arise in future investigations of the control mechanisms in rubber biosynthesis. When latex was withdrawn from the tree, enzymes and co-factors essential for the regeneration of rubber were also removed. This drastic disturbance in the physiological balance of the laticiferous tissue might mean that the rate of enzyme synthesis was of particular importance in determining the yield of rubber from a tree.

The conference sessions were devoted to tw
topics: the ultrastructure and metabolism of
latex, and the chemical structure and mechani
perties of vulcanized rubber. Great interest was s
work on the mechanism of rubber biosynthesis.
rubber is but one member of a large family of comp
the terpenoids—all of which appear to be formed
common precursor, isopentenyl pyrophosphate
distinctive molecular features of natural rubber
extremely high molecular weight and the fact
possesses a uniformly *cis*-geometrical config
Other papers presented showed that encouraging
had now been made in understanding the det
subtlety of the structure of cross-linked rubber n
and the relationship between these featur
mechanical and physical properties of the vu
rubber.

In an introductory lecture to the symposium
first topic, Prof. G. E. Blackman (Oxford) discu
reasons for the unique position of the specie
brasiliensis as a commercial source of natural
One of the most important factors is that this
possesses an anastomosing system of latex vessel
allows a considerable area of bark to be drained
at each tapping, and the regeneration of latex is suf
rapid for the tapping to be repeated every two da
without injury to the tree. Breeding and s
work at the Rubber Research Institute of Malay
the past forty years has increased the yield of
per tree more than three-fold. Further increase
obtained by applying to the bark growth regulat
as 2,4-dichlorophenoxyacetic acid or the 2,4,5-t
analogue. The use of these materials results
withdrawal of latex from a larger area of bark per t

Electron microscope studies of the ultrastru
Hevea brasiliensis latex vessels were described
P. B. Dickenson (NRPRA). The vessels reta
late stage of maturation the recognized ultrast
features of a plant cytoplasm, but with some
features. Rubber particles and two main types
rubber particle are present at an early stage in the
ment of the vessel and, as maturation procee
became increasingly predominant while the p
cytoplasm progressively degenerates. Howev
Dickenson emphasized that both structural a
chemical characteristics indicated that tappe
latex is part of a specialized cytoplasm.

The commonest non-rubber particle is the
which in young latex vessels passes through a tr
phase characterized by inclusions of proteinaceou
fibrils which have a soluble helix structure. Th
fibrils disappear from the particles as the ves
A vigorous discussion took place concerning t
of these particles.

The less-frequently-occurring Frey-Wyssling
composed of lipids and often containing car
originate as inclusions in larger organized pa
structures. Within these, there is also a separat
elle containing a complex of ramifying tubules
ordered structure. The lecturer suggested that
stant association of the lipid particles with this
implied a possible functional role of the latter
carotenoid synthesis.

The composition and stability of the lutoi
fibrils were discussed in a paper by Dr. B. G.
(NRPRA). One of their most interesting fe
the reversible break-down and re-formation of t
fibrils under suitable conditions of temperature

Temperatures below 0°C and *p*H values close to the isoelectric point of the protein favour the reassembly of the fragments into long microfibrils. The mechanism of this process aroused considerable discussion, centring mainly on the question whether separation of the double helix into single strands was a necessary preliminary to both break-down and re-formation of the microfibrils. Dr. Audley stated that the metabolic function of the microfibrils and the lutoid particles themselves was not known, but that neither contained ribonucleic acid.

Three papers were presented dealing with different aspects of rubber biosynthesis. Dr. R. G. O. Kekwick (Birmingham) discussed some of the earlier stages in the biosynthesis, with particular reference to the roles of acetate and mevalonate and the influence of ATP on their incorporation into rubber. An unexpected finding was that ATP at moderately high concentrations retarded the incorporation of acetate but not of mevalonate, suggesting that ATP-dependent side reactions might be taking place involving, as substrate, one of the intermediates between acetate and mevalonate.

Dr. D. Barnard (NRPRA) recalled that the work of Bloch, Lynen and others had established that the 'active isoprene' derivative utilized in the build-up of terpenoid chains is isopentenyl pyrophosphate. For terpenoids containing an isopropylidene end group the chains are initiated by dimethylallyl pyrophosphate, formed from isopentenyl pyrophosphate by an isomerase enzyme. So far, this enzyme has not been detected in rubber latex. However, Dr. Barnard described experiments showing that incubation of a mixture of dimethylallyl pyrophosphate and isopentenyl pyrophosphate with the (rubber-free) aqueous phase of latex resulted in the formation not of rubber but of *trans-trans*-farnesyl pyrophosphate. This product was shown to have a marked inhibitory effect on the incorporation of isopentenyl pyrophosphate into rubber. Dr. Barnard pointed out that the presence in the aqueous phase of latex of enzymes capable of forming low-molecular-weight *trans*-terpenoid compounds suggested a likely control mechanism for the utilization of isopentenyl pyrophosphate in latex.

Dr. B. L. Archer (NRPRA) described recent work on the biosynthesis of rubber (*cis*-1,4-polyisoprene) in whole latex. The incorporation of isopentenyl pyrophosphate into rubber *in vitro* appears to occur exclusively at the surface of existing rubber particles, at a rate proportional to the surface concentration of enzyme as calculated from the Langmuir adsorption isotherm. The addition of dimethylallyl pyrophosphate increased the rate of incorporation of isopentenyl pyrophosphate into rubber, provided that the farnesyl pyrophosphate synthesizing enzyme in the aqueous phase of the latex was first removed, thus indicating that rubber molecules can be formed *de novo* from dimethylallyl pyrophosphate in the presence of a rubber surface. Dr. Archer also described work carried out in collaboration with Dr. G. Popjak and his colleagues, in which the incorporation of 2-(^{14}C)-4-(^{3}H)-mevalonate into rubber had been studied. The availability of this material in two stereoisomeric forms had enabled the authors to prove that in the formation of rubber the elimination of a proton from the α-carbon atom of isopentenyl pyrophosphate was completely stereospecific. Furthermore, stereospecificity was in the opposite sense to that previously found by Popjak for the formation of *trans-trans*-farnesyl pyrophosphate in liver systems.

A speculative, but stimulating, paper on the role of rubber in plant metabolism was given by Dr. F. J. Bealing (NRPRA), whose thesis in essence was that rubber formation provides a mechanism for the anaerobic reoxidation of the TPNH produced (mainly) by operation of the pentose cycle. This reoxidation occurs during the reduction of β-hydroxy-β-methylglutaryl-coenzyme *A* to mevalonic acid, and the subsequent formation of rubber was considered by Dr. Bealing to be the means of converting relatively large quantities of the soluble acid to a metabolically inert product. However, this transformation requires the consumption of three molecules of ATP per molecule of mevalonate, and a lively discussion was held on the sources and availability of ATP in rubber latex.

The symposium on the chemical structure and mechanical properties of vulcanized rubber was opened by Prof. G. Gee (Manchester), who discussed some recent developments in the field of rubber elasticity. The main features of rubber elasticity are accounted for by a model consisting of a set of random flight chains linked together into a network. Corrections are needed for the small volume change which accompanies elongation and results in an increase of energy. Recently, account has also been taken of the effect of the energy difference between rotational isomers in the main chain. Separation of these two factors has been achieved experimentally by thermoelastic measurements at constant volume. There still remain small discrepancies between theory and experiment; these may arise from the excluded volume problem in a real chain.

In a paper dealing with the strength of rubbers, Mr. A. G. Thomas (NRPRA) pointed out that many apparently different types of mechanical failure were based ultimately on a common physical process, namely, the propagation of a tear in the rubber. This had led to the development of a unifying way of describing the strength of rubber in which the energy required to create unit area of new surface (the 'tearing energy') was a parameter of fundamental significance. The tearing energy was generally much higher than ordinary surface energies because, in the case of rubber, the energy associated with stress concentration at the tip of a flaw could be readily dissipated throughout the surrounding volume. By means of the tearing energy, cut growth could be theoretically related to fatigue and flex-cracking behaviour; the theory postulated flaws of ascertainable size as initiating this type of failure.

Dr. A. Schallamach (NRPRA), talking on tyre wear, first stressed the importance of the elastic properties of tyres on the amount of sliding, and therefore abrasion, in the area of contact with the road. These effects are as relevant to wear as the abrasion resistance of the tyre tread. The mechanism of abrasion depends on the nature of the road, and at least two processes can be distinguished. On a sharp pointed surface, tensile failure predominates; on smoother surfaces, fatigue governs the rate of wear. In practice, both processes come into operation, and relative ratings depend on the severity of service.

The chemistry of vulcanization was considered in a paper by Dr. C. G. Moore (NRPRA), who first outlined the different experimental approaches to this complex subject, including the use of model olefins and of 'chemical probes' to detect specific groups in the vulcanizates. Such methods had led to a much better understanding, in recent years, of the chemistry underlying the vulcanization process. As a result, an explanation could now be given of the way in which the nature of the vulcanizing agent affected the structure of the initially formed cross-links between the rubber chains, and of how these cross-links influenced the subsequent chemical reactions occurring during vulcanization and in service. Dr. Moore pointed out that this knowledge represented an important advance in our ability to design the type of vulcanizate best suited to particular industrial applications.

Dr. Cunneen stated that the chemistry of the oxidative ageing of natural rubber vulcanizates had been studied at the NRPRA ever since its formation. The work was initially carried out by Dr. Bateman, Dr. Bolland, Dr. Farmer and Prof. Gee. Their researches led to explanations for the characteristic features of olefine autoxidation, and showed that oxidation proceeded via a free radical chain mechanism that is now well-known.

These studies were later extended to simple organic sulphur compounds which structurally resemble the sulphurated groups present in vulcanizates, and to mixtures of sulphur compounds and olefines. The chemical results, in conjunction with those obtained directly on the ageing of vulcanizates by the technique of stress relaxation, have explained many of the hitherto puzzling features of ageing. Dr. Cunneen indicated that attention is now concentrated on the scission reactions which occur during ageing, and which must be primarily responsible for the loss of physical properties.

In a final lecture, Dr. L. Mullins (NRPRA) drew together the work which had been described in previous lectures on both the determination of detailed differences in vulcanizate structure and the understanding of mechanical properties, and he attempted to establish the present position with regard to relationships between structure and properties. He traced how the mechanical behaviour of vulcanized rubber depends not only on the chemical constitution of the base rubber and the density of cross-links introduced by vulcanization, but also on the type and distribution of cross-links and on changes produced in the base polymer during vulcanization. He showed how at least a qualitative explanation for the effects of each of these factors on the elastic and strength properties could now be advanced and how detailed differences in vulcanizate structure had important effects on chemical properties as reflected by ageing.

Closing the conference, Dr. Bateman (Malayan Rubber Fund Board) said that the rubber tree was one of the supreme agricultural colonists of all time. Unknown in the Far East eighty years ago, it now covered ten million acres and produced more than two million tons of rubber worth £400 million a year and supported, directly or indirectly, millions of people. Referring to the past work of the NRPRA, he applauded the decision of their first Board to concentrate on laying bare the underlying science of rubber technology. The immense impact of this course of attack and the transformation in thought and knowledge that ensued were widely recognized and were reflected in the papers presented at the conference.

Delegates were accommodated in St. John's College, so that after the formal sessions there was ample opportunity for further discussion and interchange of ideas. A jubilee dinner was held at the college, at which the guest of honour was Y. T. M. Tunku Ya'acob, the High Commissioner for Malaysia in the United Kingdom. The conference also provided the opportunity of paying tribute to Sir Eric Rideal for his long and valued service to the Association. The chairman read a letter from the Minister of Commerce and Industry, Federation of Malaysia, conveying deep appreciation of the service which Sir Eric had rendered, and presented to him an inscribed silver salver and a specially bound copy of NRPRA's new publication, *The Chemistry and Physics of Rubber-like Substances.*

NEWS and VIEWS

The John Fritz Medal, 1965 : Dr. F. R. Kappel

Dr. Frederick R. Kappel, chairman of the board of the American Telephone and Telegraph Company, has been awarded the 1965 John Fritz Medal, one of the highest honours to be bestowed by the American engineering profession. The award was announced by the United Engineering Trustees, Inc., custodian of the award, which is presented not more than once annually for "scientific or industrial achievement in any field of pure or applied science" by a special board appointed from the membership of five professional engineering societies. Dr. Kappel was born in Minnesota in 1902. He has had an outstanding career as an industrialist since he graduated in the University of Minnesota in 1924 as an electrical engineer with a B.S.E. degree. He began his career with the Northwestern Bell Telephone Company in Minnesota and joined the American Telephone and Telegraph Company in 1949; he was elected chairman of the board of directors in 1961. Dr. Kappel also serves as a director of the Chase Manhattan Bank in New York, and on many public bodies. He has honorary doctors' degrees from Lehigh University, Knox College, Union College, Ohio Wesleyan University, Rensselaer Polytechnic Institute, Columbia University, Williams College, the University of New Hampshire, and Michigan State University. The John Fritz Medal was established in 1902 by professional associates and friends of the noted industrialist and engineer. Among the previous holders have been Lord Kelvin, Alexander Graham Bell, Thomas A. Edison, Orville Wright, Charles F. Kettering, Vannevar Bush, Herbert Hoover, George Westinghouse, Guglielmo Marconi, and Benjamin Fairless.

Horticulture at Wye College: Prof. H. W. Miles

Prof. H. W. Miles, who will be retiring from the chair of horticulture at Wye College (University of London) at the end of the academic year 1964–65, graduated at the University of Bristol in 1923 and obtained his Master's degree in 1925. After a short period as biologist at the Kirton Agricultural Institute, he was appointed adviser and lecturer in agricultural entomology in the Zoology Department at the University of Manchester. There he made a special investigation of the sawflies of importance in Britain and was awarded a Ph.D. He obtained a D.Sc.(Manchester) in 1936. Then followed a series of investigations into the losses caused by wireworms to market-garden crops in Lancashire and Cheshire and the initiation of some of the techniques for the assessment of soil insect populations in relation to crop injury that were to prove of great value to the official advisory services from 1939 onwards. In 1942 Dr. Miles transferred to the Bristol province as advisory entomologist centred on the Long Ashton Research Station. When the National Agricultural Advisory Service was established in 1946 he was promoted to deputy provincial director in the West Midland Province, the post he was holding when appointed to the chair of horticulture at Wye College in 1947. At Wye his policy has been to ensure that equal attention should be paid to the three main sections of horticulture (fruit culture, market gardening and decorative gardening) and, with the full support of colleagues, he has secured the steady improvement in the science content of the degree course. Under Prof. Miles's guidance, the Department has developed research into fruit tree nutrition and orchard sward management, the problems of vegetable seed crop production and, latterly, the use of intermittent mist and supplementary lighting in the propagation of difficult ornamental plants. Post-graduate workers who have taken part in these investigations have now progressed to positions of responsibility in research stations at home and overseas. The demand for horticultural graduates of high calibre is likely to continue and Dr. Schwabe, who succeeds Prof. Miles, may well be called on to extend and diversify the Department's research facilities still more to meet this need.

Dr. W. Schwabe

Dr. W. Schwabe, who has been appointed to succeed Prof. H. W. Miles as professor of horticulture at Wye College (University of London), was born in 1920. He took first-class honours at Imperial College, London, in 1945, and then joined the research staff of the Institute of Plant Physiology at that College under the directorship of the late Prof. F. G. Gregory. Working on the mineral nutrition of plants in the group stationed at Rothamsted, and headed by Dr. F. J. Richards, he was awarded a Ph.D. in 1949. Later, his research was centred mainly on the physiology of flowering and the analysis of growth and morphogenesis in plants, and included a period of work at the Arctic research station of the Swedish Royal Academy. These researches have elucidated the action of environmental factors controlling flowering in the chrysanthemum and other plants, and have also revealed the dual control of flowering by internal inhibition and promotion. For these investigations he designed a new type of plant growth cabinet with natural illumination which has since been used extensively in other phytotrons. For this work Dr. Schwabe was awarded the D.Sc. degree in 1957 and the Huxley Medal of Imperial College in 1958. On the dissolution of the Institute of Plant Physiology at Imperial College he remained with Dr. Richards's research group, which was later transferred to Wye College as the Agricultural Research Council Unit of Plant Morphogenesis and Nutrition.

New Research Building for Pfizer, Ltd.

PFIZER, LTD., is increasing its research facilities at Sandwich, Kent. The new building, costing £370,000, is planned to be completed by the end of next year, but this is only the first stage of a three-stage programme which when completed will house most of the pharmaceutical research departments. This will enable the research work being conducted in various locations at Sandwich to be centralized and, at the same time, allow the Group to move into new areas of research such as tropical medicine. The major fields in which the group is already heavily engaged and which will be given even greater emphasis are those of the cardio-vascular diseases and virology. The first stage, of 30,000 ft.^{2}, will house the chemical and clinical research departments plus a lecture theatre with a seating capacity of 75, a library and research headquarters. The library, on the ground floor, will contain a fire-proof vault for storing research data and other irreplaceable documents. Four chemical research laboratories will be situated on the first story together with the research headquarters and micro-analytical and chromatography laboratories, and space for nuclear magnetic resonance investigations. On the second story will be six chemical research laboratories, a conference room, wash-up area and workshop. Special features will be a 'safe' laboratory where overnight and long-term experiments can be conducted in isolation and a large-scale laboratory for producing intermediates and compounds required in more than normal bench quantities. On the roof will be hydrogenation and isotope laboratories. The isotope laboratory will be used for 'tagging' certain new therapeutic compounds with radio isotopes so that their activity in the body can be accurately measured.

Representational Services Overseas

IN opening a debate in the House of Commons on July 29 on the Plowden Report on *Representational Services Overseas* (*Nature*, 202, 1039; 1964), Mr. H. Berkeley challenged the retention of the Commonwealth Relations Office and the Foreign Office as separate Ministries and argued cogently for a Department of External Affairs, covering both Commonwealth and non-Commonwealth countries, with a ministerial structure based on geographical areas. He also suggested a Cabinet Minister for Overseas Development, with much wider responsibilities than the Secretary for Technical Co-operation. Mr. C. P. Mayhew, who followed, questioned the wisdom of any such fusion and was inclined to support the recommendation of the Plowden Committee, and in this he was also supported by Sir Charles Mott-Radclyffe. The Under-Secretary of State for Commonwealth Relations and for the Colonies, Mr. N. Fisher, who replied on the debate, admitted that there might be disadvantages in having two departments, but thought that these disadvantages were exaggerated and that the balance of argument in practice was in favour of two departments. He did not deal with the point about development aid and the Department of Technical Co-operation, but discussed the question of recruitment at some length, particularly with regard to widening the basis of recruitment. He agreed that it was important to encourage university graduates to compete for entry into the executive branch as well as the administrative branch, but was silent as to the desirability of a larger recruitment of those with a scientific background urged by Mr. Mayhew. No speaker referred to the point at issue between the Estimates Committee and the Plowden Committee on the margin of staffing the Service (see *Nature*, 203, 797; 1964).

Industrial Training Boards

IN a short debate on the industrial training boards in the House of Commons on July 22, opened by Mr. R. E. Prentice, the Minister of Labour, Mr. J. B. Godber, said that the first four training boards established under the Act, for the construction, the wool, the engineering and the iron and steel industries, had all met at least once. In the light of the advice of the Central Training Council, he had selected shipbuilding, cotton, construction materials and public utilities (gas, water and electricity) as the next four industries for which training boards should be established. At its meeting on July 23, the Central Training Council would consider its own committee structure, with the view of dealing effectively with its varied tasks on a regional and functional basis. The Ministry now had two branches, each under an Assistant Secretary, dealing with the implementation of the Act: one was concerned with preparations for establishing further boards, and the other with relations with those already established. There was also at present a technical staff, under the chief technical adviser, with two assistant technical advisers, to advise on technical aspects of training; a deputy was shortly to be appointed. Grants of up to £10,000 had been made to assist the boards until they could raise their own funds from a levy, and they were also being assisted in compiling a register of firms in each industry. The Construction Board and the Wool Industry Board each had a deputy chairman, as well as a chairman, but the other two boards, which had chairmen from within the industries concerned, had not.

Public Libraries and Museums

IN moving the second reading of the Public Libraries and Museums Bill in the House of Lords on June 30, the Minister of State for Education and Science, Lord Newton, explained that museums and art galleries had recently been surveyed by the Standing Commission under the chairmanship of Lord Rosse, and when the Bill was introduced into the House of Commons in February it was the Government's intention to make no substantial change until the Survey had been further studied. It had been represented, however, that certain changes would command a wide measure of support and should be made without waiting for further legislation on museums. It also appeared that the existing law regarding charges for admission to museums and art galleries was full of anomalies and could not be re-enacted. For these two reasons, Clauses 12–15 dealing with museums and art galleries had been extensively revised.

Under the revised Clause 12 all library authorities would continue to have powers to provide and maintain museums, and any other authority which now maintained a museum would be able to do so. In addition, any local authority would be able to provide a museum or art gallery if it obtained the consent of the Secretary of State. The amended Clause 13 removes the original prohibition on charges for admission, but requires local authorities to take into account the need to ensure that the museum or gallery plays its full part in promoting education in the area, and to have particular regard to the interests of children and students. Lord Newton added that it was not the Government's intention to encourage local authorities to impose charges. Clause 14 allowed library authorities or authorities maintaining a museum or art gallery to contribute to the expenses incurred by any person, either in providing or maintaining a museum or art gallery, or in providing advisory or other services, or financial assistance, for it. The second power had been inserted with the needs of regional museum organizations particularly in mind. Clause 15 gave effect to the recommendation of the Standing Commission that the power taken by some authorities under private Acts to establish a fund for the purchase of exhibits should be made general.

Apart from some discussion of the clause relating to admission charges which, as Lord Newton anticipated, was not regarded as entirely satisfactory, the debate centred mainly on three points. First, on finance: Earl Attlee argued that there was need for some kind of Government grant to the large regional centres which served wide areas, although it was accepted that it would be easier to wait for the present review of central and local Government finance to be completed before changing the finance of the library service. The need for some immediate contribution from the central Government was urged by Viscount Eccles and by Lord Shackleton, who challenged in particular the Government's treatment of the National Central Library, for which he urged further consideration and support. Secondly, the question of the needs of students was urged by Earl Attlee as well as other speakers, and the importance of every library having a chief librarian and fully qualified staff with adequate remuneration and status was repeatedly emphasized. Thirdly, Viscount Eccles and others emphasized the importance of adequate research, and Lord Newton, in replying on the debate, said that under Clause 1 the Secretary of State would be able to commission research and also to draw on the services of the Architects and Buildings branch of his Department, both for research and for development work on public library buildings. He assured the House that this question would be considered when the Act came into operation. Earlier he had estimated that the improvement of standards of service might involve an increase of perhaps 50 per cent in the present cost of about £23 million, though this increase would be spread over several years.

South African Museum

The annual report of the South African Museum for the year ended March 31, 1963, breaks with a 105-year-old tradition and provides, in an attractive format, a general account of the present main activities and future plans (Pp. v+23. Capetown: South African Museum, 1964). This replaces a record of accession data, donors, etc., which in future will be prepared but not printed. The policy of the Museum is to maintain a balance between funds allocated for research and educational activities. In the year under review, 25 major field trips were undertaken in addition to numerous short ones. Major extensions to the buildings are in hand and the new library and administrative block has been completed. A new exhibition of South African birds, a diorama to show bird life at Rondevlei and a display of Cape birds have been prepared. During the next two or three years it has been decided to concentrate on research in marine biology, palæontology and entomology by teams of workers on the staff.

Transvaal Museum, Pretoria

As usual, this Museum's report is printed in English and Afrikaans (Transvaal Museum. Report for the year ending March 31, 1963. Pp. 31. Pretoria: Transvaal Museum, 1964). The study collections, which form the basic material for research, continued to be augmented through selective collecting by members of the staff, and by donations. Further accommodation is urgently needed for the storage of these collections. A number of short collecting and survey trips have been undertaken to areas of particular scientific interest. Five new large mammal habitat cases have been installed. In the Cultural History section an interesting exhibition, "Old Cape Silver and Furniture", has been arranged.

Science in the Less-well-known Languages

In the April (1964) issue of *Science East to West*, Mr. J. Garrido, deputy director of the Documentation Centre of the National Centre for Scientific Research, Paris, contributes an article on scientific and technical publications in the lesser-known languages. His analysis of articles published in 1962 indicates that English (66 per cent), Russian (13 per cent), French (11 per cent) and German (8 per cent) account for all but 2 per cent of published work in nuclear physics. In applied chemistry, the agricultural sciences and geology, other languages account for 14–18 per cent, the proportion varying slightly in the different subjects. Among these other languages, Japanese and Italian come first, each with about 20 per cent of the total in languages other than English, Russian, French and German. Polish, Romanian, Czech, Spanish and Ukrainian come next with 10–5 per cent, followed by Hungarian, Serbo-Croat, Bulgarian, Dutch, Swedish and Portuguese with 5–1 per cent. The most surprising result is the place of Chinese, which in M. Garrido's figures only stands first among 30 languages with less than 1 per cent of the output of the scientific literature in languages other than English, Russian, French and German. In applied chemistry, the proportion in English is 39 per cent, in the agricultural sciences 42 per cent, and in geology 33 per cent; for German the corresponding percentages are 20, 11 and 8 respectively; for Russian, 17, 19 and 35; and for French, 8, 10 and 10.

New Approach to National Health Service

One in ten of the adult population is probably suffering from a potentially serious illness which is unknown to his or her doctor. Not all these need medical attention; but many do, and delay in starting treatment is frequently responsible for unnecessary deaths and disablement. 2,500 deaths from cancer of the cervix and about 4,000 cases of blindness from cataract and glaucoma each year might be prevented if treatment had been started sooner. These facts are brought out in a report issued by the Office of Health Economics entitled *New Frontiers in Health: a Review of the Incidence, Impact and Implications of Minor and Unrecognized Illness in the Community* (Pp. 32. London: Office of Health Economics, 1964. 2*s*.). The report criticizes former delays in introducing measures which could demonstrably improve the health of the nation. During the 1930's about 20,000 child deaths from diphtheria could have been prevented had Britain started an immunization campaign at the same time as the Canadians. "It seems clear", says the report, "that powerful advocacy is sometimes required before even the most obvious advances in the health field are put into practice". It also directs attention to the amount of minor ill-health which is untreated. For every person who visits a doctor, there are probably three more who suffer from a minor illness without consulting him. It is estimated that minor illnesses cause the loss of about 25 million working days each year. Much of it is among women, for whom short sicknesses cause about two and a half days' absence a year. For men, short sicknesses on average are responsible for less than one day off work each year. The report concludes: "It is unrealistic to expect that everyone should enjoy 'perfect' health, and it would be unreasonable to strive for it. Man will always be faced with more or less important diseases and disorders caused by the environment in which he exists, and which, to a large extent, he has created. On the other hand, there is very much that could be done to correct disorders, to control diseases, and to raise the present general level of health. The costs which this would involve should be seen in perspective against the benefits in having as healthy a community as possible".

Bausch and Lomb Metallographs

The 'Dynazoom' series of metallographs recently introduced by Bausch and Lomb consists of a versatile set of bench-type models suitable for general metallurgical work both in industry and in schools and colleges. The instrument is based on the inverted microscope principle and there are models for both visual and photographic work, using bright field, dark field or polarized light illumination. The exclusive 'Dynazoom' system permits extended continuous magnification ranging from ×25 to ×800, at a twist of a dial. With accessory optical components the range can be extended to ×3,750. The optical elements are enclosed in a 'Power Pod' housing for protection against dirt and dust. The housing can be removed from the main body of the instrument and a different 'Pod' (monocular or binocular) substituted. The 'Power Pod' bodies are inclined, providing a comfortable viewing angle for prolonged examination and can be rotated through a full 360°. A unique feature of the metallograph is a built-in rotating reticle turret with six apertures to accommodate a variety of measuring reticles which can be quickly and easily inserted or removed through a door in the bottom of the instrument. Photomicrography is a simple procedure. The photobinocular zoom body can be obtained separately and is directly interchangeable with any other zoom body. Four cameras are at present available: 35 mm, $2\frac{1}{4}$ in. × $3\frac{1}{4}$ in. 'Polaroid', and the Bausch and Lomb '*L*' and '*N*' models. A green filter with a peak at 500 mμ is standard on all instruments. There are provisions for both black-and-white and colour reproduction of metallic structure, and colour-compensating, colour-balancing and neutral-density filters are also available.

Lalor Foundation Grants for 1965

The Lalor Foundation has announced the programme of awards for 1965, which it is offering for support of research on the fundamental biochemical and physiological mechanisms concerned with fertility and the early stages of reproduction in various forms of life. The objectives are to further the knowledge and understanding of the basic phenomena involved and to extend and develop the possibilities for effective regulation and control. The awards are open to all nationalities, and may range up to 8,000 dollars a year, depending on the scope and duration of the projects approved. Preference will be given to younger members of university and college faculties and staff, with an upper age-limit of forty-one years. The work may be carried on at the applicant's own institution or elsewhere. The Foundation will also grant post-doctorate summer or short-term research awards at the Marine Biological Laboratory at Woods Hole, Mass., or elsewhere for appropriate projects in the fields specified. These awards will normally not exceed 1,150 dollars for a single man or a woman, 1,350 dollars for a married man working at his home institution, and 1,500 dollars for a married man with principal programme at another institution. Further information and application forms can be obtained from the Lalor Foundation, 4400 Lancaster Pike, Wilkington, Delaware, 19805. The final date for receipt of executed application forms, complete with supporting data, is January 15, 1965. Notification of appointment will be on or before March 15.

North of England Soils Discussion Group

A discussion group is being formed covering broadly the North of England to further the investigation of all aspects of the soil. Membership is invited from individuals or educational and vocational bodies that have an interest in the soil. It is intended to embrace all aspects of the investigation of the soil, including the biological, cultural, physical and economic, as well as the purely pedalogical, and its bearing on land use. An annual meeting will be held, to include a field excursion, and a paper-reading session with discussion, probably in September. A specific topic will be the main feature of each meeting, and will largely determine the locality to be visited. Further information concerning membership and meetings can be obtained from: Dr. V. B. Proudfoot, Department of Geography, University of Durham; Mr. G. D. Ashley, Soil Survey of England and Wales, Kenton Bar, Newcastle upon Tyne; Mr. P. J. A. Howard, Nature Conservancy, Grange-over-Sands, Lancashire; or from the secretary, Mr. H. T. Davies, Soil Chemistry Department, National Agricultural Advisory Service, Government Buildings, Lawnswood, Leeds, 16.

The first meeting of the North of England Soils Discussion Group on "Soils and Land Use Problems in the Marginal Farming Areas of the Pennine Foothills" will be held in Lancashire during September 28–30. Further information can be obtained from Mr. H. T. Davies.

Announcements

The President's Lecture of the Institution of Electronics, entitled "The Vanishing Electron", will be delivered by Dr. R. C. Jennison at the Manchester College of Science and Technology on October 3. Further information and tickets can be obtained from the General Secretary, Institution of Electronics, 78 Shaw Road, Rochdale, Lancs.

The fourth annual meeting of the American Society for Cell Biology will be held in Cleveland, Ohio, during November 11–13. The programme will include a symposium on "The Structure, Function and Origin of Biological Membranes". Further information can be obtained from Dr. D. Green, Institute for Enzyme Research, 1,170 University Avenue, Madison 6, Wisconsin.

A symposium on "Models for the Perception of Speech and Visual Form", sponsored by the Data Sciences Laboratory of the Air Force Cambridge Research Laboratory, will be held in Boston during November 11–14. Further information can be obtained from Mr. G. A. Cushman, Wentworth Institute, 550 Huntington Avenue, Boston, Massachusetts 02115.

A symposium on "The Use of Radioisotopes in Animal Nutrition and Physiology", sponsored by the International Atomic Energy Agency and the Food and Agriculture Organization, will be held in Prague during November 23–27. Topics under discussion will include: physiology and biochemistry of milk secretion; metabolism and requirement of trace elements including magnesium; the influence of environmental factors on animal production and reproduction. Further information can be obtained from IAEA/FAO Symposium Secretariat, Kärntnerring 11, Vienna I.

A short course on "Solution Properties of Polymers", arranged by the Department of Chemical Technology of the Bradford Institute of Technology, will be held in the Institute during November 13–14. The course, which is intended primarily for graduates, will include lectures on: viscosities of dilute solutions; fractionation; determination of molecular weight distribution; solution properties in relation to polymer properties; newer methods of molecular weight determination. Further information and forms of application can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7.

THE U.K. ATOMIC ENERGY AUTHORITY

THE tenth annual report of the Atomic Energy Authority* covers the year ended March 31, 1964, in which the Authority's staff was reduced by about 5 per cent because production of uranium-235 for military purposes at Capenhurst ceased and most of the capital works programme has been completed. Of the £45 million expended on civil research and development, £36 million was current expenditure and £9 million on capital facilities; research, development and design contracts with industry, universities, etc., amounted to £4,612,000 compared with an estimated £4,223,000 in 1964–65; contributions towards International Atomic Energy Agency projects were £2·48 million and grants to the National Institute for Research in Nuclear Science £8,998,000, compared with an estimated £1·36 million and £9,648,000, respectively, in 1964–65. Of 2,830 engineers and scientists employed at March 31, 1964, 180 were doing work for other organizations on repayment, 1,900 were engaged on the reactor development programme and reactor systems research, on which current expenditure is estimated at £26 million, 470 on general research (£6 million), 200 on plasma physics and fusion research (£3 million), and 80 on isotopes research (£1 million). Attention is directed to a reduction in the balance sheet of £50 million in the value of work in progress, which is due mainly to the transfer of nearly £30 million to "loss on revaluation of fissile material", representing a writing-down of the value of material held for research purposes to bring the valuation in line with world prices. Uranium ore, no longer required for military purposes, other than supplies purchased for the nuclear power programme, will form a national stockpile in the custody of the Authority as agent for the Secretary of State for Education and Science, and the cost of the uranium in this stockpile will in future be excluded from the Authority's balance sheet. Of the £52·5 million received from the Authority's trading and other commercial activities during the year, £39·5 million was from sale of reactor fuel, fuel services, etc., £7·9 million from sales of electricity, £1·6 million from sales of isotopes and £2·7 million from sales of reactor-grade graphite.

On the production side development work on the gas centrifuge as an alternative to gaseous diffusion continued satisfactorily and productivity in the magnox fuel manufacturing process continued to increase, while the anticipated peak in production of magnox fuel at Springfields was reached. A separate chapter devoted to the reactor development programme estimates that at March 31, 1964, 19 per cent of the scientists and engineers in the Reactor Research and Weapons Group were engaged in work on the advanced gas reactor, 17 per cent on the steam-generating heavy-water reactor system, which has the advantage of lower enrichment than the advanced gas reactor and the potentiality of producing superheated steam, 38 per cent on the fast reactor, 14 per cent on the civil marine reactor and 12 per cent on the high-temperature gas-cooled reactor for the *Dragon* project at Winfrith. In the advanced gas reactor encouraging progress is reported in protecting the graphite moderator from corrosion by the carbon dioxide coolant under irradiation and much information was also obtained on the dimensional changes in graphite caused by irradiation from irradiation experiments. The operational availability of the Windscale advanced gas reactor during its first year of power operation was 87 per cent and compares favourably with any other nuclear reactor built solely as a nuclear power station or otherwise. With fast reactors the outstanding achievement was to get the Dounreay fast reactor operating at full power and to continue at full power for the maximum time to build up irradiation experience of fast reactor fuels. General predictions of the kinetics of a high-power fast reactor have been shown to be substantially accurate and steps taken in design of the core to eliminate a positive power coefficient were successful. On marine reactors little is added to what was said in the Padmore Report (*Nature*, 202, 1248; 1964); work on the integral boiling reactor was mainly confined to physics of burnable poison cores and to fuel element and core design investigations; work also continued on burnable poison cores and experimental physics studies were made of the characteristics of regular fuel element clusters, as well as investigations of the use of plutonium as a nuclear fuel for marine reactors.

A further chapter dealing with research on reactor systems records that, in the programme of nuclear measurements to provide data for calculating the physics characteristics of proposed reactors, the emphasis continues to be on measurements for fast reactors. A general investigation continued into the mechanism by which neutrons transfer their energy to liquids and solids and are thus brought into equilibrium with the atoms of a moderator, and a major advance in the established technique of transfer-function analysis for measuring the dynamic response of a nuclear power plant has been linked with the development of a neutron generating tube of high output capable of being modulated at frequencies up to 1,000 c/s. Work on fuels has included studies of solid solutions of plutonium dioxide in uranium dioxide to determine the chemical (phase) stability under various conditions, and in work on carbide fuels reactor-grade material has been obtained by heating granules of fissile oxides with carbon in a stream of argon. Other work has been concerned with canning and structural materials, moderators and coolants and the design of biological shields for power reactors.

Among examples selected to illustrate the Authority's programme of general research in this report may be mentioned work which is directed at devising mathematical methods by which computers can be used more efficiently and applied to a wider range of problems. In a programme of basic research on solid-state physics the emphasis is now moving to non-metallic solids such as oxides and carbides. The fundamental causes of changes in solids induced by radiation are also being investigated, while basic information bearing on steam-cooled systems is being sought in studies of the radiolysis of water-vapour, in which first results indicate that the net radiolytic decomposition of pure water is negligible, but with vapour containing ammonia or oxygen the primary decomposition is comparable with that of pure water. Other long-term research is concerned with the physical and chemical properties, and the fabrication behaviour, of the oxides, carbides, nitrides, sulphides and phosphides of fissile and fertile elements, especially the carbides. Some work also continues on fissile metals, and protactinium oxide has been investigated in detail for comparison with the oxide systems of the other actinide elements from thorium to plutonium. A major effort is devoted to improving existing methods of analysis and applying new ones, while an increase in the resolution with which chemists can measure the energies of α-particles has led to a tenfold improvement in the sensitivity of certain measurements in health physics. The utility of γ-ray spectra for identifying the radionuclides present in various materials has been increased by the development of an automatic method of analysing complicated spectra and an absolute hygrometer has been developed for measuring the humidity of the stratosphere in investigations on fall-out.

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* United Kingdom Atomic Energy Authority. Tenth Annual Report for the period 1st April, 1963–31st March, 1964. Pp. ix + 89 + 8 plates. (London: H.M.S.O., 1964.) 6s. 6*d*. net.

In plasma physics and fusion research the main problems are still to identify, understand and control the many instabilities which prevent magnetic fields from confining extremely hot ions and electrons for more than a relatively brief moment. While these instabilities remain as a potential source of trouble in closed systems, there are now indications that they can be overcome in the open-ended magnetic traps. The programme also includes two experiments in 'Minimum *B*' geometry designed to see if the same freedom from interchange instabilities noted by Ioffe at the Kurchatov Institute will apply at higher plasma densities and with different methods of plasma injection. Besides investigations with a cusped field trap and a theatron trap, containment with closed lines of force has been investigated and basic investigations made on plasma itself, particularly the propagation characteristics of the wide variety of wave motions which a magnetized plasma can support, and also the processes which can arise from collision between its constituent particles. At the Wantage Research Laboratory the feasibility of radioisotope X-ray fluorescence analysis for on-stream assay of zinc–lead ores has been investigated in co-operation with the Department of Scientific and Industrial Research, while investigations bearing on health and safety have led to the development and manufacture of a personal neutron dosimeter. Studies of radioactivity in the human body continued using whole-body monitors and bioassay methods to determine the metabolic behaviour of nuclides, and continued physico-chemical study of chelating agents confirmed that diethylenetriamine pentacetic acid and triethylenetetramine hexacetic acid assist the removal of plutonium from the human body, but more effective and less toxic compounds are being sought.

The Reactor School programme at the Postgraduate Education Centre set up at Harwell on April 1, 1963, has been revised, and, after transfer of four of the more elementary courses, three new courses have been added: physics for engineers; language and technical writing; and a refresher course for senior technical executives. In collaboration with the European Nuclear Energy Agency a one-week residential course was given on health and safety aspects of radiation in industry for Trade Union officials. Of 379 students attending these courses, 340 were British and 39 from overseas, while 490 students attended a new series of postgraduate courses, and another 300 the 14 courses held in the Isotope School.

While the non-industrial staff fell over the year from 20,008 to 18,864 and industrial employees from 18,350 to 17,098, the Authority made it clear to their staff that there is a large and continuing volume of work for the Authority, including civil research and development as well as production and processing. Some recruitment continued and the level will be raised in 1964, the need for young scientific and engineering graduates for research, design and development, and for training as future managers being mentioned, while 202 apprentices were recruited compared with 230 in the previous year.

The Authority has also published the tenth anniversary issue of *Atom*† including both an illustrated summary of the tenth annual report and an illustrated review of the developments during 1954–64.

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† United Kingdom Atomic Energy Authority. *Atom 1954/1964: an Illustrated Summary of the Tenth Annual Report of the United Kingdom Atomic Energy Authority from April 1st, 1963, to March 31st, 1964.* (Tenth Anniversary Issue.) Pp. 48. (London: H.M.S.O., 1964.) 2*s.* 6*d.*

THE SOUTH AFRICAN ATOMIC ENERGY BOARD

THE South African Atomic Energy Board was set up in March 1949, and at first confined its operations almost exclusively to administrative procedures related to the mining, extraction and sale of uranium concentrates, the distribution and control of radioisotopes, mineral investigations, and the study of the production of heavy water. During 1957 detailed planning of an atomic energy research and development programme for South Africa was started, and on September 5, 1959, the then Minister of Mines, Mr. J. de Klerk, announced the Government's acceptance of the proposed programme. This covered an initial period of five years. *The Way to Pelindaba* is an illustrated description of the main activities during that period 1959–64*.

The programme called for both electric power production by nuclear fission, and for radioisotope and radiation applications, and necessitated the provision of a nuclear reactor for research and a suitably located research centre. It was decided, in 1960, to construct a 20-MW high-flux light water moderated and cooled reactor of the Oak Ridge type, suitable for materials testing and for operation initially at about 5 MW. A contract for the supply of *SAFARI*-1 (*S*outh *A*frican *F*undamental *A*tomic *R*eactor *I*nstallation) was signed with the authorities of the United States. A site, on a farm formerly known as Wilgegund, was chosen for the nuclear research centre, and named Pelindaba, from the Bantu *pelila indaba*, meaning 'the discussions are finished'. Construction commenced in July 1961, and two years later, in July 1963, the administration building and part of the reactor building were occupied. The chemistry building, the Van de Graaff accelerator building, the water purification works and a portion of the reactor engineering building were completed by the end of 1963. Unfortunately, accidental damage to the reactor vessel of *SAFARI* in the United States during test delayed its delivery to South Africa, but the repaired vessel eventually arrived on Christmas Day 1963 and by March 1964 it was installed, together with most of its heavy concrete shield, within the reactor building. The Van de Graaff accelerator—a 3-MeV high-intensity beam type—which provides a source of mono-energetic neutrons, with pulsing if required for the time-of-flight method, has also been erected and is now in commission. A detailed description of the site and layout of the nuclear research centre, together with diagrams of the site buildings, the accelerator and reactor, is given in the companion booklet *Pelindaba* (Brochure No. 2).

It is well known that South Africa contains some of the world's largest reserves of uranium and has been one of its largest producers. Indeed, until recently, 17 uranium extraction plants handling about 20 million tons of residue slimes from 26 mines, eight contact-process sulphuric acid plants, and a factory for the conversion of the custard-yellow cream emanating from the extraction plants were in continuous operation. All forms of refined uranium are produced in substantial quantities, the products including uranium metal, its carbide, trioxide and tetrafluoride, and small quantities of hexafluoride. A pilot plant has been constructed to investigate the production of a nuclear-grade uranium product, containing only a few parts per million of impurity, from the uranium extraction plants at the gold mines at Bufflesfontein. This is known as the Bufflex Project and was started in October 1963.

The research staff of the Atomic Energy Board, some eighty of whom have been trained or are still training at nuclear research establishments in some ten countries outside Africa, belong to eight divisions. The Geology Division, though of primary importance, is small, because many of its activities are covered by the Geological Survey Office of the Government. The Extraction Metallurgy Division, which is housed at the Government Metallurgical Laboratory, makes use of the services provided there. The third division which relies on external facilities is the Life Sciences Division, and the number of its staff at Pelindaba is accordingly limited. The remaining five Divisions—Reactor Engineering, Chemistry, Physics, Physical Metallurgy, and Isotopes and Radiation (Protection and Application)—comprise the bulk of the research staff stationed at or attached to the National Nuclear Research Centre at Pelindaba, and at present three laboratory blocks are at their disposal. There are in addition three technical divisions. The Research Reactor Division will operate the reactor *SAFARI* for the research scientists; the Technical Services Division undertakes the provision of services such as power, water, compressed air and workshop facilities; and the Liaison and Information Division is responsible for external relations and the provision of information to the staff and outside bodies.

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* South African Atomic Energy Board. Brochure No. 1: *The Way to Pelindaba.* Pp. 28. Brochure No. 2: *Pelindaba—National Nuclear Research Centre of the South African Atomic Energy Board.* Pp. 31. (Pretoria: South African Atomic Energy Board, 1964.)

The final section of the report deals with future activities. In the late 1970's the world demand for uranium may exceed its availability and consideration may have to be given to low-grade sources of uranium. Rapid developments in enriched uranium reactors have directed attention to enrichment processes in which uranium hexafluoride is of importance, and the production of this particular compound is being investigated, together with that of zirconium, hafnium, thorium and the rare earths. The recovery of beryllium, niobium, tantalum and lithium from deposits of pegmatites is also being investigated. The power reactor project is to be pushed ahead and this will require the evaluation and testing of fuel, materials and components, together with the development of fabrication techniques. Solid-state investigations of irradiation effects and neutron diffraction investigations will be needed, and in order that this work may proceed before the neutron flux from *SAFARI* becomes available, a large γ-radiation source will be installed. The Van de Graaff building is to be extended to accommodate the whole of the expanded Physics Division, and a new library building is to be provided to relieve congestion in the administration building. For the second five-year programme to reach the target figure of expenditure of *R*.3 million per year the Government has trebled its contribution and the Electricity Supply Company its share five-fold. Heavy industry, too, has pledged an amount totalling more than twice its contribution during 1959–64.

S. WEINTROUB

PRESERVATION OF RURAL ENGLAND

THE dominant impression left by the thirty-eighth annual report of the Council for the Preservation of Rural England for the year 1963–64 * is the magnitude of the scope there still is for such independent voluntary effort, and the vigilance and vision, as well as the balance and fairmindedness, with which the Council, through its Executive Committee, diligently strives to ensure that harmful development is never allowed without question. Wherever possible, the needs of the countryside as a whole are balanced against what are often private or temporary ends. While the year has brought some evidence of increasing official awareness of the need to look well ahead in planning matters, and in estimating future demands on land and natural resources, the report notes that amendment of the National Parks Act, 1949, is still long overdue, the findings of the Royal Commission on Common Land in 1958 have still to be implemented, and the coast is still subject to piecemeal development and divided control. The circular issued to local authorities by the Ministry of Housing and Local Government in September 1963 emphasized the need for positive planning of coastal areas. It is welcomed, but it is regarded as being too local in scope. What is needed is a national policy for the preservation of the countryside based on a Land Budget, allotting land used according to definite patterns of priority.

The Council is sympathetic to the difficulties of the Central Electricity Generating Board and recognizes that sites for power stations are hard to find, and that there is still a formidable gap between the cost of overhead and underground transmission. The generation, transmission and distribution of electricity present the Council and the planning authorities with difficult problems, and the concern expressed in this report, especially with regard to overhead lines, will not be diminished by the subsequent decision of the Minister of Power to disregard the recommendations of his own inspectors and the objections of all the local authorities concerned and allow the Central Electricity Generating Board to run a supergrid power line in 160-ft. pylons over the Sussex Downs. The timing of the announcement to coincide with the parliamentary recess may well have justified the Council in thinking that the scales are weighted against amenity and that there is substance in Lord Chorley's suggestion that the replacement of the Ministry of Town and Country Planning by one of Housing and Local Government represents more than a change of name and results in a situation in which decisions against amenity are taken by one who judges his own cause. On water undertakings the Council is convinced that traditional sources of water are likely to prove inadequate before long and that a national water grid will be needed. Major reliance will eventually be placed on the distillation of sea-water and the re-use of water. It points out that the Minister of Housing and Local Government himself agrees that the Conference on Water Supplies in the north-west dismissed the distillation of sea-water too lightly.

The Council has made constructive proposals concerning many road projects. It welcomes the Buchanan Report and the South-East Study, as well as the proposals put forward towards the end of 1963 by the Standing Conference on London Regional Planning under the title *Population, Employment and Transport in the London Region: Meeting the Situation*, but the report stresses the need for research into every aspect of life in towns, of movement between towns, and of their relations with one another. This involves the consideration of regional groupings and, beyond these, of national problems. Concern is expressed at the shortage of qualified planning staff, and the Council is profoundly disturbed as to the future of the 'Green Belts', on which it finds the Minister's attitude disturbing, in spite of an explicit statement that the Government has no intention of abandoning the Green Belt policy.

Inevitably, much of the report is concerned with comments on particular topics, but the broad subjects covered—including rural housing, litter and refuse disposal, commons and open spaces, nature conservation, trees, national parks, preservation of buildings, the siting of radio and television stations, caravan and camping sites, oil pollution of the sea, mineral undertakings—illustrate its wider range of interests and the many spheres in which it has been called on for advice or action.

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* Council for the Preservation of Rural England. Thirty-eighth Annual Report 1963-64. Pp. 56+10 photographs. Vol. 17, No. 6. (London: Council for the Preservation of Rural England, 1964.)

MODERN GLASS TECHNOLOGY

BOTTLES of skin, gourd or calabash (tree or climbing plant, the fruit-shell of which has been emptied and dried), alabaster, ivory and even beaten gold, have been in use down the centuries, things both of beauty and utility; many relics are with us to-day, in museums and elsewhere, all over the world. But for several millennia the most favoured bottle material has been ordinary glass, starting with the craftsman glass-blower (still with us in many specialized pursuits, particularly concerning apparatus for scientific laboratories), graduating by moulding processes ultimately to the fully automatic bottle-making machines operating to-day. It may not be generally realized that glass as such is unique matter. It is not a true solid, but, according to C. L. Boltz in an interesting article in *Catalyst*, Vol. 12 (Industrial Journal of Shell Chemical Co., Ltd., London, Spring 1964), " . . . scientifically . . . a super-cooled solution that still flows over a long period of time . . . it is not crystalline in the sense that atoms are arranged in regular geometrical patterns . . . it is mainly amorphous, shapeless, and shatters randomly when it is abruptly broken".

In the early days glass products were usually coloured green, amber, blue or ruby, due to impurities such as iron, manganese, etc. It was not until 1908 that the first colourless glass bottles were produced in London. "The first university department in the world for the scientific investigation of glass technology was formed at Sheffield under Prof. W. E. S. Turner and has achieved international recognition. The use of polarized light for the detection of stress-lines in bottles and the importance of the glass surface in the retaining of strength are just two of the developments that have led to to-day's industrial glass technology." Apart from Sheffield, glass technology is now also the concern of the British Glass Industry Research Association (formed as recently as 1955) and the Glass Manufacturers' Federation.

Such has been the phenomenal advance in research and manufacturing techniques in recent years that it is estimated that, in Britain alone, some 4,500 million glass containers are manufactured per annum. Collaterally there has been the challenge of tin in replacing glass containers and more recently some of the thermoplastics, notably polyethylene. But in quite a different and perhaps more refined sphere of glass technology comes the highly skilled and expert precision operations of those firms concerned with manufacture of optical glass.

The word 'Jena' conjures up many memories, past and present, of lenses, microscopes and other optical instruments, and the bi-monthly *Jena Review* (VEB Carl Zeiss Jena, English translations available), now in its ninth year, makes available much technical information of present-day value to optical physicists and others concerned with precision optical equipment in this and allied fields. The issue No. 2, 1964, contains, *inter alia*, informative articles on synthetic crystals of fluorite and salt from melts, alum and quartz from solutions made in Jena; on objective photo-electronic testing or precision glass scales; preparation of fibre surfaces for electron microscopic examinations; spectrophotometry in the near infra-red with the '*UR* 10' Spectrophotometer, and linearity tests of other infra-red instruments. This review is an attractive publication and yet another example of a high-quality house journal.

KÁRMÁN VORTEX-STREETS IN EARTH'S ATMOSPHERE

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AND
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THE mechanism for the production of eddies on the planetary scale (cyclones) and those on the microscale (turbulent eddies) have been extensively investigated. The recent weather satellite results[1] have revealed the existence of the mesoscale eddies which are on an intermediate scale that is too small to be delineated by the standard weather observation network and are too large to be seen by an observer on the ground or even pictured from a high-flying aircraft. These eddies, shown in Fig. 1, are formed in the wakes of islands and have been observed to persist at downstream distances of a few hundred miles. They are made visible by patterns in the stratocumulus clouds lying beneath a strong inversion about 0·5–1 mile above the ocean surface. These eddies have a band-width of the order of the cross-stream diameter of the island, and bear a certain resemblance to the classical von Kármán vortex-street pattern shown in Fig. 2 and observed in the laboratory investigations of wakes behind obstacles to fluid flow. The purpose of this communication is to examine the properties of these eddies in the light of this apparent resemblance.

Viscosity plays two parts in the flow pattern of a Kármán vortex-street. First, it leads to the formation of the boundary layer in which vorticity is generated and the vortex-pairs are formed, the vortices being shed alternately near each edge of the obstacle. Secondly, the strength of the eddies is dissipated by the mechanism of diffusion; the size of an eddy increases and its strength decreases as it propagates downstream, until its region of influence overlaps that of a neighbouring eddy of opposite circulation. In all other respects, the fluid may be regarded as perfect (inviscid).

The rate N of shedding of the eddy pairs is given by:

$$N = u_e/\alpha \tag{1}$$

where

$$u_e = u_0 + (k/2\alpha)\tanh(\pi h/\alpha) \tag{2}$$

is the speed of propagation downstream of the eddies in a co-ordinate system fixed to the obstacle, u_0 is the speed of the undisturbed flow, α is the longitudinal spacing (pitch) between two eddies of similar circulation of strength k.

The drag per unit length on the obstacle, being equal to the rate at which momentum is transferred to the wake of the obstacle, is given by:

$$D = 2\rho h u_e\ (u_0 - u_e)\ \coth(\pi h/\alpha) \qquad (3)$$

where ρ is the density of the fluid, and h is the lateral spacing (band-width) between the two vortex-rows, approximately related[2] to d as:

$$C_D = D/\left(\frac{1}{2}\right)\rho d u_0^2 \approx h/d \approx 1{\cdot}25 \qquad (4)$$

for circular cylinders. Here C_D is the coefficient of drag. An alternative method of computing drag which takes into account the difference between the upstream and downstream mean pressure yields:

$$D = \frac{\rho k^2}{2\pi\alpha} + \frac{\rho k h}{\alpha}\ (2u_e - u_0) \qquad (5)$$

Equations (3) and (5) are identical if:

$$\coth \pi h/\alpha = \pi h/\alpha \qquad (6)$$

The diffusive decay of vorticity ζ is governed by the well-known diffusion equation (also called the heat-conduction equation):

$$\frac{d\zeta}{dt} = \nu\nabla^2\zeta \qquad (7)$$

which has a solution

$$\zeta = \zeta_0(t)\ \exp\ (-r^2/4\nu t) \qquad (8)$$

The last equation reveals that (1) the vorticity $\zeta_0(t) = k/2\nu t$ at the centre of the vortex decreases inversely with time t, and (2) vorticity spreads, in an interval of time t, over a radius r given by:

$$r^2 = 4\nu t \qquad (9)$$

At an instant of time $t = n/N$ after it is shed, a vortex would have traversed a downstream distance $n\alpha$ and it would have acquired a radius:

$$r = 2(u_0/N)\ (\beta n)^{1/2} \qquad (10)$$

where

$$\beta = \nu N/u_0^2 \qquad (11)$$

is the Lin parameter[3] and equals the ratio of the Strouhal number $S = Nd/u_0$ and the Reynolds number $R = u_0 d/\nu$. The parameter β is independent of the size of the obstacle and depends only on the parameters ν, N, and u_0, and hence it is an inherent property of the vortex-street.

A vortex would be perceptible until $r = h/2$. This yields for the eddy life-time:

$$\tau = \frac{1}{16\beta N}\left(\frac{h}{\alpha}\right)^2\left(\frac{u_e}{u_0}\right)^2 \qquad (12)$$

and the length of the wake:

$$L = \tau u_e \qquad (13)$$

In the laboratory investigations on vortex-streets, the various parameters have the following values:

$$\left.\begin{array}{lll} 0{\cdot}28 < h/\alpha < 0{\cdot}52 & u_e/u_0 \sim 0{\cdot}85 & S \sim 0{\cdot}2 \\ 35 < R < 2{,}500 & 10^{-3} < \beta < 2{\cdot}5\times10^{-3} & \end{array}\right\} \qquad (14)$$

We will now apply the above considerations to the meteorological eddies of Fig. 1. We have selected this particular photograph because it exhibits strong and distinct eddies in a pattern that is more complex than a single two-row vortex-street. A careful inspection of the eddy pattern in the light of the surface analysis 2 h before picture time reveals that there are indeed two vortex-streets generated by the interaction of the airflow with the Tenerife and Gran Canaria islands, respectively.

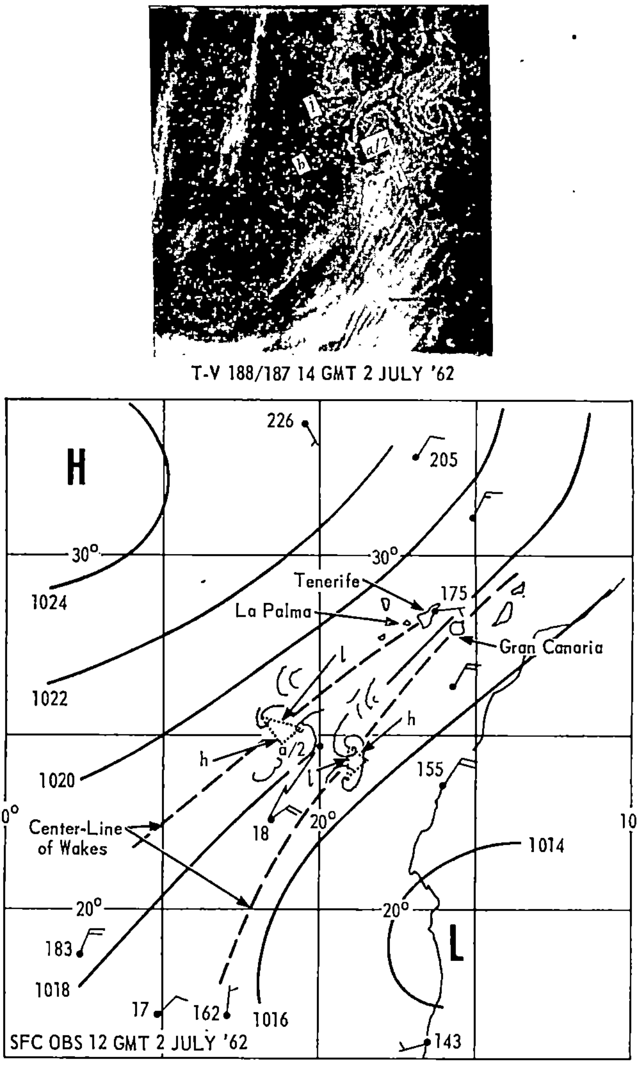

Fig. 1. Complex eddy pattern downstream from Canary Islands 1400 G.M.T. July 2, 1962, *Tiros* V pass 187

It appears from Fig. 1 that the easterly vortex-street is caused by the Gran Canaria Island with a cross-stream diameter $d \approx 35$ mi. The estimated value of the cross-diagonal distance l between two vortices of opposite circulations is 70 mi. Treating the Island as a cylindrical obstacle, it follows from equation (4) that:

$$h \approx 44 \text{ mi.} \quad \alpha = 2(l^2 - h^2)^{1/2} \approx 109 \text{ mi.} \quad h/\alpha \approx 0{\cdot}4 \qquad (15)$$

Again equations (3) and (6) lead to the relation

$$4\pi\left(\frac{h}{\alpha}\right)\left(\frac{u_e}{u_0}\right)\left(1 - \frac{u_e}{u_0}\right) = 1 \qquad (16)$$

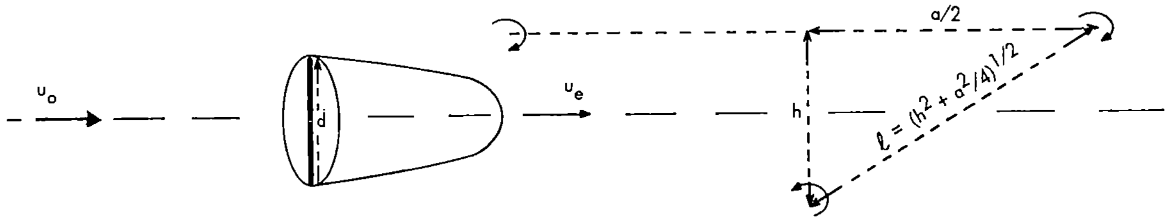

Fig. 2. Schematic diagram of a Kármán vortex-street pattern

which, with the adoption of $u_0 \approx 17$ m.p.h. and substitution for h/α from equation (15), yields:

$$u_e = 0{\cdot}73\, u_0 = 12{\cdot}4 \text{ m.p.h.} \qquad (17)$$

Hence, the frequency of shedding of the eddy-pairs is given by:

$$N \approx 0{\cdot}114/\text{h} \qquad (18)$$

The observed maximum length $L \approx 435$ mi. of the perceptible wake corresponds to $n \approx 4$, which leads to a life-time $\tau \approx 35$ h. These eddies are not always observable during their entire life-time, because certain favourable meteorological conditions, for example, low inversion layer, are essential for making the eddies visible. Therefore, it is of interest to estimate the minimum interval of time the pattern of Fig. 1 would have been discernible to an observer viewing the same area. Since an eddy passed from the upstream edge of the discernible portion of the vortex-street to its downstream edge—a length of approximately $0{\cdot}437L$—the pattern must have existed for at least an interval of time:

$$\tau_{\text{disc}} \approx 0{\cdot}437 \times 35 \approx 15{\cdot}3 \text{ h} \qquad (19)$$

Finally, the estimates of the characteristic parameters β, S, and R, and the coefficient of viscosity ν, are:

$$\left.\begin{array}{l} \beta \approx N\tau \approx \dfrac{1}{16n}\left(\dfrac{h}{\alpha}\right)^2\left(\dfrac{u_e}{u_0}\right)^2 \approx 1{\cdot}3 \times 10^{-3} \\ S = Nd/u_0 \approx 0{\cdot}235 \\ R = S/\beta \approx 178 \\ \text{and} \\ \nu = \beta u_0^2/N \approx 3{\cdot}36 \text{ mi.}^2/\text{h} \approx 2{\cdot}35 \times 10^7 \text{ cm}^2/\text{sec} \end{array}\right\} \qquad (20)$$

Similarly the westerly vortex-street appears to have been caused by the Tenerife and La Palma islands jointly offering a cross-stream diameter $d \approx 36$ mi. with $l \approx 65$ mi. On carrying out an analysis similar to that for the easterly vortex-street, we obtain the following set of values for the various parameters:

$$\left.\begin{array}{lll} h \approx 45 \text{ mi.} & \alpha \approx 94 \text{ mi.} & h/\alpha \approx 0{\cdot}48 \\ u_e/u_0 \approx 0{\cdot}8 & u_e \approx 13{\cdot}6 \text{ m.p.h.} & N \approx 0{\cdot}145/\text{h} \\ L \approx 5\alpha \approx 470 \text{ mi.} & \tau \approx 34{\cdot}5 \text{ h} & \tau_{\text{disc}} \approx 16{\cdot}7 \text{ h} \\ \beta \approx 1{\cdot}82 \times 10^{-3} & R \approx 170 & S \approx 0{\cdot}31 \\ \nu \approx 3{\cdot}6 \text{ mi.}^2/\text{h} & \approx 2{\cdot}55 \times 10^7 \text{ cm}^2/\text{sec} & \end{array}\right\} \qquad (21)$$

The foregoing estimates of the spacing ratio h/α, velocity ratio u_e/u_0, and the parameters S, R, and β are of the correct orders of magnitude for the stable vortex-street patterns observed in the laboratory (equation (14)). The estimate of the eddy viscosity also compares well with other independent estimates made, for example, from experiments on diffusion[4].

The foregoing considerations lead to the following conclusions:

(1) The mesoscale eddy pattern of Fig. 1 is an atmospheric analogue of the Kármán vortex-street observed in the laboratory.

(2) The eddies shown in Fig. 1 are caused by the Canary Islands.

(3) The considerations of the appropriate dimensionless parameters[5] indicate that the eddy viscosity in the atmosphere may be used in the dynamical equations in the same sense as the molecular viscosity is used in the laboratory model experiments to investigate atmospheric phenomena.

(4) The estimate of the eddy viscosity from the above analysis is in agreement with other independent estimates.

(5) A consideration of some practical consequence follows from the observed and expected persistence times of the eddy pattern. If the Canary eddies exist for at least 16 h, the meteorological conditions must also have been steady for that period. This would immediately suggest that if an Earth-synchronous satellite observed such an area continuously, the time-changes of the vortex-pattern would provide a very sensitive indicator of the changing meteorological conditions.

If the foregoing indications (1) and (4) are valid, the loss of momentum to the ocean surface would be smaller than that assumed by Hubert and Krueger[1], and, therefore, it may not be necessary to postulate an instability mechanism to account for the observed long life of the mesoscale eddies.

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ICE NUCLEATION AND GROWTH IN SUPERCOOLED WATER FILMS CONDENSED ON A HYDROPHOBIC SURFACE

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THE mechanism through which a substance may induce a transition of phase from supercooled water to ice[1,2] is still not fully understood, since, although hundreds of compounds have so far been investigated in cloud chambers, conditions at their surfaces seldom appear to have been rigorously monitored. In particular, some aspects of the action of silver iodide still seem to arouse great interest[3]; thus, both the size effect of silver iodide particles on ice-nucleation[4] and the sintering of silver iodide surfaces[5] were investigated in detail, and effects of some surface contaminants on the nucleability of silver iodide were evaluated[6,7]. It was shown, on the other hand, that silver iodide is de-activated by light in its properties of acting as an ice-forming nucleus, and therefore means of protecting the activity of the substance were experimentally sought[1,2].

Photolysis of a surface of silver iodide crystal will result in dissociation of the compound into elements and, what follows, in a progressive collapse of the catalysing surface structure[8]. Although this process will ultimately lead to the de-activation of silver iodide, it was thought[9] that formation of strains and lattice defects during the first moments of irradiation of the surface with light of suitable wave-length will initially activate the compound, because of enhancement of catalysis, due to surface defects and dislocations[10–13]. In order to confirm this hypothesis, we have carried out a series of measurements of ice-nucleability and related lag-times of nucleation, using both ice and silver iodide nuclei which were supported on smooth plates made of an anticorrosive aluminium alloy, 15·0 cm × 15·0 cm and 1 mm thick. The top of the plates was uniformly coated with a layer of 'High Vacuum, Apiezon *L*' stopcock grease. The plates were made to adhere to a copper block, 25 cm × 30 cm of surface, which was hollow and cooled inside to the required temperature, ± 0·2° C, by a fast-flowing stream of 'Antifreeze', which, in turn, was thermo-controlled by means of a standard refrigeration unit. Temperatures of the aluminium plates were obtained, on the basis of suitable calibrations, directly from an electric thermometer placed in the copper block. Only the upper surface of the copper block was exposed and not insulated; a stability of about 0·3° C could have therefore been achieved there, since the room in which the apparatus was mounted was kept at 20 ± 1° C and fluctuations in relative humidity were low (70 ± 10 per cent). Since temperatures at which the experiments were carried out were relatively high (> −12° C) the work was not appreciably affected by side-effects due to foreign matter nuclei in the air; such aerosol was, however, kept at a low level by means of an improvised electrostatic precipitator, which operated continuously in the laboratory.

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The development of an ice-spot on an 'Apiezon'-coated aluminium plate from water which condensed there from laboratory air could be satisfactorily monitored from a suitable angle if the plate were properly illuminated. Illumination was provided by a 30-W 6-V microscope lamp, which was situated obliquely to the plate at about 1 m distance and was switched on only for photography, or else by an ultra-violet '*HBO*-200' Zeiss high-pressure-mercury lamp of 600-W input power, which was provided with a quartz collimator and fritted-disk homogenizer, without filters, and which illuminated the plate continuously from the same distance. The experimental procedure was initiated by making a coated plate adhere to the block, after which the plate was fogged by breathing on it, and then allowed to reach thermal equilibrium for about 5 min. Nine groups of nucleation spots were thereafter symmetrically induced on the plates by placing, with a spatula, small mounds of the substance examined on the coated surface, while the procedure was timed. Photography of the growing ice-spot was carried out, using a 16-mm automatic *Ga-Mi* camera, red standard filter and fine-grain 20 *ASA* film; the dimensions of the growing spots were thereafter evaluated by direct examination of the developed film under an enlarger. Fig. 1 shows part of the photographic results of a typical run, while Fig. 2 gives the results obtained on the basis of photographs partly quoted in Fig. 1 and shows the increase of radii in the icing groups, with time.

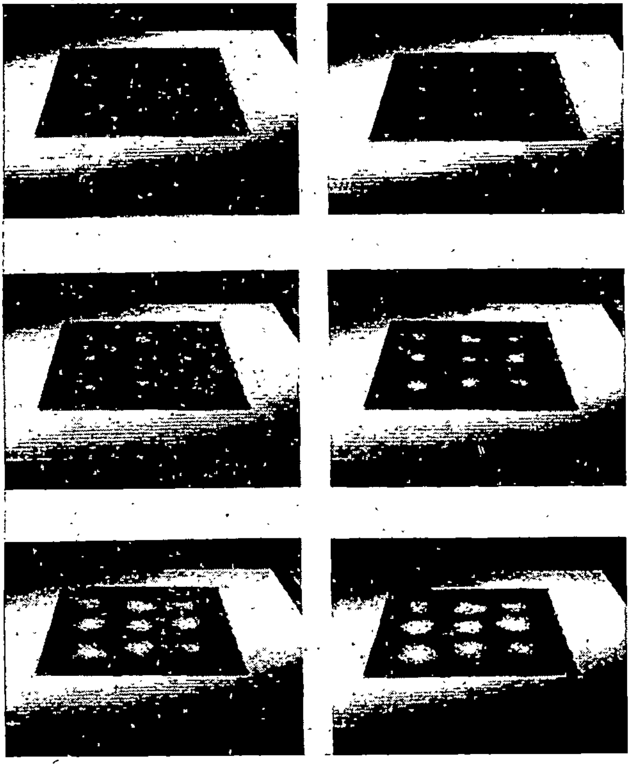

Fig. 1. Ice-spots on 'Apiezon'-coated metal plate, at −2·5° C and at different stages of growth. Timing is about: 600, 1,000, 1,400, 1,800, 2,200, 2,600 sec, respectively, from moment of seeding

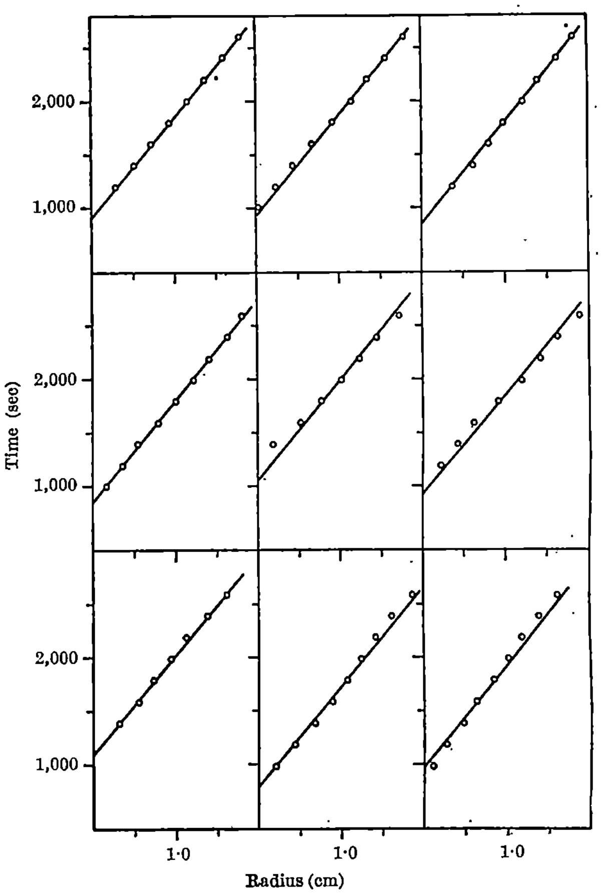

Fig. 2. Plots of times of growth versus 0·5 times diameter (as horizontally 'seen' by camera) of ice-spots shown in Fig. 1. Diagrams emphasize lag-times of nucleation of silver iodide; the experiment was carried out under ultra-violet illumination. Cumulative linear regression on all lines assumes common gradient[14]

Freshly ground, commercial silver iodide, 99 per cent pure, which was desiccated 24 h at 110° C in a stove and afterwards ground to pass through an A.S.T.M. screen of 140 mesh/in., or, alternatively, ice which condensed on the copper block, were used under conditions of illumination described here for the 'seeding' of plates.

The spread of icing after nucleation appears to be approximately linear with time in our experimental conditions; hence its rate can be obtained from the gradients of the plots, such as shown in Fig. 2, while the lag-time of nucleation inherent to the substance examined may be evaluated from the intercepts. The rates were calculated from gradients common to the lines obtained at the same conditions and were plotted against the temperature of operation in order to evaluate the change of the rates of growth with temperature (deg. C). The plots obtained from experiments where ice-growth was initiated

Table 1. TEMPERATURE DEPENDENCE OF RATES OF GROWTH OF ICE ON 'APIEZON', UNDER TWO DIFFERENT CONDITIONS OF ILLUMINATION AND WITH TWO DIFFERENT SEEDING AGENTS

Nuclei	Illumination	Rate of ice-growth (cm/sec/°C × 10^4)
AgI	Tungsten	2·46 ± 0·04
Ice	Tungsten	2·52 ± 0·07
Cumulative	Tungsten	2·48 ± 0·04
AgI	Ultra-violet	3·37 ± 0·1
Ice	Ultra-violet	3·71 ± 0·2
Cumulative	Ultra-violet	3·54 ± 0·1

either with ice or with silver iodide nuclei are shown in Fig. 3, and the results are summarized in Table 1.

While the time of lag in nucleation appeared to be approximately nil in the case of freezing initiated with ice, as substantiated by a symmetrical distribution, with a standard deviation of 50 sec, of extrapolated values about the r axis, the behaviour of silver iodide is quite different, as shown in Fig. 4. The experimental values appear to follow here a monotonic increase with temperature; the plot can satisfactorily be fitted (see later) with a function of the type:

$$t = \frac{\alpha}{\Delta T}\, e^{-\beta \Delta T} \qquad (1)$$

where t = lag-time of nucleation (sec); this quantity is taken as an average of individual lag-times of n spots which nucleated out of the total of nine spots, multiplied by $9 : n$; ΔT = the difference (deg. C) between T_0, the highest temperature at which the substance will nucleate ice, and the temperature of operation, T; α and β = constants, which can be estimated by linear regression if ΔT is known.

We are aware that the experimental method, such as described here, of estimating ice-nucleating power and lag-time of action of different substances may be subject to several refinements. However, although this work was carried out mainly with the object of finding and testing fast and reliable means of evaluating ice-nucleating properties of diverse compounds without having to disperse them as aerosols, some preliminary observations of a more fundamental nature can already be made at this time. The rates of growth of nucleated ice-layers appear, in our experimental conditions, to be in fair agreement with the order of magnitude of values derived by Burton, Cabrera and Franck, as discussed by Mason[15]; they differ, however, from values proposed by Hillig and Turnbull[16], probably due to the effects of the substrate on which such growth occurs[17] and to the structure of the growing ice-surface[18,19] and related effects[20], as substantiated by faster rates of growth under ultra-violet radiation. The lag-time of ice nucleation of a substance may, in our experimental conditions, be expressed in terms of the difference between the temperature at which the substance nucleates, and the temperature of operation. If equation (1) is resolved in series for small values of ΔT, there is approximate proportionality between t^{-1} and ΔT, and hence the value of T_0, the highest temperature at which a substance will act as a freezing nucleus, may be obtained by extrapolation (Table 2).

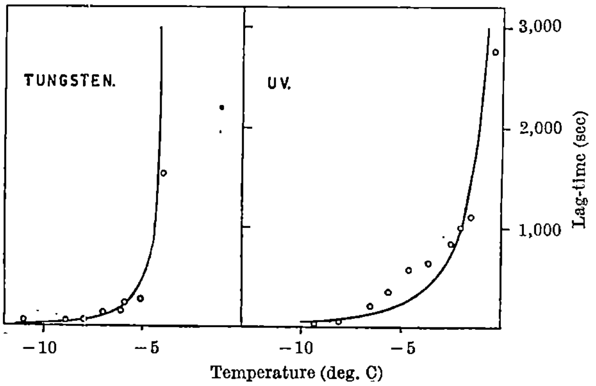

Fig. 4. Lag-times of nucleation of ice on 'Apiezon', for identical silver iodide product, under tungsten and ultra-violet light; experimental points were fitted with function of type given in equation (1); parameters are given in Table 2

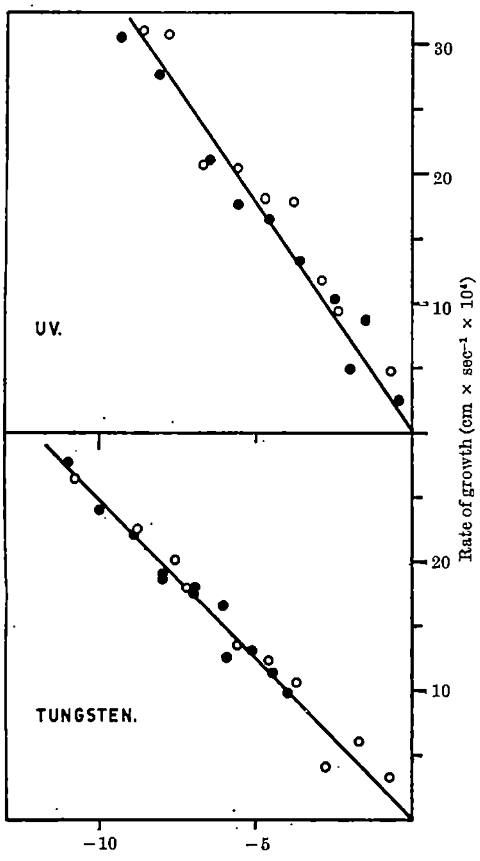

Fig. 3. Growth of nucleated ice-spots on 'Apiezon' substrate, as function of temperature. Full and empty circles stand for experiments where silver iodide and ice, respectively, were used for 'seeding' of plates. Regression was made to force lines through origin

Table 2. CONSTANTS OBTAINED FROM CURVE-FITTING PROCEDURE ACCORDING TO EQUATION (1), AND HIGHEST EXPERIMENTAL TEMPERATURES OF NUCLEATION OF SILVER IODIDE, FOR BOTH TYPES OF ILLUMINATION

Illumination	Correlation coefficient, t^{-1} versus $T(-6–0°C)$	T_0 (°C) Calculated	T_0 (°C) Used for curve fitting	T_0 (°C) Highest experimental	α	β
Tungsten	−0·97	−3·9 ± 0·2	−3·9	−4·0	726	0·260
Ultra-violet	−0·97	+0·4 ± 0·5	0·0	−0·4	2,763	0·168

Both Fig. 4 and Table 2 reveal an effect of ultra-violet radiation on the nucleating power of freshly ground and desiccated silver iodide, indicating, in agreement with what was stated in ref. 9, that the activity of a crystal of silver iodide may first be enhanced by short-wave light, and only afterwards destroyed by photolysis.

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SEGRÉ-SILBERBERG ANNULUS FORMATION: A POSSIBLE EXPLANATION

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SEGRÉ and Silberberg, working with a neutrally buoyant dilute suspension of rigid spheres, were first to report the formation of an annulus of spheres concentric with the tube axis during laminar flow through a vertical duct of circular cross-section[1]. Their result, described in detail elsewhere[2], was unexpected because prior investigations of radial particle displacements in Poiseuille flow had suggested only the possibility that particles could move radially inward, that is, from the wall toward the tube axis.

We are at present examining the radial motion of single rigid spheres in a laminar flow contained in a vertical duct and have observed particles that drifted all the way from the wall to the tube axis while others have done the reverse. Still others have exhibited no radial drift or have drifted either a short distance in from the wall or a short distance out from the axis, a condition analogous to that required by the Segré-Silberberg effect.

We believe these phenomena can be explained as follows: Rubinow and Keller[3] have shown that a rotating sphere moving with velocity V through a viscous unbounded fluid is acted on by a lift force F given by:

$$F = \pi a^3 \rho_f \Omega \times V \qquad (1)$$

where a is the sphere radius, ρ_f is the fluid density, Ω is the angular velocity, V is the difference between the transverse velocities of the fluid and particle and F is the lift force perpendicular to the plane of Ω and V. This equation was not derived for a Poiseuille flow, but, as Rubinow and Keller point out, there is reason to believe that it yields a force of the correct order of magnitude for such a flow. This assumption was supported by the results of a test we applied to the data of Goldsmith and Mason[4] for rigid spheres in a Poiseuille flow at very low Reynolds numbers. (Equations (1), (2) and (5) of this article were combined to give another describing the radial lift force on the sphere. Radial acceleration was neglected and the differential equation describing radial motion of the sphere was obtained by equating the lift force to the Stokes drag, $6\pi\mu a(\mathrm{d}r/\mathrm{d}t)$. Simultaneous solution of the differential equations of radial and axial motion resulted in predictions of particle trajectories which for the small radial migrations reported by Goldsmith and Mason resulted in predicted axial travels greater than those observed by a factor of 5·2 at most, and by a factor of 2·7 on the average. Similar results are obtained for particles in the system we are investigating (described elsewhere in this article), a system considerably different from that of Goldsmith and Mason.) Accordingly we apply equation (1) here, bearing in mind the reservations associated with its use. The required rotational and transverse velocities are consequences of the parabolic velocity distribution as is shown below for the case of a neutrally buoyant system.

The gradient of the velocity profile is responsible for the angular velocity of the particle. If one restricts consideration to spheres the diameter of which is small compared with the radius of curvature of the velocity profile, Ω can be approximated by a relationship given by Jeffery[5]:

$$\Omega = -\frac{v_m r}{R^2} \qquad (2)$$

where v_m is the maximum (centre-line) velocity within the tube, r is the distance from the axis to the sphere centre and R is the tube radius.

Goldsmith and Mason[6] have presented evidence showing that for rigid spheres the difference between actual particle velocity and the fluid velocity that would prevail at the location of the sphere centre increases as $\frac{r}{R}$ increases, with the sphere lagging behind for all $\frac{r}{R}$. Their results, obtained from several systems, when put in non-dimensional form, give a general curve, which we have approximated by:

$$\frac{v_f - v_p}{v_m} = \frac{2}{3}\left(\frac{a}{R}\right)^2 \left[A\left(\frac{r}{R}\right)^4 + B\left(\frac{r}{R}\right)^2 + C\right] \qquad (3)$$

where v_f is the fluid velocity that would prevail at the sphere centre were the sphere not there, v_p is the actual axial particle velocity, and the constants A, B and C have the values 3·04, 1·58 and 1·00. Dr. Mason has kindly furnished additional data points[7] which fall slightly above this curve, and he points out that one would expect factors such as the Reynolds number to affect the position of the curve. However, we shall see that the important feature of equation (3) is its dependence on $\frac{r}{R}$, and Dr. Mason's additional results also exhibit this dependence.

With the above means of approximating the particle velocity, a decision is now required as to what fluid velocity to compare it with in order to obtain V. Since the fluid velocity is different at various points surrounding the sphere, some form of average velocity is suggested. Consider the moving particle to sweep out a tube of fluid within the paraboloid describing the velocity distribution in the duct. The presence of the sphere will influence the fluid to some distance δ past the sphere surface. If one computes the average velocity v_{af} of the fluid within this tube of radius $(a+\delta)$ for any position of the tube within the duct, the result is:

$$v_{af} = v_m\left[1 - \left(\frac{r}{R}\right)^2 - \frac{1}{2}\left(\frac{a+\delta}{R}\right)^2\right] \qquad (4)$$

Since $v_f = v_m\left[1 - \left(\frac{r}{R}\right)^2\right]$, it follows that:

$$V = v_{af} - v_p = \frac{2}{3}\left(\frac{a}{R}\right)^2 v_m\left[A\left(\frac{r}{R}\right)^4 + B\left(\frac{r}{R}\right)^2 + C\right] - \frac{v_m}{2}\left(\frac{a+\delta}{R}\right)^2 \qquad (5)$$

The following considerations apply in the selection of a value for δ: when $\delta = 0$, V is positive for all $\frac{r}{R}$. In terms of a co-ordinate system fixed on the duct centre-line in which the positive axial direction is with the flow, a positive V together with the negative value of Ω from equation (2) would give a negative, or inward-directed force on the sphere as determined by the vector cross-product of equation (1). If δ is assigned increasingly larger values (as defined, δ cannot be negative) the value of V for any $\frac{r}{R}$ is decreased from its $\delta = 0$ value until at $\delta = 1{\cdot}15a$ V becomes zero. Further increases in δ result in negative V near the tube axis and a corresponding shift in the $V = 0$ point toward the tube wall. Negative values of V are associated with particle drift away from the tube axis, again as dictated by the vector cross-product of Ω and V. Thus, if δ were in fact greater than $1{\cdot}15a$, one would predict the existence of a region near the tube axis in which particles would seek to move away from the axis and to accumulate at the value of $\frac{r}{R}$ for which $V=0$. Similarly, particles near the wall (where V is positive) would move inward, also seeking the position for which $V=0$. When $V=0$ the radial force is zero and this consideration determines the site of the Segré-Silberberg annulus.

The choice of δ therefore influences the prediction of where the annular zone would form. (The accumulation site is also influenced by the fluid velocity distribution. In a two-dimensional parabolic field (as ideally between broad flat plates or as approximated by a duct of rectangular cross-section of large aspect ratio) the coefficient of $(a+\delta)^2/R^2$ in equation (4) is 1/4 instead of 1/2. This produces the result that, for a given value of δ, the accumulation site would occur, if at all, closer to the axis than it does within a duct of circular cross-section.) In actuality δ is possibly even a function of $\frac{r}{R}$, but is here assumed constant for simplicity. Dr. Mason's results, which demonstrated drift in either direction[7], were best correlated by assuming $\delta=a/2$. When V is plotted against $\frac{r}{R}$ for $\delta=a/2$ a curve such as Fig. 1 is obtained where $V=0$ corresponds to $\frac{r}{R}=0·53$. This value agrees reasonably well with that reported by Segré and Silberberg.

Our observation that particles could move in either direction over the entire distance between the wall and the tube axis has a similar explanation. The density of these particles was about 0·1 per cent different from that of the fluid. Consequently a buoyancy term, independent of position within the duct and approximated by the Stokes settling velocity, must be added to the value of V. A particle less dense than the fluid could therefore be described by a curve similar to Fig. 1, only shifted upward in its entirety by an amount equal to the Stokes velocity. A sufficiently large buoyant velocity could make the curve everywhere positive and thereby give a force directing the particle toward the tube axis for all values of $\frac{r}{R}$. Similarly, a sphere more dense than fluid in downflow through a vertical tube could be driven out to the duct wall.

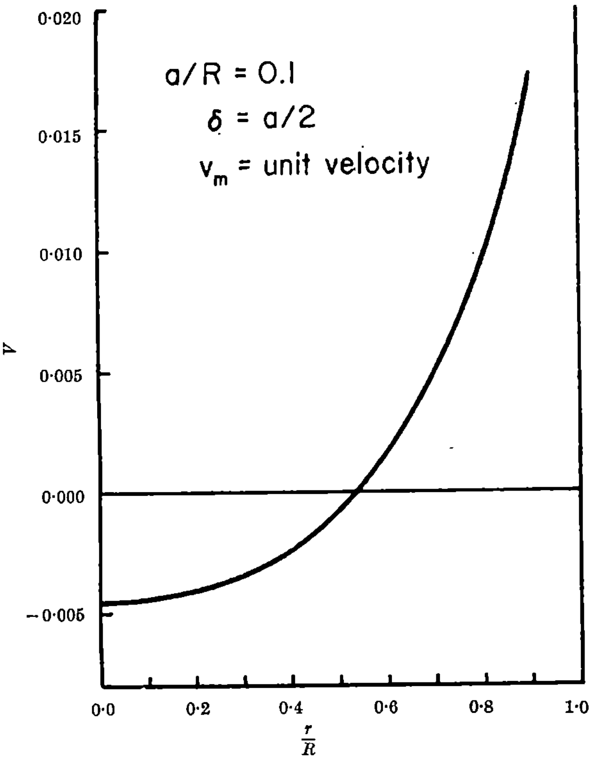

Fig. 1. Typical plot of velocity difference between sphere and fluid versus radial position. Where V is negative, particle moves faster than surrounding fluid and drifts outward, whereas for positive V the reverse is true. $V=0$ represents stable equilibrium point

Experimentally we have readily produced these phenomena in a system of single nylon spheres within a flowing aqueous sodium chloride solution. The small density differences required to change the drift direction are obtained either by changing the system temperature (usually less than 5° C) degrees for a given sphere density, or by the addition of either more salt or water to the fluid.

A typical result with three spheres of 0·476 cm diameter and only slightly different densities released at different times from a position of $\frac{r}{R}=0·2$ within a long vertical duct of rectangular cross-section showed the following: after 66-cm axial travel a nearly neutrally buoyant sphere had drifted to $\frac{r}{R}=0·47$, a second sphere 0·04 per cent more dense than the first had drifted to $\frac{r}{R}=0·66$ and a third 0·06 per cent less dense than the first had drifted to $\frac{r}{R}=0·25$ These values do not represent equilibrium positions and are cited for the purpose of illustrating the effect of small density differences. (Cross-section dimensions are 2·54 cm × 10·16 cm and duct-length is 2·5 m, the lower 66 cm of which are used for experimental observations. Drift is examined between the narrowly separated walls and is confined to the mid-plane between the other walls. In the example cited, R therefore equalled 1·27 cm and the fluid flow rate was 50 cm³/sec.)

Oliver[8] has reported similar experimental results and furthermore has concluded that particle rotation causes movement to the wall while the absence of rotation causes motion away from the wall. Equation (1) here appears to require that both particle rotation and slip be present simultaneously in order that a lift force may exist. Oliver observed drift with no rotation, however, and we have found that non-rotating particles could drift either toward or away from the duct wall. (Spheres were drilled with small shallow holes which were then filled with wax so that the particle remained spherical in shape, but with a displaced centre of gravity. The fluid density was then adjusted so that it either equalled or was slightly different from that of the particle.) The explanation of the apparent discrepancy between equation (1) and observation is that the equation is of the same type as the more general Kutta-Joukowski equation for infinitely long cylinders in which the lift is related to the cross-product of the fluid circulation and the transverse velocity difference. As Tollert[9] points out, circulation can arise with a translating sphere either by sphere rotation or by unsymmetrical streaming about the non-rotating sphere. The Rubinow-Keller stationary viscous fluid required that the particle rotate in order that the circulation be non-zero, with the circulation in effect being approximated by a function of the particle angular velocity. In a Poiseuille flow, however, the velocity distribution produces a non-zero circulation by virtue of the unsymmetrical streaming about the sphere. (This was demonstrated by filling several small shallow holes in a non-rotating sphere with potassium permanganate. As the sphere was carried along by the fluid, two easily visible permanganate streams emanated from it: one leading the sphere and which left its surface on the duct axis side of the sphere centre and one trailing the sphere and which left the surface on the wall side of the sphere centre.) Hence one would expect particle drift to be possible in either direction anywhere in the tube except at the axis where, because of symmetry, the circulation vanishes to zero. This requirement confirms the intuitive feeling regarding the importance of particle shape as a factor in influencing drift rate, because of the way in which streaming around the body and hence circulation is shape-dependent. Equation (1) is therefore to be regarded as only an approximation in which the circulation is in turn approximated by a function of the fluid velocity gradient. Dryden[10], in discussing the circulation about rotating cylinders, referred to the discrepancy between theory and experiment and concluded that accuracy requires actual measurement of the circulation, a comment that sheds light on the likelihood of developing a single accurate expression for predicting drift rates for particles of different shapes and surface conditions.

In summary, the foregoing analysis has described the factors producing radial drift and has proposed a means by which the Segré-Silberberg annular zone could be formed. Experimentally we have qualitatively confirmed all modes of drift predicted for a system flowing down a vertical duct. We are now seeking to compare quantitatively the observed trajectory with the predicted trajectory based on the radial force obtainable by a combination of equations (1), (2) and (5).

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SEPARATION OF HYDROCARBON LIQUIDS BY FLOW UNDER PRESSURE THROUGH POROUS MEMBRANES

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THE selective permeation of hydrocarbon mixtures through non-porous polymeric films has been examined extensively[1-12]. In these investigations the feed is usually in the liquid state, the film is held in the swollen state by heating and the permeated product is withdrawn as a vapour and afterwards condensed. On the other hand, Kammermeyer and Hagerbaumer[13] established the feasibility of the liquid phase separation of several hydrocarbon mixtures by pressure permeation through microporous glass having an average pore diameter of 40 Å.

The concept of preferential sorption at the fluid-membrane interface together with the flow of the interfacial fluid through capillary pores of size equal to, or less than, a critical diameter characteristic of the system offers a new approach to the development of a general industrial process for the separation of hydrocarbon mixtures. The experimental technique consists simply in letting the fluid mixture flow under pressure through an appropriate porous membrane; the membrane-permeated fluid is enriched in the preferentially sorbed constituent. No heating of the membrane and no phase change in product recovery are involved in this separation process.

The governing criteria of this process may be stated as follows. An appropriate chemical nature of the film surface in contact with the solution, together with the existence of pores of appropriate size on the area of the porous film at the interface, is an indispensable twin requirement for the success of this separation technique. Further, the rate of fluid permeation through the membrane can be increased, without affecting the degree of separation under the given operating conditions, by simply increasing the number of pores of the appropriate size on the film surface. Thus, for any given membrane material, the obtainable degree of separation and the rate of fluid permeation are two independent characteristics of the film, which can be varied by varying its porous structure.

From an industrial point of view, the application of the above technique for a given separation problem involves essentially the problem of choosing the appropriate chemical nature of the film surface and developing techniques for making films containing the largest number of pores of the required size on the area of the film at the interface. This approach formed the basis of the successful development of porous cellulose acetate membranes capable of giving both high flow rates and high degrees of demineralization of aqueous sodium chloride solutions[14]. The general applicability of the foregoing technique for the separation of inorganic and organic substances in aqueous solution, as well as for the separation of gases, has already been demonstrated[15-18]. This communication is concerned with its applicability for the separation of liquid hydrocarbon mixtures.

Three different types of porous films, designated here as *S and S*, *CA* and *PE* films, were used in these experiments. The *S and S* films are the commercially available cellulose acetate ultrafine filter membranes, type *UA*-superdense, supplied by Carl Schleicher and Schuell Co. The *CA* films are porous cellulose acetate membranes made in the laboratory by the method described earlier[14] using the following composition (weight per cent) for the film casting solution: acetone, 68·0; cellulose acetate, 17·0; water, 13·5; magnesium perchlorate, 1·5. The *S and S* and *CA* films have similar, but not identical, relative separation characteristics, and hence they are classified separately. These films were initially shrunk by immersion in hot water, and then washed in successive dishes of absolute alcohol. The *PE* films are porous polyethylene films made in the laboratory by a special method, details of which will be published at a later date. The feed solution systems tested were xylene-ethyl alcohol, xylene-*n*-heptane and *n*-heptane-ethyl alcohol. Just before use in the experiment, the films were dried between filter papers, and quickly immersed in the feed solution and again dried superficially between filter papers. The operating cell is a stainless steel pressure chamber consisting of two detachable parts. The film is mounted on a stainless steel porous plate embedded in the lower part of the cell through which the membrane permeated liquid leaves the system at the atmospheric pressure. The upper part of the cell contains the feed solution under pressure in contact with the membrane. The two parts of the cell are clamped and sealed tight using rubber O-rings. Compressed nitrogen gas was used to pressurize the system. The experiments were conducted under static conditions at the laboratory temperature. A new sample of the film was used for each experiment. The quantity of liquid removed by membrane permeation was small compared to the amount of feed solution in the pressure chamber. The compositions of the feed and the membrane permeated liquids were analysed by means of a Bausch and Lomb precision refractometer capable of giving refractive index data within an accuracy of 0·00003, which corresponds to an accuracy of better than ±0·1 per cent for the systems tested in this work.

A set of experimental results is given in Table 1 and Fig. 1. The results illustrate the fact that the separation technique was successful in all the cases tested. For the feed system xylene-EtOH, the *S and S* and *CA* films have preferential sorption for EtOH, and the *PE* films exhibit preferential sorption for xylene. Further, when the surface of the *S and S* film was sprayed uniformly with 'Fluoro-glide' (a fluorocarbon polymer supplied by Chemplast, Inc.) and used in contact with the above feed system, the direction of separation was reversed. This latter observation again shows[19] that the mechanism of the process is governed by a surface phenomenon. For the system xylene-*n*-heptane, all three types of films used exhibit a preferential sorption for xylene. For the system *n*-heptane-EtOH, the membrane permeated liquid shows alcohol enrichment for the *S and S* and *CA* films and *n*-heptane enrichment for the *PE* films ; the data also illustrate that azeotropic composition is no limitation to this separation process. The chemical nature of the membrane surface determines the direction of separation for a given feed system. The separation and flow rate data given here do not set the limits of those obtainable for the types of films used, but they illustrate that different levels of separation and flow rates are obtainable for the same membrane material depending on the porous structure of the films used. Fig. 1 shows the effect of operating pressure on the separation and flow characteristics of a *CA* film for the system *n*-heptane-EtOH. The variation of flow rate of the membrane permeated product with pressure, considered together with the corresponding alcohol-enrichment in the product, indicates that the process involves capillary flow of the liquid through the porous film.

Table 1

Area of film surface: 7·9 cm². Operating pressure: 1,000 p.s.i.g.

Exp. No.	Feed liquid composition (mole %)		Film type	Membrane-permeated liquid Composition (mole %)		Flow rate (g/h)
1	Xylene EtOH	19·5 80·5	*S and S* film	Xylene EtOH	16·5 83·5	(Not determined)
2	Xylene EtOH	19·5 80·5	*S and S* film surface sprayed with 'Fluoro-glide'	Xylene EtOH	23·0 77·0	,, ,,
3	Xylene EtOH	19·5 80·5	*S and S* film	Xylene EtOH	12·5 87·5	,, ,,
4	Xylene EtOH	14·0 86·0	*S and S* film surface sprayed with 'Fluoro-glide''	Xylene EtOH	15·5 84·5	,, ,,
5	Xylene EtOH	50·5 49·5	*S and S* film	Xylene EtOH	38·0 62·0	1·23
6	Xylene EtOH	51·0 49·0	,, ,,	Xylene EtOH	41·0 59·0	1·04
7	Xylene *n*-Heptane	50·0 50·0	,, ,,	Xylene *n*-Heptane	57·5 42·5	1·85
8	Xylene *n*-Heptane	50·0 50·0	,, ,,	Xylene *n*-Heptane	60·5 39·5	0·44
9	*n*-Heptane EtOH	33·0 67·0 *	,, ,,	*n*-Heptane EtOH	9·5 90·5	2·90
10	*n*-Heptane EtOH	33·0 67·0 *	,, ,,	*n*-Heptane EtOH	6·0 94·0	0·51
11	Xylene EtOH	51·0 49·0	*CA* film	Xylene EtOH	39·0 61·0	2·03
12	Xylene EtOH	51·0 49·0	,, ,,	Xylene EtOH	34·0 66·0	1·67
13	Xylene EtOH	51·0 49·0	,, ,,	Xylene EtOH	28·0 72·0	0·43
14	Xylene *n*-Heptane	50·5 49·5	,, ,,	Xylene *n*-Heptane	84·0 16·0	0·18
15	*n*-Heptane EtOH	33·0 67·0 *	,, ,,	*n*-Heptane EtOH	8·5 91·5	2·16
16	*n*-Heptane EtOH	33·0 67·0 *	,, ,,	*n*-Heptane EtOH	12·5 87·5	25·21
17	Xylene EtOH	51·5 48·5	*PE* film	Xylene EtOH	97·5 2·5	0·17
18	Xylene EtOH	51·5 48·5	,, ,,	Xylene EtOH	98·5 1·5	0·12
19	Xylene EtOH	51·0 49·0	,, ,,	Xylene EtOH	98·0 2·0	0·18
20	Xylene *n*-Heptane	50·5 49·5	,, ,,	Xylene *n*-Heptane	53·5 46·5	0·47
21	Xylene *n*-Heptane	50·5 49·5	,, ,,	Xylene *n*-Heptane	54·5 45·5	0·47
22	*n*-Heptane EtOH	33·0 67·0 *	,, ,,	*n*-Heptane EtOH	61·5 38·5	0·18

* Azeotropic mixture.

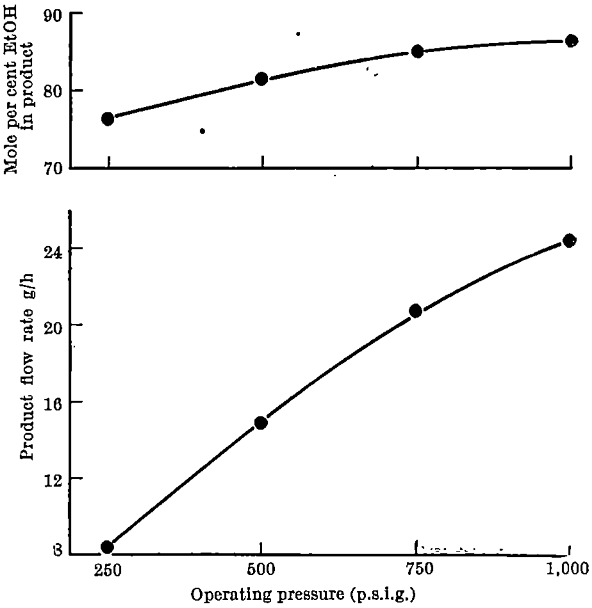

Fig. 1. Effect of operating pressure on the separation and flow characteristics of a *CA* film. Area of film surface, 7·9 cm²; feed composition (mole per cent): *n*-heptane, 33; EtOH, 67

The experimental results presented in this paper and those reported earlier[15–18] demonstrate the general applicability of the foregoing technique for the separation, concentration or fractionation of inorganic or organic substances in aqueous or non-aqueous solutions in the liquid or the gaseous state, and open a new and versatile field of separation technology in chemical process engineering. A precise understanding of the physicochemical criteria of preferential sorption (or preferential repulsion) of substances at interfaces, and the mechanism of pore formation in the process of casting high-flow porous films is, however, needed to develop this technology to its fullest industrial potential. Already it is clear that the high flow porous cellulose acetate membranes developed in connexion with saline water conversion[20] can also be profitably used in separation processes involving a wide variety of aqueous solutions as well as mixtures of hydrocarbon liquids.

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RELATION OF PROTEIN EVOLUTION TO TERTIARY STRUCTURE

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THE recent advances in the determination of the amino-acid sequences and the three-dimensional configurations of proteins have provided a great impetus to the examination of the evolution of proteins, or 'chemical palæogenetics'[1]. When one compares proteins which are closely related in configuration and function but differ significantly in their amino-acid sequences (as, for example, the several chains of hæmoglobin), it is of great interest to determine whether there are any basic principles that governed their evolution. Two stages of protein evolution may be arbitrarily defined. The first involves the assembly of amino-acids into a 'primitive protein', such as the postulated 'primitive chain' of hæmoglobin, which has a definite configuration and function (whether enzymatic, structural, transport, or other). In terms of evolutionary chronology, this is probably by far the longer stage. During the second stage of evolution, with which this article is concerned, the 'primitive protein' undergoes divergent evolution, presumably by a process of random mutations and natural selection (of the organism in which it is found), so that several distinct proteins (in the same or different species) with similar structures and activities are produced.

It is now generally believed that the specific conformation that a polypeptide chain assumes in the formation of a 'native' protein is determined principally by its amino-acid sequence[2]. This being the case, for a 'mutant' or 'evolved' protein to preserve its original function, two requirements must be met. First, it is necessary that the few critical residues directly involved in activity or function remain intact (or that adequate substitutes be provided). Second, the polypeptide chain must be capable of folding in a way that will bring these residues into the proper relationship to one another. Therefore, an enquiry into the principles which govern the evolution of homologous proteins from common precursors becomes, in a large measure, an investigation of the factors which govern the folding of proteins.

On the basis of investigations of the chemical and X-ray crystallographic properties of myoglobin[3,4], a simple but important generalization has been made: most, but not all, of the polar residues appear to be located on the surface of the molecule, while most of the non-polar (hydrophobic) residues are found in the interior of the molecule. The results obtained from the experimental modification of ribonuclease[5,6] and trypsin[7] are in agreement with these ideas. The only major exception, in the case of myoglobin, involves glycine and alanine, these residues being frequently located on the surface. On the basis of these observations, the following hypothesis was recently advanced[8]: during the evolution of homologous proteins, amino-acid substitutions (or 'exchanges' or 'transitions') will be permissible (that is, will allow for proper folding of the protein) if they involve interchanges between one polar residue and another, between one hydrophobic residue and another, or (in some cases) between a polar residue and glycine or alanine: exchanges between polar and hydrophobic residues will not be permissible. This hypothesis is derived, of course, from the supposition that during the folding process polar residues will tend to seek out the surface of the molecule while hydrophobic ones will seek out the interior. Therefore, if a non-permissible substitution is made, a residue destined for the surface might now seek out the interior, or vice versa, thereby interfering with the correct folding of the molecule.

These 'rules' must be considered in two contexts. The first involves situations in which the homologous proteins differ by only one or a very few amino-acid residues. In such cases, it is quite conceivable that a small number of non-permissible exchanges might not be able to negate the overwhelming influence of the remainder of the residues and would not, therefore, interfere with the formation of the proper tertiary structure. On the other hand, when two homologous proteins differing significantly in their amino-acid sequences are compared, it would be expected, if the hypothesis is correct and if structural and functional integrity is to be preserved, that the 'rules' should hold quite firmly for the aggregate of the amino-acid exchanges. It should also be realized that other unknown 'rules', possibly not always fully compatible with the hypothesis under consideration, may be involved in protein evolution. Furthermore, it is quite apparent that the present hypothesis represents a simplification of the process of protein evolution and of the mechanics of polypeptide chain folding ignoring, as it does, the role of factors such as electrical charge and the size and shape of the various amino-acid residues.

In order to test the hypothesis stated here, the following question was asked: in comparing related proteins, that is, proteins with similar functions and with sufficient homologies in amino-acid sequence to ensure that they are derived from common precursors, how often are the observed amino-acid exchanges of a permissible type, and how often are they not? To facilitate the analysis, the specific amino-acid differences between pairs of homologous proteins for which complete primary structure data were available were placed in one of three categories: (I) permissible: polar/polar or hydrophobic/hydrophobic interchanges; (II) possibly permissible: polar/glycine or polar/alanine interchanges; (III) non-permissible: polar/hydrophobic interchanges.

The assignment of residues to the polar or non-polar groups has followed the usage of Kendrew[3], and tyrosine and tryptophan have been considered as polar residues; cysteine, which is only rarely involved in an exchange, has been considered as non-polar. Accordingly, interchanges between phenylalanine and another aromatic amino-acid have been placed in category III, although it is quite conceivable that they could be considered as permissible in many instances (particularly phenylalanine/tyrosine interchanges).

In order to evaluate the significance of the results obtained, it is necessary to have a standard for comparison. Ideally, one would like to know the single and multistep mutations that are actually (chemically) possible on the basis of the nucleotide amino-acid 'code words'. Unfortunately, such data are not yet available. Experimentally produced single-site mutations are still too few in number and such information would, in general, not be applicable. This is because of the fact that, in most cases, only those mutations which are compatible with the recognition and isolation of the mutant proteins are observed, thereby eliminating from consideration those mutations that might be chemically feasible but structurally impossible.

Because of these difficulties it was thought reasonable, in this preliminary analysis, to compare the observed

Table 1. Analysis of Observed Amino-acid Exchanges

	Authors* (refs.)	Total ex-changes	Type of exchange			Assuming exchanges of type I + type II are permissible				Assuming exchanges of type I + 1/2 × type II are permissible			
			I	II	III	Permis-sible	Non-per-missible	χ^2	P	Permis-sible	Non-per-missible	χ^2	P
Expected random frequencies						60%	40%			54%	46%		
Observed frequencies (actual numbers)													
Hæmoglobin													
Human/horse, α-chain	15	17	9	6	2	15	2	4·53	< 0·05	12·0	5·0	1·27	—
Human/primate, β-chain	16	26	16	7	3	23	3	7·63	< 0·01	19·5	6·5	3·81	≈ 0·05
Human, α/β-chains	17, 18	72	48	15	9	63	9	21·56	< 0·001	55·5	16·5	14·53	< 0·001
Human, β/γ-chains	19	40	24	12	4	36	4	13·78	< 0·001	30·0	10·0	6·28	< 0·02
Hæmoglobin/myoglobin													
γ-chain (human)/myoglobin (whale)	4, 9, 19	107	58	23	26	81	26	10·31	< 0·01	69·5	37·5	4·72	< 0·05
α-chain (human)/myoglobin (whale)	4, 9, 17	102	57	18	27	75	27	7·23	< 0·01	66·0	36·0	4·29	< 0·05
Insulin													
Human/bonito II	20, 21, 22	7	4	0	3	4	3	—	—	4·0	3·0	—	—
Cytochrome *c*													
Horse/human	23	12	5	4	3	9	3	—		7·0	5·0	—	
Horse/baker's yeast	23, 24	39	26	3	10	29	10	2·78	—	27·5	11·5	3·64	—
Tuna/baker's yeast	24, 25	41	29	7	5	36	5	12·07	< 0·001	32·5	8·5	9·55	< 0·01
Hæmoglobin, single-site mutations (human)	26	16	11	3	2	14	2	3·96	< 0·05	12·5	3·5	2·84	—
Total		479	287	98	94	385	94	82·02	< 0·001	336·0	143·0	49·62	< 0·001

frequencies of the various categories of exchanges with the frequencies which would be expected on the basis of random exchanges between any two of the twenty amino-acids (without utilizing any specific information relating to the amino-acid 'code'). By the latter method, the 'random' frequencies of the three categories of exchanges were found to be: type I, 48 per cent; type II, 12 per cent; and type III, 40 per cent. When these data were weighted for the actual amino-acid composition of a protein such as the hæmoglobin β-chain, the frequencies obtained were very nearly the same.

Two different methods have been used to compare the random expectations with the observed frequencies. The first method (method *A*) is based on the assumption that all type II exchanges will be permissible, that is, that type I + type II = permissible exchanges, and type III = non-permissible exchanges. Thus, the frequency of randomly-expected permissible exchanges will be 48 per cent + 12 per cent = 60 per cent, and of non-permissible exchanges, 40 per cent. These values may then be compared with the observed data (in which type I and type II exchanges are similarly combined), and the results analysed statistically by the χ^2 method (with the Yates correction for continuity). The second method (method *B*) of comparison makes use of the more rigorous assumption that, on the average, only half of type II exchanges are actually permissible (for example, in specific instances, a polar-glycine might be while an alanine-polar might not). The random frequencies then become: permissible = I + 1/2 × II = 48 per cent + 1/2 × 12 per cent = 54 per cent; non-permissible = 1/2 × II + III = 1/2 × 12 per cent + 40 per cent = 46 per cent. As with method *A*, these values may then be compared with the similarly treated observed frequencies. If the observed and random-expected frequencies are significantly different, we may consider this as evidence in support of the hypothesis.

The major problem in actually testing the hypothesis by these methods is to find suitable sets of homologous proteins for examination. So far, complete sequence data are available for relatively few proteins, and for still fewer sets of homologous proteins. However, the pairs listed in Table 1 were considered to be suitable for analysis. In comparing the α- and β-chains of human hæmoglobin with one another, and the hæmoglobin chains with myoglobin, it has been necessary to compensate for deletions or additions of amino-acids. Because of the large number of identical regions in the two chains, no difficulty is encountered in the former instance, but when comparing myoglobin with hæmoglobin, it was necessary to make assumptions about homologous regions. The hæmoglobin–myoglobin analogies are based on the myoglobin sequence of Edmunds and Kendrew as quoted by Schroeder[9].

The results of the various analyses are shown in Table 1. For the hæmoglobin α/β, β/γ, and the human/primate β-chain comparisons, the observed frequencies of the various types of amino-acid exchanges are significantly different from random, by both methods of computation. For the human/horse α-chain comparison, the results are significant by method *A*, and by both methods for the myoglobin/hæmoglobin chain comparisons. In the case of the insulin comparison (human and bonito insulins being compared because they appeared to be the most divergent), the number of interchanges is small and there is a rather high proportion of non-permissible exchanges. One wonders whether these two proteins might prove to have significant differences in tertiary configuration. The total number of residues involved in the comparison of human and horse cytochrome *c* is small, and the observed frequencies are not statistically significantly different from random. On the other hand, the comparison of horse heart and baker's yeast cytochrome *c* and of tuna fish and yeast cytochrome *c* (proteins derived from species that are certainly quite distant) shows that type I exchanges far outnumber type III. The differences are highly significant by both methods for the tuna fish/yeast comparison, but do not reach statistical significance for the horse/yeast comparison.

The analysis of series of homologous proteins in which only a small number of differences are found presents considerably more of a problem. While it is easy to determine the category in which to place the single-site mutations of human hæmoglobin, it is much more difficult to handle groups of proteins in which more than two amino-acid residues are found at the same site. Examinations of such groups of proteins and also of physiological peptides and protein fragments have been made by Yčas[10], by Eck[11], and by Šorm[12]. If we evaluate the results obtained by these authors, we find that type I exchanges are by far the most common, and that type III are generally the least frequent. However, because of the difficulties inherent in interpreting these comparisons, only the results of the analysis of the human hæmoglobin single-site mutations are included in Table 1. For these exchanges, the results are significantly different from random expectation, by method *A*, even though the numbers are small. Finally, if all the data in Table 1 are pooled, the distribution of exchanges is significantly different from random, with $P < 0·001$, by both methods of comparison.

The outcomes of the various hæmoglobin and myoglobin comparisons are particularly instructive, since for these proteins we have information regarding their tertiary configurations in addition to their amino-acid sequences. As has been previously pointed out, these proteins are quite similar, although certainly not identical, in their configurations. Furthermore, myoglobin, which undoubtedly is derived from the common 'primitive' hæmoglobin, has diverged considerably from hæmoglobin in its function and structural organization. Therefore, the results obtained by the present analyses of these proteins are quite gratifying and are in good agreement with the present hypothesis. The fact that a moderate number of non-permissible exchanges do occur is quite understandable when one considers the differences between the various molecules.

In a recent discussion of the evolution of cytochrome *c*, Margoliash[13] observed that certain clusters of basic and of hydrophobic residues tended to be preserved intact (an observation in full accord with the present hypothesis), and raised the question of whether this 'conservation is based solely on evolutionary selection for structural features of functional importance' or whether mutations involving basic or hydrophobic residues tend to yield the same type of residues more often than not. This question strikes to the very heart of the problem under consideration, and it might be rephrased as follows: are the observed amino-acid exchanges governed by the present hypothesis, or are they merely the result of the restrictions imposed on mutation by the 'amino-acid code'? At the present time it is still not possible to distinguish conclusively between these two alternatives, but the analyses of the hæmoglobin and myoglobin exchanges, in which multi-step mutations are undoubtedly involved, would appear to support strongly the former alternative.

Besides the theoretical analysis just presented (which will undoubtedly be expanded as the sequences and configurations of additional sets of proteins are examined), it has been possible to approach the hypothesis experimentally. By utilizing the system in which native ribonuclease is 'unfolded' by reduction in urea and is then re-oxidized and 'refolded' to its active configuration, it has been shown that extensive modifications of 'surface' residues (such as lysine, glutamic acid, and aspartic acid) that do not convert polar side chains to hydrophobic ones do not prevent the proper 'refolding' of the unfolded proteins[6,8]. Similar results have been obtained with surface-modified trypsin[7]. On the other hand, the attachment of large aromatic rings to the surface does appear significantly to impair folding[14]. Further experiments of this type, using not only selective modifications of amino-acid side chains but also specific amino-acid substitutions within the polypeptide chain, will allow for a more extensive and definitive experimental test of the hypothesis.

In summary, it seems reasonable at the present time to assert that the available amino-acid sequence data and experimental evidence support the hypothesis that the necessity to preserve the correct tertiary configuration by the proper distribution of polar and hydrophobic residues in protein molecules is an important factor in guiding the evolution of proteins. Therefore, it may be predicted that homologous proteins which violate this principle will be found to differ markedly in their tertiary configurations, although the fact that enzymatic activity and specificity are preserved would suggest that the 'active centre' portions of the molecule may still be relatively intact.

Note added in proof. Fisher[27] has recently suggested that the combination of the chain-length and the ratio of the hydrophobic to polar residues in a protein strongly influences its tertiary and quaternary structures. His conclusions are quite complementary to those reached in the present discussion.

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A THEORETICAL MODEL OF γ-GLOBULIN CATABOLISM

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RECENTLY one of us[1] suggested that certain resemblances between the processes of the passive transmission of immunity from the gut to the circulation in suckling rats and mice and of passive anaphylactic sensitization in guinea pigs warranted the hypothesis that essentially similar cellular mechanisms were involved in both. These two processes are involved in very different and rather special functions, and if they do indeed depend on a similar cellular mechanism the possibility that this may be concerned also with some other process of more general physiological significance arises. Recent new evidence concerning the catabolism of γ-globulin in the mouse[2] and in the rabbit[3] appears to us *prima facie* to lend itself to such interpretation. It is the purpose of this article to suggest a theoretical model of the mechanism of γ-globulin catabolism, consistent with these results and with the assumptions that have been made concerning transmission of immunity in the mouse, rat and rabbit and with anaphylactic sensitization in the guinea pig.

Briefly the case which was made for assuming that a similar receptor mechanism was involved in both sensitization and transmission was as follows. Both processes are concerned with γ-globulins (or antibodies) and both are selective also in that the γ-globulins of some species are effective, whereas those of others are less effective or ineffective. The fragment of the γ-globulin molecule obtained by papainization which contains most of the antigenic sites, known as fragment III or the *F*-piece[4], determines both transmission and sensitization, whereas the other parts, fragments I and II or the *S*-pieces, are ineffective. Sensitization by, or transmission of, a given γ-globulin can be interfered with by γ-globulins of other species, but not by other serum proteins. Again, it is the antigenic part (fragment III) of the molecule, not the other part (fragment I or II), which is responsible for interference. To account for the specificity and selectivity of sensitization and of transmission, workers in both fields have postulated independently the presence of γ-globulin receptors on, or in, the cells and have regarded interference as being due to competition for them.

Transmission involves passage across the gut wall. The protein in solution apparently is taken up by the cells of the intestinal epithelium by pinocytosis, as can be demonstrated microscopically and electron microscopically. Protein so absorbed and not transmitted to the circulation as such appears to be degraded. Thus the receptors, which may be in the walls of the pinocytotic vesicles, appear to function in saving from degradation protein molecules attached to them and later releasing them to be secreted from the other pole of the cell. Sensitization, on the other hand, requires receptors that are accessible to antibodies and soluble antigens and may be on the external surfaces of the cells. It is not necessary to assume that they are in the walls of vesicles within the cells, though there does not appear to be any convincing evidence on which to exclude such a possibility.

Fahey and Robinson[2] find that for *BALB/c* mice the rate of γ-globulin catabolism increased with γ-globulin concentration from a normal half-life of $T\frac{1}{2} = 4{\cdot}1$ days at a normal concentration in the serum of 0·5 g per cent to a minimum half-life of $T\frac{1}{2} = 1{\cdot}8–2{\cdot}0$ days at concentrations of 2·0 g per cent. These mice were used at a size of 25 g body-weight when the average plasma volume was 1·25 ml., and approximately half the total γ-globulin was extravascular. Levels of γ-globulin concentration were raised by hyperimmunization and injection of normal mouse γ-globulin. Comparable reduction of the half-life of γ-globulin was observed in mice with high concentrations of γ-myeloma protein due either to bearing transplanted plasma cell tumours or to injection of the purified myeloma protein, and also in mice that had received injections of normal human γ-globulin or human γ-myeloma protein. Mouse or human β_{2A} myeloma protein, human γ_1-macroglobulin, and human albumin had no effect on the half-life. The *F*-pieces of both normal human γ-globulin and of mouse γ-myeloma protein, obtained by treatment with papain and cysteine, were as effective in shortening the half-life as the whole γ-globulins at comparable molar concentrations, whereas the *S*-pieces did not shorten, and even appeared to slightly lengthen, the half-life.

Our attention was attracted by the resemblance of this phenomenon to passive transmission and to passive sensitization in its specificity, since γ-globulin alone is effective, and the other serum proteins have no effect. Moreover, the effective part of the γ-globulin molecule is the same in all three processes, since the *F*-piece of human γ-globulin corresponds to fragment III of rabbit γ-globulin, and the *S*-pieces to fragments I and II. Such correspondence obviously suggested that a similar basic cellular mechanism might be involved.

The model we suggest to account for these findings is the isolation from the general pool in a special compartment or compartments of a part of the γ-globulin solution, some of the molecules of which become attached to specific receptors on, or in, the cells and are ultimately returned to the circulation, while the remainder are degraded. The essential point is that, of the γ-globulin so isolated, only those molecules which attach to receptors are saved and returned to the circulation. It is suggested that isolation is effected by pinocytosis and that the receptors are intracellular in the walls of the vesicles, but the principle could apply to isolation in a viscus with the receptors on the surface of the cells lining it. Such a mechanism would be consistent with that which we have found it necessary to postulate to account for the passive transmission of immunity and appears to us applicable at least to much that is known concerning passive anaphylactic sensitization. The fractional rate of γ-globulin catabolism, *F*, is the amount catabolized in unit time divided by the total amount in the system. If *V* is the total γ-globulin space, and *c* is the concentration, then the total amount in the system is *Vc*. The proportion catabolized will be the proportion isolated less the proportion saved by the receptors. If *a* is the proportion of the total γ-globulin solution isolated per day and *b* is the minimum concentration of γ-globulin that will saturate the receptors, then:

$$F = \frac{Vac - Vab}{Vc} \qquad (1)$$

Since the half-life, $T\frac{1}{2}$, is related to the fractional rate of catabolism, as:

$$T\tfrac{1}{2} = \frac{\log_e 2}{F} \qquad (2)$$

then by substitution:

$$T\tfrac{1}{2} = \frac{c \log_e 2}{a(c-b)} \qquad (3)$$

Similarly, the total amount of γ-globulin catabolized per day, *G*, will be:

$$G = Va(c-b) \qquad (4)$$

Fahey and Robinson[2] provide results for the serum γ-globulin concentrations and half-lives in normal and immunized mice of the *BALB/c* strain which are reproduced in Table 1. These authors provide further results in the form of a graph of the γ-globulin concentrations and half-lives in mice with various kinds of plasma cell tumours, or injected with large doses of mouse of human myeloma protein, or normal human γ-globulin, or human serum albumin. We have extracted these results from their graph for normal mice and for those injected with human serum albumin, which we consider strictly relevant, and reproduce them, together with those for the immunized mice, in Fig. 1, plotting half-life against γ-globulin concentration. On the simplest assumption that the amount isolated is constant irrespective of γ-globulin concentration, the values of the constants *a* and *b* can be estimated graphically, since when $T\frac{1}{2} = \infty$, $c = b$, and when $c = \infty$, $a = \log_e 2/T\frac{1}{2}$. The values determined in this way were found to be $a = 0{\cdot}34$ and $b = 2{\cdot}5$. Solving equation (3) with these values gives the curve shown in Fig. 1 with a theoretical minimum half-life of 2·05 days. The expected half-life for each γ-globulin concentration, derived from this curve, is given in Table 1 for comparison with the observed half-life. It can be seen that the curve is consistent with the data, allowing for experimental errors inseparable from determinations of γ-globulin concentrations in small samples.

Table 1. CONCENTRATIONS AND HALF-LIVES OF γ-GLOBULIN IN NORMAL AND IMMUNIZED MICE (DATA FROM FAHEY AND ROBINSON[2])

Concentration mg/ml.	Half-life (days) Observed	Expected
5·0	4·0–4·3	4·0
7·2	3·5–3·6	3·1
6·8	3·3	3·2
15·6	2·5–2·7	2·4
28·0	1·9–2·2	2·2

It was desired to test whether equation (3) was equally applicable to other species. Unfortunately few reliable data covering the necessary range of γ-globulin concentrations are available in the literature. Recently Anderson and Bjorneboe[3] have provided determinations of both half-lives and concentrations of γ-globulin in seven rabbits before and during hyperimmunization which are appropriate. These are shown in Fig. 2, together with the curve derived from equation (3), using the values $a = 0{\cdot}21$ and $b = 4{\cdot}74$. The observed values, together with the expected values of the half-life for each γ-globulin concentration

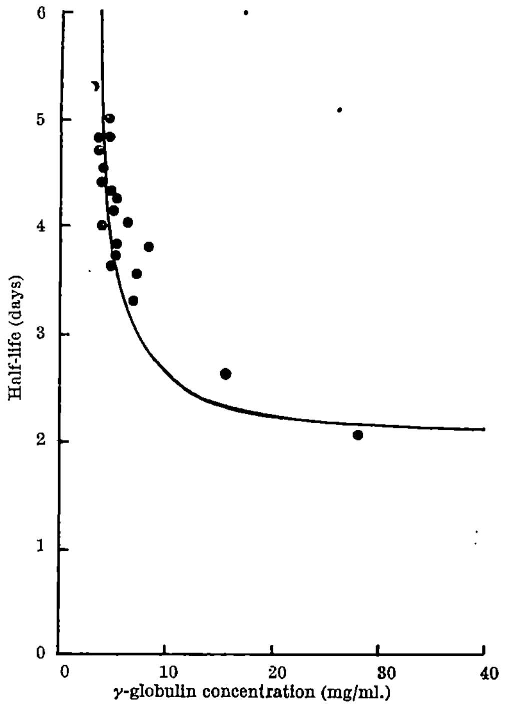

Fig. 1. Relation of half-life to serum concentration of γ-globulin in mice; $a = 0{\cdot}34$, $b = 2{\cdot}5$. Data extracted from Fahey and Robinson[2]

derived from this curve, are given in Table 2. It can be seen by inspection that the fit is reasonably good.

Table 2. CONCENTRATIONS AND HALF-LIVES OF γ-GLOBULIN IN RABBITS BEFORE AND DURING HYPERIMMUNIZATION
(Data from Andersen and Bjorneboe[3])

Before hyperimmunization			During hyperimmunization		
Concentration mg/ml.	Half-life (days) Observed	Half-life (days) Expected	Concentration mg/ml.	Half-life (days) Observed	Half-life (days) Expected
11·5	4·2	5·6	52·9	3·3	3·6
16·2	4·8	4·7	53·2	3·2	3·6
10·6	5·7	6·0	71·4	3·4	3·5
11·5	4·9	5·6	109·1	3·7	3·4
12·5	5·6	5·3	97·2	3·8	3·5
9·7	6·1	6·5	83·3	3·2	3·5
12·9	7·3	5·2	79·7	3·8	3·5
Mean: 12·1	5·5	5·6	78·1	3·5	3·5

The concepts from which equation (3) is derived have been drawn from the phenomenon of passive transmission of immunity, yet the curves defined by this equation fit remarkably well the data of the relation of half-life to concentration of γ-globulin. These results refer to two unrelated species and were obtained by two independent groups of workers. This hypothesis of the mechanism of γ-globulin catabolism is attractive because of its relative simplicity. Both the fixation of γ-globulin to cellular receptors and pinocytosis are well-recognized concepts. The hypothesis is novel in relation to γ-globulin catabolism in so far as it involves both these mechanisms and postulates that the receptors are in the walls of the vesicles and that the molecules which attach to them are saved from degradation and returned to the circulation. Such a mechanism could provide a means of automatic monitoring of the circulating γ-globulin for defects, since any distortion of a molecule that prevented its attachment to a receptor would result in its degradation. It brings into line the special processes of passive transmission of immunity and of anaphylactic sensitization with the general process of γ-globulin catabolism. As a working hypothesis it has the merit of lending itself to experimental verification.

Neither the concentration of albumin nor of the other serum globulin affects the half-life of γ-globulin, presumably because their molecules are unacceptable to the γ-globulin receptors. Hence, unless separate specific receptors for these serum proteins are present in the walls of the pinocytotic vesicles also, of which there is no evidence at present, then all these molecules taken up with the γ-globulin by pinocytosis would be expected to be degraded. On this assumption the half-life of these serum proteins should not exceed the minimum half-life of γ-globulin. It is suggestive that Fahey and Robinson[2] find the half-life of serum albumin in their mice to be 2·1 days. Since this is the theoretical minimum half-life of γ-globulin, it leaves no margin for the catabolism of albumin by any other channel.

The problem of where in the body γ-globulin is catabolized is not involved in this hypothesis. The gut is an attractive possibility, however. There is evidence that substantial quantities of γ-globulin and other serum proteins are secreted into the small intestine. Recently Andersen, Glenert and Wallevik[5] found that in dogs, in which the half-life of γ-globulin had been measured previously, 38 per cent of the total γ-globulin catabolized per day was accounted for by intestinal clearance. They used iodine-131-labelled homologous γ-globulin injected intravenously, and measured the appearance of the labelled protein in isolated washed-out intestinal loops. They assumed that any γ-globulin reaching the lumen of the small intestine is degraded there. If such quantities of γ-globulin do in fact reach the intestinal lumen in the normal animal and are there degraded *in toto*, it would be incompatible with the hypothesis of γ-globulin catabolism outlined above. Andersen *et al.*, however, provide no crucial evidence of such degradation and in fact their results provide some evidence against it. They found that about 50 per cent of the activity in the fluid aspirated from the loop was not protein bound. However, when ^{131}I-γ-globulin solution was injected into the loops in dogs which had not had it intravenously, only 1·2 per cent of the activity was non-protein bound after the same interval. This suggests that the breakdown products in the intestinal lumen of the intravenously injected dogs were not the result of degradation in the lumen but may have reached it from elsewhere. The results are consistent with the assumption that the γ-globulin exuded into the small intestine is not degraded

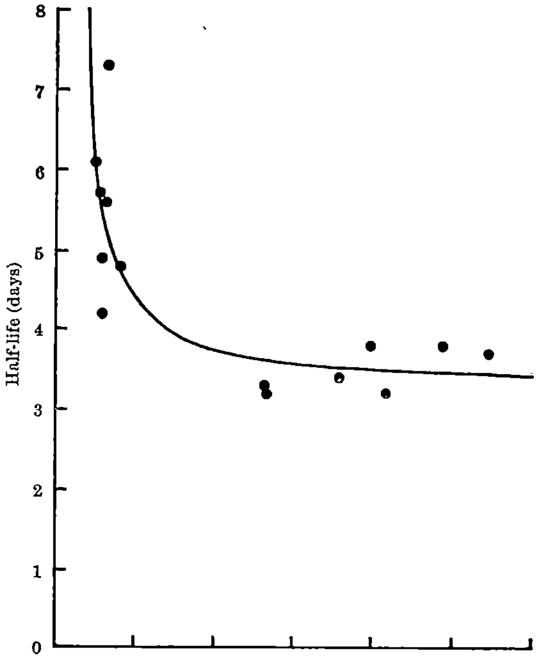

Fig. 2. Relation of half-life to serum concentration of γ-globulin in rabbits before and after hyperimmunization; $a = 0{\cdot}21$, $b = 4{\cdot}74$. Data extracted from Andersen and Bjorneboe[3]

in the lumen but may be reabsorbed by a similar method to that of the suckling rat or mouse, such molecules as are attached to the receptors being saved and returned to the circulation and such as are not so attached being degraded. Diffusion of these degradation products into the lumen could account for the appearance of non-protein bound iodine-131 there. This is equivalent to suggesting that the change in the gut that results in termination of transmission of immunity at some time after birth is due to the onset of gastric and duodenal function and that the small intestine is still capable of transmission provided that the γ-globulin reaches it without having traversed more anterior regions of the gut. The small intestine of the adult could be the site of at least a significant fraction of γ-globulin catabolism by precisely such a mechanism as is postulated by our model.

We thank Prof. C. L. Oakley for his advice.

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SYRIAN HAMSTER FIBROBLAST CELL LINE *BHK*21 AND ITS DERIVATIVES

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THE *BHK*21 line of diploid hamster fibroblasts is used widely for research on viruses and on neoplastic transformation. The purpose of this article is to describe the origin of the cells, to list viruses to which they are susceptible, and to summarize the main characteristics of some variants of the cells.

Origin. *BHK*21 cells arose from cultures of kidneys from 1-day-old Syrian hamsters[1]. Such cultures are initially mixtures of fibroblasts and epithelial cells in which fibroblasts soon predominate. They can usually be propagated for about 8 weeks before growth slows and eventually ceases, but in one series of cultures rapidly growing cells were found after 65 days in culture.

The primary cultures from which these cells were derived were prepared in March 1961 from the kidneys of 5 1-day-old hamsters from litter No. 21. After 30 days and three subcultures a sample was stored at −70° in glycerol for 8 days before continuous culture was continued. Tests were carried out for sensitivity to polyoma virus transformation on the 24th, 31st, 36th and 48th day of continuous cultivation (excluding the storage period). These involved plating the cells, and from the earliest test on the 24th day large fibroblastic colonies were noted. Nevertheless the growth rate and cloning efficiency of the cells remained low and 10^5 cells was insufficient to form a confluent monolayer in a 60-mm Petri dish in one week. Between the 31st and 41st day kanamycin sulphate ('Kannasyn', Bayer Products Co., Surbiton upon Thames) was added to the medium because contaminants were found in a few cultures.

On the 41st day some cultures were exposed for 2 days to the medium which had been used for baby hamster kidney cells from another litter, in an unsuccessful attempt to transfer resistance to polyoma transformation[2].

Finally, after 65 days of propagation a sudden increase in growth rate was noticed, with a large increase in yield of cells from each culture. This was confirmed on the 67th day, when a sample of the cells was tested for polyoma transformation and it was found that 10^5 cells formed a thick confluent growth after one week in a 60-mm Petri dish.

The cells which are the source of all *BHK*21 cells are from those cultures which had been exposed to the exchange of medium, but a control culture not so treated was later found to be growing rapidly, so the medium exchange was probably irrelevant. It should be noted that the rapidly growing cells had not been knowingly exposed to polyoma virus during their propagation, and derivatives have since been shown to lack the polyoma specific cell antigen[3,4].

Nineteen days after the change in growth rate was first observed, 8 clones were grown from single cells in small drops of medium under mineral oil. One of these, designated *BHK*21/13 (or *C*13), has been extensively examined and is the source of most cells distributed from this laboratory.

A sample of the original uncloned *BHK*21 cells was frozen at −70° two days after the rapid growth was first observed. Clone *BHK*21/13 cells were stored after about 24 cell generations, that is, when the population reached 10^8 cells. Further stocks of the clone for general use have been frozen after a total of about 30 generations since cloning.

Characteristics of BHK21 cells. *BHK*21 cells are elongated and grow in parallel orientation, like fibroblasts. In logarithmic growth they have a mean doubling time of 12 h (ref. 5) when grown in medium containing 10 per cent autoclaved tryptose phosphate broth (Difco), and 10 per cent unheated calf serum, in Eagle's medium with twice the usual concentration of amino-acids and vitamins.

Growth is maintained even after the cell sheet is confluent, so that the final saturation density of the cells is approximately 10^6 cells per cm^2 of glass, some ten times the saturation density of freshly isolated cells.

When dilute suspensions of monodispersed cells are cultured, on average 16 per cent grow to form colonies. In the presence of X-irradiated feeder cells this figure is raised to at least 50 per cent.

Cells stored in medium containing glycerol or dimethyl sulphoxide can be resuscitated with high viability after long periods at −70° or −196° C.

The karyotype of *BHK*21 cells is that of diploid male Syrian hamster cells[5] and has remained so in *BHK*21/13 for at least 450 generations. At this stage the distribution was: 43 chromosomes—4 cells; 44 chromosomes—55 cells; 46 chromosomes—2 cells. In a larger sample 0·4 per cent were in the tetraploid region.

On implantation of large numbers of *BHK*21 cells in unconditioned adult hamsters progressively growing fibrosarcomas develop after a latent period of several months. One million cells produce tumours in 50 per cent of animals in 3 months when implanted intracerebrally, and in 13 per cent of animals in 8 months when implanted subcutaneously[6]. This low transplantability contrasts strongly with that of the highly malignant variants described here.

Sensitivity of BHK21 cells to viruses. The following viruses are known to multiply in *BHK*21 or *BHK*21/13 cells. *Pox viruses*: Vaccinia. *Herpes viruses*[7]: Herpes simplex, pseudorabies, bovine rhinotracheitis, equine abortion. *Myxoviruses*[8]: Influenza NWS, NM, fowl plague, NDV, mumps, Sendai. *Arboviruses*[9–12]: Groups *A*, *B*, *C*, Bunyanwera, Guama, Simbu, Triniti, VSV, Anopheles *A* and *B* and California complex. *Encephalomyocarditis virus*. *Rabies virus*[13] (several strains). *Foot-and-mouth virus*[14] (several strains). *Vesicular stomatitis virus*[15].

In addition non-neurotropic strains of influenza virus undergo an incomplete cycle[8]. Polyoma virus induces transformation, but infective virus is not produced in detectable amount[16].

Polioviruses Types 1, 2 and 3, and several strains of common cold virus[17], have been tested and not found to multiply.

No difference has yet been found in the viral sensitivity of *BHK*21 cells after polyoma virus transformation.

'Spontaneous' variants of BHK21 cells. When uncloned *BHK*21 or *BHK*21/13 cells have been propagated for long periods or deliberately exposed to selective conditions, the following variants have been isolated. These are not due to polyoma infection and are presumably due to mutation, or to transformation, possibly by unknown viruses.

(*a*) *High transplantability.* Cell lines with increased transplantability have been isolated in several laboratories[6,18–20]. These range from highly malignant (for example, *BHK*21/*Is*3), with a minimum tumour-producing dose of less than ten cells inoculated intracerebrally, to intermediate types (for example, *BHK*21/13/150), requiring approximately one hundred cells. In transplantability these variants resemble cells transformed by polyoma virus, but, as demonstrated by Defendi *et al.*[18], this property is not correlated with random orientation, nor with the possession of the polyoma cell antigen. Nevertheless these cell lines appear to us to have less oriented growth pattern than the original *BHK*21 cells.

(*b*) *Growth in suspension.* A variant capable of growth in suspension has been described by Capstick *et al.*[21].

(*c*) *Growth in agar.* Standard *BHK*21 cells will not grow in agar, but Montagnier has isolated a variant which will form colonies with an average efficiency of 15 per cent when embedded in agar[22].

Polyoma transformed variants of BHK21 cells: (*a*) *Standard.* Neoplastic transformation of *BHK*21 cells can be produced rapidly by exposure to polyoma virus. After transformation the cells lose all trace of infective virus. Compared to the original cells the polyoma transformed cells are characterized by high transplantability in hamsters (minimum tumour-producing dose of 60 cells intracerebrally), random orientation in culture, increased glycolytic capacity, and a virus specific cell antigen. One transformed clone (*BHK*21/13/*Py*6) has been compared in detail with *BHK*21/13 cells from which it was derived[5,6].

(*b*) *Tetraploid.* The karyotype of the standard freshly transformed cell is diploid, but aneuploid cells rapidly appear and the proportion of tetraploid cells increases on propagation. A few clones, however, contained tetraploid cells exclusively when first examined soon after transformation. In these cells tetraploidy persisted on further propagation[5,23].

(*c*) *Colonial morphology.* A variant, termed Colony Type 2, may be observed in freshly transformed clones of less than 10^3 cells infected with cloned virus stock. Type 2 is characterized by a greatly increased proportion of rounded but viable cells which lie on top of the colony. This feature is maintained on propagation but soon reverts to the standard (Type 1) type of colony.

(*d*) *Increase in electrophoretic mobility.* The electrophoretic mobility of freshly transformed clones may either be unchanged or increased[24]. After continuous propagation, however, the mobility becomes heterogeneous[25].

(*e*) *Growth as ascites cell.* A particularly interesting variant of *BHK*21/*Py*6 has been obtained by Sanders and Burford[26] by adaptation to the hamster peritoneal cavity. These ascites cells, when cultured directly, will grow in agar with cloning efficiency of about 30 per cent. After passage in agar this increases to nearly 100 per cent.

(*f*) *Growth in agar.* Some freshly transformed clones will give rise to colonies in agar without adaptation as an ascites cell[27]. These include polyoma transformed derivatives of the agar growing variant[22] (see foregoing). (It is now known that the ability to form colonies in agar is rapidly acquired after polyoma transformation[37].)

(*g*) *Biochemical markers.* Spontaneous variants (not shown in Table 1) with increased resistance to purine analogues, or antibiotics, such as puromycin, have been isolated from *BHK*21/*PyY* cells and also from untransformed *BHK*21 cells by Subak-Sharpe[28].

Table 1. CHARACTERISTICS OF SOME VARIANTS OF *BHK*21 CELLS

Main characteristic		Full designation of representative clones or lines	Ref.
Original	Original uncloned *BHK*21	*BHK*21	1
	Clones of *BHK*21	*BHK*/13, etc. (or *C*13)	
Spontaneous variants	High transplantability	e.g., (*a*) *BHK*21/13/798 (*b*) *BHK*21/*Is*3 (*c*) *BHK*21/13/150	(*a*) 18 (*b*) 19 (*c*) 6
	Growth in suspension	*BHK*21/13/*Susp.*	21
	Growth in agar	*BHK*21/13/*Ag*	22
Polyoma virus transformed	Standard	e.g., *BHK*21/13/*Py*6 (previously *TC*6) *BHK*21/13/*PyY*, etc.	5 23
	Tetraploid	e.g., *BHK*21/*PyC* *BHK*21/13/*PyZ*	5 23
	Rounded (Type 2) morphology	e.g., *BHK*21/13/*PyM*	
	Increase in electrophoretic mobility	e.g., *BHK*21/13/*PyP*	25
	Ascites adapted (also grows in agar)	*BHK*21/13/*Py*6/*As*	26
	Growth in agar	*BHK*21/13/*Py*/*Ag*	27 22

A list of these variants and their sources and designations is given in Table 1. For regular use the designations may be suitably abbreviated by the use of the last letter or figures. It should be noted that the standard clone *BHK*21/13 has frequently been referred to as *C*13. Polyoma transformed clones are now designated *Py* (instead of *T* as previously) because of the possibility of transformation by other viruses.

Comparison with other cell lines. *BHK*21 cells differ from freshly isolated fibroblastic cells in their high cloning efficiency, and their rapid growth for an apparently indefinite period. They thus come into the category of a cell line rather than a cell strain[29]. On the other hand, they resemble freshly isolated cells in being diploid, a character they have retained through many generations and in which they differ from other continuous lines of cells. Though no regular abnormality has been found in the chromosome number of *BHK*21 cells, it is possible that, like some Chinese hamster cell lines[30], these cells are quasi-diploid.

It seems likely that *BHK*21 cells are derived from a variant arising after many weeks in culture. If it had been present in the original kidneys or in the early cultures from them, the high growth rate should have allowed a rapid emergence. However, the presence of unusually large fibroblastic colonies noted after 24 days in culture, and 41 days before the *BHK*21 cells were observed, may mean that two or more steps were involved. The variant is probably rare and no similar cells have been obtained from 47 separate batches of baby hamster kidney cells in our laboratory. The rapid decline of Syrian hamster cells and the dearth of variants have also been the experience of other workers[31].

It is of interest to note that the frequency of emergence of cell lines from normal (as opposed to tumour) tissue seems to be a function of the species of origin. Human fibroblasts survive in the euploid condition for a comparatively long time, but continuous cell lines rarely arise[29]. Rat cells may behave similarly[32]. On the other hand, mouse cells rapidly decline in growth rate when cultured and frequently give rise to aneuploid variants capable of indefinite growth[33,34]. A third class exists in which continuous cell lines may appear but are not markedly aneuploid. The chromosome number may not be changed or only changed by one or two. Chinese hamster[35] and perhaps pig cells[36] belong to this class, and it is possible that Syrian hamster cells should be included as well.

Though highly malignant variants arise in the absence of polyoma virus transformation it is likely that the original *BHK*21 cells are not malignant. The slow-growing tumours which can arise from large cell inocula even after cloning may be due to a few malignant variants which were already present by the time the clone was tested, or which arose during the long latent period following implantation. These malignant cells have little selective advantage under the growth conditions used in our laboratory, but in other laboratories they may rapidly replace the original cells.

Normality is only a relative term when applied to cells in culture, and whatever the status of *BHK*21 cells in relationship to the kidney cells of the baby hamster or to freshly cultured fibroblasts they should prove useful in biological research. Viruses, such as those of foot-and-mouth disease and rabies, which grow poorly in other continuous lines, multiply well in *BHK*21 cells, and the cells are sensitive to the transforming action of polyoma virus. Finally, the rapid growth and high cloning efficiency of *BKH*21 cells combined with a low chromosome number may make them especially suitable for physiological and genetic studies.

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GLOMERULONEPHRITIS AND LATE ONSET DISEASE OF MICE FOLLOWING NEONATAL VIRUS INFECTION

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NEONATAL inoculation of mice with lymphocytic choriomeningitis (LCM) virus has been shown[1] to cause a persistent infection of the animal with high virus titres lasting for many months after inoculation. During this period the mice remain perfectly well[2] and free from signs of disease, except for a brief temporary period of runting in some mouse strains; the blood causes severe hepatitis and death with convulsions if inoculated intracerebrally or intraperitoneally into an uninfected litter mate or other mouse more than 4 days old[3]. This harmless LCM virus infection has been called persistent tolerant infection (PTI)[4] and appears to parallel the carriage of human serum hepatitis virus in man. Initially it was believed that LCM–PTI mice were entirely comparable clinically to uninfected or LCM-immune controls throughout the normal life span of the mouse. However, comparison of PTI and control mice[5] showed that PTI mice weighed a few grams less than uninoculated controls or mice of the same age which had received an injection of the virus by the subcutaneous route at approximately 1 month of age. (The latter are actively immune to the virus if challenged, and contain circulating antibody against LCM but no circulating virus.) In addition, the onset of a chronic late-onset disease was noticed with the occurrence of renal lesions in some respects similar to human systemic lupus erythematosus (SLE)[5-7]. This article describes the results of a preliminary investigation of this new late-onset disease.

Mice of both Albany and Swiss strains[4] were used with the M/B_7L_{11} (ref. 8) strain of LCM virus. A large number of PTI mice were obtained from experiments concerning the effects of various factors on the induction of immunological tolerance to LCM virus; some of these have been reported elsewhere[8]. In addition, 4 large groups of mice—PTI, LCM-immune, mice inoculated with normal tissue, and uninoculated mice—were set up to follow the course of tolerant infection in later life. All mice were checked at weekly intervals for onset of clinical signs of late

Table 1. INCIDENCE OF CLINICAL LATE DISEASE AND RENAL LESIONS IN MICE

Mouse category	Incidence* of clinical late disease	Percentage of clinical late disease	Incidence of histological kidney lesions†	Percentage of kidney lesions
Albany PTI	92/109	85·0	17/50	34
Albany control	2/121	1·6	0/42	0
Swiss PTI	76/167	46·0	19/118	16
Swiss control	2/136	1·5	0/7	0
Total mice	533		Total kidney sections 217	

* The second figure denotes the number of mice in the group.

† The second figure denotes the number of mice on which renal histological examination was performed.

disease; if definite signs were noted, mice were checked daily and killed when severely ill or when death appeared imminent.

The PTI mice remained well for approximately 7–10 months, at which time they began to show the characteristic disease picture of 'late disease'. Such a mouse exhibiting the ruffled and sparse fur, hunched posture, and moth-eaten appearance is shown in Fig. 1 with normal controls for comparison; the incidence of clinical late disease and renal lesions is shown in Table 1. Of the mice examined, 85 per cent of the Albany strain showed clinical evidence of late disease at some point after their 7th month of life, whereas the corresponding figure for Swiss mice was only 46 per cent. Some of the mice in both these groups, regardless of whether or not they showed clinical late disease, were sampled at ages between 7 and 18 months for histological examination of their organs; 34 per cent of the Albany and 16 per cent of the Swiss mice showed specific renal lesions; this trend conforms with the lower tendency of the Swiss mice to exhibit clinical signs of late disease. Occasional mice showed the renal lesion but not clinical late disease. The histological examination of approximately 168 PTI and 49 control mice aged between 5 and 32 months revealed the following.

The gross appearance of the kidneys was somewhat paler and slightly larger than normal. The glomeruli showed a diffuse involvement of the capillary tufts with hyaline thickening of the basement membrane of the capillary loops, and occasionally Bowman's capsule showed thickening, splitting, or reduplication (Fig. 2). There was accumulation or deposition of material on the intraluminal surface of the capillary basement membrane which resulted in partial or complete obliteration of the lumen and in areas simulated a 'wire loop' appearance (Fig. 3). A thin layer of material presenting a laminated structure with ridges perpendicular to the basement membrane was noted on the extra-luminal surface of the capillary tufts. No lesions of this type or significant clinical evidence of late disease were found in any of the control mice. No differences have become apparent so far between any of the control sub-groups.

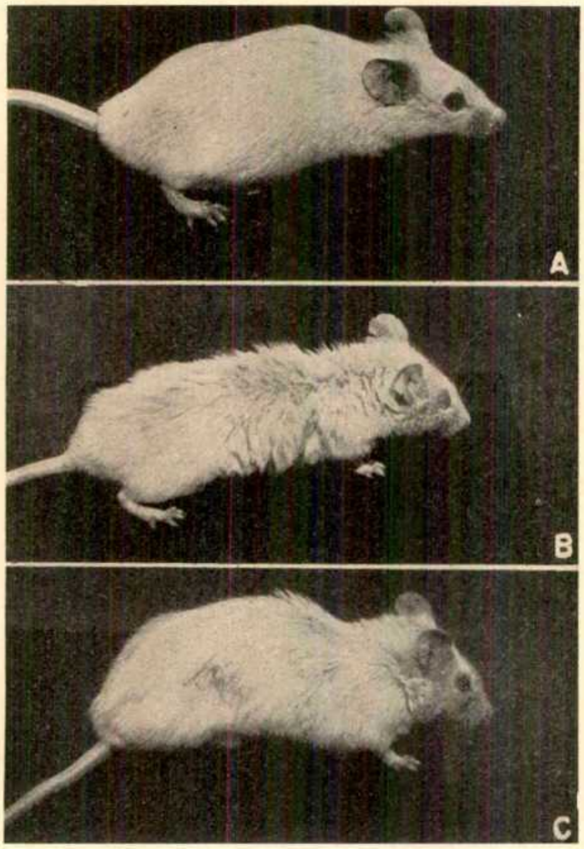

Fig. 1. *A*, control mouse inoculated at birth with normal mouse brain suspension, showing normal appearance, photographed at 14 months of age, weight 38·4 g; *B*, mouse inoculated with LCM virus at birth showing moderately severe late disease, photographed at 13 months of age, weight 28 g; *C*, control uninoculated mouse, aged 30 months, weight 29·5 g. This animal is at the extreme limit of old age, the signs of which are clinically quite distinct from those of late disease

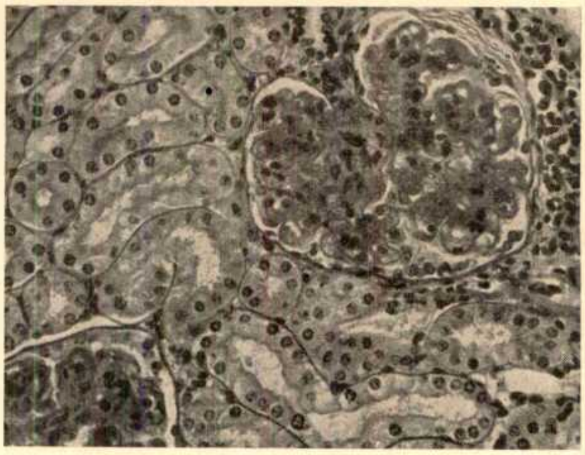

Fig. 2. Thickened basement membrane, splitting or reduplication of Bowman's capsule, and accumulation of mononuclear cells. Periodic acid-Schiff stain. (× *c*. 115)

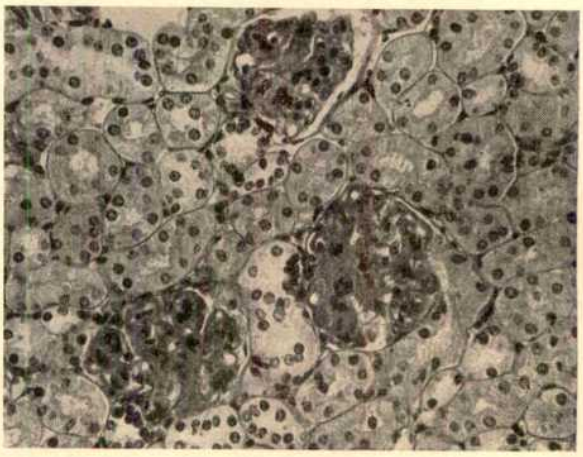

Fig. 3. Diffuse hyaline thickening of basement membrane, accumulation of material on capillary basement membrane with partial to complete obliteration of lumen. Periodic acid-Schiff stain. (× *c*. 115)

In affected kidneys, the glomeruli were uniformly involved throughout the cortices. The tubules frequently contained fine droplets and laminated concretions in the epithelial cells, but casts were rarely present at this stage of glomerular involvement. In severely damaged kidneys, the glomeruli exhibited hypercellularity, adhesions of the glomerular tufts, and occasionally epithelial crescents (Fig. 4). The tubules were dilated and contained homogenous acidophilic casts. An almost constant finding was a perivascular accumulation of lymphocytes and plasma cells about arcuate and intralobular arteries. The glomerular deposits stained with periodic acid-Schiff reagent showed an intense positive reaction; congo red and alcian blue stains were negative for amyloid. No birefringence or dichromism was noted on congo-red stained sections when examined by polarized light. Fluorochrome staining with thioflavin T exhibited no fluorescence of the glomerular deposits[9]. Glomerular amyloidosis was present in a large number of PTI and control mice in the slightly older age group; this appeared to be most marked in the Swiss strain and in the PTI mice. The deposition of amyloid

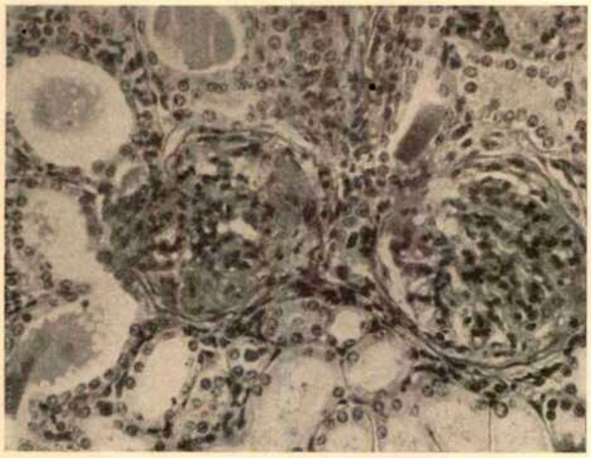

Fig. 4. Hypercellular glomeruli, adhesions and epithelial crescent. The tubules are dilated and contain casts. (× *c*. 115)

in these mice was generally confined to the glomeruli and did not involve the medulla and papillæ of the kidney, neither was it identified extra-renally.

A few of the renal sections showing typical glomerular lesions were stained by the fluorescent antibody technique using an antimouse γ-globulin fluorescent conjugate. Marked fluorescent staining of the glomerular deposit occurred; however, similar staining also occurred in sections showing severe amyloidosis, from mice uninfected with LCM virus.

The finding of severe clinical disease and renal lesions as a late sequel to neonatal virus infection raises the question of the nature of the pathogenic mechanism of this disease and its relation to acute LCM pathogenesis. There is considerable evidence[4] that the acute phase of LCM disease represents the outer manifestations of an immunological conflict occurring between the replicating viral antigen and the host's immune mechanism; however, the acute infection of adult mice with our strain of LCM shows meningoencephalitis and hepatitis, without noticeable kidney involvement. It has been suggested[5] that the host's response is essentially the same as the homograft response and constitutes a delayed hypersensitivity mechanism designed to eliminate the virus-infected tissue of the animal. This in turn suggests that the late disease of PTI mice may occur as a result of the gradual waning of immunological tolerance formerly exhibited by the mouse towards an otherwise harmless virus infection, thus constituting an example of virus-induced auto-immune disease. In this light, the late disease syndrome might be considered as a possible auto-immune reaction of the host immune mechanism towards previously tolerated tissue components. This view is compatible with the lesions found, and the fluorescent staining results, but has not been confirmed by serological or other evidence of SLE: thus, significant evidence for this view must be considered lacking at present. Alternatively the disease may arise from a very slow type of cytopathic effect on certain target tissues such as those found in the kidney. It must also be remembered that the mouse contains other latent viruses and micro-organisms which as contaminants of the LCM virus-infected tissue suspension used for inoculation could conceivably be responsible for the late lesions, although all tests for such agents have been negative.

The nature and significance of the renal lesions cannot at present be finally assessed. The glomerular changes, while exhibiting some features of lupus nephritis, rarely showed fibrinoid necrosis, and hæmatoxylin bodies were never demonstrated. That they might represent precocious changes related to senile amyloidosis as described by Dunn[10] and also by Thung[11] was not borne out in the failure to obtain positive results for amyloid using classical amyloid stains[9]. The presence of senile amyloid which was found in the older PTI mice might be expected to occur at about 18 months of age although it may vary with the mouse strain used. It is hoped to answer the question of whether the deposition of periodic acid-Schiff-positive material is a precursor of amyloidosis by a more extensive quantitative histological investigation which has been initiated. Helyer and Howie[12] have described renal lesions in an inbred strain of *NZB* mice which are remarkably similar to those seen in the PTI mice; their cross-bred strain showed the hunched posture, cachexia, and alopecia similar to the PTI mice, but in addition their cross-bred strain developed hæmolytic anæmia and jaundice, and SLE cells were demonstrated; these features were believed to be indicative of an auto-immune process. However, the possibility exists that a persistent tolerant virus infection could be responsible for the effects described by Helyer and Howie, and this does not appear to have been ruled out by their results.

Guttman and Kohn[13] described an intercapillary glomerulosclerosis in ageing mice, and a spontaneous hyaline form of degeneration has been noted in ageing mice of the *RF* strain by Gude and Upton[14]; their morphologic descriptions and photomicrographs are similar to but not identical with those in our investigation of younger PTI mice.

The very late onset of the disease in PTI mice is reminiscent of the gradual onset of scrapie infection in sheep several years after inoculation, and also in mice, which has been shown to occur after several months[15,16]; it would therefore appear to constitute an example of 'slow' virus infections as defined by Gajdusek[17]. The fact that scrapie was for a long time considered to be a non-infectious hereditary disease raises the question of the role of viruses in other 'genetic' conditions, in terms of the carriage of LCM through generations of mice in a closed colony[18], and as already mentioned, in the congenital disease described by Helyer and Howie. If similar mechanisms of chronic virus disease occur in man, serum hepatitis virus carriers should ultimately succumb to some form of chronic organic disease, possibly of the kidney or liver, in later life; a somewhat similar mechanism was proposed by Himsworth[19] to explain the progressive sequence of subacute hepatitis. The role of relatively 'harmless' viruses in the initiation of human late-onset disease has long been suspected, but proof of this remains elusive. The occurrence of late disease in LCM–PTI mice constitutes an example of an infective wasting disease, with an incubation period greater than half the normal expected life span of the host. This evidence strengthens the concept that other diseases of later life may, like some types of cancer, owe their origin, not to faulty genetics, but to a slowly acting but insidiously destructive virus.

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EVIDENCE FOR REPAIR OF ULTRA-VIOLET DAMAGED DEOXYRIBONUCLEIC ACID IN CULTURED MAMMALIAN CELLS

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AS an extension of our previous work on ionizing radiation effects on nucleic acid metabolism in cultured mammalian cells[1], we have initiated a similar investigation of the effects of ultra-violet light. Results accumulated so far strongly suggest that an active ultra-violet repair mechanism exists in mammalian cells, and is especially evident in cells that have incorporated brom-uracil deoxyriboside (BUdR) into their DNA.

Setlow and Carrier[2] and Boyce and Howard-Flanders[3] have recently reported evidence indicating that dark repair of ultra-violet light damaged DNA in *Escherichia coli* occurs by excision of damaged areas containing thymine dimers. Presumably the next step is the replacement of the nucleotides which have been removed, and Pettijohn and Hanawalt[4] have reported that a "non-conservative replication" of DNA does occur following irradiation of *E. coli*.

Repair and/or recovery mechanisms in mammalian cells are operative following irradiation with ionizing radiation[5], but the molecular basis of this phenomenon is, as yet, unknown. Little work has been done with ultra-violet irradiation of mammalian cells, *in vitro*, although Lee and Puck[6] determined the effect of ultra-violet on the survival of HeLa *S*3 cells and Bloom and Leider[7] have shown that microspot irradiation with ultra-violet causes loss of ultra-violet-absorbing materials from the chromosomes of newt heart cells in culture. Humphrey *et al.*[8] have determined the relative sensitivity to ultra-violet of the different phases in the cell cycle in Chinese hamster cells.

HeLa *S*3 cells and the Chinese hamster line *DFAF*-33, routinely maintained in Eagle's medium, were grown as monolayers in special flasks made of 'Vycor' (Corning No. 7910) or Leighton tubes with 'Vycor' windows (Corning No. 7900) 2 mm thick with a transmission of about 70 per cent at 2537 Å. Two or three days prior to irradiation, the medium of half of the cultures was replaced with one containing $1\cdot6 \times 10^{-5}$ M 5-BUdR (5 µg/ml.) (International Chemical and Nuclear Corp.) and of the other half with one containing $1\cdot6 \times 10^{-5}$ M thymidine (TdR) (4 µg/ml.) (Nutritional Biochemical Corp.). Simon[9] has shown that HeLa cells incorporate BUdR into their DNA when it is included in the growth medium; radioisotopic investigations with ^{14}C-BUdR have shown that, under our conditions, at least 3·5 per cent of the thymidine moieties of DNA are replaced by bromuracil (BU). Immediately before irradiation, the cells were washed twice with bicarbonate buffered normal saline, the flasks flushed with 5 per cent carbon dioxide–95 per cent air, and irradiated, through the surface to which cells were attached, with 2537 Å light from a low-pressure mercury arc ('Mineralight'), at a dose rate of about 5 ergs/mm^2/sec at the surface of the flasks. In the case of the Leighton tubes, the glass cover-slips, which had been placed in the tubes before inoculation and on which the cells had grown, were turned over prior to the irradiation so that the cells were irradiated directly through the 'Vycor' window. Following irradiation, the saline solution was removed and replaced with Eagle's medium containing the labelled compound(s), the flasks flushed again with 5 per cent carbon dioxide–95 per cent air, and incubated at 37° for periods up to 2 h.

Incorporation of nucleic acid precursors was measured with several isotopic compounds. Tritiated thymidine (H^{3}TdR) at a specific activity of 6·7 c./mM was used at a concentration of 0·036 µg/ml.; ^{14}C-labelled thymidine (^{14}CTdR) (30 mc./mM) at a concentration of 8 µg/ml., tritium-labelled cytidine (1 c./mM) at a concentration of 0·2 µg/ml. and ^{14}C-guanine (3 mc./mM) at a concentration of 20 µg/ml. (All labelled materials were obtained from New England Nuclear Corp.) To stop DNA metabolism simultaneously in all flasks, they were plunged into an ice bath, the medium removed, the cells washed twice with phosphate buffered normal saline, and extracted with 4 per cent perchloric acid for 10 min in the cold. RNA and DNA were extracted by either fractional extraction with perchloric acid[10] or by digestion with sodium hydroxide, reprecipitation with hydrochloric acid, and extraction of the DNA with hot perchloric acid[11]. The absorbance of the DNA extract thus obtained was measured at 267 mµ and the DNA calculated by comparison with DNA standards; the radioactivity was determined in a liquid scintillation spectrometer, and the activity calculated as d.p.m./µg DNA.

For autoradiography, incubation with ^{3}HTdR-containing medium (0·036 µg/ml.) occurred for 0·5 h, after which the cells on the cover-slips were fixed with acetic acid–alcohol (1 : 3), washed with distilled water, the cover-slips mounted on 1 in. × 3 in. microscope slides and covered with '*AR*–10' stripping film. With duplicate cultures the cells were treated with 0·02 per cent deoxyribonuclease in 0·002 M magnesium sulphate to remove DNA before the cover-slips were mounted. Some sets of autoradiograms were exposed for 1 week and others for 4 weeks before development.

When HeLa *S*3 cells were grown in normal medium or medium containing TdR the effect of ultra-violet on incorporation of radioactive precursors into DNA followed a similar pattern independent of the precursor used. Fig. 1 shows the results of a number of experiments using different labelled precursors, and, although there is some variation, in all cases the dose-dependent depression appears to consist of at least two components when plotted on a semilogarithmic plot—an initial steep decline in the lower dose range with a decreasing slope at higher doses.

When cells were grown in medium containing 5-BUdR for 2–3 days prior to irradiation the extent of incorporation varied with the type of precursor used, especially at higher doses. (It should be pointed out that just the presence of 5-BUdR in DNA depresses the rate of incorporation of radioactive precursors from 40 to 70 per cent (ref. 1).) The results are summarized in Fig. 2. When 8-^{14}C guanine (3 mc./mM) is used as tracer, the depression of incorporation with increasing dose, compared with its unirradiated control, is nearly the same as that obtained with TdR grown cells. When ^{3}HTdR and ^{14}C-thymidine were used as tracers, the depression of incorporation at low doses was similar to that obtained with cells grown in TdR; however, at doses above 200 ergs/mm^2 the uptake of these tracers began to increase, and at 800 ergs/mm^2 the incorporation was back to 40–50 per cent of the unirradiated control. Qualitatively similar results were obtained with *DFAF*-33 cells.

The results of the autoradiographic experiments with ^{3}HTdR are summarized in Table 1. One-week exposures gave practically identical results, but, of course, with correspondingly reduced grain densities. In normal, unirradiated populations, only those cells synthesizing DNA will show nuclear label after a short period of

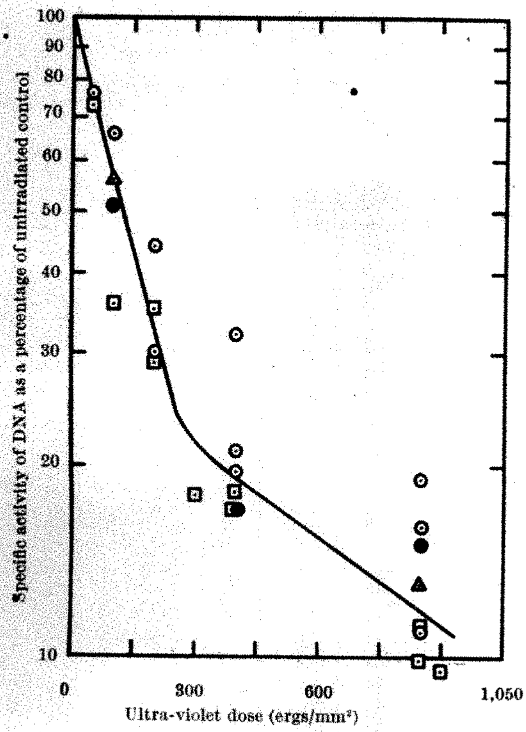

Fig. 1. Incorporation of various tracers into DNA of HeLa cells after two-days growth in Eagle's medium containing 4 μg/ml. TdR, and exposure to ultra-violet. Activity of the DNA was measured as described in the text. The ultra-violet dose was that incident at the surface of the flasks.
(⊡), 8-^{14}C Guanine; (●), 8-^{14}C adenine; (⊙), ^{3}HTdR; (△), ^{14}CTdR

incubation with ^{3}HTdR. The results with *DFAF*-33 cultures were both surprising and revealing. The thymidine-grown cells exhibited no difference in fraction of labelled cells between unirradiated and irradiated cultures, even at high doses (although the intensity of labelling did decrease with increasing dose). However, with BUdR-grown cells, the fraction of labelled cells in irradiated populations was, in all cases, 98 per cent or greater, with only an occasional cell not exhibiting grains above the nucleus. With HeLa the results were even more surprising, and showed a significantly different response from the Chinese hamster cells. With this line not only did irradiated BUdR-grown cells exhibit almost 100 per cent labelling, but so also did thymidine-grown cells. The percentage of labelled cells in unirradiated TdR-grown HeLa cells was unusually high; this may be due to the prior growth in the presence of TdR, which has been shown to affect DNA metabolism[12]. The fact that extension of exposure of labelled *DFAF*-33 cells to autoradiographic emulsion to four weeks did not significantly increase the fraction of cells showing label indicates that the difference between the two strains is really a qualitative one. Treatment with DNase in all cases resulted in depressions of grain count by more than 90 per cent, showing that the label was actually in DNA.

Separate experiments were performed in which all work beginning with the ultra-violet irradiation and thereafter was done in subdued light, using the same conditions necessary to avoid photoreactivation in bacteria. Such procedures had no effect on the results, and intentional exposures of ultra-violet irradiated cultures to 3650 Å light also failed to yield any evidence of photoreactivation of this response.

Table 1. PERCENTAGES OF CELLS SHOWING NUCLEAR LABEL AFTER ULTRA-VIOLET IRRADIATION

Cell type	Medium supplement	Percentage of labelled cells	
		Unirradiated	Irradiated
DFAF-33	TdR	67	62
DFAF-33	BUdR	56	98
HeLa	TdR	53	99 +
HeLa	BUdR	27	99 +

Cover-slip cultures, grown as described in the text, were irradiated with 500 ergs/mm² of ultra-violet at 2537 Å and incubated with ^{3}HTdR at 10 μc./ml. (0·36 μg/ml.) for 30 min at 37° C. Autoradiograms were prepared and exposed for 4 weeks. The percentages are based on counts of at least 500 cells.

These results, showing: (1) increased uptake of ^{3}HTdR and ^{14}CTdR at doses above 200 ergs/mm²; (2) incorporation of ^{3}HTdR by practically every cell in BUdR-grown *DFAF*-33 cultures and in all HeLa cultures following ultra-violet, cannot be explained on the basis of the kind of DNA synthesis occurring normally in these cells. The HeLa cultures show this most clearly. In this line, DNA synthesis (*S* period) normally occurs for 6–7 h of the average 20 h generation time[13]. Therefore about 5 per cent of the cells move into and out of DNA synthesis each hour. The incubation time for some autoradiographic experiments was 0·5 h, and it is inconceivable that a few seconds exposure to ultra-violet could drive all cells into the *S* period. This is especially exemplified by the fact that, following ultra-violet, even cells in mitosis are labelled by an immediate 0·5 h incubation with ^{3}HTdR. In no other circumstance, including after X-irradiation, has this ever previously been observed.

The fact that the increased uptake of tracer at doses above 200 ergs/mm² occurs with ^{3}HTdR at 0·036 μg/ml. and ^{14}CTdR at 8 μg/ml. clearly shows that the effect is not related to pool size changes. The failure of ^{14}C-guanine to exhibit a similar rise indicates that the effect has a certain degree of specificity. Because of the difficulty of obtaining a specific label for the cytosine moiety of DNA, it cannot be definitely concluded, as yet, that only thymine is involved in this phenomenon. However, the enhancement by BUdR of ^{3}HTdR uptake but not of ^{14}C-guanine does suggest that the effect occurs predominantly at areas of DNA containing bromuracil, and the labelling of 99+ per cent of the cells after ultra-violet of non-BU containing HeLa cultures indicates that the presence of BU in DNA makes more apparent the existence of a system that is always active following ultra-violet irradiation.

The most puzzling result is the difference between the two cell lines in extent of labelling following ultra-violet irradiation of thymidine-grown cells. Extending the exposure time of the autoradiograms failed to appreciably increase the fraction of labelled cells in the irra-

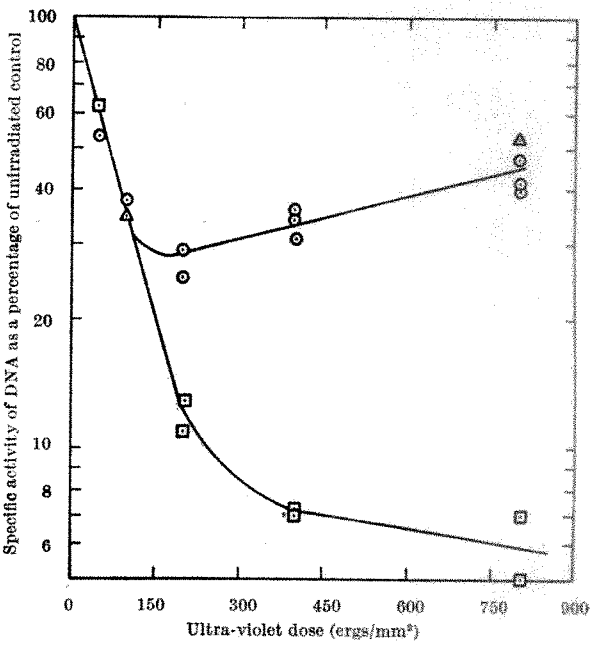

Fig. 2. Incorporation of various tracers into DNA of HeLa cells after two days growth in Eagle's medium containing 5 μg/ml. BUdR, and exposure to ultra-violet.
(⊡), 8-^{14}C Guanine; (⊙), ^{3}HTdR; (△), ^{14}CTdR

diated, thymidine-grown, *DFAF*-33 populations, and it thus appears that cells of this kind that are not in DNA synthesis at the time of irradiation do not participate in the post-irradiation synthesis unless they have previously incorporated BUdR into the DNA.* This difference in the two lines might still be only quantitative, with ultra-violet damage to normal *DFAF*-33 DNA being relatively light (compared with HeLa) but greater with BU substituted DNA; however, the evidence now available suggests that the response to ultra-violet damaged DNA differs qualitatively in the two lines.

The results reported here may involve a system similar to that reported for bacteria by Setlow and Carrier[2] and Boyce and Howard-Flanders[3]. In the case of bacteria the effect was detected by the finding that thymine dimers moved from the acid-insoluble to the acid-soluble fraction in the first hour after ultra-violet irradiation. We have attempted to determine, by pre-labelling the DNA with ^{3}HTdR and ^{14}CBUdR, if thymine or BU in the DNA of cells irradiated with ultra-violet light is lost to the acid-soluble fraction (or to medium, wash fluid, etc.). So far we have found no quantitative increase in material lost from DNA of irradiated cultures over that lost in controls. However, the fraction which would be lost, calculated on the uptake of ^{3}HTdR which occurs, would be so small that it would fall within the variability of our control values. Improved techniques may allow us to detect this loss, if indeed it occurs. To our knowledge, there has been no report indicating that the presence of BU in DNA affects the dark repair occurring in bacteria following irradiation.

We are using the approach of Pettijohn and Hanawalt[4] to determine if the density labelling of DNA following ultra-violet irradiation is different from normal. We are also examining other cell lines to determine the generality of the phenomenon, both in normal and in BU substituted DNA.

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CATALYTIC DECOMPOSITION OF GLUCOSE IN AQUEOUS SOLUTION BY THERMAL PROTEINOIDS

By Prof. SIDNEY W. FOX and Dr. GOTTFRIED KRAMPITZ

Institute for Space Biosciences, Florida State University, Tallahassee

THE thermal condensation in an initially dry state of the eighteen amino-acids common to protein[1-3] represents a synthesis by which protein molecules can be visualized as having come into existence simply in a geological matrix[4]. These thermal polymers, proteinoids, readily form membranous microparticles[5,6], have nutritive quality for bacteria[3] and rats[7,8], are split by proteolytic enzymes[2,3], and have their own catalytic activity for the hydrolysis of *p*-nitrophenyl acetate[9], in some cases at a rate many times that observed with the amount of histidine contained in the polymer[9,10].

Preliminary reports of activity for the hydrolysis of ATP at 40° by zinc-containing proteinoids are appearing[11,12]. This communication reports the catalytic activity in aqueous solution of thermal proteinoids for the decomposition of the natural substrate, D-glucose.

The activity observed concerns the decomposition of uniformly labelled ^{14}C-glucose to $^{14}CO_2$ at a low rate of conversion ; some $^{14}CO_2$ production has, however, been measured in every one of more than sixty experiments. Evidence has been obtained that one of the intermediates is glucuronic acid, and radio-glucuronic acid itself has been found to yield $^{14}CO_2$ in the presence of thermal proteinoid, at a rate many times that of the production from glucose. Glucose labelled only in the 6-position showed a comparable rate of production of $^{14}CO_2$, whereas glucose labelled in the 1- or 2-position yielded virtually no $^{14}CO_2$.

In a typical experiment, 100 mg of a 2 : 2 : 1-proteinoid[3] dissolved in 50 ml. of redistilled water was incubated at 37·5° C for 72 h with 10 μc. of uniformly labelled ^{14}C-glucose (specific activity, 28·5 mc./mM; Calbiochem) freshly purified by chromatography. The reaction mixture was maintained under a slow stream of pre-purified nitrogen and the gas was passed through a flask containing saturated barium hydroxide. The carbon dioxide traps were replaced after 1, 2, 3, 6, 12, 24, 48, and 72 h. To each trap was added 50 mg of carrier barium carbonate ; the total carbonate was collected on Whatman No. 50 filter paper after complete precipitation. The precipitate was washed with 50 ml. water and then 20 ml. ethanol. The radioactivity was estimated on a windowless, gas flow beta-detector operated in the Geiger range with helium-isobutane. The counts were determined over 10 min. Controls without proteinoid and others without glucose were run. Measurements of the evolution of carbon dioxide are represented in Table 1.

Mixtures of amino-acids in the proportions found in the analysis of the proteinoid were observed to be devoid of catalytic activity, as were direct hydrolysates (Table 1).

Some increase in rate of evolution of carbon dioxide was observed when ATP or Mg^{++} or both were added to the reaction mixture (Fig. 1). The shape of the curves constitutes part of the evidence ruling out microbial contamination as a source of enzymatic activity. Most of the experiments were at *p*H 2·5, which of course inhibited most or all microbial growth. As a rigorous check on the possibility of contamination, syntheses were carried out aseptically under the supervision of Dr. and Mrs. Lutz Wiese.

The aseptic syntheses began with amino-acid mixtures which had stood under diethyl ether for 48 h and which had

Table 1. EVOLUTION OF $^{14}CO_2$ FROM UNIFORMLY LABELLED GLUCOSE (10 μC.) ON INCUBATION WITH THERMAL PROTEINOID

Proteinoid or control	$^{14}CO_2$ produced in c.p.m.*	*S.D.*
2 : 2 : 1 (*DJ* IV 51)	117	5
2 : 2 : 1 microspheres	64	7
2 : 2 : 1 (*DJ* V 15)	70	9
2 : 2 : 1 (190° *RM*)	62	6
1 : 1 : 1-Zn (*BW* IV 79*B*)	79	5
1 : 1 : 1-Zn, purified from hot H_2O	68	5
1 : 1 : 1 (*GK* I 40)	83	5
Lysine proteinoid (*KS* IV 23 *b*, *d*)	1,023, 1,278	25, 36
2 : 2 : 1 (*GK* aseptic)	441	13
Amino-acid mixture	0–5	0
Glucose in solution	0–5	0
Hydrolysate of *DJ* IV 51	0–5	0

* Each value is the figure above background (42–55 c.p.m.).

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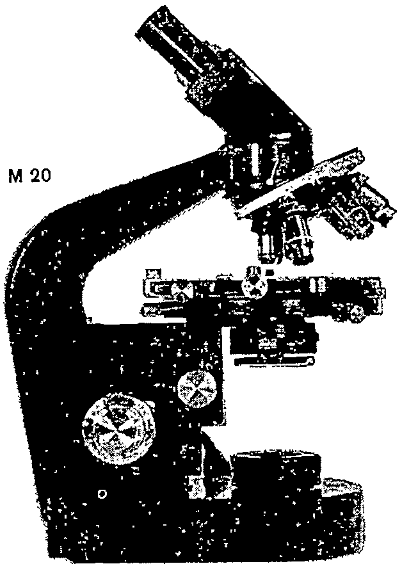
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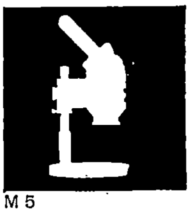
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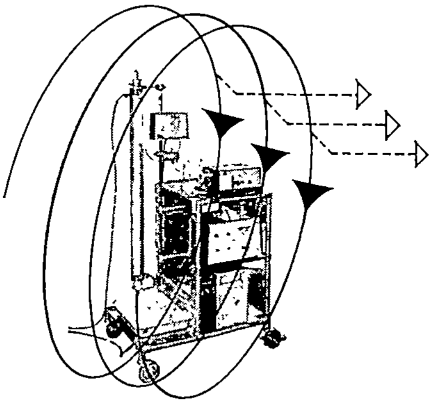

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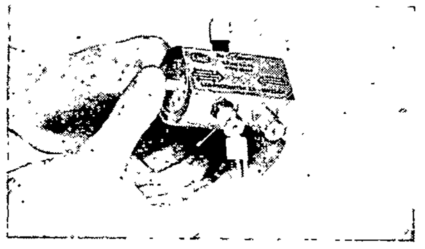

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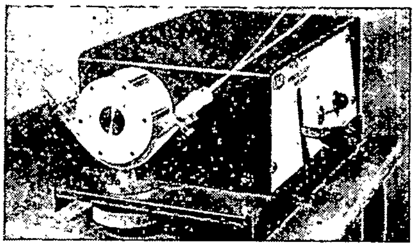

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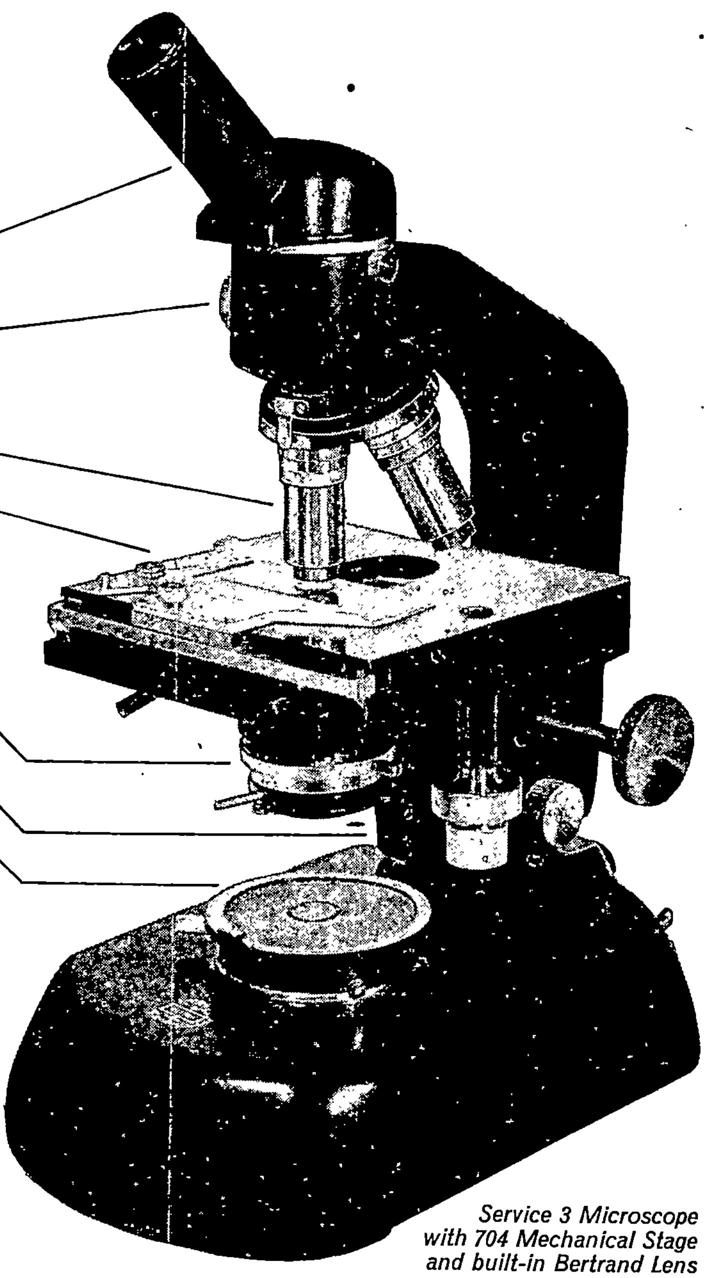

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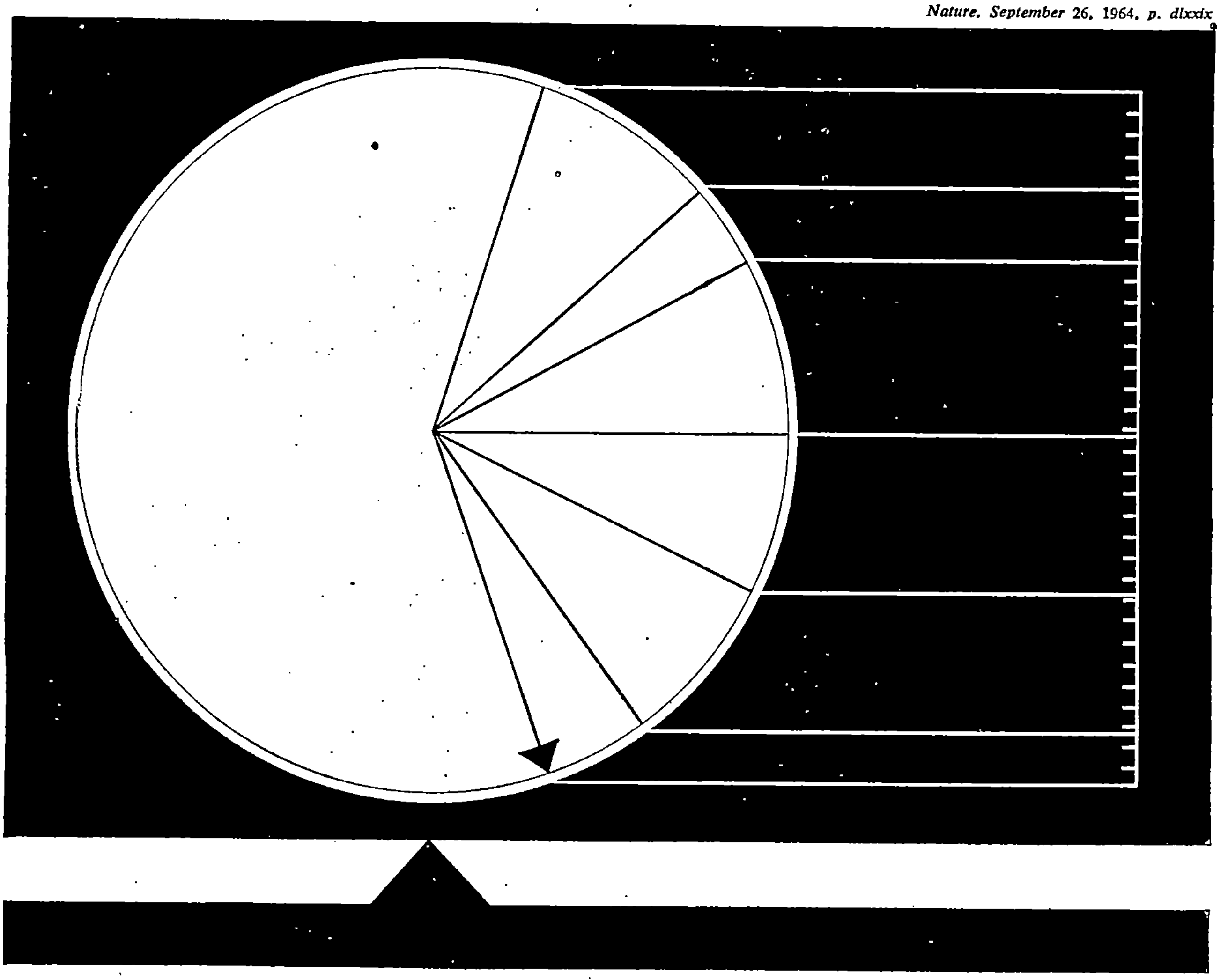

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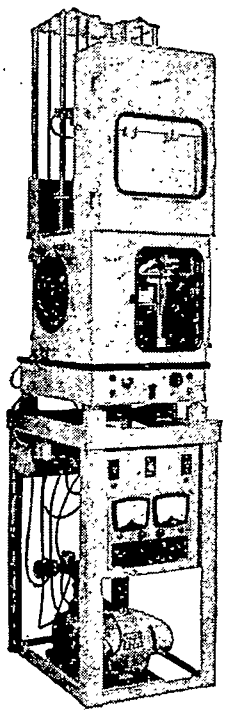

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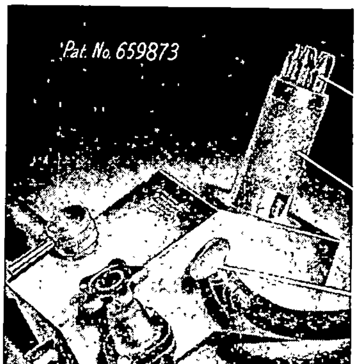

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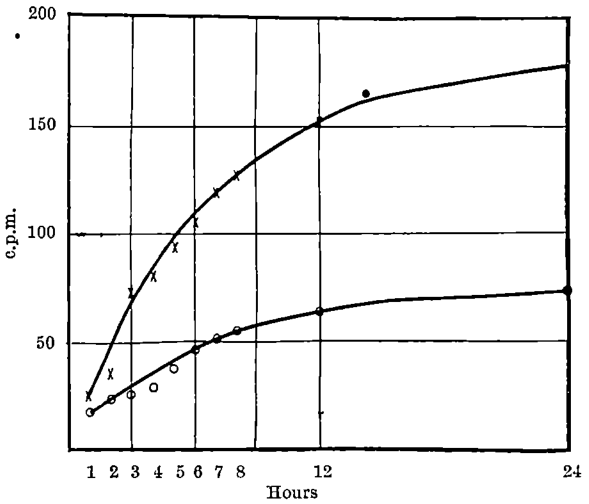

Fig. 1. Evolution of $^{14}CO_2$ from radioglucose in presence of thermal proteinoid, demonstrating the carbon dioxide formation from glucose in the presence of proteinoid. O, shows carbon dioxide evolution for the reaction mixture without any additions; ×, indicates carbon dioxide production from the reaction mixture in the presence of 2·5 μM ATP

no catalytic activity *per se*. The acid proteinoids thus prepared were each tested for sterility in tryptone glucose beef extract: (*a*) broth; (*b*) agar, in tryptone glucose yeast extract; (*c*) broth and (*d*) agar, before dialysis of the proteinoid, after dialysis for 4 days, after resolution of aseptically lyophilized polymer and at the end of the tests for catalysis. The polymers thus prepared showed at least the usual amount of activity after having given negative results for growth in the sterility tests. Lysine proteinoid was also synthesized under sterile conditions. Both the aseptically synthesized lysine proteinoid and lysine proteinoid prepared months earlier, and stored in the usual way in a bottle, were found to be bacteria-free ; this latter probably reflects the bacteriostatic activity of polylysine[13].

As Table 1 shows, thermal lysine proteinoid[9], as first prepared by Dr. Harada[9], was more active than thermal acid proteinoid.

Other sugars, radio (fructose) and radio (sucrose), also yielded $^{14}CO_2$, but in smaller proportions than did (radio) glucose. In three experiments, 10 μc. of sucrose yielded 130, 212, and 316 c.p.m. Such results with sucrose help to rule out the possibility that decarboxylation is due to a Maillard reaction between proteinoid and the sugar[14]. Study of the *p*H activity curve for lysine proteinoid also revealed *p*H optima at 2 and 5, whereas the Maillard reaction is favoured in alkaline solution[14]. The proteinoids were active over the entire range of *p*H 1–9 tested. Activity was only slowly destroyed by heating in aqueous solution. Microspheres[5,6] formed in the steam autoclave from hot water also displayed activity for the evolution of carbon dioxide. An experiment in which a second charge of substrate, added after three days, was decomposed as rapidly as the first charge represents one experiment to test whether the activity is truly catalytic.

For analytical purposes, the reaction mixture was concentrated in a rotary evaporator and finally dried in a vacuum desiccator over sodium hydroxide pellets and over sulphuric acid. The dried residue was three times extracted with dry pyridine in order to remove unreacted glucose; the pyridine extract was taken to dryness over sulphuric acid. Aqueous solutions of the residue from pyridine extract and from the pyridine-insoluble residue were separately subjected to paper chromatography. The samples were applied to Whatman 3*MM* paper. The developing solvent usually was ethyl acetate: pyridine : water (12 : 5 : 4). Chromatography was carried out by ascension (Fig. 2). Fig. 2 presents the initial results.

Following the suggestion of glucuronic acid as an intermediate from the experiment with 6-^{14}C-glucose, this acid was sought as follows. The reaction mixture was made alkaline to phenolphthalein with barium hydroxide. Carbon dioxide was bubbled through this mixture for a few minutes. The mixture was then heated to 100° for 10 min, filtered, and the precipitate was washed with boiling water. The combined filtrates and washings were concentrated to 2 ml. *in vacuo* and mixed with 100 ml. ethanol. The barium salts were collected by filtration and washed with ethanol. The radioactive precipitate was acidified with acetic acid in aqueous suspension and the barium was removed with 1 N sulphuric acid, the liquid dried, and extracted with ethanol. The extract was subjected to two-dimensional paper-chromatography[15] with phenol : deuterium oxide (100 g : 39 ml.) in the first direction and butan-1-ol : propionic acid : water in the second direction. (For this chromatography, two solutions were prepared. Solution *A* contained 919 ml. of butan-1-ol and 81 ml. distilled water. Equal volumes of solutions *A* and *B* were mixed just before use.) One of the spots had the R_F of glucuronic acid as determined with a control. The material was eluted and rechromatographed in ethyl acetate : pyridine : water (120 : 50 : 40). The R_g $\left(R_g = \frac{\text{distance substance travels from origin}}{\text{distance glucose travels from origin}} \times 100\right)$ of the sample, corresponding with that of glucuronic acid. was 13–14. This eluted spot was rechromatographed in methanol : formic acid : water (30 : 15 : 5 v/v), again indicating glucuronic acid.

From an experiment on 3·0 g of glucose (100 mg proteinoid), the isolated glucuronic acid fraction was subjected to reaction with carbazole[16] to give a positive colour test. The maximum absorbance was at 530 mμ, the same maximum being found with the carbazole reaction product of authentic glucuronic acid.

In another reaction of 100 mg of 2 : 3 : 1-proteinoid. 10 g of D-glucose and 10 μc. of U-^{14}C-glucose the extract, following barium sulphate formation and ethanol extraction, was dried in a desiccator. The residue was dissolved in 10 ml. of 0·2 N sodium acetate and 100 mg *p*-bromophenylhydrazine hydrochloride was added. The suspension was heated to boiling for 5 min and 50 mg authentic non-radioactive glucuronic acid *p*-bromophenylhydrazone was added. The suspension was chilled for 2 days. The solid was filtered, dissolved in hot 60 per

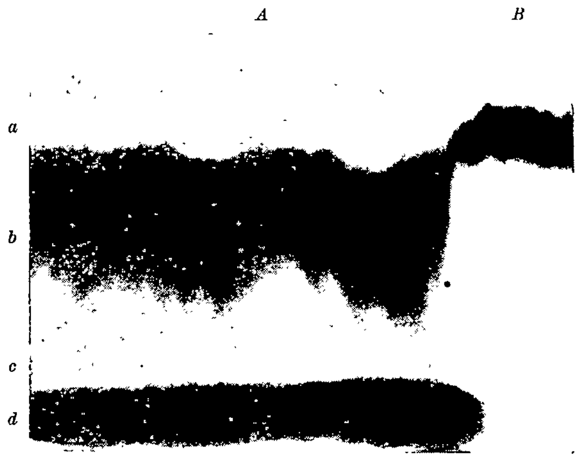

Fig. 2. Autoradiogram of decomposition of radioglucose catalysed by thermal proteinoid. Fig. 2 demonstrates the decomposition products of U-^{14}C-glucose, after incubation with a lysine proteinoid, in a solvent system of ethyl acetate : pyridine : water (12 : 5 : 4) ascending technique; Whatman No. 3*MM* paper. *A* is the water-soluble extract of the reaction mixture. *B* is the water-soluble extract of the reaction mixture before incubation. Bar *a* corresponds to glucose; *b* is unidentified, but does not contain free glucose after rechromatography since it is insoluble in pyridine. On rechromatography, R_F of glucose = 0·27, R_F of major spot = 0·22. Bar *c* has R_F identical with that of glucuronic acid after rechromatography in other solvent systems. Bar *d* is unknown, contains no free glucose or free glucuronic acid

cent ethanol and left in the refrigerator for 48 h. This recrystallization was repeated 4 times. The yellow needles m.p. 236° C unc.[17] mixed m.p. 236° C unc. When examined in a planchet with a window-less counter, 3,700 c.p.m. were recorded. Following a sixth recrystallization, 3,700 c.p.m. were again recorded.

The radioactive *p*-bromophenylhydrazone was chromatographed in water saturated with butan-1-ol. The R_F for this material on an autoradiogram and for cold material observed visually was in each case 0·89. A mixed chromatogram showed also $R_F = 0·89$.

The evidence thus indicates that at least a small fraction of the glucose is first converted to glucuronic acid, which is then decarboxylated, and that each of these reactions is catalysed in aqueous solution by proteinoid prepared by thermal condensation of dry amino-acids mixed in appropriate proportions. Fig. 2 is an autoradiogram which is part of the evidence that the glucose is more than 90 per cent converted to other materials.

These results may explain many earlier findings in this laboratory in which were observed activities for other natural substrates; the variability of such results since 1958 was such that no report had been submitted for publication. This variability now appears to be explainable in part as due to the presence of several weak activities. The evolutionary value of weak catalytic activities, such as compared with those of contemporary enzymes and as here demonstrated for production of polymers active in producing carbon dioxide and glucuronic acid, has been expounded in theoretical treatments particularly by Calvin[18].

The finding of catalytic activities for natural substrates and of integrated nutritive qualities in thermal proteinoids entitles them to be considered both as synthetic general protein and as a model of primitive abiotic protein. In the latter context a catalytic proteinoid which forms organized units[4–6] is conceptually consistent with an evolution which led to diversified, specialized, and quantitatively enriched catalysts.

We thank Dr. and Mrs. Lutz Wiese for help with the aseptic preparations; Dr. David H. Durant for repeating the key experiment demonstrating production of carbon dioxide and intermediates from written directions only; and David Joseph and Sylvia Steen for repetitions of the key experiment. We also thank Dr. Harry Lipner for the use of counting equipment.

The investigation was supported by grant *NsG*–173–62 of the National Aeronautics and Space Administration.

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A VARIATION IN THE ELECTROPHORETIC PATTERN OF HUMAN ERYTHROCYTE CARBONIC ANHYDRASE

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CARBONIC anhydrase is the most abundant soluble non-hæmoglobin protein in human erythrocytes[1,2]. At least two and perhaps three distinct proteins in red cells have carbonic anhydrase activity[1,3,4]. The two principal forms, now designated by agreement as *B* and *C* (ref. 4), differ markedly in amounts present and in activity[1,3–5]. Enzyme *B* occurs in 5–10 times greater amount than enzyme *C* (refs. 4 and 5). The specific activity of enzyme *C* is 20–30 times as great as that of enzyme *B* (refs. 1 and 4). The molecular weights of the two forms are quite similar, but the amino-acid compositions differ significantly[3,4,6,7]. Enzymes *B* and *C* are easily separated by starch-gel electrophoresis at alkaline *p*H, enzyme *B* moving more rapidly towards the anode[4,8].

During the course of an investigation of abnormal hæmoglobins, blood samples from approximately 1,400 adults and 1,000 neonatal infants were examined. The hæmolysates were examined by starch-gel electrophoresis[9–11] and the gels were stained with benzidine and amido black 10*B*. As has been previously reported[2], the carbonic anhydrase bands were absent or markedly reduced in intensity in all the hæmolysates made from cord blood (Fig. 1). On electrophoresis of hæmolysates from the adults, using a *tris*-EDTA-borate buffer system[11], at *p*H 8·6, enzyme *B* moved slightly more slowly towards the anode than hæmoglobin A_2 (Fig. 1). Enzyme *C* moved a short distance from the origin towards the cathode (Fig. 2). The only variation noted in the 1,400 adult specimens was an almost complete absence of enzyme *B* in the hæmolysate from a 31-year-old Negro woman (R. M.) (Fig. 1). Unlike the cord bloods, the hæmolysate from R. M. contained carbonic anhydrase *C* and hæmoglobin A_2 (Figs. 1 and 2).

Fig. 3 is the pedigree of the family of R. M. An investigation of the family revealed no other person with the abnormal carbonic anhydrase pattern (Fig. 4). The peripheral blood smear of R. M. was characteristic of thalassæmia minor and the percentages of hæmoglobin A_2 (4·0 per cent) and hæmoglobin F (4·4 per cent) were elevated. The father of R. M. and her three daughters had peripheral blood smears suggestive of thalassæmia minor. The blood of the mother of R. M. was normal except for a hæmoglobin A_2–B_2 electrophoretic pattern (Fig. 4). No other family member had thalassæmia minor. Extensive blood grouping investigations revealed no incompatibility between R. M. and either parent.

Total carbonic anhydrase activity in the erythrocytes of R. M. was assayed by an electrometric method[12] and found to be normal. As has been previously reported[13] enzyme activity was quite low in cord blood. The hæmolysate of R. M. was subjected to starch-gel electrophoresis and the gel serially sectioned, frozen and thawed, and extracted with 0·85 per cent sodium chloride. Carbonic anhydrase activity was found only in the region

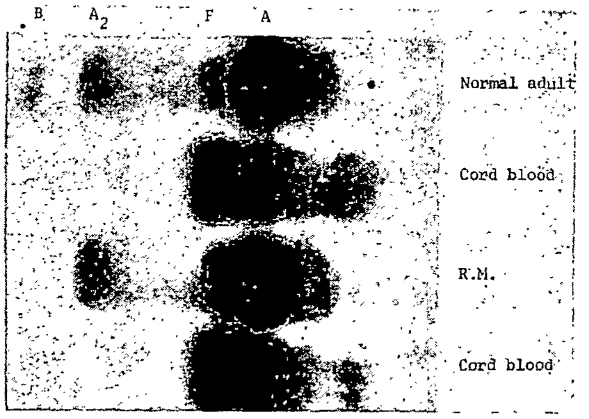

Fig. 1. Vertical descending starch-gel electrophoresis showing marked reduction in carbonic anhydrase *B* from hæmolysates prepared from the blood of R. M. and a normal umbilical cord blood (amido black stain)

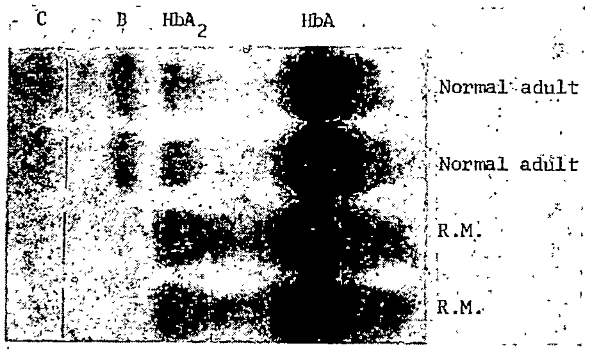

Fig. 2. Vertical descending starch-gel electrophoresis of hæmolysates prepared from the blood of R. M. and a normal individual showing marked reduction in the amount of zone *B* and normal amount of zone *C*, migrating behind the origin (amido black stain)

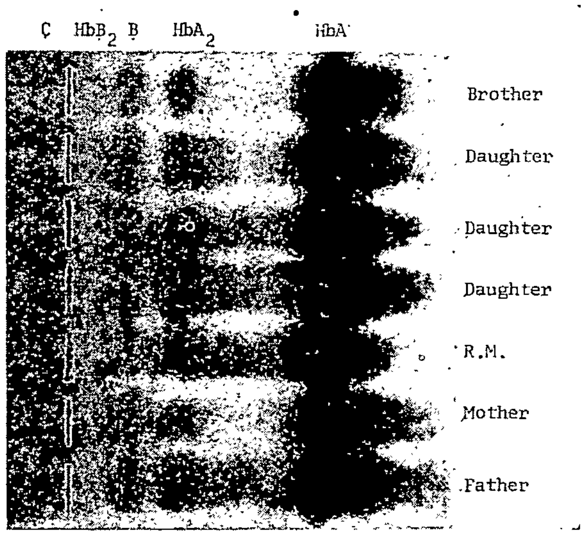

Fig. 4. Vertical descending starch-gel electrophoresis of hæmolysates prepared from the blood of R. M.'s near relatives. In each case carbonic anhydrase zones *B* and *C* are normal. The sample from R. M.'s mother shows hæmoglobin A$_2$ and B$_2$ (amido black stain)

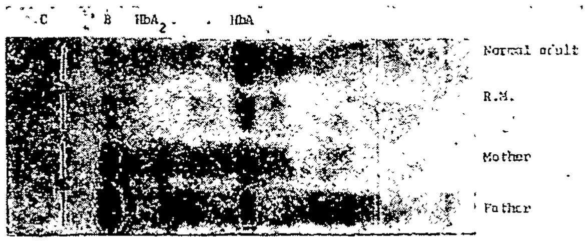

Fig. 5. Non-hæm protein concentrates prepared by 'Amberlite *CG*-50' column chromatography of hæmolysates prepared from the blood of R. M. and relatives (amido black stain)

corresponding to the position of enzyme *C*. In contrast, a normal hæmolysate demonstrated activity in the regions corresponding to the position of *B* and *C*.

The erythrocyte non-hæmoglobin proteins from R. M. and her parents were separated from hæmoglobin by 'Amberlite *CG*-50' column chromatography[14] or by ethanol–chloroform precipitation of hæmoglobin[4]. The non-hæmoglobin protein from each person was concentrated ten-fold by ultrafiltration in 'Cellophane' bags and subjected to starch-gel electrophoresis using both *tris*-EDTA (*p*H 8·8) and phosphate (*p*H 7·0) buffer systems (Fig. 5). No protein band was found in any of these materials that was not present in the concentrated non-hæmoglobin proteins derived from normal hæmolysates. The electrophoretic pattern of the concentrated protein of R. M. did reveal a faint band in the position of enzyme *B*. Serial sections and assays for carbonic anhydrase showed slight activity in the region of *B* in the pattern of R. M. No new region of activity was found for either R. M. or her father. Staining of the gel for esterase activity[15] also failed to reveal a new band of carbonic anhydrase.

Genetically determined variant forms of human erythrocyte carbonic anhydrases[16,17] and baboon erythrocyte carbonic anhydrases[18] have been previously reported. The mutants observed in those investigations showed mobility changes in one of the two enzyme bands. In the work recorded here one band was almost completely absent and no new band was detected. The normal total activity in the red cells of R. M. probably reflects the high activity of the remaining carbonic anhydrase *C* (ref. 4). The genetic relationship of *B* and *C* is not known but they do not appear to be simple chemical derivatives of each other[7]. Isolated complete absence of single proteins has previously been reported for hæmoglobin A$_2$ (ref. 19) and for β$_{2A}$-globulin (ref. 20). In neither instance was an example of inheritance of the trait found. The genetic mechanism of the presently reported variation is not at all clear, but it is possible that R. M. is homozygous for a deficiency in synthesis of carbonic anhydrase *B*. The faint protein band found may represent the minor component of enzyme *C* mentioned by Rickli *et al.*[4], or it may represent a very small amount of enzyme *B*. This situation may therefore be analogous to thalassæmia in that the protein formed is structurally normal but is produced in markedly decreased quantities.

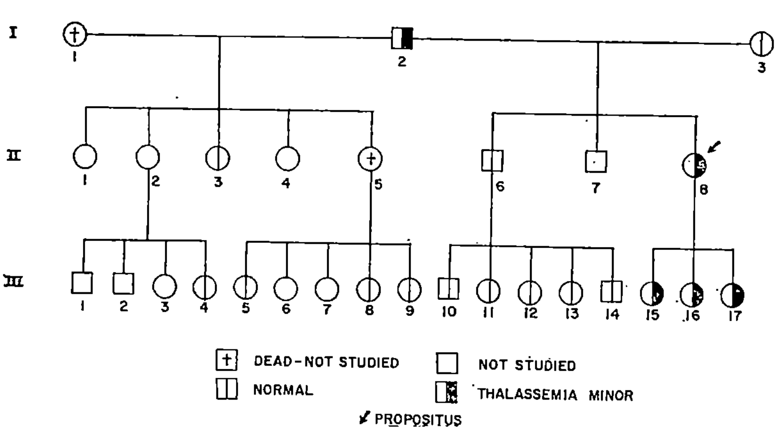

Fig. 3. Family pedigree of R. M. and near relatives

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MALARIA ANTIBODY CONTENT OF GAMMA$_2$-7*S* GLOBULIN IN TROPICAL POPULATIONS

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A GREAT deal of evidence has been accumulated to demonstrate that the raised γ-globulin levels observed in many tropical populations[1-3] are due in part at least to malarial infection, and it has been shown that species-specific protective antibodies are associated with the γ_2-7*S* globulin fraction of sera from immune adults[4]. It has also been found that malarial infection is associated with non-specific precipitins[5] and complement-fixing antibodies and with the production in rats of auto-antibodies to uninfected erythrocytes[6]. It may also be possible that in the course of the intense activity of the reticulo-endothelial system during malarial infection the production of antibodies to other non-malarial endemic pathogens is enhanced. We have attempted to determine the proportion of the γ_2-7*S* globulin representing parasite antigen-binding antibody in individuals living in holoendemic malarial areas in New Guinea and New Britain.

Two groups of Melanesians living in very different regions, both holoendemic for malaria, were chosen for the work recorded here. One was from the Sause linguistic group in the Sepik River district of New Guinea, the other from the Kilengi linguistic group in the Gloucester Peninsula of New Britain. Anticoagulated whole blood was obtained sterilely from individuals (mostly children) with parasite counts (*P. vivax* and *P. falciparum*) exceeding 10^5/mm^3 and flown, packed in ice, to Melbourne, where cells were separated from plasma by centrifugation and washed three times with 0·9 per cent sodium chloride. The packed cell suspensions were pooled and subjected to sonic disintegration at 20 kc/s for 20 min at 4° C. The resulting mixture was dialysed against 0·1 M sodium borate, *p*H 8·3 and then conjugated to azo-benzyl-'Sephadex *G*200' which had been prepared by the method of Campbell, Leuscher and Lerman[7]. 'Sephadex *G*200' was used instead of cellulose because of its lower non-specific adsorption. The conjugated column material was washed six times by decantation with 0·9 per cent NaCl and packed with stirring[8] into 36 cm × 0·9 cm columns.

The sera to be tested were applied in 1-ml. aliquots to the top of the columns and eluted with 0·9 per cent sodium chloride using the precautions usual to conventional gel-filtration[8]. The progress of elution was followed with a 2570 Å ultra-violet recording photometer. Parallel experiments were carried out using columns prepared with unparasitized erythrocytes. All the column effluents were concentrated to 1 ml. using dry 5 per cent cross-linked acrylamide sticks[9]. These and the original sera were then examined for the presence of malaria antibody by an indirect fluorescent antibody test[10] using acid-washed parasitized blood films, obtained from both children and adults in various areas of New Guinea to ensure a range of parasite species and strains. It was found that a single passage through a column of the specified dimensions, prepared with parasitized erythrocytes, of serum containing malaria antibody detectable by this test was sufficient to remove all that antibody. No discernible change in malaria antibody content occurred after passage of such sera through a column prepared with non-parasitized erythrocytes.

The γ_2-7*S* globulin content of each serum sample, before and after passage through the column, was then measured using a highly specific rabbit antibody[11] calibrated with ^{131}Iγ_2-7*S* globulin using carrier-free isotope and a low level of iodination[12]. The γ_2-7*S* globulin content of an aliquot of each serum sample before passage through the column was also estimated in this way. The proportion of the total γ_2-7*S* globulin represented by malaria antibody is given for different age groups and areas in Table 1.

It can be seen that malarial antibody detectable by the fluorescent antibody technique represents quite a small proportion of the total γ_2-7*S* globulin (6–11 per cent) although it may well be large in relation to the amount formed as specific antibody against other individual types of pathogens. The rise in γ_2-7*S* globulin with age shown in Table 1 is in accord with the age-dependence of γ-globulin levels in New Guinea populations[11]. Thus it appears that the amount of γ_2-7*S* globulin synthesized as malaria antibody remains fairly constant while the total γ-globulin rises with increasing age.

The adsorption of γ_2-7*S* globulin to the columns prepared from the control, non-parasitized erythrocytes was low—0·5 per cent of the total—and there was no increase in adsorption with increasing age of subjects whose

Table 1. MALARIA ANTIBODY AND γ_2-7*S* GLOBULIN

District	Age	Total γ_2–7*S* globulin (g/100 ml.)	Malaria antibody γ_2-7*S* globulin (g/100 ml.)	No. of subjects
New Britain (Kilengi linguistic group)	0–5	1·86 (0·36)	0·21 (0·07)	18
	5–10	2·07 (0·27)	0·23 (0·09)	21
	10–	2·69 (0·21)	0·29 (0·11)	21
Sepik (Sause linguistic group)	0–5	1·79 (0·29)	0·17 (0·06)	26
	5–10	2·11 (0·18)	0·21 (0·12)	12
	10–	2·86 (0·24)	0·19 (0·06)	23

The figures in brackets are standard deviations.

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* Postal address: Commercial Road, Prahran S.1, Victoria, Australia.

sera were tested. On this basis, then, antibodies to blood cells do not represent a large part of the very high concentrations of γ_2-7S globulin found in people living in holoendemic malarial areas of New Guinea. This is in contrast to the greater production of anti-erythrocyte antibodies in the rat[6].

In our detailed analyses of serum protein characteristics of Melanesian people in New Guinea[13] we have concluded that the high γ-globulin levels observed are mainly the result of infections. Data accruing from comparisons of children with high and low rates of malarial infection, with respect to globulin-levels and levels of malarial antibody[14], suggest that malaria may play a relatively minor part and that the total infective load may be more important in the ætiology of the hypergammaglobulinæmia. This conclusion is supported by the present quantitative data demonstrating the small contribution of malarial antibody to total γ_2-7S globulin and the absence of increase in malarial antibody γ_2-7S globulin with increasing age. It is in contrast to the conclusions drawn by other workers whose observations have been made in Africa[1-3].

It is possible that the intense activity of the reticuloendothelial system in response to infections results in the production of 'non-specific' γ-globulin, including antibody directed towards autologous tissue determinants. In respect of this possibility it is interesting that we have observed in several New Guinea populations a high proportion of hypergammaglobulinæmic individuals with human tissue antibodies[15], while at least one group (the Tolai in New Britain) exhibit a high incidence (10 per cent) of cold hæmagglutinins[16]. The possibility that the production of auto-antibodies may be one result of high rates of γ-globulin synthesis in tropical populations is worthy of further investigation.

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SIMULTANEOUS RECORDING OF ELECTRICAL AND MECHANICAL ACTIVITY OF THE UTERUS IN THE UNANÆSTHETIZED ANIMAL

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EXTENSIVE research has been devoted to elucidating the mechanisms involved in smooth muscle function[1]. Most of this work has been carried out under *in vitro* conditions. The results obtained in such investigations have led to a variety of hypotheses which are often extrapolated to explain the physiology of the tissue in its *milieu interieur*. In order to aid in testing and integrating the various hypotheses resulting from *in vitro* investigations, it is desirable to perform *in vivo* investigations, provided the latter can be carried out without distorting the normal function of the organ.

This article concerns a method of recording electrical and mechanical activity from the myometrium in the unanæsthetized dog. The method was adapted from investigations performed on the gastro-intestinal tract[2-4]. The animal is prepared for chronic examination by implanting strain gauges and electrodes on the surface of the uterus and exteriorizing the wires to an electrical (female) connector which is sutured to the ventral surface of the abdomen. These animals may be monitored for several hours on a given day and have remained useful for up to four months without impairment of the blood supply, function, or continuity of the uterus. Thus, uterine activity may be recorded in the same animal under a variety of experimental conditions.

The combination electrode-transducer unit used in these investigations is illustrated in Fig. 1. The lower right portion of this figure shows a connecting wire soldered to a platinum electrode which is mounted on the underside of a copper beryllium clip, while the middle portion illustrates the strain gauge mounted on the upper surface of the same clip. In preparing these units, the electrode is placed in a groove in the bottom of a rectangular mould. The assembled strain gauge is then centred over the electrode and insulated from the electrode by a layer of silicone rubber (Medical 'Silastic 372', Dow Corning Corp., Midland, Michigan). The entire unit is then covered with 'Silastic' with the exception of the bottom surface of the electrode. The upper drawing indicates the final size and encapsulation of the electrode-transducer unit. Two or more units may be implanted on each uterine horn of a given animal. Individual electrodes[2] and transducers[3] have also been used successfully to record myometrial activity from both pregnant and non-pregnant dogs and rabbits for prolonged periods of time. The recordings were made on an 8-channel 'Offner' (Type *R*) recorder. A time-constant of 0·03 sec was used to record the electrical activity.

While evaluating the effect of various agents on the myometrium, it was necessary to test a variety of suspending vehicles. Fig. 2 illustrates various types of myometrial

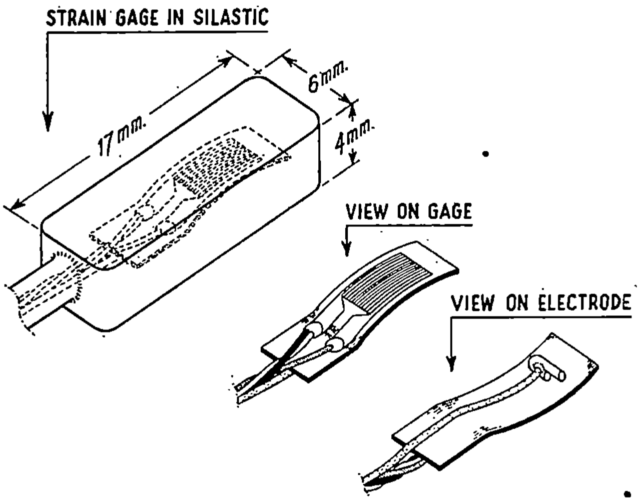

Fig. 1. Combination electrode-transducer unit for recording myometrial activity

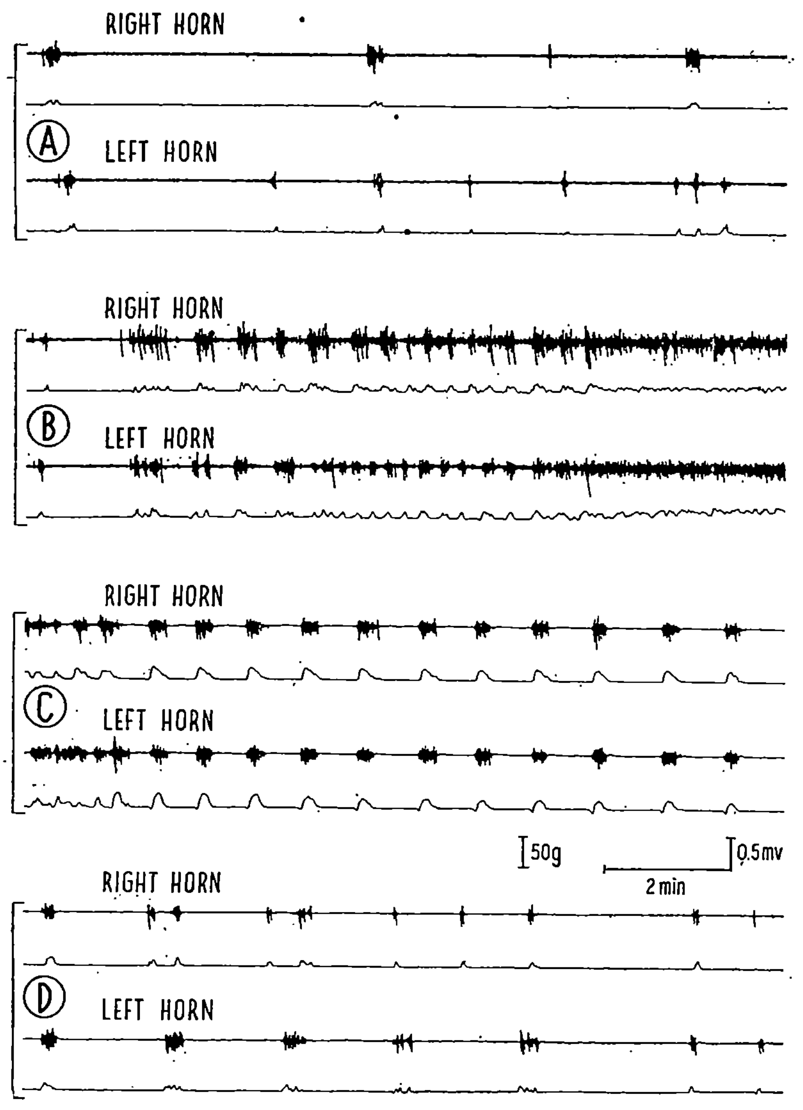

Fig. 2. Effects of a suspending vehicle on electrical and mechanical activity of the uterus of the unanæsthetized dog; mv, mV; g, grams of tension

activity recorded when an aqueous suspending medium was infused into the radial vein of œstrogen-primed dogs. A pellet of 17β-œstradiol (10 μg/kg) was implanted subcutaneously at surgery. The suspending vehicle consisted of sodium chloride (0·9 per cent), polysorbate-80 (0·4 per cent), carboxymethylcellulose (0·5 per cent), and benzyl alcohol (0·9 per cent)[5]. Records similar to that shown in Fig. 2 were observed repeatedly in a total of nine dogs. Record *A* (Fig. 2) shows a control pattern prior to infusion of the above vehicle. The electrical activity of each uterine horn was observed to precede the corresponding mechanical activity of that horn. In addition, myometrial activity of a given horn was independent of the other horn, although occasionally the two horns were synchronized. In general, the frequency and amplitude of uterine activity were related to the level of exogenous œstrogen administered. In this series, the average frequency of uterine contractions was once every 50 sec (record *A*, Fig. 2).

Record *B* (Fig. 2) was recorded within seconds following the beginning of vehicle infusion (1·2 ml./min). It is apparent that both the electrical and mechanical activity of each horn increased dramatically during the first few minutes of infusion. Infusion of the vehicle progressively caused a more asynchronous pattern than was observed during the control period. In addition, the mechanical activity recorded from both horns was observed to go into contracture which was accompanied by excessive depolarization. This contracture normally lasted approximately 6 min.

Record *C* (Fig. 2) illustrates the pattern of myometrial activity obtained after 40 min of continuous infusion of the aqueous suspending medium. In contrast to record *B*, the electrical and mechanical activity of each horn became synchronous. This contractile pattern is similar to, but not identical with, that observed during parturition in the dog. It is obvious that the frequency of the bursts of action potentials and also the magnitude (amplitude and duration) of mechanical activity was greater than during the control period. Moreover, record *C* emphasizes the correlation observed between the electrical and mechanical activity of the myometrium. The contracture pattern (record *B*) was always followed by the synchronous activity (record *C*) whether the infusion of the vehicle was stopped during the contracture or allowed to continue.

Record *D* (Fig. 2) illustrates the return to a pattern similar to the control 18 min after infusion of the vehicle was stopped. The myometrial activity of the two horns has again become more or less asynchronous and the magnitude of both the electrical and mechanical activity has decreased.

The effect of the above suspending vehicle on the electrical and mechanical activity of the myometrium of the unanæsthetized dog was selected to illustrate a variety of uterine contractile patterns. Most of these patterns have been observed in various physiological states in the dog. The contracture and excessive depolarization shown in record *B* (Fig. 2) have been observed with oxytocin. Although no behavioural or nociceptive responses were observed during infusion of the vehicle, this agent was toxic to the animal. Repeated infusion of the vehicle eventually caused considerable hæmorrhage of the lungs and hypertrophy of the kidneys.

The foregoing method of recording uterine activity has several advantages, which include: (1) the preparation is physiological; (2) recordings can be made in the same animal under a variety of conditions over a prolonged period of time; (3) activity is recorded from a localized area of the uterus; (4) the method is applicable to several species. These advantages provide a useful approach to the elucidation of physiological mechanisms.

Electrical activity of the uterus has previously been recorded *in vivo* in the cat[6], rabbit and man[7]. In the latter investigation, Csapo and Takeda also recorded intraluminal pressure of the uterus by means of a small balloon attached to a catheter. The balloon technique records the net pressures within a hollow organ in contrast to our technique which records the tension exerted by the muscle in a localized area. However, both methods of recording are useful in determining and evaluating uterine activity.

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LETTERS TO THE EDITOR

PHYSICS

Effect of Gas Flow on the Properties of a Plasma Column

THIS communication describes some effects of gas flow on the properties of a weakly ionized d.c. discharge in argon at pressures of 2–30 mm mercury, with discharge currents of 15–200 m.amp, and gas flow velocities ranging from zero to $1\cdot5 \times 10^4$ cm/sec, corresponding to a Mach number approximately 0·5.

Fig. 1 is a schematic drawing of our apparatus. The discharge is maintained between a hot oxide-impregnated cathode (C) and anode (A). Both electrodes can emit thermionically and thus the roles of cathode and anode can be interchanged. Langmuir probes inserted in the plasma column are used in the investigation of its electrical properties. The static gas pressure is measured at a point midway between the two probes.

Fig. 2 shows the characteristics of the plasma column in the absence of gas flow for a fixed current of 100 m.amp. The electric field E is obtained from the potential difference between the two Langmuir probes of Fig. 1; the electron density N is determined from the ion saturation current drawn to one of the probes when it is biased negatively relative to the plasma potential; and the electron temperature T is obtained from measurements of microwave bremsstrahlung from the plasma.

In Fig. 3 the axial electric field is shown as a function of gas flow at a constant current and pressure. As the flow velocity is increased from zero, E decreases at first, then remains almost constant and rises sharply at a certain flow speed. This speed is independent of the direction of the flow and discharge current, and is inversely proportional to the gas pressure. The corresponding Reynolds number (based on a tube diameter equal to 0·6 cm) is approximately 1,800, a value that is consistent with the critical value for the onset of turbulence in neutral gas flow in a tube. The sharp upward break in E appears to be of the same origin as that observed by Cottingham and Buchsbaum[1] in their investigation of the effect of turbulence on the r.f. breakdown of gases. The reason for the initial decrease in E (indicating an increase in electrical conductivity) is not understood.

It is seen from Fig. 3 that the value of the electric field depends on the direction of flow, at least at low values of discharge current, in our case less than approximately 100 m.amp. This effect appears to be intimately related to the influence of the flow on the travelling striations in the discharge. As many investigators have observed in discharges formed in non-flowing gases, the most intense and commonly observed moving striations (henceforth referrred to as P-waves) are those travelling from anode to cathode. The reported wave-velocities of the striations lie between 10^3 and 10^5 cm/sec, with wave-lengths typically of the order of several tube diameters.

In our experiments the strongest influence of flow is obtained when its direction is from cathode to anode, that is, in opposition to the direction of the P-waves. In fact, at a definite flow speed that depends on the pressure but only weakly on current, the plasma column breaks up into stationary striations (striation spacings $\sim 1\cdot5$ cm). For example, at pressures of 2 and 12 mm mercury and discharge current of 100 m.amp, these flow speeds are $1\cdot9 \times 10^4$ and $0\cdot32 \times 10^4$ cm/sec, respectively. These values are in good agreement with the speeds of P-waves measured[2,3] in non-flowing gases under similar conditions of discharge current, gas pressure and tube radius. They are also in fair agreement with proposed[4] empirical formulæ for P-wave velocities. It appears, therefore, that the stationary striated state of the plasma column occurs when the flow velocity is equal and opposite to the P-wave velocity, at which point the P-wave is effectively brought to rest with respect to the discharge tube.

The audio noise spectrum of the discharge is also influenced by the flow. When the flow is directed toward the cathode, the noise on the Langmuir probes is essentially 'white' for frequencies between 0·5 and 30 kc/s and decreases monotonically with increasing frequency. When the flow direction is toward the anode, however, the signal is periodic with characteristic frequencies of 0·8–2 kc/s, the frequency depending on flow speed, pressure, and current. The amplitudes of the signals range from 1 to 10 V. These effects are observed for gas-flow speeds between approximately 1,000 and 7,000 cm/sec. In this range the average electron density also exhibits a marked increase (as much as a factor of two) and the average electron temperature a 5 15 per cent decrease.

The effects described are influenced only slightly by an axial magnetic field (range, 0–5,000 gauss). Through the

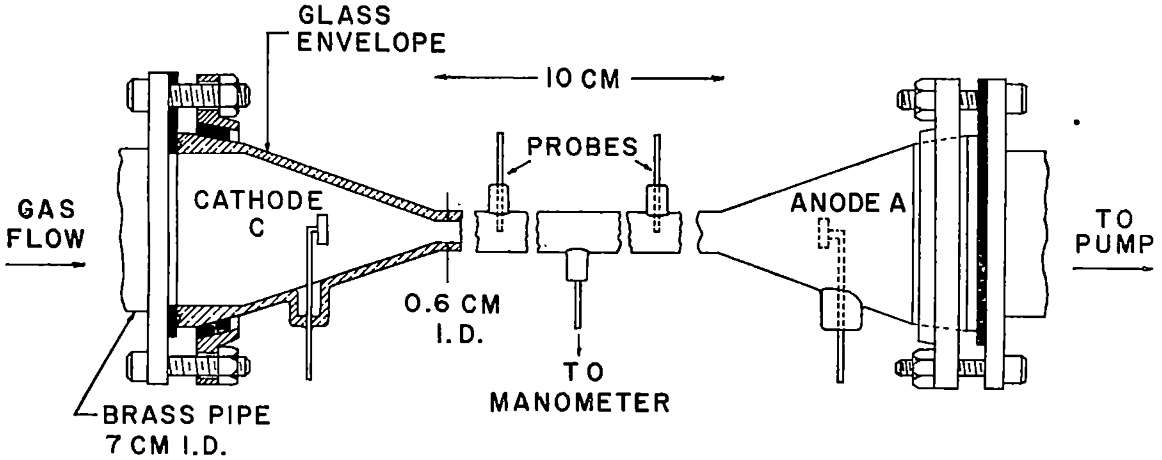

Fig. 1. Experimental arrangement. The role of cathode and anode can be interchanged so that the influence of the direction of the gas flow can be easily studied

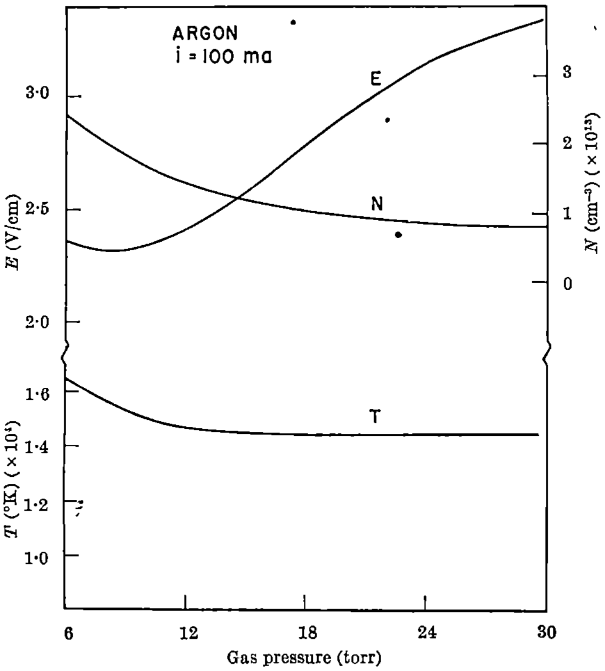

Fig. 2. Discharge characteristics when the flow velocity is zero

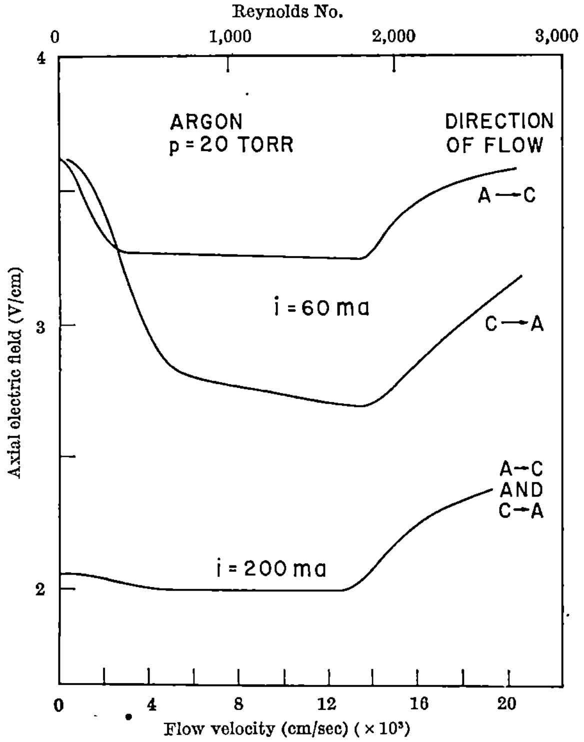

Fig. 3. Influence of gas flow on the axial electric field in the plasma column when the flow is directed from anode to cathode ($A \to C$) and from cathode to anode ($C \to A$)

combined influence of flow and magnetic field, however, a spiral instability can be produced in the plasma column, and at the appropriate flow velocity the plasma spiral can be brought to rest.

Thus, the experiments show that in addition to the expected influence of turbulence on the characteristics of a discharge, gas flow produces more subtle effects that appear to be related to the interaction between the flow and travelling striations and other instabilities in the discharge.

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Recognition of Simple Forms

G. E. HAM[1] has examined the hypothetical scanning mechanism of the brain with reference to its expenditure of energy in identifying forms of varying degrees of symmetry. His approach has important implications both for investigations in mental fatigue associated with perceptual tasks (for example, in industry or transport) and also, more fundamentally, for investigations of the seemingly more abstract categorizing processes involved in learning and thinking. Considerations of symmetry have often played a crucial part in the development of scientific and mathematical thought (as conspicuously demonstrated in the recent Ω^- discovery).

But in Ham's treatment there is an implicit model of quasi-mechanical brain-storage of 'sub-shapes' which would be difficult to harmonize with known neurological mechanisms. His general thermodynamic approach would not be invalidated by the adoption of a more plausible model. His storage of sub-shapes does not take account of the possibility of coding, though the coding theorem itself should clearly be related to his thermodynamic concept of form-recognition. This theorem as stated, for example, by Khinchin[2] defines the "compression coefficient μ" for all possible codes thus: "If the entropy of the given text is H, then the greatest lower bound of the compression coefficient μ for all possible codes is H/lg *m*, where *m* is the number of different symbols in the text".

Ham's treatment implicitly postulates an 'inner eye' which visually compares a perceptual image with a stored sub-image, scanning the store to find the best approximation. This must lead to an infinite regress of scanning-mechanisms, the same fallacy as that of the genetic 'homunculus'. It assumes that a visual form can be identified only by comparison with a second visual form. This assumption is neither warranted nor needed. There is certainly no introspective evidence to require it or to support it. Recognition has a quality of immediacy. There is no awareness of scanning or comparison. If we may take it that the 'stored sub-shapes' are completely unconscious they need not be regarded as 'shapes' at all in any visual sense, but rather as distributions of modifications in the brain tissue. As distributions they have spatial extent and pattern but need not have any geometrical isomorphism with visual shapes. They may be as different from a visual shape as a photographed spectrum is from an observed colour, but having an inverse ratio of complexity. Visible colour may be regarded as the brain's coding of the physical spectrum, and, by the economy of the code, contains considerably less information than the physical spectrum. Storage represents a second coding, back into a physical state but with still further economy of information. (We call the intermediate visual experience 'psychological', but it, too, has its physical distribution of neural excitation.)

Thus perceptual coding and memory coding represent two processes of economizing information. The second will be based on certain parameters of the first. The neural excitation involved in perception constitutes a 'pulse-spectrum'[3] and the storage is an abstract of this spectrum. In this interpretation Ham's implicit model of internal quasi-visual comparison is replaced by a process of resonance[4] between parameters of the pulse-spectrum responsible for perception and corresponding spectral features in the stored system of brain-modifications. Owing to the rapidity of resonance (requiring only the time for neural impulses to travel across the brain from the visual cortex to the region responsible for storage) the recognition has a conscious quality of immediacy.

Group-theory provides an invaluable instrument for classifying the parameters for such features as symmetry in the perceptual image, thus providing a measure of information. Tachistoscopic experiments on the immediacy of recognition of forms having different degrees of symmetry could test the coding theorem as applied to compression of perceptual parameters into the code of storage. The very short time-factor would be a measure of the energy involved in the resonance-process. The code, however, may prove more difficult to 'crack' than a simple geometrical analysis suggests. For perceptual recognition depends on more than the focal image. It invokes numerous marginal cues, all of which provide parameters important in producing specificity of resonance. There is always a certain urgency in perceptual recognition and this indicates a thermodynamic factor in the implicit strategy of coding. It will be governed by a law of least action.

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THE foregoing comments of Prof. Meredith are most pertinent with respect to suggestions about the particular modes of information storage utilized by the brain. Unfortunately, in trying to explain the thermodynamic approach to recognition of forms in as simple a way as possible I may have left the impression that the brain may actually store 'visual' sub-shapes, as such, for use in comparison with a perceptual image for recognition of the parent shape. Certainly, this implication was not intended. The possibilities for coding of information for use in memory and decision-making have been previously considered by me[1] and are pertinent to the present subject.

Prof. Meredith states that, "There is always a certain urgency in perceptual recognition and this indicates a thermodynamic factor in the implicit strategy of coding". Certainly, this statement is not contested and proceeds directly from my papers. High-speed memory devices have already demonstrated the practicality of immediacy in information storage and retrieval. His suggestion that tachistoscopic experiments could test these conclusions concerning symmetry and immediacy of form recognition is certainly worth while.

In my previous communication I observed that, in its most primitive operation, the brain recognizes simple forms with increasing difficulty. First, as the number of required rotational operations increases and, secondly, as the maximum angle of each rotation increases for superimposition of observed sub-shape and stored parent shape. Using conventional thermodynamic symbols (since we are here dealing with actual energy changes), as we proceed from a rotational requirement of zero for a sphere to two rotational requirements of $\leqslant 180°$ and $\leqslant 180°$ for the most simple of the more complex shapes, we have traversed entropy increases from Δs_1 (equivalent to the entropy change required for recognizing a sphere) to ΔS_s, the incremental entropy increase involved in the recognition of the simplest of the more complex shapes.

Such behaviour suggests a simple relationship between entropy and rotational requirements:

$$\Delta S = \Delta s_1 + \Delta s_2 + R\Delta s_a \qquad (1)$$

where ΔS is the entropy change in recognizing any simple form, Δs_1 has already been defined, Δs_2 is the entropy change associated with invoking a second rotational requirement, R is the sum of the individual maximum angular rotations required, measured in degrees, and Δs_a is the entropy change per degree of rotation. ΔS is, of course, a measure of the difficulty of form recognition.

The equation relating entropy and probability[2] is:

$$S = k \ln W \qquad (2)$$

where S is the entropy of a system; W, the thermodynamic probability, and k, the Boltzman constant. The thermodynamic probability is defined as the ratio of the probability of an actual state to one of complete order.

It seems likely that the relative time required for recognition of a simple object or shape is directly related to W, the thermodynamic probability. In other words, the more probable it is that a form will be readily recognized after a requisite number of 'trials' the shorter the recognition time. Accordingly, the following equation for perception of simple forms is offered:

$$S = k \ln \frac{T_2}{T_1} \qquad (3)$$

where T_2 is time to recognition of a given simple shape and T_1 is time to recognition of a sphere. It is noted that if a sphere is being observed, the equation reduces to $S = 0$. Of course, the equation is limited to entropy changes in excess of those for recognition of a sphere, hence Δs_1 must be added. In addition, the upper limit of equation (3) would be determined by the time required to recognize the most complex shape with which the brain is acquainted. The upper limit of equation (1) is $\Delta S_s + \Delta S_1$, the entropy change involved in the recognition of the simplest of the more complex shapes.

Experimental confirmation of the foregoing equations offers some problems. However, since:

$$\Delta S = \frac{\Delta H}{T}$$

where ΔH = the change in heat content in a given transformation, and T is the absolute temperature, measurement of the change in heat content of the appropriate brain region involved during recognition is suggested.

It seems likely that the brain scans stored 'sub-shapes' in sequence according to increasing complexity. Such a result would be consistent with the foregoing treatment, as well as with introspective conclusions about the nature of symmetry.

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Optical Correction in Micro-electrophoresis

AN optical correction in the microscopic measurement of the electrophoretic mobility of particles was proposed by Henry[1] and has been discussed more recently by Hunter and Alexander[2]. The correction concerns measurements made in the type of cell similar to that used by Mattson[3]. Such cells consist essentially of a thick-walled capillary, the outer face of which is ground flat to within a short distance of the inner bore. The foregoing authors discussed the apparent position of a point within the cell resulting from refraction through the circular cell wall.

However, these authors did not make it clear that the optical system presented by cells of this type show a complex form of astigmatism[4]. A point within the cell gives rise to two virtual images, both of which are in the form of caustic surfaces, having cuspidal edges. These edges, which are mutually perpendicular and non-intersecting, may be the only parts of the image which are visible in practice. When the aperture of the microscope is small, the length of the edges may be sufficiently short for a point image to be observed when the microscope is focused on any point between them. The results of astigmatism, therefore, may appear only as a lengthening of the depth of field of an observed image.

Let us consider an emitting point source at a depth d from the inner wall of the cell of radius a. Suppose the refractive indices of the immersion medium, the cell and the dispersion medium are n_1, n_2 and n_3 respectively. The depth $d_1^{\text{app.}}$ of one caustic edge is given by :

$$d_1^{\text{app.}} = \frac{n_1 d}{n_3 + \dfrac{d}{a}(n_2 - n_3)} \qquad (1)$$

while the position of the other is obtained by putting $a = \infty$, that is,

$$d_2^{\text{app.}} = \frac{n_1 d}{n_3} \qquad (2)$$

The depth of field resulting from this effect is therefore :

$$d_2^{\text{app.}} - d_1^{\text{app.}} = \frac{\dfrac{d}{a}(n_2 - n_3)}{n_3 + \dfrac{d}{a}(n_2 - n_3)} \qquad (3)$$

This field-depth depends not only on the radius of the cell but greatly increases with the depth within the cell of the observed point. For example, when $n_1 = n_3 = 1{\cdot}33$, $n_2 = 1{\cdot}51$, $\dfrac{d}{a} = 0{\cdot}293$ (near stationary layer) and $a = 1{\cdot}0$ mm, the length of the image along the microscope axis is 11μ. However, the image of the far wall of such a cell would have a length of 430μ.

The formulæ presented by Henry, and also Hunter and Alexander, relate to only one of the caustic edges, namely, that which is a function of the cell radius. The burden of Henry's argument was that it is not possible to select a given level in the cell by linear interpolation between the apparent positions of the near and far walls.

When the system's astigmatism is considered, it is evident that while one can focus with reasonable accuracy on the apparent position of a point at the near stationary layer, it is usually quite impracticable to focus on the far wall of the cell. There appears to be no valid circumstance, therefore, where the Henry correction could be applied.

If a microscope objective has a sufficiently large numerical aperture to enable the caustic elements to be seen as edges, it should be possible to choose the edge which conforms to equation (2). A given level would then be found by linear interpolation. Where this is not possible, it seems essential to measure the cell bore by an alternative method and apply a simple refractive index correction, where necessary.

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Moving Helium Film Measurements

Measurements on the profile of the moving helium film by Gribbon and Jackson[1] have given qualitative, but not quantitative, support to Kontorovich's theory[2] of the moving film. This theory was based on the postulates of the two-fluid model of liquid helium, and it predicted that the moving film would be thinner than the static film, while an alternative theory by Franchetti[3] predicted a thicker moving film. Tilley[4] has reconciled the two approaches using arguments based on the thermodynamics of a system in which relative motion occurs and has confirmed Kontorovich's result. Recently Franchetti[5] has proposed several reasons for the contradiction between the measurements and the simple theory.

Franchetti has suggested that disturbances, such as evaporation, were present which altered the expected magnitude of the change in thickness of the film as it flows out of a beaker. Now if it is accepted that there are two competing processes which could affect the film thickness, then a superfluid flow process could decrease, and a downward temperature gradient[3] could increase, the film thickness. The latter effect is negligible in a film at a typical temperature not too close to the λ-point, and superfluid flow alone need be considered. The film would be disturbed if evaporation took place above or at the measuring region on the outer wall of the beaker. Evaporation would occur if there were an unbalanced heat input to the film from either thermal vibrations in the helium vapour[6], absorption of thermal radiation from the cryostat surroundings[7], or absorption of incident radiation from the sodium lamp source[8] used in the optical method of film thickness measurement. There was no evidence for a heat input due to thermal vibration while the estimated heat input from thermal radiation was at least an order of magnitude less than the maximum heat input of about $100\ \mu\text{W cm}^{-2}$ from the absorbed sodium lamp radiation[9,10]. However, since the influence of evaporation applied equally to both the moving and the static film, measurements made to find the difference in thickness between these films would be independent of any thickness change due to evaporation. Since in practice this was the experimental technique used, the contradiction between experiment and theory is not resolved by taking account of any evaporation.

Secondly, Franchetti has pointed out that the theory only applied to a reversibly flowing film and is not strictly applicable to an irreversible film transferring out of a beaker. However, when the initial high transfer rate had decreased to the more characteristic rate, local equilibrium should have been reached in the measuring region and the theory should hold. The thickness measurements were made under these conditions, the film flowing at the steady characteristic rate of about 12×10^{-5} cm^3 cm^{-1} sec^{-1} and the measurements being taken well below the irreversible region close to the beaker rim[3,11].

Finally, Franchetti suggested that the film flow also would be irreversible if vorticity were present. Allen[12] and Kuper[13] considered that an array of vortex lines in the film might be responsible for the fast flow rates found under certain conditions ; but recent work[14] has shown that these enhanced flow rates seem more likely to be due to the presence of an unstable thick film draining slowly to the normal film thickness on the inner surface of the beaker. Nevertheless if a regular array of rolling vortex lines were present the thermodynamic free energy and hence the film thickness would be altered, but the contribution of this additional vorticity free energy would be negligible compared with the superfluid kinetic energy contribution. A further small free-energy contribution might arise due to the absorption of radiation energy by the beaker surface with subsequent heat flow into the film producing a turbulence energy density in the superfluid.

In conclusion it may be said that Franchetti's suggested reasons for the disagreement between the experiments and the theory do not resolve the problem, and indicate that further experimental and theoretical work is required on the moving helium film.

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Additional Observations of Probable Stimulated Emission of a Terbium Ion Chelate in a Vinylic Resin Matrix

The experimental results presented here were to have been included as an addition to my first communication[1]. However, the results were not submitted in time to be included in it. I should like at this time to present these additional results, as they provide a more definite indication of probable stimulated emission of terbium thenoyltrifluoroacetonate Tb(TTA) in a polymethyl-methacrylate (PMMA) matrix than the results presented in the previous communication[1].

Hereafter, in this communication I shall refer to the Tb(TTA) doped (PMMA) fibres, some of the properties of which were investigated in the experiments presented in my first communication, as belonging to the first group[1]. The fibres investigated, for which the results are presented in this communication, will be referred to as the second group.

These new experimental results are presented in the form of oscilloscope recordings of the fluorescence emission (≈5450 Å) versus time of the second group of fibres. These fibres are of higher optical quality than those of the first group. This second group of fibres is doped with a Tb(TTA) concentration of ≈0·08 per cent of the weight of the PMMA, and have diameters and straight lengths of approximately 0·75 mm and 65·0 mm respectively. The ends of these fibres were polished, but not silvered. The second group of fibres possesses a smaller degree of strain, bubbles, surface voids, and other surface imperfections than the fibres of the first group, because of improved fibre fabrication technique. Other physical parameters of the second group of fibres are essentially the same as those measured for the first group.

The experiments with the second group of fibres were performed with the fibre cooled to 77° K, and with the same experimental apparatus (except for a change in the pump light filter from a Corning 7–54 to a Corning 7–51 filter glass) as was used in the investigation of the first group of fibres[1]. The photomultiplier output load resistor also was changed to a smaller value in order to improve the time resolution of the observation.

The more pronounced spiking, and the suggested lifetime shortening of the fluorescence decay time observed in the second group of fibres as compared to the same characteristics in the first group, I attribute primarily to the improved optical quality of the second group of fibres (Figs. 1–5*b*), which resulted in lower fibre cavity losses, and therefore in a larger degree of spiking of the fluorescence emission. However, the increased time resolution of the photomultiplier is also responsible in part for the difference in the characteristics of the oscilloscope recording between the first and second group of fibres. In these experiments with the second group of fibres, as in the experiments with the first group, blank, undoped fibres of polymethyl-methacrylate were substituted for the Tb(TTA) doped fibres in the experimental apparatus in order to determine the degree of pump light leakage, and to detect if any spurious responses due to unusual pump light output, pump light filter fluorescences, or polymethyl-methacrylate fluorescences occurred. No spurious responses were noted in the oscilloscope recordings for the blank, undoped fibres (Figs. 2*b* and 5*a*), and only a small amount of pump light leakage was observed.

The results represented by Figs. 1–5*b* are for single, Tb(TTA) (≈0·08 per cent of wt. PMMA) doped fibre of the dimensions described here, under the condition of increasing pump power. Observation of several other Tb(TTA)-doped fibres of the second group indicates quite similar behaviour.

Due to the small amount of (≈5450 Å) output power obtainable from both groups of these short fibres of Tb(TTA)-doped polymethyl-methacrylate, it was not possible to observe, with the spectrograph available, whether or not line-narrowing due to stimulated emission occurred. Observation of coherence properties was also thwarted for the same reason. Fibres of longer length needed to provide increased output power necessary to

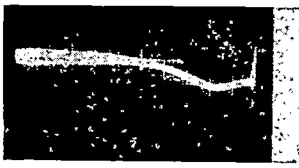

Fig. 1. Near threshold emission at 400 joules input

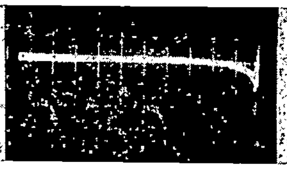

Fig. 2*a*. Blank sample (pump light leakage) at 600 joules input

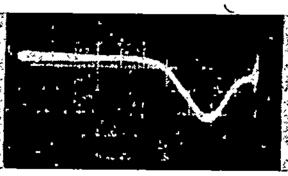

Fig. 2*b*. ≈5450 Å emission at 600 joules input

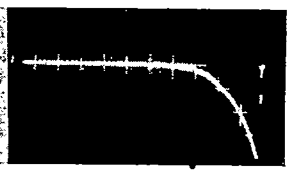

Fig. 2*c*. Pump light emission at 600 joules input

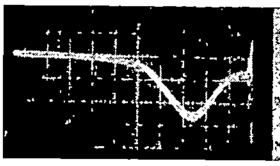

Fig. 3. ≈5450 Å emission at 800 joules input

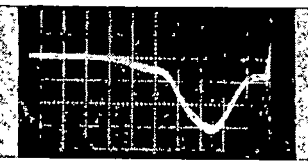

Fig. 4. ≈5450 Å emission at 1,000 joules input

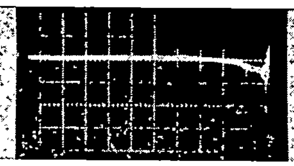

Fig. 5*a*. Blank sample (pump light leakage) at 1,200 joules input

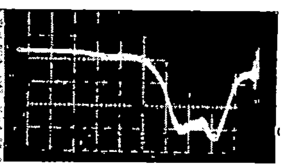

Fig. 5*b*. ≈5450 Å emission at 1,200 joules input

Figs. 1–5*b*. In the Figs. 1–5*b* increasing time is from right to left, and the sweep time 500·0 μsec for all figures. The photomultiplier voltage and anode load resistance are the same for Figs. 1–5*b*. The oscilloscope vertical amplifier sensitivity is the same for all figures, except Fig. 2*c*, which is 100 times less than the sensitivity for all the other figures. The pump light outputs for Figs. 1, 3, 4 are not shown, but are similar in shape to the flash lamp output of Fig. 2*c*. The sharp spikes at the beginning of the trace are due to trigger spike leakage make these observations were not available, because of difficulties encountered in obtaining the necessary optical quality in fibres of greater than approximately 75·0 mm in length.

The increased spiking amplitude to exponential spontaneous emission amplitude ratio, the suggested lifetime shortening, and the indicated spike sharpening under increased pumping as is shown in Figs. 4 and 5*b* are also indications of probable stimulated emission. However, since all proofs are not available at this time, the conclusion must remain that the phenomena observed in these experiments for both groups are only to a moderate degree of probability-stimulated emission.

In conclusion, I wish to note a divergent interpretation of the results presented in my previous communication[1], based on conclusions drawn from observations of the fluorescence emission of other rare earth, beta diketone chelates, in various solutions[2].

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ENGINEERING

Stress Concentration in Concrete

In carrying out some experimental work in the torsion of hollow concrete box beams, the authors were surprised to discover that the size of the fillet radius at the internal corners has little or no effect on the strength of the section. That is to say that in practice, although not in theory, there was no measurable stress concentration effect. This point has also been noted by Turner and Davies[1], who tested *T*-beams in torsion with a sharp re-entrant corner.

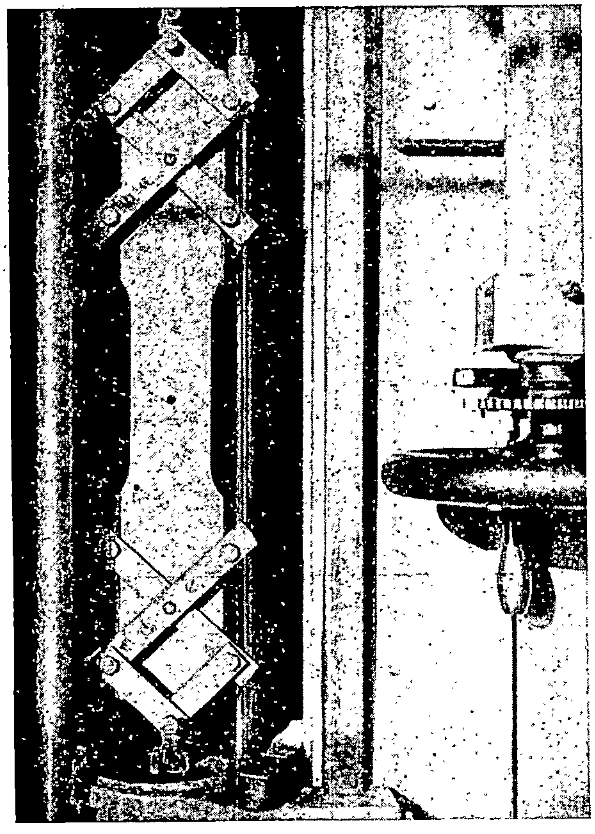

Fig. 1

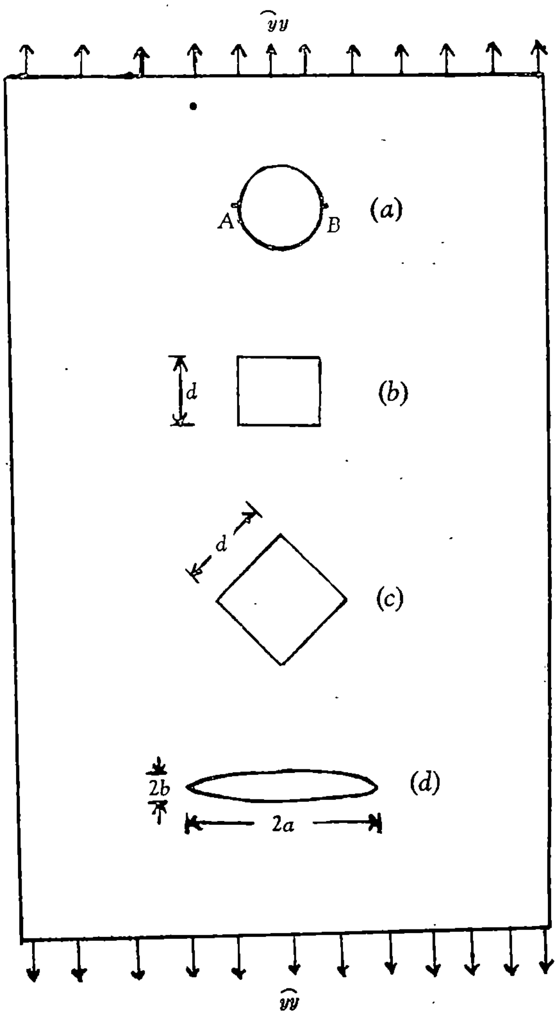

Fig. 2

In an attempt to find an explanation, tensile tests were carried out on plain thin concrete specimens, using a lazy tongs gripping device developed in the Engineering School and described previously elsewhere[2] (Fig. 1). The purpose was to ascertain the effects of holes of various shapes on the tensile strength. Tests were first of all carried out on sections with circular holes (Fig. 2*a*) for which the stress concentration factor is 3, that is, the stress at points *A* and *B* is 3 times the applied stress, that is, $3\widehat{yy}$. Allowance was made for the reduction in area due to the hole and no stress concentration effect was found, that is to say the failure stress was the same as that for a specimen without a hole. A square hole with a corner radius of 0·014*d* was then used (Fig. 2*b*), but again the effect was negligible in spite of the fact that the stress concentration factor as computed by the complex variable method was 5·9. In fact the section with the hole gave slightly higher values for the tensile stress, as one would expect from statistical theory. A diamond hole with a corner radius of 0·014*d* was then resorted to (Fig. 2*c*) and a 10 per cent decrease in strength was found, with a computed stress concentration factor of 14·8. This small decrease could be entirely due to experimental error since only three tensile specimens were used. As there still seemed to be no appreciable effect thin slits were tried; these were meant to simulate ellipses, for which the formula for the stress concentration factor is $\left[1 + \frac{2a}{b}\right]$, where *a* and *b* are the semi-major and semi-minor axes of the ellipse (Fig. 2*d*). The stress concentration factor varied from 40 to 80, but still no effect was noticed. However, the ellipses were not true ones and the stress concentration factor probably mainly depends on the radius of curvature at the end of the major axis, and the radii on the specimens tested could well have belonged to ellipses of smaller a/b ratio. The results for elliptical sections should therefore be treated with some caution, though clearly they confirm the general trend.

The fact that a brittle material like concrete is not reduced in strength by the presence of such high stress concentrations calls for some explanation. A possible hypothesis is to assume that there is a redistribution of stress due to plastic flow as in the case of ductile materials like mild steel. Although this is put forward by Turner and Davies[1] by way of explanation, it is not considered by the authors as very probable because of the peculiar structure of concrete.

Concrete is a macroscopically homogeneous and isotropic material which is full of air and water voids. The latter are usually very small, of spherical shape and closely packed together. The air voids, which are due to incomplete compaction, are of all shapes and sizes. Since concrete is a non-linear visco-elastic material the stress distribution for instantaneous loading follows the elastic theory. The stress concentration factor inherent in concrete due to all the voids, and also the stress concentrations which occur at the interface of the cement and the sand grains, must be of large magnitude. For glass, which seems to have considerably fewer voids than concrete, a stress concentration factor of 600 has been calculated. A. A. Griffith[3] showed that there was an enormous difference between the theoretical molecular strength of glass and the actual tensile strength of ordinary glass. By carefully making some glass rods as free as possible from voids and surface scratches he increased the tensile strength to about half the molecular strength. In glass it seems that the dominant factor is the state of the surface, while in concrete the internal structure is likely to have just as much, if not more, effect. The authors believe that concrete fails at very low loads because the inherent stress concentration factors are very high, much higher than the stress concentration factors artificially introduced in their experiments.

Alternatively, concrete could be regarded as being of honeycomb structure with a very small mesh size, so small that it could not be affected by any artificial stress raiser such as, for example, a hole or sharp change in angle.

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CRYSTALLOGRAPHY

Structure of Strontium Caprylate Hydrate

The strontium caprylate was prepared by metathesis from sodium caprylate and strontium chloride. The material crystallized from aqueous solution in the form of very small fragile plates. All the crystals examined were twins and all the X-ray photographs showed streaking of the spots, indicating some disorder in the long axis direction.

The crystals were triclinic with unit cell dimensions a = 4·7 Å, b = 8·0 Å, c = 25·8 Å, α = 94°, β = 91° and γ = 100°. The space group was assumed to be $P\bar{1}$. The position of the strontium atom was determined from the Patterson syntheses of the [100] and [010] projections. Both projections were refined by Fourier and Fourier-difference methods. The final value of the residual, R, was 0·16 for both projections. Because of the twinning the estimates of some of the intensities of some of the reflexions in both zones are uncertain and the structure cannot be regarded as very accurate.

There are some very unusual, non-crystallographic symmetries in the structure. The [100] projection exhibits symmetry about the lines perpendicular to the b axis through the points $\frac{3}{4}$,0, and $\frac{1}{4}$,0. The Fourier transform of the unit cell appears to be symmetrical, or very nearly so, but the reciprocal net is non-orthogonal and so the sampling is not symmetrical. Fig. 1 shows the final Fourier synthesis of the electron density projected along [100] and a diagram of the structure. The accidental symmetry is apparent in Fig. 1.

The [010] projection appears to have the symmetry *pmg*. In this projection not only is the Fourier transform of the unit cell contents symmetrical but also the reciprocal net is orthogonal and the sampling is orthogonal too. This projection showed no difference in intensities between reflexions related by the apparent symmetry in spite of the two halves of the twin being very different in size (as shown by the [100] projection). The projection was therefore refined assuming that the plane group was *pmg*. Fig. 2 shows the electron density projected along [010] and a diagram of the structure.

The structure consists of an ionic layer containing the strontium ions with eight oxygen atoms as nearest neighbours in the form of a distorted octahedron, six from the carboxylic acid groups and two from the water molecules. The hydrocarbon chains are normal to this layer. The

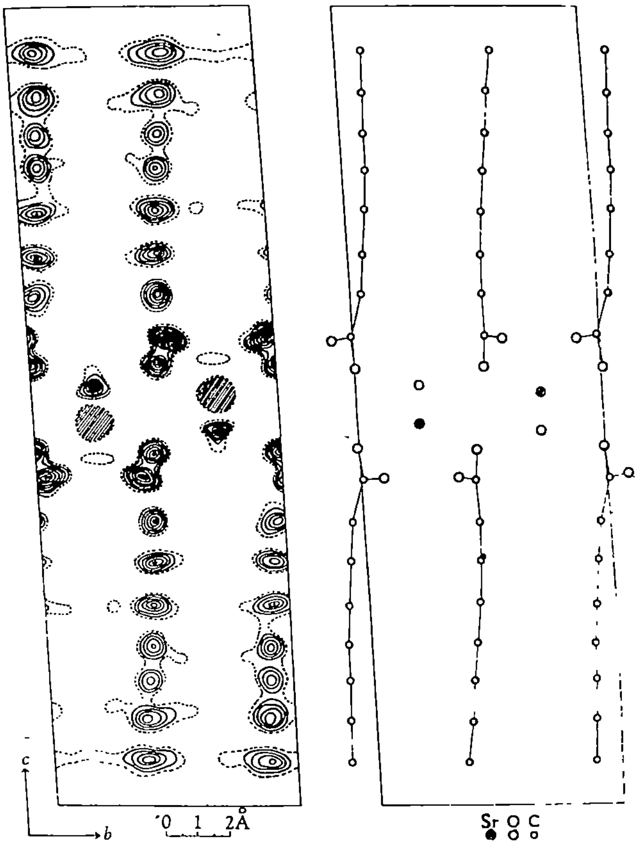

Fig. 1. Left, Fourier synthesis of the electron density projected along [100]. The contours are at intervals of one electron per Å^2. The zero contour is broken. The strontium ion has been shaded. Right, diagram of the structure projected along [100]

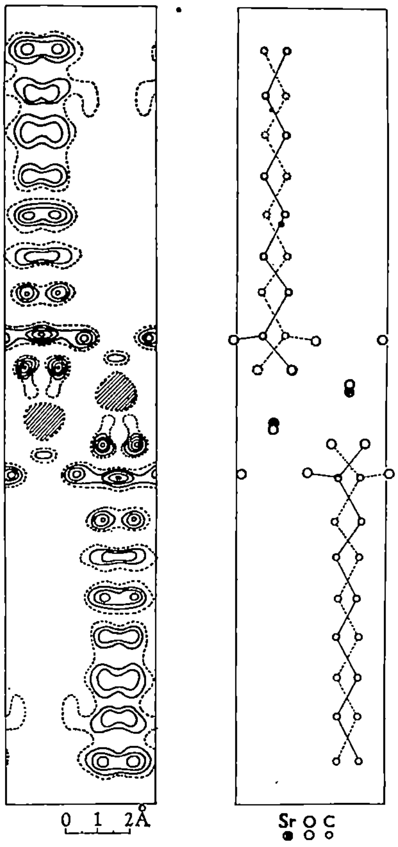

Fig. 2. Left, Fourier synthesis of the electron density projected along [010]. The contours are at intervals of two electrons per Å^2. The zero contour is broken. The strontium ion has been shaded. Right, diagram of the structure projected along [010]

mean Sr—O distance is 2·62 Å, which agrees with the ionic radii. The C—O bonds in the carboxylic acid groups are not of the same length. One is 1·31 ± 0·08 Å, the other is 1·16 ± 0·08 Å. These are of the same order of magnitude as those found by Vand, Morley and Lomer[1] in lauric acid. The mean C—C distance in the hydrocarbon chains is 1·48 ± 8 Å, which is not significantly shorter than the value of 1·51 Å found by Turner and Lingafelter[2] in tetradecamide. The average distance between alternative carbon atoms in the chain is 2·59 ± 0·3 Å, which is the same as that found by Vand, Lomer and Lang[3] in potassium caprate but is longer than the value of 2·53 found by Turner and Lingafelter[2] in tetradecamide. The structure appears to be quite different from that of strontium laurate proposed by Morley and Vand[4].

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Calcium Fluoride : Slip and Polygonization during Crystal Growth

Calcium fluoride occurs in Nature as the crystal form known as fluorite. It can, however, be grown artificially as single crystals, and when doped with suitable impurities it is widely used as a laser material[1,2]. For this purpose,

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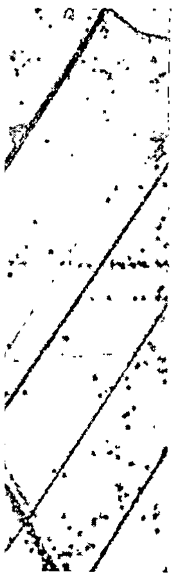

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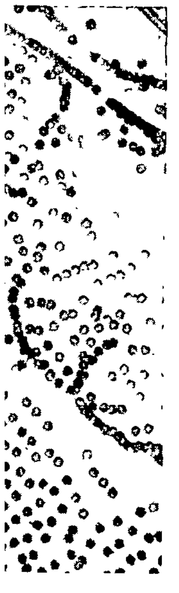

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during deformation experiments[5]. After annealing for a period of one week at a temperature of 950° C in an atmosphere of pure argon, the dislocations in the slip bands polygonized to give more low-angle boundaries (Fig. 2); the boundaries formed during growth remain unaltered by this annealing treatment.

Slip bands and low-angle boundary arrays are also observed on Stockbarger-grown crystals (Fig. 3) and the slip vector is again <110>. The number of dislocations and the amount of slip which occurs are, however, far less than in the Czochralski-grown crystals. Typical dislocation densities are respectively ~ 10^5 cm^{-2} and ~ 10^3 cm^{-2} for Czochralski and Stockbarger crystals. Furthermore, the latter crystals show the highly polygonized arrays of Fig. 4 without further annealing.

It is well established in semiconductor materials, such as germanium[7,8], and in laser materials, such as calcium tungstate[9], which have been grown by the Czochralski technique that dislocations are formed as a result of plastic flow which occurs in order to relieve thermal stresses in the cooling material. The evidence presented here suggests that calcium fluoride crystals, grown by the same technique, also fit into this general pattern of behaviour as the slip bands along <110> directions are very similar to those observed on {111} surfaces of germanium and the arrangement of low-angle boundaries is similar to that observed in calcium tungstate crystals[9]. This hypothesis also explains why the dislocation density in crystals grown by the Stockbarger method is much lower than in Czochralski-grown crystals, namely, because of the lower temperature gradients and correspondingly lower stresses experienced in this process. The slow cooling associated with the Stockbarger technique also permits dislocations to move into the observed highly polygonized configurations.

The slip bands observed in calcium fluoride crystals remain unmodified on crossing a low-angle boundary, thus suggesting that the slip occurred after the formation of the boundaries. This behaviour is in contrast to that of calcium tungstate crystals grown by the same method[9] where, although regions of high dislocation density are observed in addition to low-angle boundaries, there is no evidence of massive slip or of slip bands crossing the boundaries. The *a* [100] slip vector for dislocations in calcium tungstate[10] is, however, much larger than the slip vector in calcium fluoride and also involves the movement of the complex $(WO_4)^=$ ion rather than the movement of single ions. It is likely, therefore, that dislocations are more easily generated and moved in the fluorite lattice than in the calcium tungstate (Scheelite) lattice; this could explain the more extensive slip observed in calcium fluoride crystals pulled from the melt. However, as both these materials lose heat during growth by transmitting radiation from the solid/liquid interface along the crystal[11], it is possible that the calcium tungstate crystals will cool more slowly because this material absorbs most of the radiation at wave-lengths greater than 3·6μ whereas calcium fluoride is transparent to radiation at wave-lengths as high as 10μ. As the crucible and melt approximate to a black body and the peak of the black-body radiation curve at the melting-points of these materials is at approximately 2·5μ, it is likely that calcium fluoride absorbs very little radiation whereas approximately 15 per cent could be absorbed by calcium tungstate. The consequent lower cooling rate in the latter case may permit dislocations to start polygonizing, thus masking any slip that has occurred.

The dislocation patterns observed in artificially grown calcium fluoride crystals can thus be explained in terms of the thermal stresses present in the growth process. The differences in the dislocation patterns of calcium fluoride and calcium tungstate can be interpreted in terms of the different crystal structures and transmission properties of these materials.

We thank Dr. W. Bardsley for his advice.

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CHEMISTRY

Effect of Micro-crystal Formation on the Electron Spin Resonance Spectra of αα-Diphenyl-β-picrylhydrazyl in Various Frozen Solvents

Despite the very numerous investigations of the electron spin resonance spectra of DPPH (αα-diphenyl-β-picrylhydrazyl) a number of perplexing problems remain. Those relating to its behaviour in various solvents are important because DPPH is used very commonly as a standard for concentration measurements in electron spin resonance and as a radical 'scavenger' in the examination of radiation chemistry.

During an investigation into the suitability of deaerated frozen solutions of DPPH in various solvents as concentration standards at low temperatures[1], it was found that there were wide differences in line width, hyperfine splittings and saturation behaviour, not all of which could be explained. In particular, frozen solutions of DPPH in benzene (10^{-3}–10^{-4} M) gave a strongly exchange-narrowed line about 2 gauss wide, while the very similar solvents, toluene and xylene, gave broad, poorly resolved five-line spectra like broadened versions of the solution spectra.

It was also found that the frozen benzene solutions showed little microwave power saturation, whereas the others all saturated heavily at 77° K. Further investigations showed that the electron spin resonance spectra could be divided into three groups: (*a*) strongly exchange-narrowed (peak to peak line width $\Delta H_{pp} \sim$ 2–4 gauss), for example, benzene, naphthalene, quinoline (Fig. 1*a*); (*b*) broader, unresolved lines ($\Delta H_{pp} \sim$ 10–20 gauss), for example, acetone, diethyl ether, toluene, carbon disulphide, thiopen (Fig. 1*b*); (*c*) poorly resolved five-line spectra with hyperfine splittings greater than those observed in solution, for example, pyridine, tetrahydrofuran, xylene (Fig. 1*c*).

The spectra of group (*c*) are similar to those observed by Schneider[2] for DPPH in solidified 'Perspex' or polystyrene, and by Lord and Blinder[3] for DPPH and carbazyl in a plastic cement. These latter authors show clearly that the increase in hyperfine splittings and line-width is the result of including anisotropic contributions which are averaged out in solution. It has been suggested[4] that the exchange narrowing of the spectra of group (*a*) results from 'super-exchange' throughout the whole lattice or from crystallization of the solute in the form of micro-crystals.

In order to test this suggestion we have examined thin films of frozen solutions on the cold stage of a high-powered polarizing microscope. A concentration of about 10^{-2} M DPPH was needed for proper observation, and at this concentration micro-crystals of DPPH could clearly be seen in frozen benzene by means of their characteristic

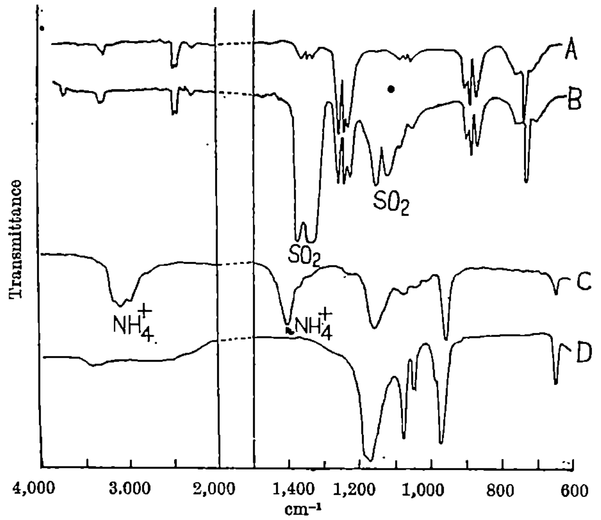

Fig. 1. Infra-red absorption spectra of: (*A*) HNSO; (*B*) gaseous product and (*C*) solid product obtained by reacting ammonia with excess sulphur dioxide; (*D*) $K_2S_2O_5$

under completely anhydrous conditions[6] occurs rapidly with evolution of heat. The infra-red spectrum of the gaseous product was shown to be thionylimide, HNSO[7], and the spectrum of the simultaneously formed solid product showed the presence of NH_4^+, HSO_3^- and $S_2O_5^{2-}$ immediately after the reaction, but the latter gradually changed into $S_2O_5^{2-}$ with the lapse of several hours. The infra-red spectra of this solid indicated no existence of SO_3^{2-}, $S_3O_6^{2-}$, $S_4O_6^{2-}$, etc. (Fig. 1).

$$3NH_3 + 3SO_2 \longrightarrow HNSO + (NH_4)_2S_2O_5$$

The reaction between methylamine and sulphur dioxide (as would be expected, occurrence of a similar reaction in view of the proceeding one) produced methylthionylamine, CH_3NSO, and pyrosulphite, $(CH_3NH_3)_2S_2O_5$. Likewise, the reaction between ethylamine and sulphur dioxide produced ethylthionylamine, C_2H_5NSO, and $(C_2H_5NH_3)_2S_2O_5$.

The reaction between dimethylamine and sulphur dioxide produced almost quantitatively a yellowish oily liquid, which was purified by vacuum distillation without decomposition; m.p. 17° C. The formula was found to be a molecular complex, $(CH_3)_2NH{\cdot}SO_2$, by infra-red and Raman spectra[8] and also by mass spectrum. The strong hydrogen bonding in this molecule did not show a remarkable effect on the absorption band by increasing dilution of non-polar solvents. An extensive examination has been also made on far infra-red and Raman spectra of molecular complexes, $(CH_3)_2NH{\cdot}SO_2$ and $(CH_3)_3N{\cdot}SO_2$. New bands, 205 cm⁻¹ for the former and 186 cm⁻¹ for the latter, could be found as intense Raman line and weak infra-red absorption band also. It may be considered that the bands indicate the bond of $N \cdots S$ caused by charge transfer force.

We thank Prof. R. Hara for his advice.

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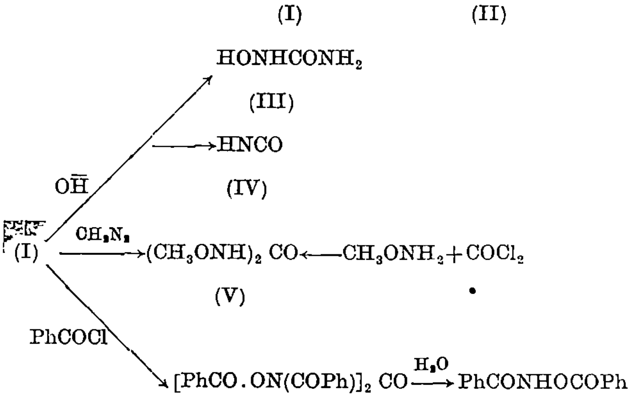
BIOCHEMISTRY

Dihydroxyurea

SEVERAL derivatives of hydroxylamine[1], including hydroxyurethane, *N*-methylhydroxylamine and hydroxyaminoacetic acid, cause chromosome abnormalities in mouse embryo and Chinese hamster cells similar to those induced by hydroxylamine[2]. Similar chromosomal abnormalities are also induced in anaphase and metaphase chromosomes of the root tips of *Vicia faba* by hydroxylamine, methyl-, ethyl-, *n*-propyl-, and *n*-butyl-*N*-hydroxycarbamates (S. H. Revell and K. S. Peggie, personal communication). Urethane appears to produce many radiomimetic effects through the metabolite *N*-hydroxyurethane to which it is converted by animals[3]. Although urea and urethane are inactive in these systems, hydroxyurea[1] and *N*-hydroxyurethane[3] are active; hydroxyurea is also an anti-tumour agent which is active against the *L*1210 leukæmia in mice[4]. It has been proposed[1,3,5,6] that these hydroxylamino derivatives may exercise their effects by a common chemical mechanism involving the production of nitroxyl or hydroxylamine *in vivo*.

In view of the effects on chromosomes and anti-tumour properties of hydroxyurea, dihydroxyurea has been prepared with the view of its possible use as a cancer chemotherapeutic agent. So far as we are aware, this compound has not been described in the literature. Hydroxylaminolysis of hydroxyurethane in ethanolic potassium hydroxide yielded hydroxyurea as the main product, which was also obtained from the reaction of phosgene (in toluene) with hydroxylamine in aqueous sodium carbonate. When phosgene (in dioxan) was allowed to react with an aqueous-dioxan suspension of hydroxylamine and potassium acetate at 0°, two main products were obtained: (I) colourless prisms which decomposed with partial melting at 102°–106° and (II) colourless plates which exploded at 95° or at room temperature after several hours. Both compounds reduced ammoniacal silver nitrate, gave red and unstable mauve to purple complexes with sodium pentacyanoammine ferroate and ferric chloride, respectively, and red condensation products with *p*-dimethylaminocinnamaldehyde. Compound (II) was probably the metastable hydroximic acid form (II) of dihydroxyurea (I).

$$\underset{}{COCl_2 + NH_2OH} \rightarrow \underset{(I)}{CO(NHOH)_2} + \underset{(II)}{HONH.C(OH) = NOH}$$



The structure (I) of the compound is indicated by the following: (*a*) The compound had the correct elemental (C, H and N) analysis. (*b*) The infra-red spectrum showed a strong carbonyl absorption near 6μ, and in the 3μ region a very broad, intense and featureless absorption centred around 3,200 cm⁻¹. The 3μ absorption appears to be typical of compounds containing the $-NHOH$ group: the spectrum of the solid $CH_3OCONHOH$ showed a similar absorption centred at 3,270 cm⁻¹, while liquids of the general type $R\mathrm{OCONHOH}$, where $R = \mathrm{C_2H_5}$, $\mathrm{CH_3(CH_2)_2}$, and $\mathrm{CH_3(CH_2)_3}$, gave similar bands centred near 3,300 cm^{-1}. Hydroxyurea showed a similar absorption, but with the bands due to the asymmetric and symmetric modes of the $\mathrm{NH_2}$ group superimposed at 3,422 cm^{-1} and 3,315 cm^{-1}, respectively. (*c*) The compound was unstable in alkaline media yielding hydroxyurea (III) and cyanic acid (IV) (detected and estimated via its blue Co^{2+} complex) as two of the decomposition products. (*d*) On treatment with diazomethane in methanol–ether, the compound yielded *N,N′*-dimethoxyurea (V) as a major reaction product which was identical with the authentic substance synthesized by reaction of phosgene with *O*-methylhydroxylamine. (*e*) With benzoyl chloride in pyridine at 0°, it yielded *N,N′,O,O′*-tetrabenzoyldihydroxyurea (VI), which, in boiling aqueous ethanol, yielded *O*-benzoyl benzohydroxamic acid (VII).

Preliminary experiments indicate that dihydroxyurea is more than five times as active as hydroxyurea in the induction of chromosomal aberrations in the root tips of *Vicia faba* (S. H. Revell, personal communication).

We thank Dr. S. H. Revell and Miss K. S. Peggie for the experiments with *Vicia faba*, and Dr. R. Lumley Jones for the infra-red determinations. This work was supported by grants to this Institute from the Medical Research Council and the British Empire Cancer Campaign for Research, and by the Public Health Service Research grant *CA*-03188-08 from the National Cancer Institute, U.S. Public Health Service.

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Concentrating Protein Solutions

The earliest method of concentrating solutions of labile proteins, drying from the frozen state *in vacuo*, has several disadvantages, some of a technical and others of a fundamental nature such as increase in salt concentration and the proneness of complexes of proteins with lipids and some other substances to denaturation on freezing and drying. The alternatives are removal of water by dialysis of the protein solution against concentrated solutions of dextran or polyethylene glycol[1] of molecular weights sufficiently high not to penetrate the walls of the 'Cellophane' dialysis sac, or absorption of the water by shaking the protein solution with dispersed, cross-linked dextran ('Sephadex')[2]. These methods avoid freezing and drying and allow the salt concentration to be maintained at any desired value. However, dialysis is time-consuming to set up if many small samples are involved, and considerable losses can occur on the walls of the sac when the removal of 0·1–0·5 ml. of viscous protein solution is attempted. Removal of the water with 'Sephadex' is simple to carry out, but there is sometimes considerable loss of trace proteins by adsorption on to the large surface presented by the gel particles. This led me to consider the possibility of using rods of dried hydrophilic gel to remove the water from protein solutions while maintaining constant ionic strength.

The rods used in the experiments were prepared by polymerizing an aqueous solution containing acrylamide (15·2 g/100 ml.) and *N,N*-methylenebisacrylamide (0·8 g/100 ml.) in silicone-coated 0·5 cm × 20 cm glass tubes. In the course of diffusion experiments it has been established that substances with molecular weights greater than 10,000 do not significantly penetrate acrylamide gels of this degree of cross-linking and concentration (Curtain, unpublished results). Polymerization was initiated by adding 0·4 g of ammonium persulphate and 0·2 ml. of dimethyl amino-propionitrile to each 100 ml. of monomer solution. For convenience in handling the activated mixture, polymerization could be retarded for 15 min by adding 100 mg of potassium ferricyanide to each 100 ml. of solution. The silicone-coated glass tubes were prepared by dipping air-dried, acid-washed glass tubes in undiluted General Electric 'Drifilm' chlorosilane for 30 sec. The tubes were then washed for 10 min in running distilled water and dried in an oven at 150° C for 2 h. The life of the coating appears to be indefinite.

The rods of polymerized gel were removed from the tubes by pushing with a close-fitting glass rod and washed with six twelve-hourly changes of excess distilled water. They were then cut into convenient lengths (3–6 cm) and exposed in an open dish to the draught from the cooling unit of a 4° C cold room. After 96 h the rods had dried to 20 per cent of their original diameter and 90 per cent of their original length. It was found that more rapid drying at higher temperatures produced cracks in the rods. The rods may be kept indefinitely in a desiccator over phosphorus pentoxide. On equilibration with water for 18 h the rods attained their original volume, indicating a water regain of approximately 5·2 g/g.

For drying, protein solutions were placed in a test-tube of a diameter 3–4 mm greater than that of the swollen diameter of the rod (0·75 cm). A length of dry rod was then dropped into the protein solution. The amount of rod required to effect a given reduction in volume may be calculated readily from the water regain of the gel. In practice it was found that a ten-fold concentration could be obtained easily with volumes as low as 1 ml. For smaller volumes thinner rods (0·4 cm) were tried and in this case it was found practicable to concentrate volumes of 0·4 ml. down to 0·04 ml.

Possible loss of protein or denaturation was measured in two ways. 2 ml. of serum was concentrated to 0·2 ml. and then diluted to 2 ml. with 0·9 per cent (w/v) sodium chloride solution. Samples of the original serum and samples of the concentrated and diluted serum were examined by moving boundary electrophoresis and by micro-immunoelectrophoresis. The moving boundary electrophoresis was carried out in a Tiselius apparatus fitted with the phase-plate gradient interferometric optical system of Moore and Opperman[3] using pH 8·6, $I = 0{\cdot}1$ sodium veronal buffer. Duplicate runs of each sample were carried out and the ordinates of the gradient curves obtained by measurement of the interference fringes[4] directly compared for each run. Recovery of all components averaged 100 per cent within the limits of precision of the techniques used (0·7 per cent). The micro-immunoelectrophoretic patterns of the two samples were enantiographic, suggesting that no alteration had occurred in any of the components resolved by this technique.

I thank Dr. A. Baumgarten for carrying out the immunoelectrophoresis run.

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Purine- and Pyrimidine-specific Antibodies: Precipitation with Denatured Deoxyribonucleic Acid

ANTIBODIES capable of reacting with thermally denatured DNA can be elicited by immunization with antigens prepared by coupling purine and pyrimidine derivatives to proteins[1–3]. In this way antibodies specific for the purinoyl moiety[1], for 5-acetyluracil[2] and for the five major bases[3] of DNA and RNA have recently been produced. Their reaction with thermally denatured DNA was demonstrated by means of a quantitative complement-fixation test, but initial attempts to obtain precipitation reactions with them were unsuccessful. We now offer evidence that precipitation reactions can occur between purine- and pyrimidine-specific antibodies and denatured DNA.

The methods of obtaining the antisera have been described[1–3]. Calf thymus DNA was obtained from Nutritional Biochemicals, Inc. A solution containing 1 mg/ml. in 0·015 M sodium chloride, 0·0015 M sodium citrate was denatured by heating at 100° C for 10 min in the presence of 1 per cent formaldehyde, followed by rapid chilling in an ice bath. Chick embryo DNA was prepared from 10-day-old embryos by a modification of Marmur's procedure[4]. Quantitative precipitin analyses were performed as described by Kabat and Mayer[5] using the Folin–Ciocalteu method[6] for analysis of specific precipitates. Rabbit γ-globulin purified by chromatography on DEAE-cellulose and assayed by Kjeldahl analysis was used as a standard. DNA in the precipitate was determined by ultra-violet spectrophotometry after extraction with 10 per cent perchloric acid at 70° for 20 min, a procedure used by Deicher *et al.*[7] to extract DNA from precipitates formed with the sera of some patients with lupus erythematosus.

The reactions between calf thymus DNA, denatured in the presence of formaldehyde, and antisera with four different specificities (anti-adenosine, anti-ribosylthymine, anti-purinoyl, anti-5-acetyluracil) are illustrated in Fig. 1. Native DNA and DNA denatured by heat in the absence of formaldehyde did not precipitate with any of the antisera. Sera obtained from the rabbits before they were immunized did not react with DNA denatured in the presence of formaldehyde. Unlike the antisera shown in Fig. 1, antibodies specific for cytidine, guanosine and uridine failed to give a precipitate with this DNA preparation.

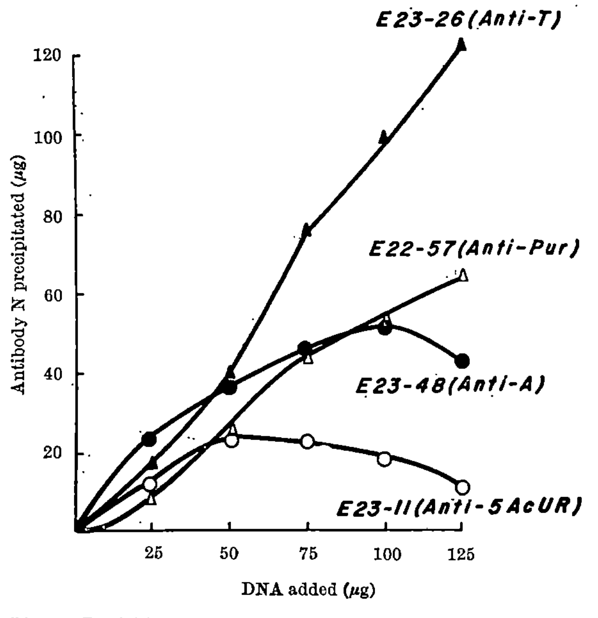

Fig. 1. Precipitin reaction between rabbit antisera (0·25 ml.) and calf thymus DNA thermally denatured in the presence of 1 per cent formaldehyde

The failure of anti-cytidine, anti-guanosine, and anti-uridine sera to yield a precipitate with formaldehyde-denatured DNA does not result from a low titre of specific antibodies, because precipitation with their homologous conjugated proteins showed that anti-cytidine, anti-guanosine, and anti-uridine sera contained, respectively, 329 µg, 270 µg and 254 µg of antibody N/ml. The anti-adenosine serum (E–23–48), which precipitated with formalinized calf thymus DNA (Fig. 1), contained only 149 µg antibody N/ml. Since the rabbits produced almost no antibody that was precipitable by the unconjugated serum albumins, these values are comparable with respect to concentration of specific antibody.

It is interesting in the case of the anti-adenosine and anti-ribosylthymine sera that essentially all the antibody which was precipitable by the conjugated protein also precipitated with DNA. On the other hand, only 22 per cent of the anti-5-acetyluracil antibody that reacted with the homologous conjugate was precipitable by DNA. Although the total antibody content of the anti-purinoyl serum has not yet been determined, it seems plausible to attribute the differences between the anti-5-acetyluracil, anti-ribosylthymine and anti-adenosine sera to the fact that the latter two are specific for bases present in the DNA molecule.

Only about 30 per cent of the formalinized DNA added to the antisera could be recovered from the specific precipitates, even in the zone of antibody excess. It would be of interest to determine whether the overall base composition of the DNA precipitated by each of the antibodies is the same as or different from that of the original DNA added; the latter finding would suggest that the antisera had effected a fractionation of the DNA. Our results are in contrast to those obtained by Deicher *et al.*[7], who, using the same procedure, found that all the DNA added to an excess of serum from a patient with lupus erythematosus could be extracted from the precipitate. Similarly, Murukami *et al.*[8], using antibody to phage *T* 4 DNA, found all the added DNA in the precipitates formed in the region of antibody excess. Although the antigen responsible for the antibody in the sera of lupus erythematosus patients is not known, it may be postulated to be DNA. Our results are explicable, then, if the reaction of DNA with lupus erythematosus serum and with anti-phage DNA serum is considered to be a reaction of an antigen with its homologous antibody, while, on the other hand, the reaction of the sera examined here is a cross-reaction, not necessarily leading to complete precipitation. Other factors, such as possible degradative effects of formaldehyde and heat on DNA, should also be considered. It may be noted, however, that Murukami *et al.*[8] used thermally denatured DNA in the investigations previously cited, and that Stollar and Grossman[9] have reported that formaldehyde increased the serological activity of thermally denatured *T* 4 DNA as measured by complement-fixation, presumably by preventing renaturation and/or intramolecular hydrogen bonding.

The observation that anti-guanosine and anti-cytidine antisera do not precipitate with formaldehyde-denatured calf thymus DNA may be ascribed either to the relatively low guanine-cytosine content (about 42 per cent) of calf thymus DNA[10] or to the fact that formaldehyde reacts most rapidly with the cytidylic and guanylic residues of DNA[11]. It may also be that the formaldehyde has caused a chemical change in the DNA which then enables it to precipitate with the other antisera. It would appear, however, that a reaction with formaldehyde is not required for precipitation since chick embryo DNA, which has been thermally denatured in the absence of formaldehyde, precipitates with the same antisera that react with formaldehyde-denatured calf thymus DNA. The failure of calf thymus DNA denatured in the absence of formaldehyde to precipitate with antisera may be due to some renaturation. Thermal transition curves[12] of such DNA show a hyperchromicity of 19·5 per cent, whereas none is seen with DNA denatured in the presence of formaldehyde. Thermally denatured chick embryo DNA, on re-heating, resembles formaldehyde-denatured calf thymus DNA and shows only a slight hyperchromic effect. These observations, together with the fact that chick embryo DNA also has a relatively low guanine-cytosine content (41 per cent)[10], support the suggestion made above, that the composition of DNA influences its reaction with antibody. Therefore, DNA's of varying base composition are being prepared in order to examine their precipitability with the antisera. If a correlation between the base specificity of an antibody and its reaction with DNA molecules of varying G–C content were found, such studies could provide a basis for using immunochemical methods in more subtle investigations of the structure of DNA.

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A $C_{15}H_{22}O_4$ Compound produced by the Fungus *Aspergillus fischeri* var. *glaber*

IN late August 1962, Dr. C. W. Hesseltine of our laboratory collected a dry soil sample from under a Douglas fir in House Rock Camp Grounds near Corvallis, Oregon. From this sample was isolated a strain of *Aspergillus fischeri* var. *glaber* Fennell and Raper, now maintained in the A.R.S. Culture Collection as *NRRL* 3088.

During our culture work with this strain, we observed abundant crystal formation after about 6 days when the fungus was grown on malt extract agar. In still culture, as much as 4·87 g of crystalline product per litre of culture liquor was produced in 45 days at 28° C. The medium had the following composition: malt extract, 20 g; peptone, 1 g; *d*-glucose, 20 g; and distilled water, 1,000 ml.

The new fermentation product was isolated by filtration of the culture liquor and by acetone extraction of the dried mixture of mycelium and crystals. The compound separated from the acetone–hexane as colourless needles which melted at 49°–50° C. On cooling, molten samples crystallized and then melted at 54°–55° C. The compound has $[\alpha]_D^{20} - 40°$. Elementary analyses and a Signer molecular weight determination were in agreement with a molecular formula of $C_{15}H_{22}O_4$. Preliminary chemical and spectroscopic investigations indicate that the substance is a dilactone.

When compared with the type strain of *A. fischeri* var. *glaber* (described by Fennell and Raper[1] and deposited in our collection as *NRRL* 2163), strain *NRRL* 3088 differed only in being somewhat darker in colony colour, in lacking a faint pinkish reverse in the medium, and in having slightly larger cleistothecia. One sub-culture of the type strain also produced a few scattered crystals in malt extract agar after repeated transfer. Several other isolates of the variety produced no crystals.

Note added in proof. Since submission of this manuscript it has come to our attention that Brookes, Tidd and Turner[2] recently reported that *Aspergillus avenaceus* produces a $C_{15}H_{22}O_4$ product apparently identical with our lactone, judging from its double melting point, rotation and nuclear magnetic resonance spectrum.

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Pyridoxine Deficiency in the Rat produced by D-Penicillamine

D-PENICILLAMINE is used in the treatment of Wilson's disease[1] and in cases of lead poisoning[2]. Recently, Crawhall *et al.*[3] reported that the administration of D-penicillamine to two cystinuric patients resulted in a marked fall in the excretion of cystine in urine. This was believed to be due to disulphide exchange resulting in the formation of the mixed disulphide of D-penicillamine and cysteine. It has therefore been suggested that D-penicillamine might prove useful in the management of this condition.

In a series of investigations, Du Vigneaud and his collaborators[4–6] showed that the inclusion of L-penicillamine in the diet of white rats caused a depletion of vitamin B_6, resulting in inhibition of growth, reduced transaminase activity and abnormal excretion of xanthurenic acid following the administration of a test dose of tryptophan. They also showed that these effects could be prevented by inclusion of large amounts of pyridoxine in the diet. It was suggested that vitamin B_6 deficiency was the result of a chemical combination between L-penicillamine and pyridoxal phosphate to form a thiazolidine structure.

Similar results on transaminases were observed by Heddle *et al.*[7], who, however, investigated the effect of DL-penicillamine on white rats.

Ueda *et al.*[8] demonstrated that, like the L-isomer, D-penicillamine reacts non-enzymatically with pyridoxal phosphate to form a thiazolidine compound. It was therefore considered of interest to examine whether B_6 deficiency resulted in the rat following the administration of D-penicillamine.

Six male albino rats (300–340 g wt.) were maintained on Oxoid rat diet (Oxoid, Ltd.). Before starting the administration of D-penicillamine the rats were placed in metabolic cages and 24 h urine was collected before and after subcutaneous injections of a test dose (40 mg/rat) of L-tryptophan[9]. A solution of 50 mg D-penicillamine hydrochloride (Dista Products, Ltd.) was given, by stomach tube, twice daily to each of three rats (Group *A*). The other three rats received the same dose by intramuscular injection (Group *B*). Since solutions of penicillamine were strongly acidic, adequate amounts of sodium bicarbonate were always added to bring the *p*H to neutrality. After a period of four weeks 24 h urine was collected again before and after injections of 40 mg L-tryptophan.

Aliquots of all urine collections containing 3·5 mg of creatinine were chilled in ice, acidified (*p*H 2) and extracted three times with equal volumes of ethyl acetate. The combined ethyl acetate extracts were evaporated to dryness under reduced pressure and the residue in each case was dissolved in 1 ml. of absolute ethanol. Fifty µl. of each sample was then chromatographed on Whatman No. 1 paper using butanol/acetic acid/water (4 : 1 : 5) as the solvent[10]. The dried papers were sprayed with diazotized sulphanilic acid and the results are shown in Fig. 1.

The intense spots (R_F 0·58) level with the xanthurenic acid marker consisted predominantly of xanthurenic acid, but smaller amounts of kynurenic acid were also present. This was established by two-dimensional chromatography of the ethyl acetate extracts using 20 per cent potassium chloride as the second solvent[11], when the mixture in each case was resolved into two substances both having a characteristic blue fluorescence. Their R_F values were 0·25 and 0·42 respectively[11], and both reacted with Pauly's reagent to produce a red colour.

In order to estimate xanthurenic acid the appropriate spots, located on another chromatogram in ultra-violet light, were cut out and eluted with 5 ml. of *tris*-buffer (*p*H 7·8). An aliquot was then used for the colour reaction with ferric ammonium sulphate as described by Wachstein and Gudaitis[12]. Values are shown in Table 1.

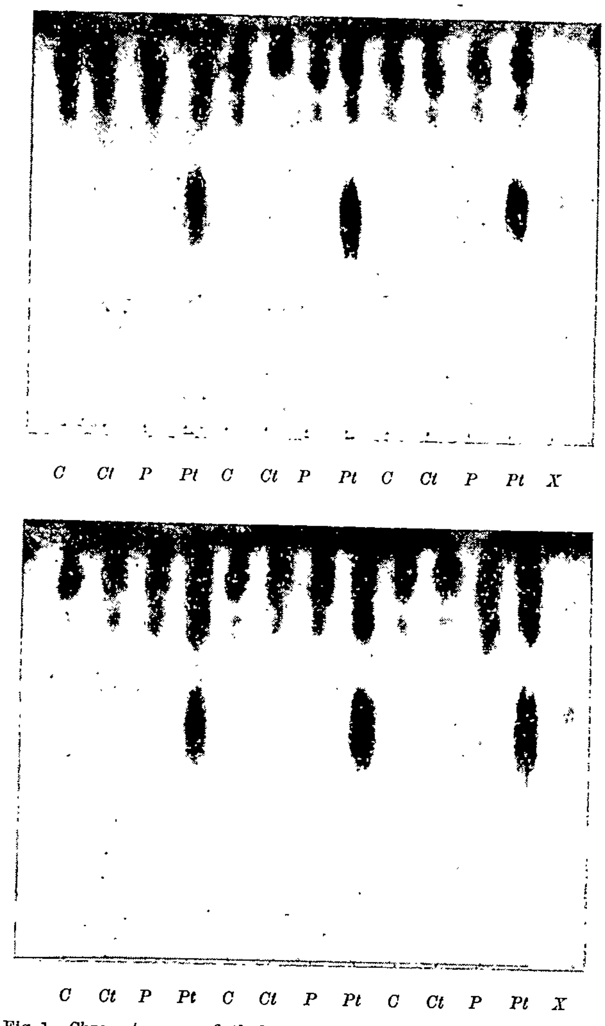

Fig. 1. Chromatograms of ethyl acetate extracts of urine run in butanol/acetic acid/water and sprayed with diazotized sulphanilic acid. Above, 3 rats oral D-penicillamine; below, 3 rats parenteral D-penicillamine. *C*, control urine before tryptophan; *Ct*, control urine after tryptophan; *P*, urine after D-penicillamine without tryptophan load; *Pt*, urine after D-penicillamine with tryptophan load; *X*, xanthurenic acid marker. Only urine *Pt* in each case shows a gross rise in xanthurenic and kynurenic acids

Table 1

Mode of administration of D-penicillamine		Xanthurenic acid excretion µg/mg creatinine			
		C	*Ct*	*P*	*Pt*
Oral (Group *A*)	1	0	0	0	86
	2	0	0	0	114
	3	0	0	0	100
Parenteral (Group *B*)	1	0	0	0	97
	2	0	0	0	100
	3	0	0	0	185

For abbreviations see legend of Fig. 1.

Table 2

R_F		Fluorescence in ultra-violet light	Ehrlich's reagent	Ekman's reagent	Pauly's reagent
Butanol: acetic acid: water (4 : 1 : 5)	20% KCl				
(*a*) 0·75	0·73	Green	Orange	Brown	Red (fades)
(*b*) 0·80	0·82	Blue	Orange	Magenta	—

Two other metabolites excreted in increased amounts, judging from their intense fluorescence in ultra-violet light, were further investigated by two-dimensional chromatography[11] followed by use of spray reagents as described by Dalgliesh[13]. The characteristics of the two metabolites are noted in Table 2.

These observations suggest the probable identity of the metabolites as (*a*) *N*α-acetyl-3-hydroxykynurenine; (*b*) *N*α-acetyl kynurenine, two intermediate metabolites of tryptophan[13].

From the results of experiments described here it is evident that following the ingestion of D-penicillamine over a period there is a considerable increase in excretion of certain intermediate metabolites of tryptophan after a test dose of the amino-acid. The abnormal excretion of xanthurenic and kynurenic acids as an indication of pyridoxine deficiency[9,13,14] has received further support from enzymatic work by Weber and Wiss[15], who studied the influence of vitamin B_6 depletion on various enzymes involved in the metabolism of tryptophan. They showed that in rat liver kynureninase is much more affected than kynurenine-2-oxo-glutarate-transaminase and concluded that the gross excretion of xanthurenic acid observed in pyridoxine deficiency could be attributed to the different behaviour of the two enzymes. It is therefore concluded that D-penicillamine, like the L-isomer, causes a depletion of pyridoxal-5-phosphate in the rat.

The results of these experiments on rats emphasize the prudence of including large amounts of pyridoxine in the diet of patients receiving D-penicillamine. Furthermore, as suggested by Evered[16], these patients need to be screened periodically for possible signs of vitamin B_6 deficiency.

I thank Prof. M. D. Milne for his advice.

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Cytochemical Investigations of Deoxyribonucleic Acid-mediated Control of Ribonucleoprotein Metabolism

THE interdependence of various types of macromolecular synthesis in the cell has been demonstrated by the use of various antimetabolites. Synthesis of some ribonucleoproteins (RNP) in the nucleus is dependent on continued formation of deoxyribonucleic acid (DNA)[1–3]. One of these ribonucleoproteins is present in the nucleolus in the form of spherules called 'nucleolini'[3]. When synthesis of DNA was inhibited by 5-fluorodeoxyuridine (FUDR), the nucleolini were at first enlarged and later disappeared. The pars amorpha of the nucleolus was not reduced during this process[3]. Further cytochemical observations and biochemical investigations of HeLa cells, grown as previously described[3], and treated with 2×10^{-5} M FUDR are presented here.

Cells were stained by the toluidine blue–molybdate method[4]. Nuclear and cytoplasmic fractions were prepared by the citric acid procedure[5]. Nucleic acids were estimated by a modified Schmidt–Tannhauser procedure[6], Burton's modification of the Dische reaction[7] and the orcinol reaction[8]. Protein was determined by the method of Lowry *et al.*[9]. The results presented in Table 1 show that, during a period of 48 h, synthesis of DNA, nuclear RNA and protein was inhibited by FUDR. Examination of stained preparations made at intervals during the 48-h period indicated that the nucleolini and the chromosomal, perichromosomal and granular cytoplasmic RNP[4] gradually disappeared. The cytoplasmic changes occurred later than those in the nucleus. Cytochemical investigations have indicated that the nucleolini, chromosomal and so-called 'perichromosomal' RNP appear to exist as hybrids with DNA[10]. The changes were not due to conversion of FUDR to fluorouracil, since they could be prevented by incorporation of equimolar thymidine, but not by uridine in the medium[11].

Table 1. EFFECTS OF FLUORODEOXYURIDINE (FUDR) ON NUCLEIC ACIDS AND PROTEIN PER CULTURE DURING 48-H INCUBATION*

Cultures	Percentage of initial amount per culture (nuclear fraction)		
	DNA	RNA	Protein
Untreated	407	390	506
Treated with FUDR (2×10^5 M)	95	86	159
Difference ± *S.E.*	312 ± 61	304 ± 53	347 ± 12

* Mean of four experiments.

Table 2. EFFECTS OF ACTINOMYCIN ON NUCLEIC ACIDS AND PROTEIN PER CULTURE DURING 24-H INCUBATION

Cultures	Percentage of initial amount per culture		
	DNA	RNA	Protein
Untreated	197	224	208
Treated with actinomycin (0·01 μg/ml.)	190	104	153
Difference ± *S.E.*	7 ± 14	120 ± 27	55 ± 25

* Mean of four experiments.

The results of similar investigations of HeLa cells treated with actinomycin *D* (0·01 μg/ml.) for 24 h are presented in Table 2. Treatment with actinomycin produced a pronounced inhibition of synthesis of RNA, some reduction of protein formation and no significant effect on DNA metabolism. Cytochemical investigations indicated that, after 24 h, the nucleolini and pars amorpha of the nucleolus had almost completely disappeared and that the granular cytoplasmic RNP was decreased. Sequential observations revealed that the nucleolar changes preceded the decrease in cytoplasmic RNP. Chromosomal and perichromosomal RNP were unaffected.

In summary, the results with FUDR show that inhibition of synthesis of DNA results in a failure of synthesis of DNA–RNA hybrids in the chromatin and nucleolini; granular RNP in the cytoplasm is also reduced. Inactivation of DNA-primed RNA synthesis by actinomycin[12], on the other hand, inhibits the formation of RNA in all parts of the nucleolus, and later results in the reduction of granular RNP in the cytoplasm. The work of Perry indicates that low concentrations of actinomycin, such as those used here, may selectively inhibit the formation of rapidly labelling high molecular weight ribosomal-precursor RNA in the nucleolus, and later lead to a decrease in ribosomal RNP[13]. This would suggest that at least one of the ribonucleoproteins of the nucleolus is a precursor of the granular cytoplasmic RNP which is, therefore, probably of the ribosomal or polyribosomal type. Since the RNP of the nucleolini and not the pars amorpha was affected in the FUDR system, in which the granular cytoplasmic RNP was also reduced, the RNP of the nucleolini may be of the ribosomal precursor type. On the other hand, as previously suggested[3,6,14–16], it may be of the messenger type, since the latter is necessary for the formation of ergosomes or polyribosomes[17] and only large aggregates of ribosomes could possibly be visualized by cytochemical techniques for light microscopy.

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Anomalous Ionization of Methyl Linolenate by Metastable Argon Atoms: Possible Linolenic Acid Participation in Photosynthetic Reactions

DURING the past few years there has been an increasing number of reports pointing to the possible specific role of α-linolenic (*cis*-9,12,15-octadecatrienoic) acid in photosynthetic reactions of green plants. In algal cells grown in the dark α-linolenic acid is virtually absent; if the cells are exposed to light, the concentration of linolenic acid increases in parallel with that of chlorophyll[1]. The same process has been observed during the greening of etiolated *Citrullus vulgaris* seedlings[2].

Approximately 85 per cent of the cellular α-linolenate is accounted for by the chloroplast fraction[3] as an acyl component of galactolipids of the chlorophyll–lipoprotein membranes[4]. According to Bloch *et al.*, the presence of linolenic acid in *Euglena gracilis Z* cells seems to be necessary for the Hill reaction and the evolution of oxygen[3].

The present-day concept of photosynthesis involves the absorption of light quanta by a chlorophyll molecule followed by the conversion of the latter to a triplet excited state. The excitation energy is transferred by a mechanism as yet obscure to a photochemical reaction centre to effect photolysis of water and simultaneous oxygen evolution[5]. It has been suggested that the linolenic acid of chloroplasts is in some way related to the dark reactions of electron transfer[3].

Having at present no means of devising a model system of photosynthesis containing linolenic acid, we decided to compare the capability of linolenic acid and other fatty acids commonly present in plant tissues to be ionized in a more simple model system by excited metastable atoms. It is known that in the ionization detector for gas chromatography introduced by J. E. Lovelock[6] the organic substances are ionized as a result of the following reactions:

$$\text{Ar} + \beta\text{-particles } (\text{Sr}^{90}) \rightarrow \text{Ar}^{+} + e^{-} \quad (1)$$

$$\text{Ar} + e^{-} \rightarrow \text{Ar}^{*} \quad (2)$$

$$\text{Organic substance} + \text{Ar}^{*} \rightarrow \text{organic substance}^{+} + \text{Ar} \quad (3)$$

where e^{-} are free electrons, Ar^{*} are metastable argon atoms and organic substance^{+} are ionized molecules of organic substance[7].

A standard mixture of palmitic, stearic, oleic, linoleic and α-linolenic acid methyl esters (0·25–0·50 µl.) was introduced into the ionization detector of an argon chromatograph (W. G. Pye and Co., Ltd., Cambridge, Great Britain) containing ^{90}Sr (20 mc.) at 190° ± 1°, 60 ml. Ar/min and × 10 or × 3 amplifier sensitivity. In various experiments the detector voltage was 750, 1,000, 1,250 or 1,500 V. Detector responses proportional to the ionization of different esters were determined by multiplication of the respective peak heights by width at mid-height, or by triangulation[8], and expressed in arbitrary units in relation to methyl palmitate: the mass response for methyl palmitate being equal to 1 in each experiment.

The effect of detector voltage on the ionization of fatty acid methyl esters is shown in Fig. 1. These results demonstrate that detector voltage has almost no effect on the relative response for palmitic, stearic, oleic and linoleic acid esters. Therefore, these acids do not vary in their ionization pattern. On the contrary, the ionization of methyl linolenate rises continuously when β-particle energy and metastable atom concentration are increased.

It is suggested that the unique properties of linolenic acid with regard to its ionization pattern and predominant role in photosynthetic reactions are related to the structure of this acid having three methylene-interrupted double bonds at the 'methyl end' of the aliphatic chain. This suggestion is all the more probable as there are other instances when the structure of unsaturated fatty acids controls their biological function. Linoleic (*cis*-9,12-octadecadienoic) and arachidonic (*cis*-5,8,11,14-docosatetraenoic) acids are essential to animal organism (vitamin F) (ref. 9). These acids can be replaced by the intermediate of their interconversion, γ-linolenic (*cis*-6,9,12-octadecatrienoic) acid[10], which is, however, completely inactive in photosynthetic systems[3].

Thus the anomalous ionization of methyl linolenate by excited metastable atoms does not explain the mechanism of α-linolenic acid participation in 'primary' photosynthetic reactions. Nevertheless, we may hope that the found relationship will be useful in planning and carrying out further experiments in this field.

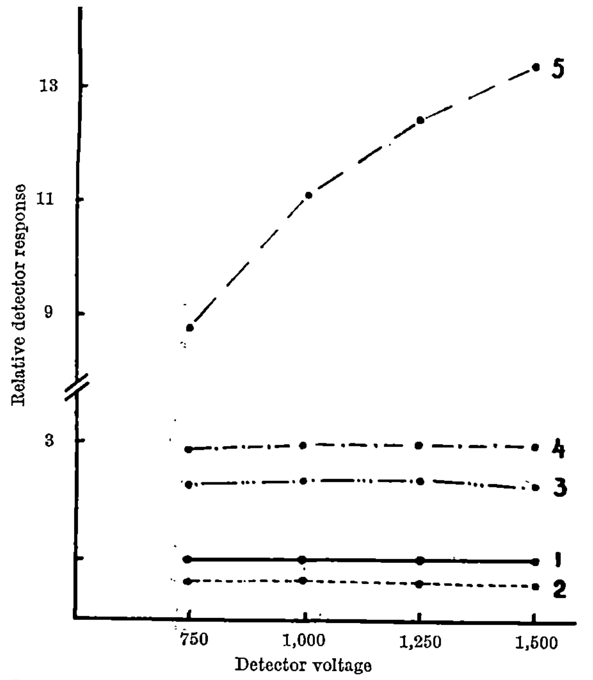

Fig. 1. Relative ionization detector response for fatty acid methyl esters as a function of detector voltage. The response for methyl palmitate is arbitrarily equal to 1 at each voltage. 1, methyl palmitate; 2, methyl stearate; 3, methyl oleate; 4, methyl linoleate; 5, methyl linolenate

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PHYSIOLOGY

Inhibition of Ovulation in the Rabbit by Actinomycin *D*

In the rabbit, an induced ovulator, sufficient gonadotropin is released in the first hour after coitus to result in ovulation 9 h later[1]. This represents an interesting example of a stimulus-response system. Although the stimulus pathway has been reasonably elucidated, the response is not well understood. The subsequent steps in the response beyond the level of release of gonadotropin, involving interaction of hormone and ovary and culminating in rupture of the Graafian follicle, are unknown. For about 8 h after the coital stimulus the only apparent change in the follicle is an increased protuberance of the follicle above the surface of the ovary. It is during the 2 h prior to rupture of the follicle that a rapid increase in volume occurs. The increased volume is followed by stigma formation, usually at the apex of the follicle, and finally the release of the ovum.

Several hypotheses have been suggested to account for the changes which have been seen. These vary from the suggestion that smooth muscle in the theca externa facilitates rupture to the suggestion that an increase in pressure in the follicle is the causative mechanism via a depolymerization and an ischæmia of the stigma followed by necrosis[2]. More recently the concept of increased pressure was made untenable by the demonstration that existent pressures remain unchanged through ovulation[3]. A model of the mechanism of ovulation has been described in which it was shown that bacterial collagenase or protease injected into the antrum of unstimulated follicles could cause swelling, stigma formation and rupture[3]. The presence of proteinases with optima at *p*H 4 and 8 in the ovaries of cycling rats in concentrations above those seen in pseudopregnant and pregnant rat ovaries also fits into the picture in which enzymes play a part in ovulation[4].

Actinomycin *D* appears to inhibit the action of several hormones[5]. It is at present believed that actinomycin *D* is an extremely specific inhibitor of protein synthesis and that it acts by blocking DNA-directed synthesis of messenger RNA, perhaps by binding with the guanine in the minor groove of the DNA primer and thus preventing the action of RNA polymerase[6]. Therefore, in the context of contemporary ideas of how actinomycin *D* acts, we explored the effects on the ovulatory mechanism subsequent to injection directly into stimulated Graafian follicles. We have determined both the minimal amount of actinomycin *D* necessary to inhibit ovulation and the most effective period after a coital stimulus for the action of the minimally effective dose.

Virgin female rabbits, individually caged and about 6 months old, were stimulated by mating with a vigorous buck. The rabbits were anæsthetized 1 h after coitus with approximately 25 mg of sodium pentobarbital/kg body wt. Bilateral laparotomies were performed and the ovaries pulled out without embarrassment of the circulation so that the follicles might be injected and examined. The ovaries were supported with a 'Plexi-glass' rod and kept warm and moist with a drip of 37° C Ringer's solution[3]. The micropipettes were inserted into mature œstrus stage follicles with a DeFonbrune micromanipulator[3]. Randomly selected follicles were injected with either actinomycin *D* or saline so that each ovary contained, frequently in close proximity to each other, both treated and control follicles. The follicles were examined for ovulation 11 h after coitus and re-examined periodically for as long as 24 h to check for delayed ovulation in follicles that had failed to ovulate initially. The data for the entire ovary were discarded if all saline-injected follicles failed to ovulate. In no case did actinomycin-*D*-injected follicles ovulate when the saline-injected follicles failed to ovulate.

Eighty-five per cent of the 33 follicles injected with 1 µl. of isotonic saline ovulated. Fifty-seven follicles in 13 rabbits in œstrus that were stimulated by mating were then injected with 1 µl. of actinomycin *D* in saline to determine the minimum concentration necessary to inhibit ovulation. The follicles were all injected 1–3 h after coitus with one of the several doses of actinomycin *D* indicated in Fig. 1. Doses of 1 µg–10^{-3} µg actinomycin *D* completely inhibited ovulation while doses less than 10^{-3} µg actinomycin *D* caused only occasional inhibition (Fig. 1).

Post-coital inhibition time was determined by injections of actinomycin *D* into follicles at various times after the coital stimulus. A dose of 10^{-3} µg was used in these experiments. Thirty-seven follicles were injected in 9 stimulated œstrus rabbits. There was complete inhibition of ovulation when the follicles were injected 1–3 h after coitus; later injections resulted in only occasional or no inhibition (Fig. 2).

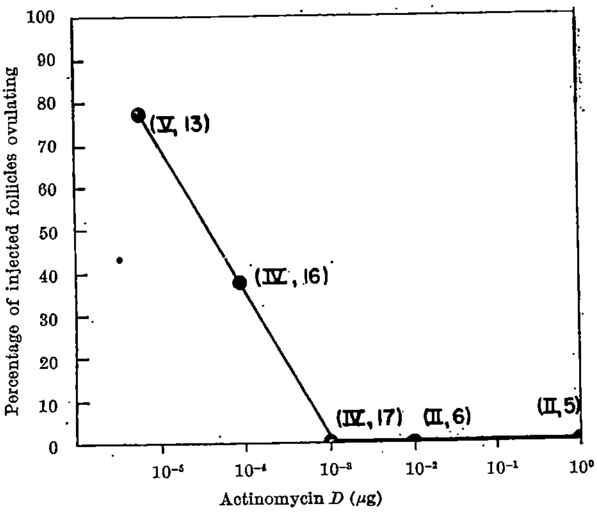

Fig. 1. Effect of actinomycin *D* on ovulation. The drug was injected directly into the œstrus stage follicle 1–3 h after a coital stimulus to the rabbit. Saline was always injected into several follicles in the same ovary and the response was ovulation of these follicles. 85 per cent of the saline-injected follicles ovulated. The response was recorded 11 h after the stimulus had been administered. Roman numerals, No. of animals; Arabic numerals, No. of follicles

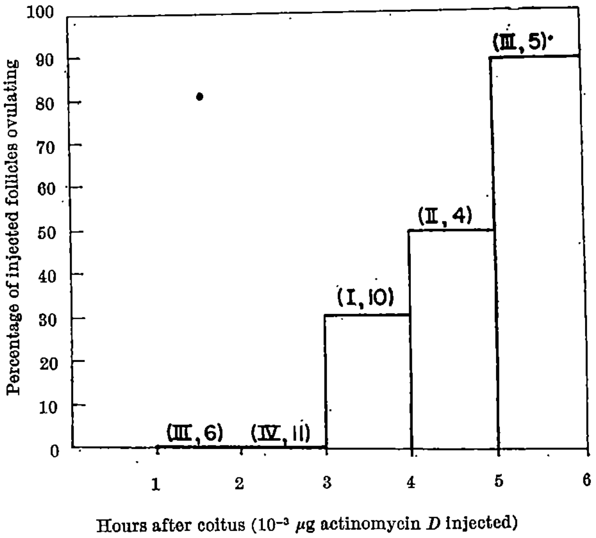

Fig. 2. Post-coital inhibition time. The rabbits were anæsthetized 1 h after coitus and the follicles injected with actinomycin *D* at the times indicated. The ovaries were examined after 11 h and after 24 h. Saline was injected into control follicles in each ovary. Evidence of inhibition of ovulation by actinomycin *D* was based on ovulation occurring in saline-injected follicles in the same ovary. Roman numerals, No. of animals; Arabic numerals, No. of follicles

Preliminary examination of the duration of inhibition of ovulation has been considered. Twenty-five I.U. of human chorionic gonadotropin was injected into 2 rabbits mated 26 h previously. The only non-ovulated Graafian follicles on the ovaries were those that had been injected with 10^{-3} µg actinomycin *D* 25 h earlier. Ten hours after the human chorionic gonadotropin had been administered the ovaries were again examined. Eleven inhibited follicles appeared greatly enlarged and hæmorrhagic and 2 follicles had ovulated. Therefore it appears that actinomycin *D* inhibition is transient and that such follicles are capable of response to a later second dose of ovulation inducing hormone.

Actinomycin *D* is an effective inhibitor of ovulation when injected into mature œstrus stage follicles in high enough concentrations and soon enough after the coital stimulus. Furthermore the inhibition is transient. In view of the effectiveness of actinomycin *D* as an inhibitor of ovulation and in keeping with present-day ideas of the mechanism of actinomycin *D* action we suggest that gonadotropin acts at the level of the nucleus of some as yet unspecified cells to induce the synthesis of a specific *m*-RNA. This *m*-RNA forms a template for the synthesis of a protein which may be intermediate in the synthesis of a hormone or may possibly be a proteolytic enzyme which then causes ovulation. It may be that sufficient messenger RNA is produced in the 3 h after coitus to allow adequate protein synthesis for ovulation to occur, since injection of 10^{-3} µg actinomycin *D* was completely inhibitory if given up to 3 h after coitus, but was only partially inhibitory or had no effect if injected later than 3 h.

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Modification of Visual Critical Flicker Fusion Frequency by Intermittent Auditory Stimuli

KRAVKOV[1,2] found that visual critical flicker fusion frequency was modified by auditory stimuli, but other workers failed to confirm this[3,4].

The experiments to be described here were suggested by the demonstration of a modifying effect of intermittent light of varying frequency on critical flicker fusion frequency[5,6]. The relationship between critical flicker fusion frequency and the adapting frequency was almost linear between adapting frequencies of 24 and 54 c/s. Because the exposure of one eye to the adapting light altered the critical flicker fusion frequency of the other, the phenomenon is probably central in origin.

Visual critical flicker fusion frequency was measured by Turner's technique[6]. It was defined as the fastest rate at which the neon light source was considered to be flickering as opposed to being steady. The threshold was determined either by an approach from a lower to a higher frequency (ascending threshold) or vice versa (descending threshold). Audible 'clicks' of varying frequency were derived from an oscillator constructed on the 'module' system[7] and were presented to the subject through a loud speaker.

The ascending (*A*) and descending (*D*) thresholds of critical flicker fusion frequency of six subjects were tested after exposure for 1 min to auditory 'clicks' at 25 or at 50 c/s., or after a period of silence. The order in which the various frequencies of sound were given and ascending and descending thresholds were measured was randomized in a latin square arrangement. The results are given in Table 1.

Table 1. EXP. 1. EFFECT OF INTERMITTENT SOUND AT 25 AND 50 C/S ON ASCENDING (*A*) AND DESCENDING (*D*) THRESHOLDS OF CRITICAL FLICKER FUSION FREQUENCY (c.f.f.f.)

$n=6$ 5 per cent critical difference $=0\cdot74$ c/s

		Mean c.f.f.f. (c/s)	
		A	*D*
No sound		35·5	35·0
Adapting sound	25	36·0	35·0
frequency (c/s)	50	36·25	35·75

Table 2. EXP. 2. EFFECTS OF INTERMITTENT SOUND (0–80 C/S) ON ASCENDING (*A*) AND DESCENDING (*D*) THRESHOLDS OF c.f.f.f.

$n=8$ 5 per cent critical difference $=0\cdot54$ c/s

		Adapting sound frequency (c/s)			
		0	20	40	80
C.f.f.f.	*A*	35·25	35·5	37·0	36·75
(c/s)	*D*	35·25	35·75	35·75	36·5

The mean ascending threshold of critical flicker fusion frequency after exposure to sound at 50 c/s was significantly higher than that without sound, while that after 25 c/s had an intermediate value. The mean descending threshold after 50 c/s was significantly higher than that after 25 c/s or that without sound, these latter two having identical values.

In a second experiment using the same method but in a sound-proof room, adapting frequencies of 20, 40 and 80 c/s were used. Determinations were randomized to eliminate any order effect. Table 2 shows that visual critical flicker fusion frequency rose as the adapting auditory frequency increased. The ascending threshold rose sharply between 20 and 40 c/s while the descending threshold rose steeply between 40 and 80 c/s.

These preliminary experiments suggest that there is an intersensory effect of intermittent sound of varying frequency on critical flicker fusion frequency.

We thank Mr. P. M. G. Bell for the design of the apparatus.

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PHARMACOLOGY

Effect of Snake Venom and Endotoxin on Cortical Electrical Activity

THE effects of a lethal injection of cobra venom, rattlesnake venom, or *E. coli* endotoxin on the cardiovascular system of the dog and monkey have been described previously in detail. The administration of endotoxin results in hypotension, acidosis, hæmolysis, and renal failure[1–4]. A similar effect is seen following the administration of snake venom[5,6]. No reports, however, describe the effects of these compounds on the central nervous system. The purpose of work recorded here, therefore, was to determine if a change in cortical electrical activity follows a lethal injection of these toxins.

Thirty adult mongrel dogs and fifteen monkeys of the Cynapthecoid group (sooty mangabey) were used. All animals were anæsthetized with pentobarbital sodium (30 mg/kg). Arterial blood pressure was continuously recorded using a Statham strain gauge and an *E* and *M* six-channel physiograph. The cortical electrical activity of the right and left hemispheres of the brain was monitored using bipolar silver electrodes placed directly on the dura of each hemisphere. Continuous electroencephalograms were recorded prior to and for up to 10 h after the intravenous administration of the toxin. The animals were divided at random into the following three groups:

Group I: 10 dogs and 5 monkeys receiving 0·5 mg/kg crude cobra venom (*Naja naja*); Group II: 10 dogs and 5 monkeys receiving 0·5 mg/kg crude rattlesnake venom (*Crotalus admanteus*); Group III: 10 dogs and 5 monkeys receiving 1·0 mg/g endotoxin (*E. coli*) (Difco).

Table 1 presents a composite of the results obtained from the three groups of animals used in this work. The effect of each toxin on cortical electrical activity is indicated. The change observed in EEG is of an all-or-none nature, being characterized by either a complete loss of activity, or no alteration at all. All animals in Groups I and II showed complete or almost complete EEG silence within 1 min after having received either venom. In contrast, none of the dogs or monkeys in Group III, which received endotoxin, showed any alteration in

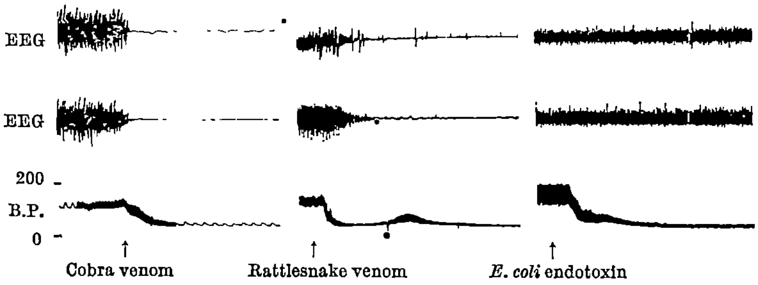

Fig. 1

EEG. Average survival time, as well as the range for each group, is indicated.

Actual tracings, representative of the changes observed in the three groups, are shown in Fig. 1. The immediate effect of cobra venom, rattlesnake venom, and *E. coli* endotoxin on EEG and blood pressure is presented. Within 30–60 sec following the administration of cobra or rattlesnake venom, a complete loss of all EEG activity is seen. This occurs prior to the initial fall in blood pressure. Although the hypotension is usually followed by partial recovery, no return of cortical activity is noted during the subsequent course of the experiment. All animals eventually expire exhibiting signs of respiratory paralysis and cardiovascular failure. In conjunction with the initial change in EEG, a loss of eyelid and corneal reflexes occurs shortly after venom, which, like the change in EEG, is irreversible. In the animals receiving endotoxin, a fall in blood pressure similar to that observed with snake venom is noted; however, no change in cortical electrical activity is observed. As both the dogs and monkeys progress into the later stages of endotoxin shock and the blood pressure once again falls to hypotensive levels, some changes in cortical activity are noted. These alterations are probably associated with and attributable to progressive anoxia and not to any direct action of endotoxin on cortical tissue *per se*.

Table 1

	Dogs			Monkeys		
	No. of animals	EEG change	Average survival time (h)	No. of animals	EEG change	Average survival time (h)
Group I (cobra venom)	10	10/10	1·4 (0·3–2·2)	5	5/5	2·0 (1·1–3·1)
Group II (rattlesnake venom)	10	10/10	1·1 (0·2–2·0)	5	5/5	1·7 (1·0–2·8)
Group III (*E. coli* endotoxin)	10	0/10	7·6 (5·1–9·6)	5	0/5	5·6 (4·1–10·0)

Results of this work indicate that the venoms of the hooded cobra and of the eastern diamondback rattlesnake have a unique and severely depressant effect on cortical electrical activity. No comparable change is observed after the administration of endotoxin, even though the resulting fall in blood pressure is in many instances greater than that produced by snake venom. This observation, in conjunction with data obtained from hæmorrhagic shock investigations, in which prolonged hypotension does not produce changes in EEG[7], eliminates the probability that the observed change in EEG is the result of either cardiovascular hypotension and/or cerebral hypoxia. In addition, changes in cortical activity consistently appear before the observed fall in blood pressure, indicating that the toxin exerts a more immediate and profound effect on brain tissue than on peripheral components. The relationship of this change to that occurring at the neuromuscular junction of the diaphragm is not clear[8,9]. It is possible that the two are in some way related. The permanent loss of eyelid and corneal reflexes in conjunction with the change in EEG supports the hypothesis that both venoms are capable of severely disrupting not only the normal spontaneous electrical activity but also the functional organization of the brain. An investigation of the mechanisms by which this change is produced, as well as a fractionation of the venoms into their various components, is now being carried out in these laboratories.

The cortical electrical activity of the brain has been shown to be severely depressed by the administration of cobra and rattlesnake venoms. This change is not seen in animals administered a lethal dose of endotoxin, even though a fall in blood pressure similar to that observed with venom is consistently noted. The relationship of the EEG change to the lethal outcome of exotoxin shock is not clear but may in some way be related to its blocking effect on neuromuscular transmission.

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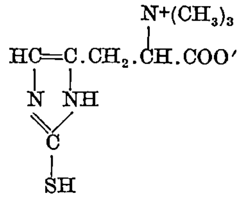
Identity of the Cerebellar Factor

EXTRACTS of mammalian cerebellum, suitably injected into the cerebral circulation of the decerebrate rabbit, produce an increase in the electrical activity of the cerebellum. This effect, first described by Crossland and Mitchell[1], is due to a hitherto unidentified substance, provisionally called the cerebellar factor. The cerebellar factor has been found in some other localized regions of the nervous system, particularly the optic nerves. It is present in small amounts in the cerebral hemispheres, but absent from the dorsal columns of the spinal cord[2]. A characteristic feature of the cerebellar factor is its greater stability in alkaline than in acid solutions. In alkali (pH 14) biological activity is still present after 5 min boiling, but it gradually disappears thereafter; in acid (pH 1) no detectable activity remains after boiling for 5 sec[2]. Although active on central neurones, the cerebellar factor appears to be inactive on smooth or striated muscle and on autonomic ganglia.

A study of the known chemical properties of the cerebellar factor suggested a similarity with ergothioneine, the betaine of thiolhistidine:



Ergothioneine shows the same relative stability towards acid and alkali as does the cerebellar factor. In acid solution its sulphydryl group is very easily oxidized but is quite resistant to oxidation in an alkaline medium. Under alkaline conditions, fission eventually occurs elsewhere in the molecule with the formation of thiolurocanic acid and the release of trimethylamine[3].

Ergothioneine is known to occur in some mammalian tissues, although earlier workers have reported its absence from brain[3], and Melville *et al.* have developed a highly specific and relatively sensitive method for its quantitative determination in tissue extracts[4]. We have applied the method to brain with the results, for the rabbit, given in Table 1.

Table 1. ERGOTHIONEINE CONTENT (IN μg/g) OF PARTS OF THE RABBIT CENTRAL NERVOUS SYSTEM

		Mean
Cerebellum	4·3, 4·6, 5·0, 5·5	4·9
Cerebral hemispheres	0·9, 1·2, 1·2, 1·3, 1·5	1·2
Optic nerves	21·0, 36·0, 29·0	28·7
Dorsal columns	< 1·0, < 1·0	< 1·0

A similar distribution has been found in other mammalian species: it corresponds to that found for the cerebellar factor. We have also found that ergothioneine has an action on the electrical activity of the cerebellum similar to that produced by the cerebellar factor. In sensitive preparations, 0·1 µg is sufficient to cause a marked increase in cerebellar activity. In their original experiments, Crossland and Mitchell were able to produce cerebellar excitation by the injection of an extract of 20 mg of rabbit cerebellum and this amount of tissue would have contained approximately 0·1 µg of ergothioneine (Table 1). Thus, the excitatory action of cerebellar extracts can be completely accounted for by their contained ergothioneine.

The actions of ergothioneine are probably not confined to the cerebellum. Preliminary experiments, in which ergothioneine has been applied to small groups of cells in the parietal cortex, using the technique developed by Mitchell[5], have indicated that it also excites some cortical cells. Outside the central nervous system it appears to be without detectable action.

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Guanethidine as an Anti-fibrillatory Agent

CATECHOLAMINES have for many years been known to produce cardiac arrhythmias[1]. The influence of acetylcholine or vagal stimulation on arrhythmias, especially of the atria, has overshadowed the influence of the sympathetic mediators[2]. Sharma[3] has reported that administration of epinephrine or norepinephrine simultaneously with acetylcholine enhanced the fibrillatory activity of the latter. Thus, it would seem appropriate to examine further the relationship between acetylcholine and catecholamines in their effect on heart rhythm.

The introduction of guanethidine, a drug which acts at adrenergic nerve terminals by preventing release of norepinephrine on nerve stimulation, suggested the possibility of studying the susceptibility to experimental atrial fibrillation in animals with deficient adrenergic mechanisms.

Healthy male dogs weighing about 10 kg were premedicated with morphine sulphate (2 mg/kg subcutaneously). In 30 min, the dogs were anæsthetized with sodium pentobarbital (35 mg/kg intraperitoneally). An endotracheal tube was inserted and the dogs immobilized in the prone position. They were given guanethidine at various doses and intervals before and during the experiment (Table 1). The method of Leveque was used for the production of atrial fibrillation[4]. With this procedure during control tests, all the experimental dogs demonstrated susceptibility to acetylcholine-induced atrial fibrillation at doses of 0·3 mg/kg or less. A total of 26 experiments were performed on 14 dogs.

Table 1. DOSE AND DURATION OF ACTION OF GUANETHIDINE AS AN ANTI-FIBRILLATORY AGENT

Experiment No.	Dose of guanethidine (mg/kg) and route		Time delay before challenge (h)	Percentage protection versus acetylcholine-atrial fibrillation (0·5 mg/kg intravenous)	
121	1·0	Intravenous	1	0	(2)
125	1·0	,,	1	100	(3)
127	1·0	,,	1	100	(2)
141	1·0	,,	1	100	(3)
147	1·0	,,	1	100	(3)
73	1·0	Subcutaneous	4	0	(3)
65	2·5	,,	4	100	(2)
69	2·5	,,	4	100	(3)
77	2·5	,,	4	50	(4)
95	2·5	,,	18	0	(1)
109	2·5	,,	18	66	(3)
113	2·5	,,	18	0	(2)
129	2·5	,,	18	0	(2)
135	4·0	,,	18	0	(2)
39	10·0	,,	24	100	(2)
41	5·0	,,	24	100	(2)
43	5·0	,,	24	100	(2)
45	5·0	,,	24	100	(2)
137	4·0	,,	24	100	(3)
139	4·0	,,	24	100	(3)
79	2·5	,,	24	100	(2)
81	2·5	,,	24	0	(3)
97	2·5	,,	24	0	(2)
115	2·5	,,	24	0	(2)
117	2·5	,,	24	0	(2)
131	2·5	,,	24	0	(2)

The usual protective dose of guanethidine within 20 min appeared to be about 1·0 mg/kg although 0·5 was sometimes adequate. These low doses were protective for at least 1–2 h, but the effect did not usually last as long as 4 h. With increasing doses of guanethidine (up to 10·0 mg/kg), the anti-arrhythmic effect of this drug was prolonged up to 24 h.

Anti-fibrillatory drugs are in general related in structure to local anæsthetics and antihistaminic agents. The chemical structure of guanethidine shows similarities to this general structure, and Green has reported that guanethidine possesses a highly persistent local anæsthetic action[5].

Using the method described here for testing the anti-fibrillatory action of guanethidine, other drugs such as procaine, diphenhydramine, chlorpheniramine, tripelennamine and quinidine have been found previously to be protective[4].

It is interesting to note that the anti-arrhythmic action of guanethidine is apparent with doses that are smaller than those generally used for adrenergic blockade[6]. This might suggest that the anti-arrhythmic effect of this drug does not depend on changes in catecholamine stores. Investigations to elucidate the mechanism of action of guanethidine in preventing cardiac arrhythmias are now in progress.

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IMMUNOLOGY

Suppression of Antibody to Endotoxin with 6-Mercaptopurine : Effect of Antigen Dose

THE selection of antigen, the type and dose of antimetabolite administered, and the species of recipient animal are all important factors in the evaluation of the immunosuppressive properties of antimetabolites[1]. Using as an antigen sheep red blood cells treated with tannic acid, Nathan *et al.* reported that suppression of antibody response by 6-mercaptopurine (6-MP) became greater as the dose of antigen was increased[2]. Furthermore, in investigations of drug-induced immunological tolerance, Schwartz and Dameshek have shown that using a constant dose of 6-MP, suppression of antibody to bovine serum albumin is proportional to dose of antigen administered[3]. We have used 6-MP to suppress the antibody response to the somatic antigens (endotoxins) of Gram-negative bacteria[4]. In contrast to the foregoing reports we have noted that suppression of antibody to bacterial polysaccharide antigens is not greater when larger doses of antigen are administered.

The endotoxin of *Salmonella typhosa* 0901 (Difco), diluted in sterile buffered saline, was administered intravenously to New Zealand albino rabbits of either sex weighing approximately 2 kg. Doses of 2, 20 or 200 μg in 2-ml. volumes were injected into three groups of experimental animals ; quantities greater than 200 μg were found to have prohibitive toxicity. 6-Mercaptopurine was prepared for injection immediately prior to use by dissolving a maximum of 150 mg of 6-MP for each ml. of 1 N sodium hydroxide. The solution was then diluted with buffered saline to a concentration of 18 mg/ml. and *p*H 10–11. All animals treated with 6-MP received 18 mg of drug/kg body wt. Control animals, not receiving 6-MP, were injected with identical volumes of a buffered saline solution adjusted to *p*H 10–11 by addition of 1 N sodium hydroxide. Both injections and bleedings were via marginal ear veins with all bloods obtained at least 24 h after the final injection of 6-MP and 5 days after the endotoxin injection, since peak antibody titres to endotoxins have been reported to occur at this time[5]. Sera, separated by centrifugation, were stored at −20° C until antibody determinations were made by the bentonite flocculation technique using serial two-fold dilutions of sera[6]. Numerous determinations of antibody to *S. typhosa* in normal rabbit sera previously made with this technique have revealed only rare positives with low titre ; therefore, antibody levels prior to endotoxin injection are not reported.

Animals were divided into three experimental groups : the first received 2 μg of endotoxin (55 animals), the second 20 μg (49 animals) and the third (111 animals) 200 μg. Half of each of the above groups received four daily injections of 18 mg of 6-MP/kg body-wt. beginning at the time of antigen injection while the other half received four daily injections of the diluent solution, thus serving as controls.

The results of these experiments are given in Table 1. Each 6-MP treated group had significant reduction in titre when compared to its respective control ($P < 0{\cdot}01$ for each, *t* test). Antibody titres of sera from both control and drug-treated rabbits were greater for the higher doses of antigen. However, the immunosuppressive action of the drug was not greater as the dose of antigen was increased ; this finding is clearly different from those with tanned red cells and bovine serum albumin as antigens[2,3]. In fact, if anything, there was a tendency toward less-pronounced suppression at the highest dose-level, 200 μg. The differences of the means of the three groups were tested for homogeneity using the *F* test, and the results were $F = 0{\cdot}7$, $P > 0{\cdot}25$.

Table 1. EFFECT OF DOSE OF ENDOTOXIN ON ANTIBODY LEVELS IN 6-MP-TREATED RABBITS

Dose of endotoxin (μg)	Drug	Antibody titre
2	6-MP*	4·2† (2·6–6·8)‡
2	Control	16·0 (10·6–24·3)
20	6-MP	9·4 (6·0–15·0)
20	Control	49·0 (35·3–68·0)
200	6-MP	22·8 (15·4–33·6)
200	Control	72·8 (55·2–96·0)

* 18 mg/kg of 6-MP daily for 4 days.
† Geometric mean reciprocal titres.
‡ Parentheses equal 95 per cent confidence limits.

While the results of the work recorded here are clear, it would not be logical to generalize from the effects of 6-MP on this immunologic system. It has recently been reported that 6-MP is capable of suppressing the development of 7*S* γ-globulin antibody to bovine γ-globulin but not 19*S* macroglobulin antibody to this antigen[7]. This work illustrates the hazards of generalizing from a single system, since the typical antibody produced in response to this dose of endotoxin is of the 19*S* variety and the suppressive action of 6-MP is readily manifested[4].

The differences between the present results and those previously reported[2,3] are probably due to the type of antibody response elicited by endotoxins. Both the previous reports of enhanced suppression by 6-MP with increasing doses of antigen were concerned with primary immune responses. Since normal rabbits have 'natural' (that is, bactericidal) antibodies to most endotoxins, including that used in these investigations, the elicited antibody responses are perhaps of the secondary type. Another possible explanation of the described effects is the distinctive character of the polysaccharide antigens when compared with that of proteins. That the results reported in these studies are probably typical for this category of complex polysaccharides is attested to by the fact that 6-MP will suppress the antibody response to the antigens of *S. enteriditis* and *Escherichia coli*[4]. Work on antimetabolites may thus help to delineate differences in immune response to different types of antigen (that is, protein versus polysaccharide) as well as the characteristics of primary and secondary immune responses.

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Permeability Factor in Pollen Extracts Active in Non-sensitized Mice

MOST of the recent work on hypersensitivity to pollens has been concerned with the isolation and characterization of the pollen allergens. The presence of biologically active materials in pollens, other than allergens, has been overlooked. The results presented here, however, demonstrate that pollen extracts contain a factor which increases vascular permeability in normal non-sensitized mice. Permeability factor was first detected by injecting Swiss Webster 20 g female mice intravenously with a mixture

Table 1. ATTEMPTS TO PRODUCE INCREASED VASCULAR PERMEABILITY IN THE EARS OF MICE BY INJECTING THE ANIMALS INTRAVENOUSLY WITH 0·2 ML. OF 0·5 PER CENT EVANS BLUE DYE AND VARIOUS AGENTS

Agent	Dose (μg)	Blueing of the ears*
Bradykinin	10†	–
E. coli endotoxin (Difco)	2·5–250	–
Crotalus atrox venom	1·5–12·5	–
Trypsin	1,000	–
Alcohol-precipitable *Lolium multiflorum* (APL)	20‡	+
48/80	35‡	+
Histamine	30‡	+
Serotonin	10‡	+
Antigen (BSA in BSA-sensitized mice)	10	+

* Maximal blueing occurred within 15 min and persisted for days.

† Maximal amount that could be tested due to the toxicity of the preparation.

‡ Approximate ED_{50}.

of 0·1 ml. of rye grass pollen extract and 0·2 ml. of 0·5 per cent Evans blue dye. Five min after the administration of dye plus pollen, a few blue spots appeared near the base of the ears; within 15 min large areas of the surface of the ears appeared deep blue. Control animals injected with dye alone showed no blueing of the ears. The most active batch of permeability factor (ED_{50} 20 μg) has been prepared from Italian rye grass (*Lolium multiflorum*). The permeability factor is precipitated from an aqueous extract of this pollen by ethanol containing 6 per cent potassium chloride. The permeability factor present in alcohol-precipitable *Lolium* is a non-dialysable very stable material. Its permeability promoting activity is unaffected by treatment with the following agents: boiling water for 30 min; 8 M urea, 4 h; conc. ammonium hydroxide, 1/2 h; α-amylase, 37° C, 4 h; pronase, 37° C, 4 h. Lyophilized preparations of alcohol-precipitable *Lolium* have retained their activity for months. The data in Table 1 compare the permeability enhancing ability of alcohol-precipitable *Lolium* with that of known permeability factors using the ear blueing technique. It can be seen that blueing did not occur in mice injected with dye plus one of the following: bradykinin, *E. coli* endotoxin (Difco), *Crotalus atrox* venom, and trypsin. Ear-blueing occurred in mice sensitized with bovine serum albumin and afterwards challenged with bovine serum albumin, as well as in mice injected with dye plus either of the following: serotonin, histamine, or 48/80. Serotonin produced a marked blueing within seconds after its administration, while the blueing produced by histamine was faint and diffuse. After the injection of 300 μg of alcohol-precipitable *Lolium* the histamine content of the ears of mice dropped from 150 μg/g to 40 μg/g. Ear-blueing was inhibited by 10 μg of the antihistamine 'Neoantergan' or 50 μg of the antiserotonin drug '*UML* 491' (Sandoz). Although an antihistamine inhibits ear-blueing due to alcohol-precipitable *Lolium* in normal mice, it does not inhibit ear-blueing produced in bovine serum albumin-sensitized mice challenged intravenously with bovine serum albumin. It thus seems unlikely that ear-blueing from alcohol-precipitable *Lolium* is due to the presence of antibodies to alcohol-precipitable *Lolium* in the sera of normal mice. No hæmagglutinating antibodies to alcohol-precipitable *Lolium* have been found in the sera of normal mice, and the incubation of alcohol-precipitable *Lolium* with normal mouse serum prior to its injection does not result in neutralization of the ability of alcohol-precipitable *Lolium* to increase vascular permeability.

Preparations of alcohol-precipitable *Lolium* contain pollen allergens as well as permeability factors. Allergenic activity is demonstrated by the ability of alcohol-precipitable *Lolium* to induce passive cutaneous anaphylaxis in mice injected intradermally with a mouse antiserum to rye grass pollen. Thus far it has not been possible to separate the permeability factor from the components of alcohol-precipitable *Lolium* which are allergenic in mice. Treatment of alcohol-precipitable *Lolium* with acid, alkali, heat, enzymes, 8 M urea, etc., has affected allergens and permeability factors in a similar manner. Chromatographic fractions of alcohol-precipitable *Lolium* obtained from DEAE and DEAE-'Sephadex' columns gave positive biuret and orcinol reactions and produced both blueing of the ears and positive passive cutaneous anaphylactic reactions. It is known that grass pollen allergens are polypeptides with a high carbohydrate content[1], and it is possible that the permeability-promoting activity of alcohol-precipitable *Lolium* resides in molecules of a similar composition. Sehon and Richter[2] have suggested that complexes of pollen allergens are continually breaking down during fractionation. The permeability factor may have indeed been produced by such a process. The presence of a permeability factor in rye grass pollen which acts by releasing histamine suggests that 'specific' desensitization to pollens in man may to some extent be a non-specific procedure dependent on the induction of tissue refractoriness to histamine. Feinberg *et al.*[3] demonstrated that the repeated injection of 48/80 in human skin resulted in a local refractoriness to the allergic response to ragweed or grass pollen.

The role of permeability factors in the unreliability of skin tests to pollens in humans has not yet been determined. Alcohol precipitable *Solium* will increase vascular permeability when injected directly into unsensitized mouse skin (ED_{50}, about 50 μg). However, it must be admitted that this dose far exceeds the amounts needed to produce a positive skin test in an allergic individual.

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Immunofluorescent Detection of Proliferating Human Antibody-forming Cells

DIFFUSION chamber cultivation studies with human lymph-node tissues have shown a high proliferative activity of the immune cell population immediately before and during antibody production as indicated by the incorporation of tritiated thymidine *in vitro*[1]. It became of interest, therefore, to determine the proliferative capacity of the antibody-containing cells as compared with the cells in the same population which were not forming antibody. This was done by using both *in vitro* and *in vivo* exposure of the cultivated cells to tritiated thymidine and subsequent staining of the cells for antibody by immunofluorescence followed by autoradiography.

Human lymph-node cells stored at liquid nitrogen temperatures and known to have the capacity to form antibody to *S. typhosa* antigen by previous tests[2] were used for this investigation. Nucleated cells obtained from storage were thawed and washed 3 times with Medium 199 to remove the preservative, dimethyl sulphoxide. They were then incubated *in vitro* with *S. typhi* antigen in a cell : antigen ratio of 1 : 2. Aliquots of this mixture containing 10×10^6 lymph-node cells were placed in diffusion chambers for subsequent cultivation in X-irradiated (600 r.) mice. One group of chamber recipients was injected with 4 doses of 5 μc. of tritiated thymidine intraperitoneally, beginning 6 days after implantation and at 12–24 h intervals over a period of 2·25 days. These intervals of injection were chosen because it had previously been observed that antibody is not detected in the chamber fluid in this system until day 7 and that the blast cells reach their peak at 6 days after cultivation[2]. A second group received no *in vivo* exposure to tritiated thymidine, but the cells obtained from these chambers at the time of removal were incubated *in vitro* with 0·5 μc. tritiated thymidine for 1 h at 37° C. The animals in each group were killed at 10, 11, and 12 days post-treatment, chamber fluid was recovered, and the cells were stained with immunofluorescent reagent. Immunohistochemical staining was performed by the double-layer technique[3] as follows: Cells from the chambers were smeared over a 10-mm diameter in the centre of a glass slide with a Pasteur pipette, air-dried, and fixed in acetone for 15 min. A drop of a 0·03 per cent flagellin solution *p*H 7·0 extracted from the flagella of *S. typhi* cultures was placed on the cells and the slide was incubated in a moist chamber for 45 min at 25° C. (The extracted flagellin protein from typhoid H flagella was kindly supplied by Dr. Frank F. Fitch, Medical School, University of Chicago.) The slides were washed in 0·01 M phosphate buffered saline *p*H 7·2 and then layered for 45 min with 1 drop of a globulin fraction of serum, which had been obtained from rabbits hyperimmunized with multiple injections of *S. typhosa* vaccine and conjugated with fluorescein isothiocyanate according to the method of Riggs *et al.*[4]. The conjugated reagent had been absorbed twice with mouse liver and spleen powder immediately before use to avoid non-specific staining. The slides were washed as before, mounted in 0·01 M phosphate buffered glycerol *p*H 7·2, and observed for fluorescence with the standard Zeiss fluorescence microscope. After immunofluorescent treatment, autoradiograms of the cells were made with exposures of about 1 week and then stained with Giemsa[5].

Table 1. FREQUENCY OF TRITIATED THYMIDINE (^{3}HT) INCORPORATION BY HUMAN ANTIBODY-FORMING LYMPH-NODE CELLS CULTURED IN DIFFUSION CHAMBERS IN X-IRRADIATED (600 r.) MICE AFTER *in vitro* AND *in vivo* EXPOSURE TO TRITIATED THYMIDINE (^{3}HT)

Time after *Ag* stimulation (days)	*In vitro*			*In vivo*		
	$\log_2$ titre*	^{3}HT-positive *Ab* cells (%)	Total ^{3}HT-positive cells (%)	$\log_2$ titre*	^{3}HT-positive *Ab* cells (%)	Total ^{3}HT-positive cells (%)
10	9	14·3	2·0	4	75·0	18·6
11	9	—	0·7	8	87·5	32·5
12	9	0·0	0·7	10	92·3	30·8

* Titration of chamber fluid involved 2-fold serial dilutions in 0·1 ml. saline, addition of 0·1 ml. typhoid *H* antigen, incubation at 56° C for 2 h in a water bath and at 4° C for 18 h. Titre is expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible agglutination.

Ab = antibody; *Ag* = antigen.

All chambers had fluids that were positive for agglutinins against typhoid *H* antigen and yielded cells the cytoplasm of which stained a brilliant green with the immunofluorescent reagent, indicating the presence of precipitating antibody against the extracted protein of typhoid *H* flagella antigen in the cells. Subsequent autoradiographic analysis of the cells showed that not only were antibody-containing cells capable of incorporating the radioactive compound in their nuclei after *in vitro* incubation at 10 days, but also that they did so at a higher frequency than the cells of the total population, the frequency being 7 times greater in the antibody-containing cells (14·3–2 per cent) (Table 1). At 12 days, however, none of the antibody-containing cells incorporated tritiated thymidine, this being comparable to the amount of incorporation (0·7 per cent) seen in cells of the total population at the same time interval. After *in vivo* exposure to the radioactive compound the frequency of tritiated thymidine labelling in the antibody-containing cells increased from 75 to 92 per cent on days 10–12 and was 3–4 times greater than the cells of the total population (Table 1). Fig. 1 shows a representative human antibody-forming cell labelled with tritiated thymidine.

These results indicate: (*a*) that the competent human antibody-containing cells proliferate at a greater frequency than the incompetent cells during the early phases of antibody synthesis and that the rate of proliferation is reduced to normal levels during the later phases of the antibody response; (*b*) that these mature antibody-containing cells are derived from precursor cells through somatic division. These findings are compatible with those of Urso and Makinodan[5], who used the rabbit anti-BSA : BSA system with pre-immunized spleen and lymph-node cells cultured in diffusion chambers. The present investigation shows that the diffusion-chamber technique in conjunction with immunofluorescent analysis is feasible for studying cellular dynamics of human antibody-forming tissue after stimulation with an antigen.

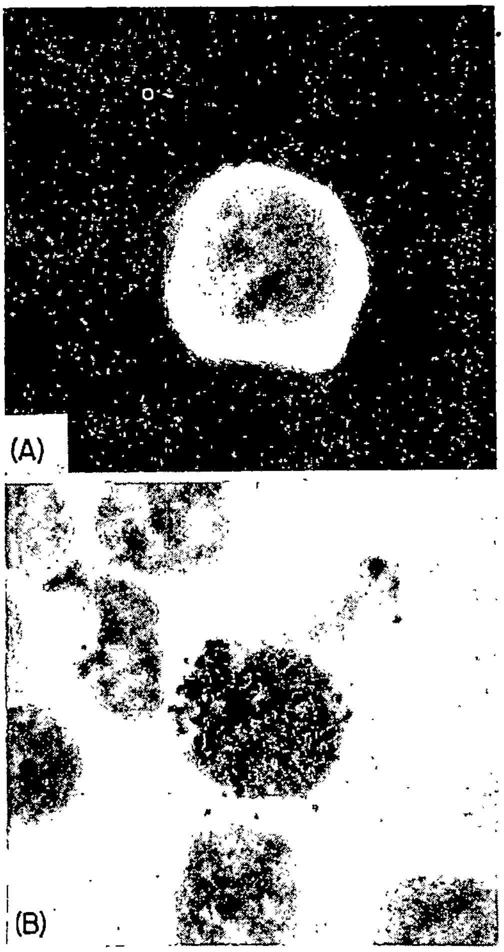

Fig. 1. Photomicrograph of antibody-containing cell stained with immunofluorescent reagent (*A*) and labelled with tritiated thymidine (*B*)

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RADIOBIOLOGY

Effect of Chemical Protective Agents (Cysteamine and β-Aminoethylisothiuronium (AET) Cl HCl) and Homologous Testes-DNA on the Death-rate of the Progeny of a First Generation originating from Male Rats irradiated in the Infantile Period

It is well known that irradiation causes death of the progeny of adult irradiated animals. For example, Osipovskii[1] found that the death-rate of the progeny of males irradiated with an X-ray dose of 360 r. reaches 53 per cent. Hertwig[2] found that the percentage of dead progeny in the period from their birth to the seventy-fifth day of age is considerably higher than later. Strandskow[3] also found an increased death-rate in the progeny of irradiated male guinea pigs for the period up to 30 days of age. Henson[3] also observed the death-rate of the progeny of male rats irradiated with an X-ray dose of 100–1,000 r. during their lactation period. It has been found that 46 per cent of the progeny live until the end of the lactation period. Observing the changes on the progeny of male mice irradiated with 25 MeV through five generations, Proshina[4] found the highest death-rate in the first generation and in the first month of life.

In our experiments, Wistar male rats were X-irradiated in the infantile period (8 and 17 days old) with a dose of 600 r. and mated with normal females when they became sexually matured. The death-rate of the progeny of the first generation was observed up to four months of age. The percentage of deaths of the progeny of normal parents observed in the course of one year was 3 per cent.

The death-rate was observed in the progeny of males: (*a*) irradiated with 600 r.; (*b*) treated with cysteamine and irradiated; (*c*) treated with AET and irradiated; (*d*) irradiated and treated with homologous testes-DNA.

The results are shown in Fig. 1.

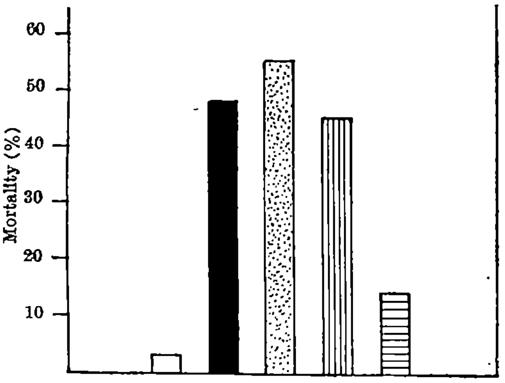

Fig. 1. Death-rate of the progeny of the first generation

Number of the progeny observed: 130 irradiated with 600 r.; 184 treated with cysteamine and irradiated; 185 treated with AET and irradiated; 326 irradiated with 600 r. and treated with homologous testes-DNA

White, normal; black, controls; stippled, cysteamine; vertical hatching, AET Cl HCl; horizontal hatching, DNA

Fig. 1 shows a high percentage of deaths in the progeny of males irradiated in the infantile period mated with normal females, namely, 48 per cent of irradiated males; 55 per cent of males protected with cysteamine before irradiation; 45 per cent of males protected with AET before irradiation; 14 per cent of males treated with testes-DNA after irradiation.

Sometimes it happens that all the animals of the litter die and sometimes only some of them. Autopsy did not reveal the cause of death. It was noticed that the main progeny which died were dwarfs.

If the death of the progeny of irradiated animals and those protected with chemical agents in the F_1 generation is considered to be the result of dominant lethal mutations, then our results show that neither cysteamine nor AET can prevent the occurrence of mutations, which in the F_1 generation are expressed as dominantly lethal. The results obtained in this respect are contradictory. Namely, examining cysteamine, Kaplan and Lyon[5] found that it does not influence the induction of dominantly lethal mutations in different post-meiotic stages. On the other hand, Lüning and Frölen[6] show that cysteamine can be a protective agent against genetic injury because it reduces the death-rate in the post-implantation period.

In addition, our results show that testes-DNA reduces the percentage of deaths and the values are closer to normal.

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In vivo Migration of Colony-forming Units from Shielded Bone Marrow in the Irradiated Mouse

The early bone marrow and spleen shielding experiments of Storer[1], Kaplan[2] and Jacobson[3] first indicated that hæmatopoietic stem cells were capable of migration and repopulation of X-irradiation depleted hæmatopoietic tissues. Popp[4] and Goodman[5] then showed that buffy coat obtained from the peripheral blood of mice contained cells capable of producing mature erythrocytes, lymphocytes and granulocytes. Trobaugh[6] has recently demonstrated that peripheral blood of the mouse contains cells capable of forming colonies in the spleen of irradiated recipients. He finds peripheral blood contains about 1 per cent of the number of colony-forming units (CFU) found in bone marrow, when comparing total nucleated cells injected. Hellman[7] reports granulocyte counts in the peripheral blood of the mouse recovered more slowly when partial body irradiation is given during 2 h, when compared with similar dosage delivered in a few minutes. He concludes this is due to killing of circulating stem cells.

Utilizing the ability of stem cells to form nodules in the irradiated spleen as initially shown by McCulloch and Till[8], combined with short-term shielding of a portion of the bone marrow, it has been possible to obtain a more quantitative estimate of the rate of release of stem cells to the circulating blood in the thigh shielded irradiated mouse, and to determine the changes in this release process following sub-lethal whole-body irradiation.

Two separate experiments were performed with each of two inbred strains. These were 8–10-month-old retired *L* female breeders and 14–16-week-old *LAF*-1 females. In the first experiment, groups of mice were anæsthetized with intraperitoneal pentobarbital and given 900 r. whole-body irradiation with an 8-mm wide, 1·5-mm thick circular lead thigh shield held in place by masking tape. At times of 2–7 h later the mice were again anæsthetized and given 900 r. to the shielded extremity. Eight days following irradiation, survivors were killed and their spleens removed and fixed for counting of nodules with the aid of a dissecting microscope.

In the second experiment, the mice were anæsthetized and given 300 r. whole-body irradiation with a 16-mm wide lead thigh shield in place. Approximately 48 h later, they were again anæsthetized and given 800 r. whole-body irradiation with an 8-mm wide lead thigh shield in place. Two to 3 h later they were given 900 r. to the shielded extremity, then maintained for 8 days after which their spleens were gathered for nodule counting. The larger thigh shield was used initially to avoid misplacement of the thigh shield used in the second part of the experiment. Irradiation factors were 250 kVp, 35 cm TSD, 1·28 mm copper, half-value layer, 225 r./min.

Bone marrow was thoroughly removed from 7-mm sections of femur that corresponded to the area shielded in the irradiated groups. Twenty femurs obtained from ten 15-week-old *LAF*-1 female mice, and twenty from ten retired *L* female breeders, were examined. The 7-mm segments of these were found to contain a similar mean of $1·9 \times 10^7$ nucleated cells (*L* female range $1·2 \times 10^7$–$2·7 \times 10^7$, *LAF*-1 female range $1·5 \times 10^7$–$2·4 \times 10^7$).

Fig. 1 shows the number of spleen colonies counted 8 days after a portion of the thigh was shielded for an interval of between 1 and 7 h in lethally irradiated adult *L* female mice. The mean for each time interval is plotted; the bar includes two standard deviations, and the total number of spleens used in determining a point is given in parenthesis. The control value of 0·3 nodules per spleen is also shown. This plot indicates that approximately 1·8 CFU appear in the spleen during each hour following thigh shielding, up to a total of 7 h.

Fig. 2 is a similar plot of the results obtained in young adult *LAF*-1 female mice. The control count of 0·2 nodules is indicated. This rate of CFU appearance in the spleen also appears to be constant at approximately 1·9 CFU/h of exposure to shielded thigh bone marrow.

Fig. 3 indicates the values obtained for CFU migration to the spleen from the bone marrow of a shielded thigh 48 h after an initial dose of 300 r. whole-body irradiation. The values for *L* female and *LAF*-1 female mice are combined. The rate of appearance of CFU in the spleen is 1·6 nodules/h of exposure to shielded thigh bone marrow.

The *in vivo* migration of stem cells in the normal and irradiated mouse is well established. The rate of migration to the spleen from a shielded population of $1·9 \times 10^7$ nucleated marrow cells is constant during the first 7 h following whole-body irradiation, and is the same for the two strains of mice tested. The rate of release of CFU to the circulating blood from shielded marrow does not appear to have changed 48 h after an initial whole-body dose of 300 r. This implies that repopulation of irradiation-depleted bone marrow sites in the partially shielded mouse is not the result of increased release of stem cells by the shielded area.

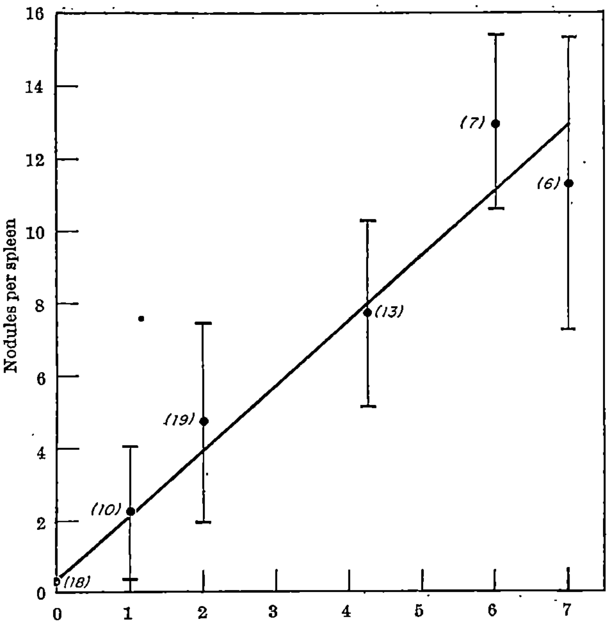

Fig. 1. Spleen nodules eight days after thigh shielding for various time intervals in lethally irradiated *L* female mice

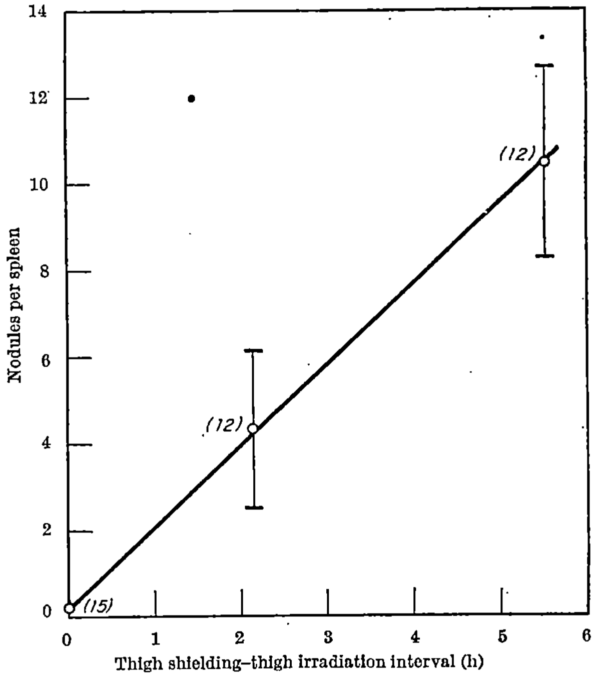

Fig. 2. Spleen nodules eight days after thigh shielding for various time intervals in lethally irradiated *LAF*-1 female mice

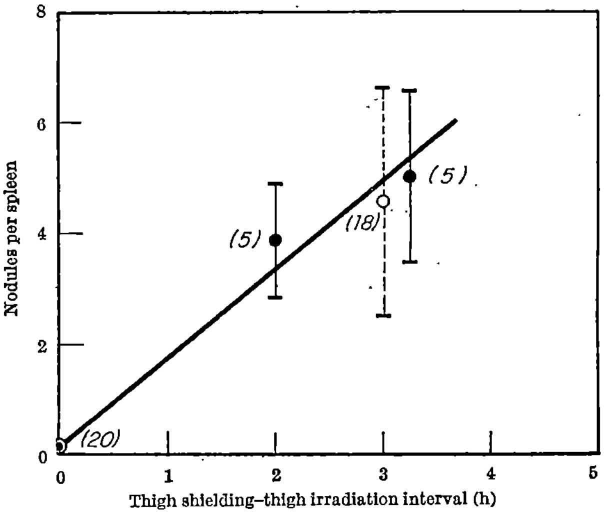

Fig. 3. Eight-day spleen nodules formed after thigh shielding for various time intervals, 48 h after 300 r. sub-total whole-body irradiation in lethally irradiated *L* female (●) and *LAF*-1 (○) female mice

There are two possible sources of error in determining these rates of release. The thigh shield used was not sufficient to attenuate the beam totally (4 per cent transmission) and the portion of shielded marrow that received side scatter cannot be calculated. Both these would result in migration rates lower than actually exist for a given shielded population. The values of 1·8 and 1·9 CFU/h for approximately $1·9 \times 10^7$ shielded cells must be regarded as minimum estimates.

The estimated shielded population is about 5 per cent of the total hæmatopoietic tissue. This means that for the total bone marrow 36 CFU would migrate to the spleen per hour. The only result available estimating the proportion of injected cells which settle in the spleen is that of McCulloch and Till[9]. They found that 1/6 of the injected CFU appeared in the spleen. If this proportion applies to the *in vivo* migration of CFU, it would indicate that more than 200 CFU enter the peripheral blood during any given hour.

Female *LAF*-1 mouse bone marrow contains 11 CFU per 10^5 nucleated cells[10], a value in good agreement with those reported by McCulloch and Till[11] for several other strains. Calculations from these results indicate that 1·9 × 10⁷ nucleated marrow cells would contain about 2,100 spleen CFU. Calculating from the percentage of injected CFU that settle in the spleen, it indicates this marrow population contains a total of about 12,600 CFU. The data show 1·8 spleen colony-forming units are released from this pool per hour, or 43 a day. The same calculation of CFU released but not deposited in the spleen indicates that a total of about 250 CFU (2 per cent of the marrow CFU content) enter the circulating blood each day.

Colony-forming units released *in vivo* by the bone marrow are expected to have considerable mechanical advantage for free circulation over cells injected in a single bolus. They will also be free of the damage which must accompany preparation and injection of single cell suspensions *in vitro*. This may result in a greater efficiency of viable colonization of the spleen, with corresponding changes in the estimates of total CFU entering the circulation per unit time, and the percentage of marrow CFU circulating during a 24-h period.

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Artefactual Chromatid Aberrations in Untreated and X-ray-treated Human Lymphocytes

RECENTLY, there has been a disquieting number of reports of observations of chromatid aberrations in human lymphocytes as a result of various treatments and conditions varying from measles to X-ray[1–4]. Although there has been no good reason to doubt the nature of these chromatid aberrations, neither has there been a good test of whether or not their induction could be attributed to phenomena other than the agents indicated in the various reports.

During an investigation of the relative *in vivo* radiosensitivity of the chromosomes of infants we observed chromatid aberrations in the chromosomes of their cultured lymphocytes following diagnostic X-ray procedures. These observations afforded us the opportunity for testing the interpretation of these and other similar reported observations as well as the relative sensitivity of the chromosomes of infants.

All chromosome investigations were carried out on samples of capillary blood using an adaptation of the method of Moorhead[5]. Replicate specimens were examned whenever possible and preparations were analysed n respect of numbers of cells containing chromosomal aberrations and the type of aberration, chromosome or hromatid.

Table 1 represents the results of the primary observaions on the chromosomes of infants following diagnostic X-ray procedures which included chest films, cinecystourethrogram, angiocardiogram and aortogram with a dosage range of approximately 2–20 r. The predominant aberration type observed was the chromatid deletion as seen in Fig. 1.

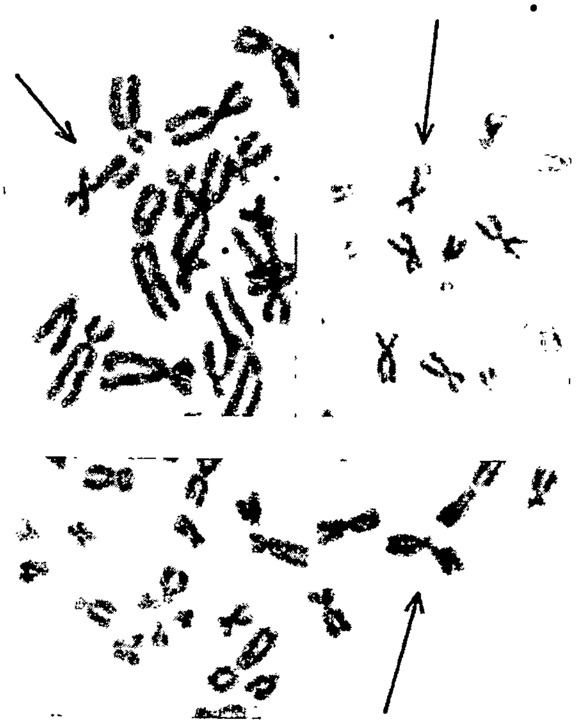

Fig. 1. Chromatid aberrations from metaphase lymphocytes from irradiated infants

A group of infant controls (Table 2) evidenced no comparably high frequency but showed considerable variability, ranging from 1·6 to 8·0 per cent. A subsequent examination of adult controls (Table 2) also showed considerable variability and, in addition, high frequencies of chromatid aberrations.

Three things are immediately obvious: (1) The frequencies of chromosomal aberrations in the X-rayed infants (Table 1) are higher than one might expect for the dosage of X-ray received[6,7]. (2) There are considerable numbers of aberrations in the control groups (Table 2) especially among the adults. (3) The predominant aberration type, the chromatid deletion, is totally unexpected, since previous investigations using tritiated thymidine[8] have demonstrated that, in adults, circulating lymphocytes are in a mitotic stage prior to DNA synthesis, that is, G_1. It is at this stage that we would expect the induction of chromosome type aberrations in cells exposed to X-ray[9]. One can explain the observation of chromatid deletions

Table 1. CHROMATID ABERRATIONS IN LEUCOCYTE CULTURES FROM IRRADIATED INFANTS

Subject	Approximate X-ray dosage (r.)	Cells scored	Chromatid type aberrations (%)
Infant 1, 13 days (♂)	20	313	24·3
Infant 2, 2 months (♂)	20	211	19·9
Infant 3, 5 weeks (♀)	Pre X-ray	130	16·9
	18	82	35·4
Infant 4, 10 days (♀)	Pre X-ray	125	4·8
	2–4	151	7·9

Table 2. CHROMATID ABERRATIONS IN LEUCOCYTE CULTURES FROM UNIRRADIATED, HEALTHY NEW-BORNS AND ADULTS

Subject	Cells scored	Chromatid type aberrations (%)
Infant 5, 5 days (♂)	64	1·6
6, 1 day (♀)	57	7·1
7, 3 days (♀)	37	2·7
8, 1 day (♂)	102	4·9
9, 2 days (♀)	150	8·0
Adult 1, 30 yr. (♂)	49	10·2
2, 40 yr. (♂)	100	12·0
3, 23 yr. (♀)	51	21·6
4, 32 yr. (♀)	95	17·9

in two ways: either they are artefacts or they are derived from cells representing a population of cells, which, in infants, are in G_2, that is, subsequent to DNA synthesis. The presence of chromatid deletions in the adult controls tends to negate the latter argument for reasons presented here. Nevertheless, it is not a satisfactory demonstration that no substantial population of lymphocytes, in infants, is in a stage subsequent to DNA synthesis.

To examine this possibility, we offered tritiated thymidine (specific activity 6,000 μc./mM in a concentration of 1 μc./ml. of media) to the cultured lymphocytes of both infants and adults for 1 h at various times (48, 54, 64 h) following incubation. In both infant and adult more than 90 per cent of the interphase nuclei and metaphases showed evidence of thymidine uptake. In addition, infant lymphocytes were not sufficiently different from adult in rate, amount or timing of uptake to support any theory that they are in fact different. This demonstrates clearly that the majority of lymphocytes of the infant had not reached the G_2 period prior to incubation. Therefore, the chromatid deletions that we have observed were most probably not induced by X-ray treatment, nor were they likely to be induced in lymphocytes by any other previously implicated agent prior to the initiation of leucocyte culture, nor for 48 h following the initiation of culture.

Table 3. CHROMATID ABERRATIONS IN HUMAN LEUCOCYTE CULTURES IRRADIATED *in vitro*

		Cells scored	Chromatid type aberrations (%)	Chromosome type aberrations (%)	Iso. frag. (%)
Adult (♂)	30 r. G_1	100	9	—	—
	30 r. G_2	100	14	—	—
Adult (♀)	30 r. G_1	100	5	—	—
	30 r. G_2	100	15	—	—
Infant (♂)	30 r. G_1	50	14	—	2
	30 r. G_2	100	19	—	—
Infant (♀)	30 r. G_1	100	11	—	2
	30 r. G_2	200	17	—	4

A further demonstration of the artefactual nature of the chromatid deletions which we have observed is presented in Table 3. These are the results of an *in vitro* investigation of cultured lymphocytes of both adults and infants which have been exposed to 30 r. prior to incubation, and 5 h prior to gathering. These stages we designate G_1 and G_2 respectively on the basis of our tritium investigations and those of German[8]. In G_1, where no chromatid type deletions would be expected, there is a frequency constantly present but consistently lower than that in G_2, the stage in which chromatid aberrations would be expected to be induced. Consequently, the frequency of chromatid aberrations seen in G_1 must be interpreted as background and considered as a constant threat to the interpretation of these kind of results.

It should also be noted at this point that all the evidence indicates that there is no difference in relative radiosensitivity between lymphocyte chromosomes of four-day-old infants and forty-year-old adults, all of which exhibit a reasonably high sensitivity to insult by irradiation.

An investigation of the origin of the background aberrations is being completed at the time of writing. The exact process is as yet unknown; but they are probably initiated by technical procedures. Their presence in the absence of adequate controls should not be considered sufficient evidence of a response to previous insult by any agent. Moreover, merely showing localization of the breakage to specific areas of specific chromosomes does not lend credence to the observation since sensitivity to mechanical stress can be just as specific and may be the result of differential coiling and/or heterochromatic areas.

The kind of chromatid aberrations which can be differentiated from background and were never seen in the unirradiated control population were chromatid exchanges and iso-chromatid deletions with sister chromatid reunion. Assay of these aberration types provides much more reasonable evidence of treatment-induced damage.

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BIOLOGY

Effect of a Diet containing Sodium Deoxycholate on the Intestinal Mucosa of the Mouse

THE life-span of the mucosal cell of the intestinal villi is short; reported values range from about 1 to 2 days depending on the region and the species examined[1-5]. Neither the reasons for the extremely short life-span nor the factors that influence the life-span are understood.

For many years the importance of bacteria as a factor in the rate of intestinal cell attrition has been discussed. Dixon[6] demonstrated the importance of peristalsis in flushing the intestinal flora towards the cæcum. Paulley and Dixon[7] found that a period of reduced peristalsis, induced by injections of mecamylamine, resulted in partial denudation of the upper intestine. Abrams *et al.*[8] and Lesher *et al.*[9] have shown that the life-span of the intestinal cells is about twice as long in the germ-free mouse as in the conventionally raised mouse. However, it is not known how the presence of bacteria reduces the life-span of the cells.

Although the detergent properties of the bile acids have been known for a very long time, their possible role in influencing the rate of cell turn-over in the intestine appears to have been neglected. It is perhaps significant that the ileum, which is the site of absorption of the conjugated bile acids, is the region of the small intestine in which the mucosal cells have the shortest life-span. Of greater importance, particularly in relation to clinical conditions, is the fact that highly detergent and cytotoxic substances such as deoxycholic acid are produced in the intestinal tract by the microbial action on the conjugated bile acids. Norman and Grubb[10] showed that certain members of the intestinal flora hydrolysed the conjugated bile acids and that this occurs mainly in the cæcum. In the germ-free animal and with neomycin treatment deoxycholic acid is not produced[11,12].

While it has been shown that *in vitro* incubation with deoxycholic acid caused marked damage to the villi[13], and Sullivan demonstrated the importance of the bile acids in post-irradiation diarrhœa[14] and the release of mucin from goblet cells, there is little information on the *in vivo* effects of varying the quantity or type of bile acid in the diet. The preliminary results of an investigation of the effects of feeding mice a diet containing sodium deoxycholate are reported here.

BCF$_1$ mice, about 100 days of age, were maintained on: (1) a synthetic diet (casein, 18 per cent; corn oil, 10 per cent; sucrose, 68 per cent; salt mixture No. 2, U.S.P., 4 per cent; complete vitamin fortification mixture, Nutritional Biochemicals Corporation); (2) the same diet with 2 per cent sodium deoxycholate added. Mice were kept under observation for more than one month, and were killed at 2, 8 and 39 days after starting the diet. The mice fed the control synthetic diet showed no gross or microscopic changes. Mice in the experimental group appeared sick within two days; they were listless and their fur was ruffled. Two days later the mice were active and appeared normal except their fur was now oily. This change in the appearance of the fur gradually disappeared in about three weeks.

At death on the second day the upper intestine was more translucent and thinner than normal. The gall bladder was distended and at least three times the normal size. At eight days the gross appearance of the lower intestine was similar to that of the upper intestine at the early death interval. There was a striking hyperæmia of the entire length of the small intestine, but otherwise the appearance of the upper intestine was almost normal. The gall bladder was still markedly enlarged. At 39 days the intestine appeared normal, the gall bladder had decreased in size but the wall was thickened.

The microscopic appearance of the duodenal villi at the second day was abnormal (Fig. 2). There was separation of the mucosal cells from the underlying structures with œdema and proteinaceous material in the lamina propria. In the ileum at eight days there was partial denudation and a decrease in the number of goblet cells (Fig. 4). At later death intervals the appearance of the villi was almost normal and there was evidence of increased proliferation in the crypts. In the duodenum at three days there was an increase in the number of cells initially labelled with tritiated thymidine. There was marked increase in cell proliferation of the epithelium of the gall bladder. The results of the changes in cell proliferation will be published at a later date.

In general, the histological findings are similar to those reported by Nissim[15] on the effects of some surface-active compounds. We presume that the histological changes are due to the direct action of the deoxycholate on the surface of the mucosa. As the animals continue eating, the normal control mechanisms of bile flow presumably are active. Therefore, the marked increase in the size of the gall bladder is probably due to the choleretic effect of the deoxycholate.

In the germ-free animals no free acids are produced and conjugated bile acids are excreted. It has been found that the half-life of cholic acid was about five times longer than in the conventional animal[16]. The intestinal mucosa of the germ-free animal is exposed to less bile and to no bile acid metabolites and the life-span of the mucosal cells is considerably longer than in the conventional animal.

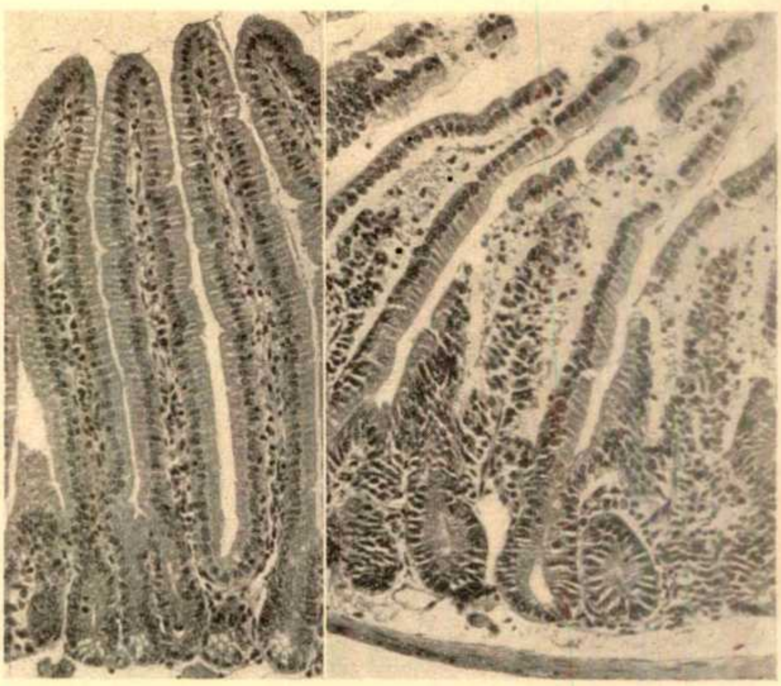
Fig. 1 Fig. 2

Fig. 1. Duodenum of mouse on control diet (× c. 160)

Fig. 2. Duodenum of mouse on diet containing 2 per cent sodium deoxycholate for two days (× c. 160)

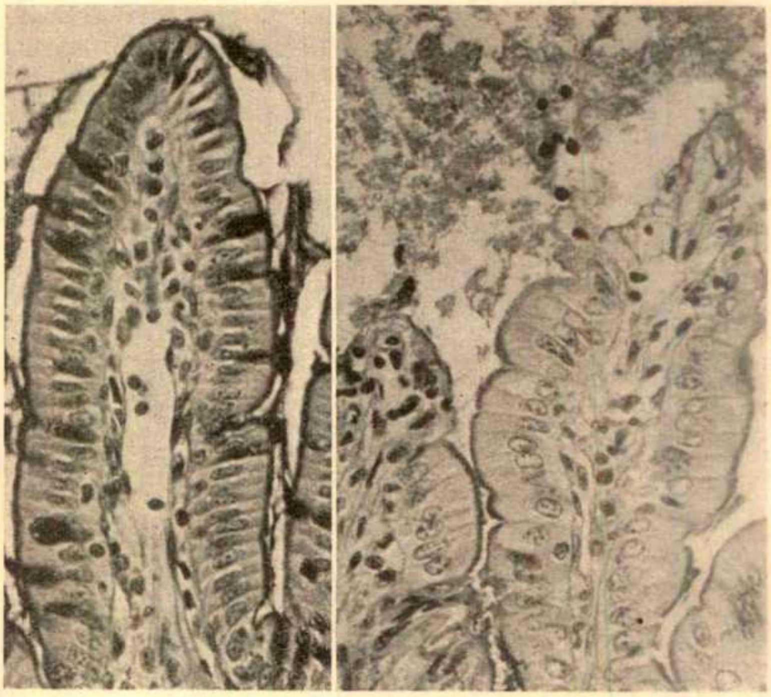
Fig. 3 Fig. 4

Fig. 3. Ileum of mouse on control diet (× c. 400)

Fig. 4. Ileum of mouse on diet containing 2 per cent sodium deoxycholate for eight days (× c. 400)

It seems a definite possibility that in conditions in which diarrhœa and partial denudation occur, and when no specific organism can be incriminated, the pathogenesis may be the following:

For some reason the motility of the gut is reduced; this results in a change in the distribution of the flora. The presence of sufficient numbers of bacteria capable of splitting the bile acids in the upper intestine results in amounts of metabolites that cause diarrhœa and in some cases damage to the mucosa. If these abnormal conditions were maintained for any length of time, steatorrhœa might occur because the amount of conjugated bile acids would be reduced due to the bacterial hydrolysis and the increased cell loss would add to the amount of fat in the lumen. Dawson and Isselbacher[11], and more anish[16], have suggested that the changes found he blind loop syndrome might be due to the production of bile salt metabolites by bacteria.

It is hoped that the use of the type of diet described will be useful for investigating the control of cell proliferation in both the mucosa of the small intestine and the gall bladder.

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Extension of Mammary Glands to the Popliteal and Cubital Fossæ

THE development and histology of the mouse mammary gland have been studied in detail[1-3]. The glands develop along the milk line and branch in the subcutaneous fat between the skin and skeletal muscle. The quantity of fat in the subcutaneous fat pad is greatest where the milk ducts later develop[4]. During lactation the distribution of the mammary glands visibly follows the subcutaneous fat tissue, the second inguinal mammary gland being reported to spread only into the inguino-genital area and the cubital only to the level of the fore-limbs.

During histological examination of the popliteal lymph nodes of *C57*, *BALB*, *Ajax* and *Swiss* normal female mice, mammary gland elements were observed in the surrounding fat and this finding led to an examination of both the popliteal and cubital fossæ in lactating mice. Seven lactating mice two weeks after delivery and five non-pregnant, non-lactating females were studied; in addition, four males were also examined.

Tissues were fixed in Zenker-formol and embedded in paraffin ; sections were cut at 7μ and stained by hæmatoxylin-eosin ; however, alum carmine stain was utilized for the 100-200μ sections.

Gross observation during lactation showed that the first pectoral mammary gland extends into the cubital fossa, and that the second inguinal mammary gland extends into the popliteal fossa along the lateral posterior part of the femur (Fig. 1).

This observation becomes reasonable when it is noted that the cranial end of the subcutaneous fat pad extends into the cubital fossa and the caudal end into the popliteal fossa.

Besides the mammary glands, sebaceous and sweat glands can be seen, and these extend into the connective tissue of the sub-cutis layer above the connective tissue lamina.

Generally, when the skin is dissected from the muscles, sebaceous and sweat glands are removed with it. The structures found in the cubital and popliteal fat pads, however, remain on the muscle layer, that is, they are located in the subcutaneous fat pads below the connective tissue lamina and above the skeletal muscle. The sweat glands, moreover, are only seen in the palmar aspect of the paws.

In the lactating animals numerous tubulo-alveolar structures were observed in tissue from both fossæ, each being lined by large cuboidal epithelial cells (Figs. 2 and 3*A*). The epithelium contained numerous small vacuoles and stained an uneven purple, depending on the amount of secretory granules and RNA in the protoplasm. The nuclei were spherical in shape and stained rather lightly; each contained chromatin clumps and a nucleolus. The lumen contained an eosinophilic granular substance in the centre surrounded by foamy matter. The histological appearance is identical to that of the mammary gland in the inguino-genital area.

During lactation the mammary gland is directly surrounded by capillaries[5] or collagenous fibres, the latter sometimes being seen in the spaces around the tubulo-alveolar components. However, most of these spaces are occupied by lipid depleted fat cells[6]. In lactation the capillary bed between the fat tissues is also clearly seen and plasma cells are found in the walls of the capillaries. Plasma cells are rarely present, however, in the non-lactating animals.

In the non-lactating female group, similar tubulo-alveolar structures were also observed in the popliteal fossa (Fig. 3*B*) but not always in the cubital fossa. The mammary epithelium in such instances is much smaller and nuclei stain more deeply compared to those in the lactating group ; in addition, the longer axis of the nucleus parallels the surface of the lumen and the cytoplasm stains lightly with eosin. Homogenous eosinophilic substance is occasionally seen in the lumen. The tubulo-alveolar structures were surrounded by normal fat cells, each containing a single large fat vacuole ; in such cases there is no lipid depletion (Fig. 3*B*). Tubulo-alveolar structures were not observed in the four normal males examined.

The relationship in lactating mice between morphological activation of the mammary gland epithelium, lipid depletion and the appearance of plasma cells is interesting and finds certain parallels in stress. Selye and Timiras[7] have described the discharge of lipid from the brown fat tissue as a characteristic reaction during stress.

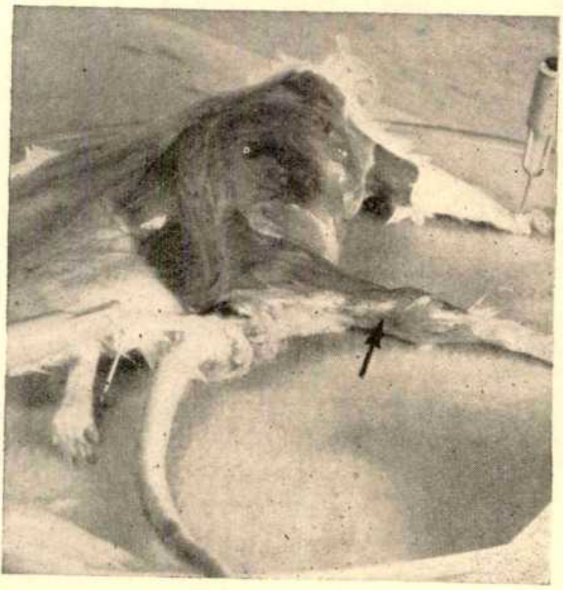

Fig. 1. Gross observation of the second inguinal mammary gland extending to the popliteal fossa. Lactating mouse

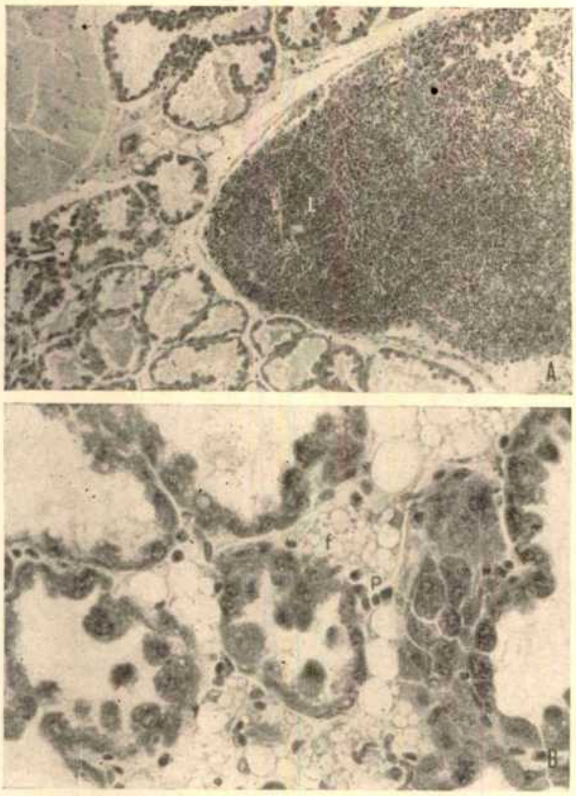

Fig. 2. Mammary glands in the popliteal fossa. Lactating mouse. (*A*) *L*, popliteal lymph node. (× *c*. 66.) (*B*) *f*, lipid depleted fat cell, *p*, plasma cell. (× *c*. 270.) Paraffin section fixed with Zenker-formol, stained with hæmatoxylin-eosin

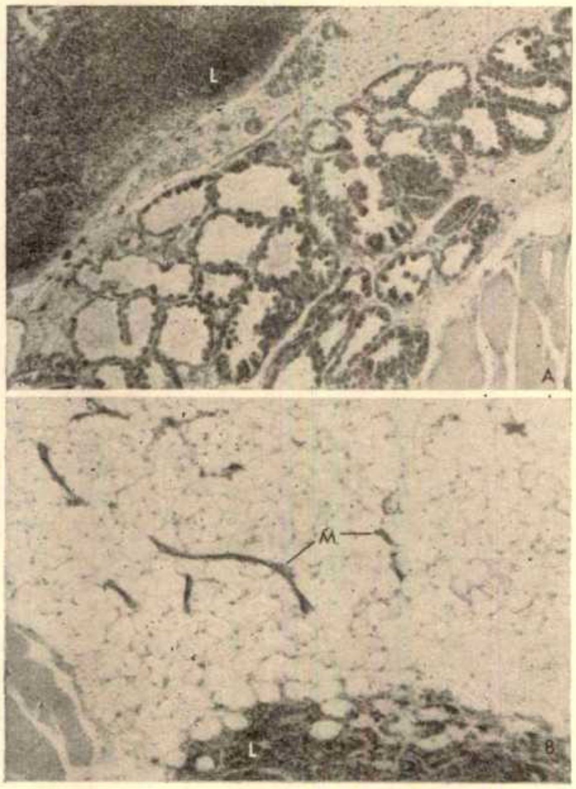

Fig. 3. (*A*) Mammary glands in the cubital fossa. Lactating mouse. *L*, lymph node. (× *c*. 66.) (*B*) Mammary glands in the popliteal fossa. Non-lactating female mouse. *L*, lymph node, *M*, mammary gland. (× *c*. 66.) Paraffin section fixed with Zenker-formol, stained with hæmatoxylin-eosin

White fat cells of mice show lipid depletion following persistent electric shock[8]; at the same time, plasma cell reaction and some activation of mammary gland epithelium are also seen[9]. This inverse relationship between plasma cell and fat cell in lactation and electric stress may be due, in part, to their common origin from the adventitial cells of the small blood vessels[10,11].

I thank Profs. W. R. Lyons, I. Monie, H. A. Bern, S. Nandi and Dr. L. Sparks for their advice; Drs. W. J. Fessel and O. Rambo for support; and Mrs. E. Brennan, Mr. D. Akers and Miss M. Kasahara for their assistance.

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Trichostrongylus colubriformis (a Nematode Parasite of Sheep and Other Ruminants) as a Test Organism in Screening for Sheep Anthelmintics in the Laboratory

THE development of anthelmintic screening tests has been guided by the need for a laboratory model of the host–parasite system for which new anthelmintics are being sought. Up to the present time, use has been made of nematodes naturally parasitic in rats and/or mice.

We felt that a screening test using a sheep nematode as a test organism in a small laboratory animal host would be more relevant to finding a new sheep anthelmintic.

We have carried out a series of experiments on the host–parasite relationships of *Trichostrongylus colubriformis* in rats, mice, guinea pigs, rabbits and Libyan gerbils (*Meriones libycus*). The initial source of infective larvæ was a pure line strain maintained in sheep. The experimental animals were exposed to the infestation by oral intubation of suspensions of known numbers of infective larvæ.

Guinea pigs were found to vary greatly in their susceptibility to infestation; a finding in agreement with Herlich *et al.*[1], Herlich[2], and Sturrock[3]. Rats and mice were found to be very susceptible, but insufficient numbers of ova were recovered from their fæces to maintain the parasite indefinitely in these animals. Rabbits, as shown by El Rawi[4] and Wood[5], proved to be suitable hosts for the laboratory maintenance of the life-cycle and we have serially passaged the infestation through more than fifty generations during the past thirty months.

As rabbits are not ideally suited to screening large numbers of compounds, we investigated the infestation in rats and mice more fully with the view of establishing an anthelmintic screening test, and experiments were carried out to determine the optimum conditions for infesting rats and mice. Young male animals were found to be the most susceptible. The infestation lasted only three to four weeks and the maximum numbers of adult worms were recovered from the small intestine 14 days after inoculation. The optimum size of inoculum was found to be 1,000 infective larvæ per animal. On the basis of these data we devised anthelmintic screening tests using *T. colubriformis* as a test organism in rats and mice. Male rats (weighing 50 g) and male mice (weighing 20 g) were each given 1,000 infective larvæ. Twelve days after inoculation the animals were pooled and randomly divided into dose groups. The test compounds were administered orally in aqueous solution or suspension at a single dose of approximately three-quarters of the acute oral LD_{50}. Forty-eight hours after dosing the test animals were killed and post-mortem examinations were carried out. The first quarter of the small intestine was removed, inverted over a length of glass capillary tubing and wholly immersed in a test-tube of warm saline. The preparations were incubated at 37° C for 2 h during which time the worms detached themselves from the mucosa. The contents of the test-tube were emptied into a squared dish and the number of worms was counted under a dissecting microscope.

The mouse and rat tests were evaluated by comparing their responses to a number of known sheep anthelmintics. The rat test appeared to be somewhat more sensitive than the mouse test and was chosen for comparison with the anthelmintic screening tests in general use, namely, the *Strongyloides ratti*- and *Nippostrongylus braziliense*-rat tests and the *Oxyuris*- and *Nematospiroides dubius*-mouse tests. The techniques used were essentially those quoted by Standen[6]. Five reference anthelmintics were tested as if they were unknown compounds in each of the screening tests shown in Table 1.

The response to phenothiazine was negative in all but the *Oxyuris*-mouse test and even here the results were variable. The *S. ratti*-rat test failed to select bephenium and the *N. dubius*-mouse test missed thiabendazole.

We have used all the foregoing tests at one time or another in screening several thousand compounds for anthelmintic activity. Compounds active in any of these tests have been selected for testing in sheep. Only 59 compounds, however, have been tested so far in all five screening tests as well as in sheep. False positives are a common feature of screening tests and, although they may result in wasted follow-up work, they are not such serious deficiencies as false negatives. A false negative means that a useful compound has been missed. Eight of the 59 compounds were found to be active in sheep; of these eight, only two are of the same chemical series.

Results of tests in small animals with these compounds are shown in Table 2. From these results, both the *N. dubius*-mouse test and the *T. colubriformis*-rat test are more relevant than the other rodent screening tests in selecting anthelmintics for use in sheep, having one and two false negatives respectively. The *N. dubius* test, however, does not respond to thiabendazole (the best of the sheep anthelmintics) whereas the *T. colubriformis* test does. *T. colubriformis* and *N. braziliense* tests were found to be somewhat complementary. By using both organisms in the same rat, the number of false negatives was reduced to one, and the system was sensitive to thiabendazole.

Table 1

	Screening test activity:				
	v. *Oxyuris*	v. *N. dubius*	v. *S. ratti*	v. *N. brazil.*	v. *T. col.*
Bephenium	–	+	–	+	+
Phenothiazine	±	–	–	–	–
Haloxon (0,0 di-(2-chloroethyl) 0-(3-chloro-4-methyl coumarin-7-yl) phosphate	+	+	+	+	+
Promintic (2-(2-methoxyethyl) pyridine)	–	+	+	+	+
Thiabendazole (2-(4-thiazolyl)-benzimidazole)	–	–	+	+	+

+ = active. – = inactive.

Table 2

Activity (+ = active; – = inactive)

	Small animal tests						
Compound	v. *Oxyuris*	v. *N. dubius*	v. *S. ratti*	v. *N. brazil.*	v. *T. col.*	v. combined *T. col.* *N. brazil.*	Sheep tests
A	–	+	–	–	+	+	+
B	+	–	–	–	–	–	+
C	–	+	–	+	–	+	+
D	–	+	–	–	+	+	+
E	–	+	–	+	+	+	+
F	–	+	–	–	+	+	+
G	–	+	+	–	+	+	+
H	+	+	+	+	+	+	+

Table 2 also shows that no one test is completely satisfactory.

Preliminary investigations using the Libyan gerbil have shown that this animal is a suitable laboratory host for both *T. colubriformis* and *T. axei*. This host–parasite combination could be a further improvement in that both a sheep abomasal and a small intestinal worm could be maintained and used for screening in the laboratory.

We thank Miss J. Crowley for supplying the initial pure line larval culture and for carrying out the tests in sheep.

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Circadian Control of Photoperiodism in the House Finch demonstrated by Interrupted-night Experiments

ALTHOUGH the reproductive cycle of many temperate zone birds is known to be dependent on photoperiod, the way in which birds measure the duration of the daily photoperiod is still uncertain[1]. In the house finch, *Carpodacus mexicanus*, testicular maturation can occur when birds are exposed to only 6 h of light in each cycle if the cycle durations are 12, 36, or 60 h, whereas no maturation occurs when the cycle durations are 24, 48, or 72 h[2]. These data appeared consistent with the hypothesis that a circadian rhythm of recurring phases of sensitivity to light is involved in the photoperiodic response[3]. If this is so, brief light periods administered during the darkness of non-inductive 24-, 48-, and 72-h cycles should also stimulate testicular maturation provided that the brief light periods are in appropriate relation to the basic circadian rhythm. Indeed, although the interpretation of interrupted-night experiments with animals has been controversial, recent experiments with plants show that flowering can be controlled by brief light periods in such a manner[4]. The following experiments, therefore, were designed to test the hypothesis of circadian control by exposing birds to non-stimulatory cycles of 24, 48, or 72 h with a main light period of 6 h during each cycle and interrupting the ensuing long dark periods with 1-h photoperiods.

Male house finches collected in August and early September, 1963, caged, and maintained as previously reported[2], were subjected to at least two months of daily cycles of 6 h of light and 18 h of darkness before experimentation. Ambient temperatures varied from 20° to 28° C. Birds were not in sound-proof chambers. Three experiments were conducted (Fig. 1). Exp. *A* began on November 22, 1963, and lasted 20 days. Control birds received repeated cycles of 6 h of light and 18 h of darkness, whereas the experimental birds received cycles of 6 h of light and 18 h of darkness plus an additional hour of light 12 h after the onset of the main 6-h photoperiod. Exp. *B* began on November 21, 1963, and lasted for 22 days. Control birds received repeated cycles of 6 h of light and

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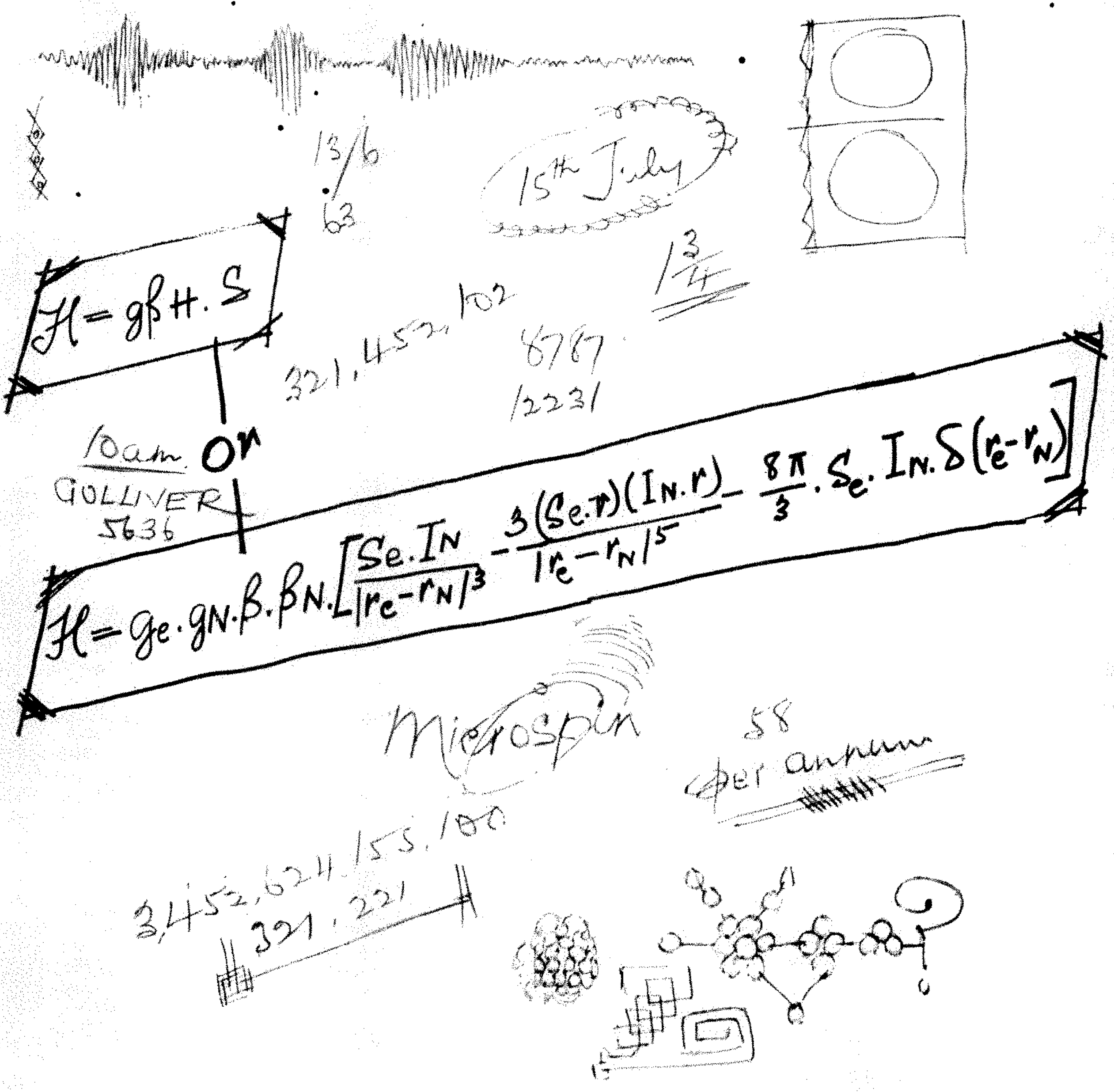
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42 h of darkness, comprising a basic 48-h cycle. The different groups of experimental birds received an additional hour of light 12, 24, or 36 h after the onset of the main 6-h photoperiod. Exp. *C* began on January 3, 1964, and continued for 54 days. Control birds received 6 h of light and 66 h of darkness in repeated 72-h cycles. The experimental groups received an additional hour of light 24, 36, or 48 h after the onset of the main 6-h photoperiod. At the termination of each of the above experiments the birds were unilaterally castrated, and released. Other procedural details are the same as has been reported elsewhere[2].

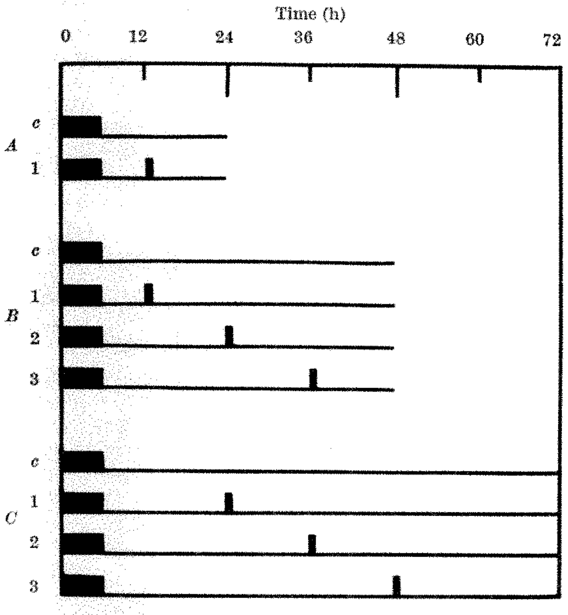

Fig. 1. Scheme of 3 experiments, *A*, *B* and *C*. The vertical bar represents the light period, while the horizontal line represents the dark period in each cycle. One hour of light was given during the dark periods at the time indicated

Exp. *A* is a typical interrupted-night experiment (Table 1). The control birds (maintained on a short-day cycle) did not show testicular enlargement. However, when one additional hour of light was given during the middle of the 18-h dark period the experimental birds behaved as if they had been exposed to long days even though the total amount of light (7 h) was substantially shorter than their critical day length (10–12 h; W. M. Hamner, unpublished results). This response is typical of many photoperiodically sensitive organisms in that the long-day response is obtained when the long dark period of an otherwise short day is interrupted with a brief exposure to light. Such a response does not require the introduction of the circadian rhythm hypothesis for its explanation, although the results are compatible with such an explanation. An alternative hypothesis, equally valid on the basis of these data, involves the postulation that the 1-h photoperiod interacts with the preceding 6-h light period which began the cycle[1].

In Exp. *B* the control birds received no additional light during the 42-h dark period of the repeated 48-h cycle and, consequently, testicular maturation did not occur. In the experimental groups, those which received a supplementary hour of light at the 12-h point exhibited the rapid testicular recrudescence seen in Exp. *A*. In contrast, light at the 24-h point was not stimulatory. On the basis of the circadian rhythm hypothesis this would be expected since this light, falling 24 h after the onset of the main 6-h photoperiod, was presumably in appropriate relation to the circadian rhythm entrained by the main photoperiod. According to this same argument, light at the 36-h point should be stimulatory, but only one of the 11 birds on this treatment showed testicular maturation (left testis weight, 13·2 mg). Although seemingly insignificant, the testicular enlargement of this single bird might be considered evidence that light at the 36-h point is mildly stimulatory since during those 22 days of treatment the birds actually received only 11 inductive cycles. Even if one assumes that all these 11 birds would have had larger testes with longer treatment, this 48-h cycle experiment would still not be a convincing demonstration of the circadian control hypothesis because, as has been argued for the 24-h cycle, light interactions with the preceding or succeeding main light periods could be adequate signals.

Table 1. WEIGHTS (MG) OF THE LEFT TESTIS OF HOUSE FINCHES FROM THREE EXPERIMENTS, *A*, *B* and *C*

c, control. No statistical treatment of these results has been presented, since it could be misleading

A		B				C			
c	1	*c*	1	2	3	*c*	1	2	3
1·0	11·5	1·1	16·0	1·2	3·1	3·2	0·5	4·6	1·7
0·8	15·9	0·8	13·4	0·8	2·2	20·3	0·3	1·4	2·0
0·6	8·6	0·6	7·2	0·2	2·0	0·9	1·9	3·9	0·7
0·5	5·3	1·2	0·9	0·4	0·3	0·5	0·8	8·2	2·1
1·2	6·9	0·6	5·2	0·5	1·1	3·0	0·8	10·3	2·7
1·0	1·6	1·6	7·5	1·6	2·0		0·8	37·1	4·1
0·5	15·7	0·5	2·8	0·9	0·7			22·6	
0·4	12·4	0·6	12·0	0·5	1·0			5·8	
0·6	13·5	0·3	14·1	0·4	0·4			31·4	
0·2	8·3	0·2	8·0	0·3	13·2				
0·6					2·4				

If light at the 36-h point is indeed weakly stimulatory, then an experimental treatment repeated for more than 22 days should be more effective. Exp. *C*, therefore, was continued for a longer time (54 days) than either Exp. *A* or *B*. The control birds received no supplemental light during the 66-h dark periods of the repeated 72-h cycles, and, with one exception, did not show overt testicular enlargement. Additional light at the 24- and 48-h points was apparently not stimulatory either, because none of the birds on these cycles had enlarged gonads. But when light was given at the 36-h point in the cycle testicular maturation occurred. The stimulation of testicular activity with 1 h of light at the 36-h point in a 72-h cycle, in contrast to the lack of stimulation at the 24- and 48-h points, seems to eliminate the possibility of a simple light interaction with the previous or succeeding main light periods and seems to provide a convincing demonstration of the validity of the circadian rhythm hypothesis.

According to the circadian hypothesis the control birds on the 72-h cycle in Exp. *C* should have shown no testicular maturation. However, one of these birds did respond (left testis weight, 20·3 mg). A possible explanation of this single deviation is inherent in this hypothesis. These rhythms are called circadian because they are not exactly 24 h in periodicity. Let us assume that the rhythm of this particular bird, entrained by the main photoperiod, was running a little faster or a little slower than 24 h during the 66-h dark period. It seems possible that its rhythm might accumulate this daily deviation during the almost 3-day dark period so that the succeeding main light period could actually interact with the recurrent light-sensitive phase of the rhythm to stimulate testicular maturation.

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Life-cycle of *Ectocarpus siliculosus* from Naples, Italy

During an attempt to grow sexual plants of *Ectocarpus siliculosus* in laboratory cultures, some observations were made which are not consistent with the present-day view of the life-cycle of this species.

Ectocarpus siliculosus was obtained from Naples in 1959. No unilocular sporangia were present on these plants. Swarmers from the plurilocular sporangia were isolated and developed to plants showing a distinctly different morphology: they had a well-developed basal system of rhizoids from which rises a great number of erect unbranched filaments up to 15 mm long (Fig. 1). When cultivated at 20° C these plants form plurilocular sporangia only, and their spores again give rise to the same plants. This cycle can be repeated indefinitely. When grown in 13° C, however, unilocular sporangia are formed[1,2]. One unilocular sporangium was isolated. The swarmers did not leave the sporangium but germinated inside, the germlings rupturing the sporangium wall in a later stage. From this accumulation of germlings eight filaments, each about 10 cells long, were cut off and isolated. These fragments regenerated to adult plants. Four of the regenerates were of the small, unbranched type, and cultivation at 13° C induces them to form unilocular sporangia. The other four regenerates were plants growing up to 20 cm long, with no noticeable basal system and typical pseudodichotomous branching (Fig. 2). These plants closely resemble the material originally obtained from Naples. Cultivation at 13° C does not induce the formation of unilocular sporangia on these plants. Plurilocular sporangia are readily formed, and the swarmers of these sporangia develop to plants of the small, unbranched type, which are again able to form unilocular sporangia at 13° C.

Three workers[3-5] have been concerned with the cytology of *Ectocarpus siliculosus*. They agree that the first nuclear division during the formation of the unilocular sporangium is the meiosis, and therefore the plants on which unilocular sporangia occur are diploid. At Woods Hole[5] and Naples[4] the swarmers leaving the unilocular sporangium are considered to be neutral and to give rise to the sexual generation. At the English coast[3] and at Rovigno, Yugoslavia[4], they are believed to act as gametes, the zygotes developing to new diploid plants. Where there is an alternation of generations in *Ectocarpus siliculosus* the two generations are believed to be identical in their morphological appearance[6]. At Naples, however, only the sexual plants are well known[7].

There are only two reports on *Ectocarpus siliculosus* with unilocular sporangia at Naples. Berthold[8] derived small plants with unilocular sporangia from zygotes. Schussnig and Kothbauer[4] found unilocular sporangia on plants which were only a few mm high. Thus it does not seem surprising that *Ectocarpus siliculosus* from Naples is able to perform two morphologically different phases in culture. As the large, branched plants were derived from a unilocular sporangium and as they closely resemble the well-known gametophytes at Naples[7], it seems likely that they represent the haploid gametophytic phase. To certify this, a large number of combinations of the swarmers from the plurilocular sporangia of these plants were made, but no sexual fusion has been observed so far. There was only one occasion when a combination of swarmers from all four clones resulted in about 1 per cent cells having two plastids and two eyespots. This might be taken as indirect evidence that the large branched plants are really gametophytes and their swarmers potential gametes. However, these swarmers readily germinate without any detectable fusion and develop exclusively as plants of the unbranched type, forming unilocular sporangia at 13° C. It would be interesting to know the cytological processes which accompany these events. The observations reported here suggest that the life-cycle of *Ectocarpus siliculosus* at Naples is considerably more complicated than was believed until now.

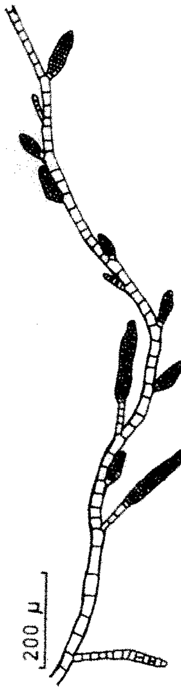

Fig. 1

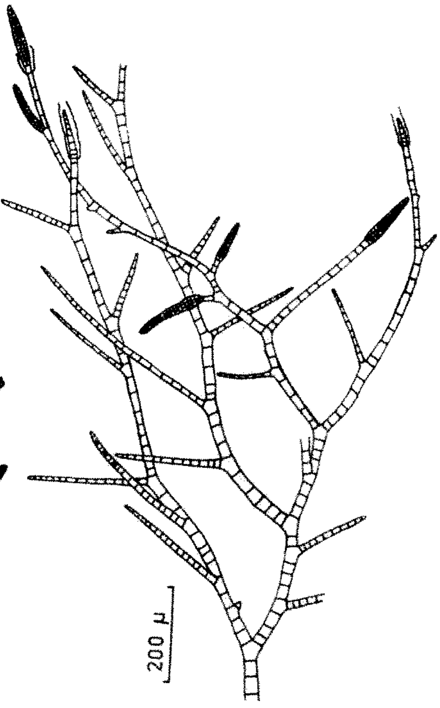

Fig. 2

Fig. 1. Part of a plant of the small, unbranched type, grown at 20° C, with plurilocular sporangia

Fig. 2. Part of a plant of the large, branched type, derived from the unilocular sporangium

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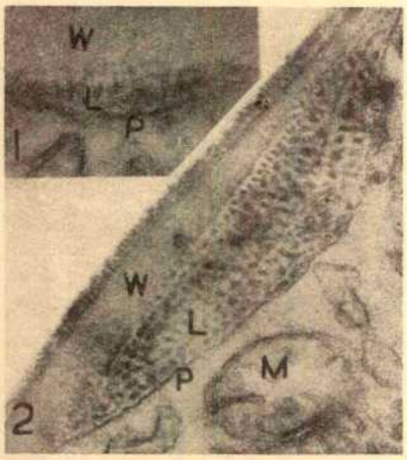
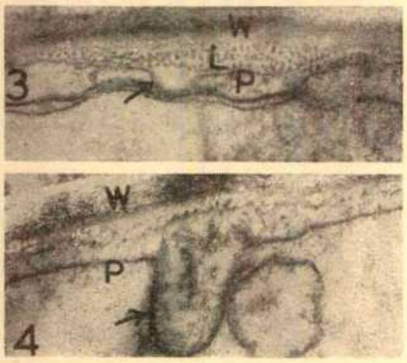
Occurrence of Lomasomes in Mesophyll Cells of 'Khapli' Wheat

Moore and McAlear coined the name 'lomasome' (λωμα—border; σωμα—body) to describe the sponge-like structures which they observed between the cell walls and plasmalemmas in the hyphæ of several species of fungi under the electron microscope. They considered that lomasomes were characteristic of, and peculiar to, the fungi and suggested that they might be of potential value in determining the phylogenetic origin of the Eumycota. This appears to be unlikely since we have found apparently identical structures in the mesophyll cells of wheat, *Triticum dicoccum* var. 'Khapli'.

Healthy primary leaves about 15 days old and free of any fungal infection were cut into small pieces, fixed in 2 per cent unbuffered potassium permanganate for 4 h at 0° C, dehydrated in an alcohol series and in propylene oxide and embedded in 'Epon'. Sections were cut, using a diamond knife, stained with lead acetate and examined in a Philips 100*B* electron microscope. Granular bodies between the cell walls and the plasma membranes of mesophyll cells were identified as lomasomes (Figs. 1 and 2). They appeared to originate from the fusion of intracellular vesicles with the plasmalemma and the formation of an opening resulting in a discharge of the contents of the vesicles from the protoplast, as suggested by Moore and McAlear[1]. Evidence of such a process was observed repeatedly and is illustrated in Figs. 3 and 4.



Figs. 1 and 2. Lomasomes (*L*) in mesophyll cells of 'Khapli' wheat. *M*, mitochondria; *P*, plasma membrane; *W*, cell wall (× *c*. 42,700 and 30,700 respectively)



Figs. 3 and 4. Fusion of vesicles (arrows) with plasma membranes and discharge of contents. (× *c*. 19,000 and 27,300 respectively)

We believe that these observations are the first to record the occurrence of lomasomes in a higher plant. It is noteworthy that so far we have not found either secretory vesicles or lomasomes in the mesophyll cells of another species of wheat, *Triticum compactum* var. 'Little Club'. This is surprising but suggests that their occurrence in this species is at least infrequent. 'Khapli' wheat is resistant, whereas 'Little Club' is susceptible to most of the known races of stem rust of wheat. Whether or not the rapidity and efficiency with which foreign substances can be excreted from mesophyll cells via vesicles have any connexion with rust resistance is not known, but the possibility is an intriguing one.

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ENTOMOLOGY

Guanine in the Excreta of the Boll Weevil

The common purine found in insect excreta is uric acid. Reports of other purines in Coleoptera are rare. Xanthine and hypoxanthine have been reported in Lepidoptera[1,2] and hypoxanthine in Diptera[3]. Ordinarily guanine would be expected to deaminate oxidatively to xanthine and undergo further oxidation to uric acid. Although we have found uric acid to be the principal nitrogenous excretory product (24 per cent of fæcal nitrogen) in the boll weevil, *Anthonomus grandis* Boheman, we have also located and identified the compound guanine.

Fæces were collected from adult weevils fed on a modification of Vanderzant's diet[4]. These were pooled, the lipids removed by washing three times with chloroform : methanol (2 : 1), then extracted with 0·5 N perchloric acid at 0°, neutralized with potassium hydroxide, and filtered. Aliquots of the extract were chromatographed singly and two-dimensionally on Whatman No. 1 filter paper. The systems used were aqueous 65 per cent (v/v) isopropanol 2 N with respect to hydrochloric acid[5], water *p*H 10 (Levenbook, ref. 6), and the upper phase of a mixture of *sec*-butanol and water[7]. Spots were located by ultra-violet light, and tentative identification was made by comparison with standard compounds in each system.

Spots were eluted with 0·1 N hydrochloric acid and again with phosphate buffer, *p*H 12·7, and their spectra determined by a Beckman *DK2A* spectrophotometer. Corroborative identifications were made from maxima,

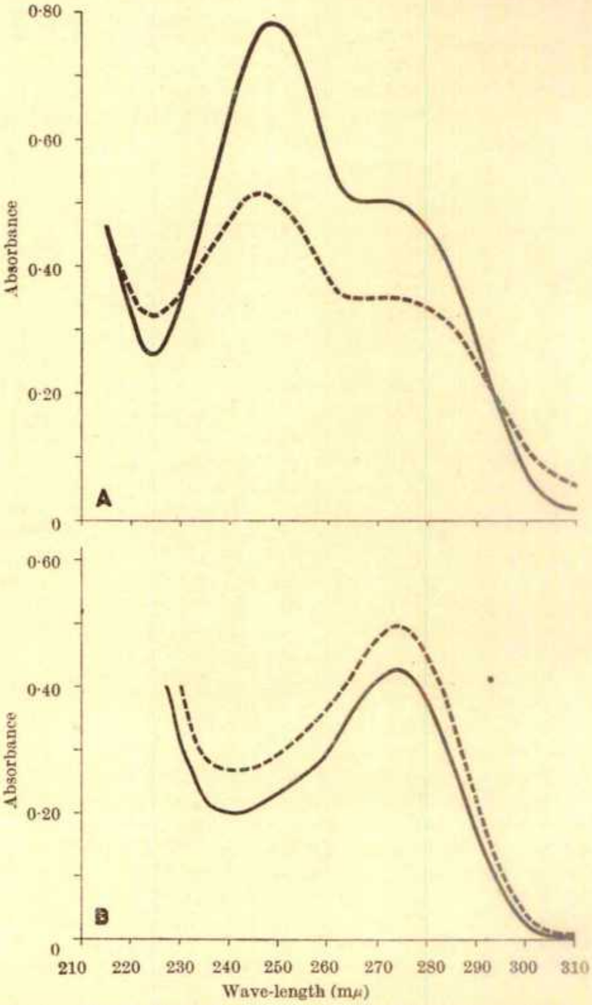

Fig. 1. Ultra-violet spectra of guanine derived from boll weevil fæces and a guanine standard. Solvents: *A*, 0·1 N hydrochloric acid; *B*, phosphate buffer, *p*H 12·7. ——, Standard; - - - -, extract

minima, and ratios of E 250/E 260 and E 280/E 260 at pH 1 and 12·7. These spectra, along with those of an authentic compound, are shown in Fig. 1.

Quantitative determination of the eluates at pH 1 was made spectrophotometrically by using differential extinction values[8]. Guanine accounted for about 3 per cent of the fæces nitrogen. This is an approximate value, since complete chromatographic separation was not achieved with any of the systems used.

In a determination of whether guanine might be excreted by other curculionids, the fæces of the plum curculio, *Conotrachelus nenuphar* (Herbst), fed on apples, were examined in exactly the same manner described here. No guanine, but xanthine, was found. Hence the finding of guanine in the boll weevil appeared to be unique and probably could not be ascribed to an artefact of the extraction procedure.

Accumulation of hypoxanthine in the excreta of *Drosophila melanogaster* Meigen has been ascribed to a deficiency of the enzyme xanthine dehydrogenase (oxidase)[9]. One could speculate that appearance of guanine might be the result of a deficiency of the enzyme guanase.

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GENETICS

Ethylation versus Methylation in Mutation of *Escherichia coli* and *Drosophila*

THE monofunctional ethylation reaction has been established as an inducer of genetic mutation in a number of test organisms. In particular, ethylation appeared to be unique among the alkylation reactions in its ability to induce mutation in the first lytic cycle following treatment of extracellular phage, since methylation, propylation, and other analogous reactions were not effective[1]. More recently, however, it was reported[2] that the methylation and propylation reactions do produce slight increases in mutation on extracellular phage, but at considerably lower frequencies than the ethylation reaction. Although such a distinct competence of the ethylation reaction does not appear to be preserved in bacteria and higher organisms, ethylation is nevertheless regarded as a more efficient inducer of mutation than other alkylation reactions.

The present communication reports on experiments designed to compare the relative mutagenic effectiveness of the ethylation and methylation reactions on the bacterium, *Escherichia coli*, and a multicellular organism, *Drosophila melanogaster*. On both test organisms, the ability of both alkyl groups to produce forward mutation (from wild-type to mutant) has been studied, since back-mutation (from mutant to wild-type) may involve molecular changes only capable of reversion by one of the reactions. The ethyl group was introduced via diethyl sulphate, the methyl group via dimethyl sulphate; both reagents were supplied by British Drug Houses Ltd. (B.D.H.) and contained 1·117–1·180 g/ml. and 1·32–1·33 g/ml., respectively.

(1) *Escherichia coli*. The ethylation reaction has been described as a potent inducer of genetic mutation in bacteria[3] under conditions where virtually all the cells survive the treatment, and the methylation reaction has been shown[4] to induce the reversion of certain bacterial mutants.

Comparison of the mutagenic effectiveness of the ethylation and methylation reactions on *E. coli* reported here is consequent on the treatment of logarithmically-growing cells of *E. coli* with dimethyl sulphate or diethyl sulphate, and scoring mutation from phage-sensitivity to phage-resistance.

E. coli B was grown in broth from an overnight culture to about 2×10^8 cells/ml. and resuspended for treatment in *M*9-buffer at about 8×10^8 cells/ml. Since neither diethyl sulphate nor dimethyl sulphate is miscible with water, dilution of the reagent was into 80 per cent ethyl alcohol before addition to the culture. At the concentration used for treatment (1×10^{-3} M), this involved diluting the reagents 100 times (0·1 ml.–9·9 ml.) and adding either 0·13 ml. (diethyl sulphate) or 0·095 ml. (dimethyl sulphate) to 10 ml. of the cell suspension; the control culture was identically handled and 0·13 ml. of the diluent was added. Treatment of the cells was for 15 min at 37° C, giving 100 per cent survival in the control and diethyl sulphate cultures, and 2 per cent survival in the dimethyl sulphate culture.

After treatment, the cells were diluted tenfold, washed, resuspended in broth at about 1×10^8 viable cells/ml., and aerated at 37° C. Samples were withdrawn immediately (zero time), and after various times of aeration, for viability counts and for scoring mutant phage *T*4-resistant (*B*/4) cells. Mutants were assayed by adding *T*4 phage at a multiplicity of between 50 and 100 for 5 min to a cell sample, spreading appropriate aliquots on to broth plates pre-spread with about 10^{10} phage particles, and incubating for at least 24 h at 37° C. Logarithmic growth of cells was preserved throughout the experiments by diluting the culture 20 times at zero time and after each two-hour period of aeration.

Mutation to phage-resistance is not expressed immediately on the cell (zero-point mutation), but only after several generations of growth. Usually at least six divisions of the cell are required for the complete phenotypic expression of phage-resistant cells (end-point mutation).

Table 1 illustrates the results of a typical experiment when the control, the diethyl sulphate-treated cells, and the dimethyl sulphate-treated cells are allowed to grow in broth over a period of 8 h; samples are taken at zero time and after each 2-h period of growth. While there is no indication of an increase in zero-point mutation (*B*/4 cells per 10^7 cells before treatment was 25), the end-point mutation is significantly higher after treatment with dimethyl sulphate compared with the control and treatment with diethyl sulphate. Since the expression of end-point mutation appears complete and constant after about 6 generations of growth, the mutation fre-

Table 1. THE MUTAGENIC ACTIVITY OF DIETHYL SULPHATE (DES) AND DIMETHYL SULPHATE (DMS) ON *Escherichia coli* STRAIN *B*
Molarity of DES = molarity of DMS = $1{\cdot}0 \times 10^{-3}$

Per cent survivors of treated cells: Control 100
DES 100
DMS 2

Time (h) of aeration in broth	No. of divisions (approximately)			*B*/4 per 10^7 cells		
	Control	DES	DMS	Control	DES	DMS
0	0·0	0·0	0·0	21	28	41
2	4·6	4·8	1·2	13	35	20
4	9·1	9·7	6·9	17	37	94
6	12·8	14·2	12·7	22	48	95
8	16·4	17·5	20·0	32	31	80

Table 2. THE MUTAGENIC ACTIVITY OF DIETHYL SULPHATE (DES) AND DIMETHYL SULPHATE (DMS) BY ADULT FEEDING OF *Drosophila* MALES (FIRST BROOD SAMPLE)

	Control	DES	DMS
Molarity of reagent	—	$3{\cdot}8 \times 10^{-4}$	$3{\cdot}8 \times 10^{-4}$
Duration of treatment (h)	24	24	24
Per cent survivors	98·0	84·0	78·0
Total number of sex-chromosomes examined	792	810	1,044
No. of lethal chromosomes	2	96	22
Percentage of sex-linked recessive lethal mutations	0·25	11·85	2·11

quencies ($B/4$ cells per 10^7 cells), averaged over the 4-, 6-, and 8-h periods of aeration, are control (24), diethyl sulphate (39), and dimethyl sulphate (90) ; both alkylating agents stimulate the rate of division of the cells compared with the control cells. The increase in the frequency of $B/4$ cells by dimethyl sulphate does not appear to be due to selection as a consequence of the higher kill, since a spontaneous and a 'dimethyl sulphate-induced $B/4$ mutant' were equally sensitive to treatment with dimethyl sulphate as the experimental culture.

Although a mutagenic effect of diethyl sulphate on *E. coli* at this concentration (1×10^{-3} M) may be questioned, its low toxic effect enables much higher doses to be tested. For example, the concentration of diethyl sulphate can be increased 100 times (to 0·1 M), giving 20 per cent survival of cells, and an end-point phage-resistant mutation frequency of about 500 $B/4$ cells per 10^7 cells ; there is no increase in zero-point mutation under these conditions.

At the test concentration (1×10^{-3} M), however, the methylation reaction produces a higher mutagenic effect than the ethylation reaction. The higher toxicity of the methylating compound does not permit much higher doses to be tested ; increase in the dimethyl sulphate concentration (to $1·5 \times 10^{-3}$ M) to give a greater kill (99·9 per cent) does not increase the mutation frequency much above 100 $B/4$ cells per 10^7 cells.

(2) *Drosophila melanogaster*. Previous work has shown that in *Drosophila melanogaster* both diethyl sulphate and dimethyl sulphate are mutagenic by a larval feeding method[5,7], and diethyl sulphate is mutagenic by an adult feeding method[6,7]. Work reported here on the relative mutagenic effectiveness of the methylation and ethylation reaction on *Drosophila* has been carried out using the adult feeding method.

The method is as follows : Several one-pint milk bottles, the bottoms of which were covered with a double thickness of filter paper, had the filter paper saturated with a solution containing 2 parts of distilled water to 1 part of absolute alcohol, 5 per cent glucose, and either diethyl sulphate or dimethyl sulphate to give a concentration of $3·8 \times 10^{-4}$ M. Since neither alkylating agent is miscible with water, alcohol was present in the treatment solution to disperse the globules otherwise formed. The filter paper was kept saturated during the period of treatment by adding 3 drops of the treatment solution 4 times daily.

Fifty newly-emerged wild-type 'Oregon-K' adult males were starved for 24 h, and placed for 24 h in one of the treatment bottles ; the treatment solution was the sole supply of nourishment during this time. After treatment, the males were examined for sex-linked recessive lethal mutations by the Muller-5 (*Basc*) method, using a brooding pattern where each treated male was individually mated to 2 females for 3 days.

The first brood samples recorded in Table 2 represent the mutational responses to adult feeding of diethyl sulphate and dimethyl sulphate on germ cells which were predominantly present as mature spermatozoa at the time of treatment. Diethyl sulphate is seen to be 5–6 times more effective than dimethyl sulphate in inducing sex-linked recessive lethal mutations on mature *Drosophila* spermatozoa at similar levels of survival of the treated flies.

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Activities of Pseudocholinesterases (Acylcholine-Acylhydrolase, EC 3.1.1.8) in *Macaca mulatta* (*rhesus*) and *Cercopithecus aethiops*

LEHMANN *et al.*[1,2] and Kalow *et al.*[3,4] first pointed out that the existence of some different pseudocholinesterase-variants in human serum is based on a genetical polymorphism. These investigations had been induced by the observation that some patients show an abnormally prolonged period of apnœa after injection of the muscle-relaxant succinyldicholine. In order to co-ordinate the results and hypotheses which have been published in recent years a 4-allele-model has recently been discussed[5-10]. Liddell *et al.*[11] demonstrated the presence of the same pseudocholinesterase variants which can be found in the sera of normal homozygous, heterozygous and atypical homozygous individuals, also in liver, kidney, brain, ileum and skin of man. Tashian and Shaw[12] were the first to show the heredity character of variants of human acetylcholinesterases. The same has been shown for various esterases of other species[13-16]. Cholinesterases with a different affinity from a number of cholinesters could be detected in the plasma of nearly all animals examined[17-21]. Except for human sera, those of other primates have not been tested to any extent. In our own experiments, and in contrast to the results of Kalow[22], the pseudocholinesterases of *Macaca mulatta* (*rhesus*) have quite different values from that of normal human serum in case of inhibition with dibucaine.

Examining the sera of *Macaca mulatta* (*rhesus*) and *Cercopithecus aethiops*, we obtained the following results: the protein-levels in the sera of *Macaca* and *Cercopithecus* were somewhat higher in comparison with human serum. The activity was measured with benzoylcholine as substrate in the optical test as described by Kalow[3]. The values of activity (and the specific activities) of *Macaca* have been found to be of the same order as that of man, while the sera of *Cercopithecus* show no or, at the most, very small activities. Table 1 shows the estimated activities, specific activities and protein values of 41 sera from *Macaca* and 14 sera from *Cercopithecus*. The enzyme proteins of pseudocholinesterase, which show nearly the same values of activity in the sera of *Homo* and *Macaca*, seem to be very different in protein structure: as demonstrated in Table 2 the inhibitor constants differ essentially (using dibucaine and sodium fluoride as inhibitors). In the case of human normal homozygotes (about 96 per cent of the population) an accumulation of DN about 80 and FN about 61 has been found[3,4,9,23-25]. In contrast

Table 1. ACTIVITIES, PROTEIN AND SPECIFIC ACTIVITIES OF PSEUDOCHOLINESTERASE IN THE SERUM OF *Macaca mulatta* (*rhesus*) AND *Cercopithecus aethiops*

	Macaca mulatta	*Cercopithecus aethiops*
Activity/ml. serum*	$\bar{x}$ = 6,008·0 s = 2,862·4 $s\bar{x}$ = 470·6	$\bar{x}$ = 157·1 s = 370·5 $s_{\bar{x}}$ = 60·9
mg protein/ml. serum	$\bar{x}$ = 86·8 s = 34·8 $s\bar{x}$ = 6·3	$\bar{x}$ = 96·0 s = 11·6 $s\bar{x}$ = 5·2
Specific activity (activity/mg protein)	$\bar{x}$ = 68·6 s = 25·7 $s\bar{x}$ = 4·6	$\bar{x}$ = 1·6 s = 2·2 $s\bar{x}$ = 0·6
n	41	14

* Decrease of extinction from the first to the third minute at 240 mμ; $t = 24°$ C; total volume in the cuvette 2·0 ml.; light pathway 1·0 cm; the serum was diluted 1 : 200; 5×10^{-5} M benzoylcholine as substrate; $6·6 \times 10^{-2}$ M phosphate buffer (pH = 7·4).

Table 2. INHIBITOR CONSTANTS (DIBUCAINE NUMBERS AND FLUORIDE NUMBERS) OF PSEUDOCHOLINESTERASES FROM *Macaca mulatta* COMPARED WITH THE VALUES IN MAN

	Macaca mulatta	*Homo*		
		Normal homozygote	Heterozygote	Atypical homozygote
Dibucaine No.	$\bar{x}$ = 98·4 s = 3·1 $s\bar{x}$ = 0·5	71–85	43–70	≤20
Fluoride No.	$\bar{x}$ = 3·0 s = 2·8 $s\bar{x}$ = 0·4	≥55	40–55	20–27
n	41·0	See refs. 3, 4, 9, 24, 25, 26		

to these values, dibucaine inhibits the transformation of benzoylcholine by serum of *Macaca* at equal concentrations at the rate of 97–100 per cent; sodium fluoride exerts only a very small inhibiting effect on the pseudocholinesterase of *Macaca*. In the agar-diffusion test[26] also (α-naphthylbutyrate as substrate), there are great differences in the behaviour of human serum and that of *Macaca* and *Cercopithecus*, on the other hand[27]. In view of the foregoing results, it cannot be decided whether there is a polymorphism of pseudocholinesterases in *Macaca* and *Cercopithecus*. Further investigations (probably using other substrates and more suitable inhibitors) may clarify this problem.

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VIROLOGY

Hæmagglutination Reaction with Vaccinia Virus: Agglutinability of Red Cells from Turkeys

Some authors[1–3] have reported that saline preparations of vaccinia virus, when mixed with suitable suspensions of red cells from certain adult fowls, caused hæmagglutination, which could be inhibited by antiserum to vaccinia. Only 50 per cent of the fowls were found to have cells agglutinable with vaccinia virus and the phenomenon appeared to operate independently of breed, sex or age.

Later work[4,5] indicated that red cells from birds of the Leghorn breed showed a higher incidence of agglutinability than did cells from other breeds.

Recent work[6] has confirmed the results of Clark and Nagler[2] in respect of both fowl and mammalian cells. An interesting finding of the work has been the observation that red cells from all turkeys were agglutinated by vaccinia virus.

Red cells (0·5 per cent saline suspension) from 108 'Broad Breasted Bronze' and 106 'Beltsville' turkeys were tested by titrating vaccinia antigen prepared from lymph from infected rabbits; cells from known positive and negative fowls served as controls. The tests were carried out in tubes using the technique of McCarthy[4]. All the turkeys except one had cells which showed complete (+ +) agglutination. This agglutination was proved to be specific, since it could be inhibited by antiserum to vaccinia. The results of the experiments are shown in Fig. 1.

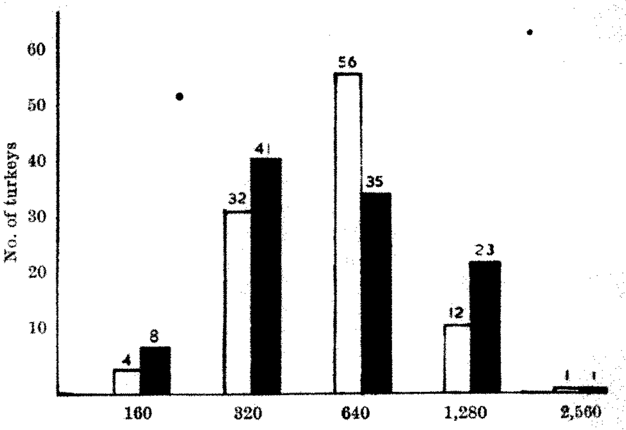

Fig. 1. Hæmagglutination titres (+ +) obtained with vaccinia virus against red cells from bronze (■) and white □ turkeys

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SOIL SCIENCE

Electro-osmosis in Frozen Soil

Water can be transported in unfrozen soil under an electrical gradient. This process, which is called electro-osmosis, is due to an excess of positive ions over negative ions in the water near a solid surface. The amount of water which can be transported by electro-osmosis is such that in some instances it has been used to stabilize soft clay embankments. The purpose of our experiment was to measure the extent to which water can be moved through the films of unfrozen water in a frozen soil.

Ice and adsorbed water can be in equilibrium in a soil at temperatures well below freezing. The adsorbed water in a frozen soil has been called unfrozen water. This term is used to indicate that the adsorbed unfrozen water in frozen soils is different from that of ice. For example, in sodium Wyoming bentonite at − 5° C as much as 0·8 g of unfrozen water per g of clay has been reported by Kolaian and Low[1], using a calorimetric method.

This experiment indicated that some water remains unfrozen, but it does not give information about the mobility of water and ions in the film of unfrozen water. Moreover, even if ions and water can migrate in the unfrozen film, the film might not be continuous through the soil matrix. Vershinin *et al.*[2] measured electrical a.c. conductance of frozen ground. Since the conductance of the frozen ground was higher than one would expect for dry soil or ice, their results suggested current flow in continuous films of unfrozen water.

When water freezes to ice most ions are expelled from the ice phase and remain in the water phase. As the salt concentration rises the ions are not incorporated in the ice lattice, but instead form brine pockets in the ice. The same principle applies to the water in soils. On freezing a soil, ice forms in the large pores or, in some instances, ice segregates as lenses. The exchangeable cations surrounding the soil particle are crowded in a film of unfrozen water on the surface. In this unfrozen film the conditions for electro-osmosis are satisfied; there is an excess of cations over anions near a solid surface.

The following describes our experimental method to investigate the occurrence of electro-osmosis in frozen soils. A rectangular slab of soil was frozen between two brass plates in a refrigerated room at −30° C. Freezing a soil rapidly at low temperatures has the advantage in that little water movement occurs during freezing. The frozen slabs of soil were cut to size and placed in 'Lucite' cells (1 cm × 5 cm × 5 cm). Electrodes (1 cm × 5 cm) were frozen to the sides of the sample. A thermistor was used to record the temperature, while an electrical potential gradient of 1 V/cm was applied across the frozen soil.

Table 1. INITIAL AND FINAL WATER CONTENTS (WEIGHT OF WATER PER WEIGHT OF CLAY × 100 PER CENT) AFTER THE FROZEN SAMPLE WAS EXPOSED TO AN ELECTRICAL GRADIENT OF 1 V/CM FOR 24 H

Soil	Temperature (° C)	Initial water content	Final water content Within 1 cm from anode	Within 1 cm from cathode
Wyoming bentonite	−2·0° C	341	275	456
	−1·5° C	265	134	310
New Hampshire silt	−1·5° C	30	27	32
	−1·0° C	28	21	40

Water was transported in the frozen soil towards the cathode. The initial and final water contents (unfrozen water and ice) of two soils after electro-osmosis are presented in Table 1. Initially, the film of unfrozen water is in equilibrium with ice in the sample, but when the unfrozen water is transported from the anode regions, the film of unfrozen water is depleted. It is replenished by the melting of ice in that region. In the final state all ice is removed from the anode region and large bodies of ice are formed in the vicinity of the cathode. Shrinkage cracks form at the anode.

The experimental results show that a considerable amount of unfrozen water can be transported in frozen soil under an electrical gradient. For unfrozen soils the migration of water under electrical and temperature gradients has been related. One expects this also to be valid for frozen soils. The amount and mobility of unfrozen water are such that they can be of practical significance in redistributing moisture under temperature gradients in perennial frozen ground.

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Silcretes of Central Australia

THE widespread occurrence in central Australia of silcrete which there caps numerous mesas and forms extensive stony pavements on the dissection slopes below them has long awaited a satisfactory explanation. A recent recording[1] of the nature and disposition of certain lateritic and desertic soils, taken in conjunction with the location of major drainage divides and the pattern of endoreic streams to the Lake Eyre depression[2], throws new light on the problem and indicates the following sequence of pedologic and geomorphic events : (1) A regional development of Lateritic Red Earths occurred across what is now the watershed of eastern and northern Queensland. The lateritic weathering was accompanied by the usual loss of silica in the drainage water, one part going to the Pacific Ocean and the Gulf of Carpentaria and the other to the precursors of the present-day ephemeral and endoreic streams, the Georgina, Diamantina, Barcoo Rivers and Cooper's Creek, the courses of which then extended up to 200 miles further south-west. (2) The silica which moved south-westerly was deposited in a large array of rocks and softer deposits of widely different chronology, including variably weathered sandstones, shales and quartzites, and calcareous, gypseous, saline and alunitic formations. To-day these materials are to be found beneath the silcrete caps of the mesas, and the later group at least indicate aridity and/or restricted drainage at the time of their deposition. (3) The large, gently sloping silcrete surface so formed was disrupted and eroded following the development of the down-warped Lake Eyre basin. This depression and its compensating upwarp to the west and south caused differences in elevation of the order of 1,000 ft. with the lowest point below sea-level. This provided a new, internal, and very low base level for erosion and caused rejuvenation and down-cutting of the streams coming from the north-east and reversal of the drainage in the silcrete area to the west and south of the newly formed depression. The combination of earth movement and erosion caused the break-up of the extensive silicified surface into numerous mesas and associated larger areas of fragmented silcrete represented to-day by the pavement on the Stony Desert Tableland Soils.

There thus appears to be in Australia a continental example of absolute accumulation of silica. The distant edge of the silcrete area is about 900 miles from the original source, that is, the Lateric Red Earth tableland from which the Great Dividing Range has also since been sculptured. So far as is known, this genetic linking of the two now dismembered landscapes with once active processes of release and deposition of silica is on a uniquely large scale.

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PSYCHOLOGY

Stereoscopic Shadow-images

THE following simple arrangement makes it possible to project, in stereoscopic depth, three-dimensional objects such as wire models of molecular or crystal structures. Small models may be presented enlarged in three dimensions, magnifications of ten or more times being possible. The optical arrangement consists of nothing but a pair of small bright light sources, separated horizontally by a few inches. The sources are placed behind 'Polaroid filters', set at orientations differing by 90°. The point polarized sources give a pair of shadow images of an object, such as a wire model, placed between them and a silver screen. Alternatively, back-projection with a ground-glass screen can be used; but the screen must not de-polarize the light. When the shadows are viewed through crossed 'Polaroid' glasses, they are fused by the brain to form a single stereoscopic shadow-image lying in space.

There is no problem in explaining this effect. The point sources cast shadows on the screen which are flat projections from slightly different positions. The shadows have the disparity of retinal images for eyes placed at the sources. The observer's brain fuses the two disparate shadows into a single stereoscopic shadow, looking incredibly like a real, but jet black, object. It lies in space either in front of or behind the screen, depending on which eye accepts which shadow. By using polaroids oriented at 45° from horizontal, the stereo shadow-image may be placed before or behind the screen simply by reversing the spectacles.

Suitable light sources are miniature filament, 12-V, 100-W tungsten-iodine lamps. The filaments should be oriented vertically, to give maximum horizontal resolution, which is important for giving maximum information of depth through disparity.

Magnification of the normal x- and y-axes is given simply by the ratio of the distance of the object from the lights to the object from the screen. Magnification in depth (z-axis) can be controlled by the horizontal separation of the lamps. In practice a magnification of at least times 10 may be given; but the apparent size of the three-dimensional shadow-image is generally less than might be expected, because of perceptual size constancy.

When the object appears to be in space before the screen it looks smaller than when behind it, though the physical size is of course identical with either orientation of the polaroids. A further perceptual effect is most marked: when the observer moves his head, the stereo shadow-image appears to move with him, to slide across the screen, and rotate to follow the observer as though continuously aimed at him. This is because there is no motion parallax although the image lies perceptually in three dimensions: this corresponds to a normal object rotating to keep the same aspect to the observer though he moves. So he sees movement though his retinal images remain unchanged. When the observer moves away from the screen, the shadow-object does not shrink or lose its depth as might be expected; the reduced angle of convergence of the eyes evidently re-scales the retinal disparity mechanism, so that a given disparity between the retinal images gives greater visual depth. This is indeed fortunate, for the shadow-image appears very similar over a wide range of distance, and so it can be used with effect in a large lecture hall for demonstrating suitable objects. They may be moved or touched by the demonstrator, which makes this a technique having advantages over photographic stereo projection. It is also a useful tool for investigating perception of depth while the observer is moving, and can form the basis of simulators for experiments on the guiding of aircraft and space vehicles.

This technique was devised for experiments supported by U.S. Air Force grant *AF-EOAR* 63–93, monitored by the European Office, Office of Aerospace Research.

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Retroactive Interference in Short-term Memory

In investigating short-term memory (a term generally used to cover the period of from one to sixty seconds) an important question is whether or not retention is influenced by factors known to be important in long-term memory. One such factor is retroactive interference. Broadbent[1] has asserted that retention depends on the duration of the delay rather than the nature of any activity during the delay. But he rests his case on evidence that retention is impaired by increasing the delay while keeping the activity as constant as possible. Direct evidence that variation of the intervening activity is ineffective has been lacking, but Baddeley and Dale[2] have shown that in minimal paired-associate learning[3] meaningful similarity fails to affect performance significantly, and this supports Broadbent's contention.

Recently Wickelgren[4] has produced evidence suggesting that acoustic similarity does affect retroactive interference in short-term memory. His technique was to present rapidly (2/sec rate) four letters which had to be remembered, then eight which were to be copied down. Immediately the eight had been copied the subjects had to write out the first four from memory. The crucial variable was the similarity of the eight letters which had to be copied to the four which were being remembered. The letters used were taken from two groups *F L M H S X* and *B C D G P T V* (*Z*). Conrad[5] has shown that within each group acoustic confusions are common, whereas between the groups they are rare. (*Z* does not confuse with *B C*, etc., in English; but with its American pronunciation it is obviously similar to the others and Wickelgren added it.) Wickelgren's results indicate that when the letters to be copied came from the same group as those being remembered, memory was poorer than when they came from the other group. They are not clear-cut, however, since he had to introduce repetition. To illustrate this: if *B C D G* were to be remembered and similar letters had to be copied, the only four letters available were *P T V Z*, so subjects were required to write each twice. With the '*F*' sub-group the problem was even more acute since there were only two spare letters.

The experiment reported here was carried out in order to confirm Wickelgren's findings. To avoid the problems already outlined here the subjects were given three letters to remember and six to copy down. The six always consisted of three different letters each repeated once. For this experiment the '*B*' group was pruned to *B C D G P V*. Forty trials were run, 10 with '*F*' letters to be remembered and '*F*' letters copied; 10 with '*F*' letters to be remembered and '*B*' letters copied; 10 with '*B*' letters to be remembered and '*F*' letters copied; and 10 with '*B*' to be remembered and '*B*' copied. Two series of trials were used. The first was prepared by randomizing the order of the 40 trials. The second was derived from the first by keeping the messages to be remembered constant and altering the messages to be copied, so changing *F–F* to *F–B*, *F–B* to *F–F* and so on. Any influence of order of presentation was in this way controlled. All items were read out by the experimenter in time with a metronome beating at a 2/sec rate. A pause of one beat separated the memory message from the copy message. Ten seconds separated the end of one copy message from the beginning of the next memory message. Subjects were tested in two groups of 17. They wrote out all messages on a specially prepared form. An invigilator checked that they put down the copy message before the memory one.

Performance was scored by counting the number of letters in each memory message which were correctly recalled in both substance and position. The results showed a mean score of 59·4 per cent when the copied message came from a different group of letters to the memory message but only 38·7 per cent when the copied message came from the same group. The individual records of 31 of the 34 showed this effect; its statistical significance is, therefore, extremely high ($P < 0{\cdot}001$). Thus Wickelgren's observation is confirmed and we must conclude that the principle of retroactive interference does apply to short-term memory. The form of the similarity which affects performance, however, would seem to differ from that which influences interference in long-term memory. Meaningful similarity is unimportant over these short periods, but similar-sounding items cause interference. Presumably the basis of coding for normal verbal learning with repeated presentations and with opportunity for rehearsal differs from that used for short-term storage following a single presentation.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Tuesday, September 29

Linnean Society of London, Palæobotany Group (at Burlington House, Piccadilly, London, W.1), at 3.15 p.m.—Scientific Papers.

University of London (in the Wright-Fleming Institute Theatre, St. Mary's Hospital Medical School, Paddington, London, W.2), at 5 p.m.—Prof. R. D. Wright (University of Melbourne): "Actions and Interactions of Aldosterone".*

Institution of Mechanical Engineers, Applied Mechanics Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "What Data do Designers Need in the Applied Mechanics Field ?"

Society of Chemical Industry, Oils and Fats Group (at the Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 6 p.m.—Prof. W. O. Lundberg: "Some Recent Developments in Fat Nutrition".

Tuesday, September 29—Wednesday, September 30

Cement and Concrete Association (at the Royal Institute of British Architects, London, W.1)—Symposium on "Lightweight Concrete in Building".

Tuesday, September 29—Thursday, October 1

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2)—Conference on "Lasers and Their Applications".

Wednesday, September 30

Institution of Electronic and Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. J. R. Blakey: "Near Field Measurements and the Determination of Aerial Patterns".

Television Society (joint meeting with the British Kinematograph Society, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 7 p.m.—Mr. G. H. Askew, Mr. D. Boston, Mr. P. Lowry and Mr. T. C. Nuttall: "A New 16 mm. Flying Spot Colour Telecine Machine".

Thursday, October 1

Royal Society of Medicine, United Services Section (at 1 Wimpole Street, London, W.1), at 5 p.m.—Surgeon Cdr. A. F. Davidson: "Studies of the Principles of Flotation by Life Jackets"; Surgeon Cdr. D. C. McNutt: "Protection from Cold in Air and Water".

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Use of Chains in Handling Machinery".

Television Society (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. D. J. Basinger: "Microphones and Sound Effects for Television".

Royal Society of Medicine, Neurology Section (at 1 Wimpole Street, London, W.1), at 8 p.m.—Prof. J. N. Cumings: "Some Lipid Diseases of the Brain" (Presidential Address).

Thursday, October 1—Friday, October 2

Eugenics Society (in the Botany Lecture Theatre, University College, Gower Street, London, W.C.1), at 10.30 a.m. daily—Symposium on "Biological Aspects of Social Problems".

Friday, October 2

The Ciba Foundation for the Promotion of International Co-operation in Medical and Chemical Research (in the Institute of Physiology, The University, Glasgow), at 5 p.m.—Dr. Donald M. Watkin: "The Impact of Nutrition on the Biochemistry of Ageing in Man" (8th (Annual) Ciba Foundation Lecture).*

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Should Basic Craft Training be done at a Technical College or at a Works ?"

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. R. S. Cahn: "The Future of Chemical Publications".

Saturday, October 3

Nutrition Society (at the London Hospital, Whitechapel, London, E.1), at 10.30 a.m.—Symposium on "Availability of Minerals in Foods of Plant Origin".

Monday, October 5

Institution of Electronic and Radio Engineers, Joint I.E.R.E.-I.E.E. Medical Electronics Group (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m.—Discussion on "Transistorized Biological Amplifiers".

British Society for the Philosophy of Science (at University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. R. Harre: "The Limits of Formalization".

Society of Chemical Industry, London Section (at Shell Centre Theatre, Shell Centre, London, S.E.1), at 6.30 p.m.—Scientific Film Evening.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Students (2) (with a good honours degree) **in the Department of Physiology and Pharmacology**—Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (October 1).

Assistant (preferably with experience in a medical or scientific library or publishing) for the *British Medical Bulletin*, for duties which will include detailed checking and preparation of material for publication in the bulletin—The Recruitment Division, The British Council, 65 Davies Street, London, W.1 (October 2).

Assistant Lecturer or **Lecturer in Textiles and Clothing in the Department of Domestic Science**, University College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 8).

Assistant Lecturer in the Chemistry Department—The Secretary, Royal Free Hospital School of Medicine (University of London), Hunter Street, London, W.C.1 (October 9).

Assistant (with an interest in applied mechanics and/or materials science, a good honours degree in engineering or in an appropriate pure or applied science, and postgraduate experience) **in Mechanical Engineering**—The Secretary of University Court, The University, Glasgow (October 10).

Lecturer or **Assistant Lecturer** (preferably with an interest in petrology) **in Geology**, at Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee (October 10).

Chair of Mechanical Engineering in the Faculty of Engineering, University of Malaya, Kuala Lumpur—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, October 15).

Lecturer in Geology and Pedology—The Secretary, Wye College (University of London), Near Ashford, Kent (October 17).

Chair of Anatomy—The Registrar, The University, Leeds, 2 (October 19).

Lecturer in the Department of Mechanical Engineering, University of Cape Town—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (London and Cape Town, October 19).

Reader in Agricultural Biology at Makerere University College, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 19).

Chair of Applied Mathematics at Bedford College—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 22).

Associate Professor of Physiology at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 23).

Lecturer or **Assistant Lecturer** (with a degree in electrical engineering, and an interest or postgraduate experience in the field of electronics, preferably microwaves or semi-conductors) **in the Department of Electrical Engineering**, University of Melbourne, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 24).

Professor of Organic Chemistry—The Registrar (Room 22, O.R.B.), The University, Reading (October 24).

Lecturer/Senior Lecturer in Agricultural Entomology at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 26).

Chair of Pathology at the University of Tasmania, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 30).

Lecturer in Physics; and a **Senior Lecturer in Physics** at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 30).

Lecturer (with a university degree in chemical engineering, plus five years' approved industrial experience) **in Chemical Engineering**—The Director, The Swinburne Technical College, John Street, Hawthorn, E.2, Victoria, Australia (October 31).

Chair of Horticulture at Lincoln College, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 14).

Post-doctoral Fellows (2) (physical chemists or physicists) to carry out research on atomic reactions—The Registrar, University of Canterbury, Private Bag, Christchurch, New Zealand (November 30).

Plant Pathologist (national of the United Kingdom or the Republic of Ireland, with an honours degree in natural science or agricultural botany and at least two years' experience in plant pathology) in Tanganyika, for duties at the Coffee Research Station, largely in connexion with the breeding programme for rust resistance—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/173/08.

Post-doctoral Research Fellow in Protein Physical Chemistry—Dr. C. M. Kay, Department of Biochemistry, University of Alberta, Edmonton, Canada.

Research Student (with a first- or second-class honours degree of a British university in physics or metallurgy) **in the Metal Physics Group**, for work on the slip and twinning modes of solid mercury, and will be expected to register for a Ph.D. degree of the University of London—Head of the Physics Department, Battersea College of Technology, Battersea Park Road, London, S.W.11.

Research Chemist (with a good honours degree and several years' research experience, and preferably postgraduate qualifications), to take charge of a laboratory investigating the physical and chemical properties of egg in relation to baking quality—The Secretary, British Baking Industries Research Association, Chorleywood, Rickmansworth, Herts.

Research Student (chemist, physicist or chemical engineer) **in the Department of Chemical Engineering**, for work leading to a higher degree, on the oxidation of free radicals in combustion reactions using a specially designed mass spectrometer—The Secretary, University College, Gower Street, London, W.C.1.

Research Students in Organic and Organometallic Chemistry—The Professor of Chemistry, University of Sussex, Brighton, Sussex.

Senior Pharmacist—The Secretary, Royal Eye Hospital, St. George's Circus, London, S.E.1.

Senior Technician for duties which will include lecture demonstrations and the organization of laboratory classes for students (previous experience in such work essential)—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1.

Silvicultural Ecologist (national of the United Kingdom or the Republic of Ireland, with a degree in forestry, at least ten years' field experience and a thorough knowledge of silviculture in tropical evergreen rain forest, preferably in South East Asia), in Malaya, to plan a system of silvicultural management for Hill Dipterocarp forests—The Appointments Officer, Department of Technical Co-operation, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 324/105/01.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletin of the British Museum (Natural History). Entomology. Vol. 15, No. 3: Notes on the Epitolinae, Part 2. By T. H. E. Jackson. Pp. 57–80 + 8 plates. 22*s*. Vol. 15, No. 4: On the Diptera of Nepal (Stratiomyidae, Therevidae and Dolichopidae). By David Hollis. Pp. 81–116. 13*s*. Vol. 15, No. 5: New Species of *Ugyops* (Fulgoroidea: Delphacidae) from South America and South-East Asia. By R. G. Fennah. Pp. 117–132. 10*s*. (London: British Museum (Natural History), 1964.) [148

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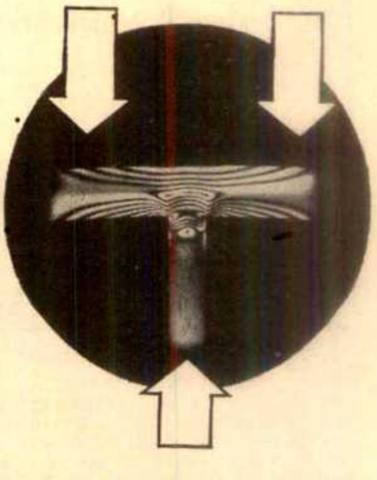

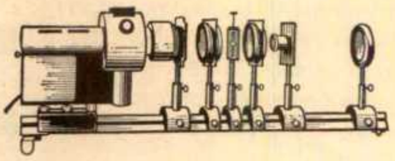

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